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BY
LAURA SINI SOFIA HULKKO

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AALBORG UNIVERSITY
DENMARK

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by

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Preface

This thesis was submitted in accordance with the requirement for attaining a PhD degree at the Faculty of Engineering and Science, Aalborg University. I was affiliated with AAU Energy, and the work was carried out from July 2020 to July 2023 as a part of the AQUACOMBINE project. This project received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 862834. Any results of this project reflect only this consortium's view, and the European Commission is not responsible for any use that may be made of the information it contains.

The research was conducted mainly on the Aalborg University campus in Esbjerg. During the project period, I spent some time in the Centre of Marine Sciences of the University of Algarve in Faro, Portugal, for three months during spring 2022 and one additional month in February 2023. This interdisciplinary PhD project includes the elements of process engineering with a focus on green biorefinery systems and bioprospecting, which are complimented with elements from the fields of applied microbiology, botany, pharmacology, and phytochemistry.

First and foremost, I want to thank my supervisor Prof. Mette Hedegaard Thomsen, for her valuable support, mentoring, and advice. Thank you for giving me the opportunity to be a part of such a great project and trusting me to follow my instincts and interests throughout these three years. I also want to thank my co-supervisor Associate Prof. Tanmay Chaturvedi, who has been a great support in navigating the academic world and helping me to stay positive when things have not gone as planned.

I want to express my gratitude to Dr. Luísa Custodio for welcoming me to her laboratory in Portugal, as well as Dr. Catarina Guerreiro Pereira for guiding me through the work with cell lines and Riccardo Trentin and José Paulo Da Silva for technical assistance. With their support, I was able to carry out experiments outside of my field of expertise I never thought I could do.

I am grateful that I got a chance to collaborate with AQUACOMBINE project partners, who are true experts in their respective fields. Most sincere thanks to Julaine, Lilian, and Birgit, as without their technical knowledge and assistance, many experiments would have been hard to carry through. To my fantastic colleagues at the Bioenergy and Bioproducts research group, thank you for creating such a warm, friendly, positively challenging, and supportive working environment where I had a chance to grow. Evelyne Kabemba Kaniki and Henrik Tribler, thank you for seeing the value in my work and giving me an opportunity to keep going.

Last but not least, my deepest gratitude goes to my partner, family, and friends for their endless support. They always believed in me and encouraged me through the moments when I doubted myself and was my own biggest enemy. Thank you.

Laura Sini Sofia Hulkko
September 2023 in Esbjerg, Denmark

Summary

> "*It's surely our responsibility to do everything within our power to create a planet that provides a home not just for us, but for all life on Earth*" (Sir David Attenborough, 2016).

As a part of the response to challenges related to climate change, it is necessary to transition from fossil-based linear economies towards circular bioeconomies. This requires the development of technologies to use the world's biomass resources efficiently, minimising waste production. However, another challenge, soil and water salinisation, is also becoming more prevalent worldwide. Indeed, we are required to enhance the use of biomass, and, at the same time, soils are becoming toxic for the majority of plants to sustain their growth and accessible freshwater resources are depleting. This is happening at an increasing rate due to salinisation and the higher occurrence of droughts and heat waves. Only approximately 1 % of the world's known flora are halophytes, naturally salt-tolerant plants that thrive in saline environments, and harnessing these plants for agricultural use has been suggested as one of the key implementations to face the upcoming challenges.

In this explorative PhD project, halophyte species suggested for biosaline agriculture, namely, *Crithmum maritimum*, *Salicornia europaea*, *Salicornia ramosissima*, and *Tripolium pannonicum*, were evaluated for their potential towards green biorefinery applications. In green biorefineries, which have been developed to produce feed protein from grass biomass and agro-residues, biomass is fractionated into green juice and fibres, and these fractions are processed separately. In this project, the effect of the cultivation salinity on the fractionation performance and distribution of primary metabolites in halophytes was assessed, and significant effects were observed.

After fractionation with a screw press, the protein precipitation from saline halophyte green juice was investigated using methods established for forage-based green biorefineries: heat coagulation, acidification, and lactic acid fermentation. Afterwards, the production of protein-enriched concentrate by lactic acid fermentation of *S. ramosissima* juice with probiotic bacterial strain was studied further in a bioreactor setting. The use of halophytes for the production of functional animal feed supplements could help to diversify the source of protein and decrease the dependency on imported feed by providing a local source, especially in the arid and semi-arid regions, where the cultivation of forages and crops is limited.

Halophytes produce high concentrations of bioactive secondary metabolites as their response to environmental stress, such as high salinity, UV radiation, extreme temperatures, drought, and waterlogging. These compounds, also known as phytochemicals, have various biological activities and health benefits, which have made them interesting for biopharmaceuticals, nutraceuticals, food additives, and cosmetic applications. Therefore, bioactive compounds found in halophytes were reviewed. Phytochemicals can be extracted from the fibre residue, and the extracts obtained from the screw-pressed halophyte fibres were analysed for their content of

phenolic compounds using different absorption spectroscopy assays and chromatographic methods. Bioprospecting towards high-value bioproducts was done by analysing the extract for their *in vitro* antioxidant properties, cytotoxicity, and inhibition activity towards enzymes linked to neurodegenerative diseases, metabolic diseases, and hyperpigmentation. Targeting products for these sectors can improve integrated biorefinery economics, provide maximum feedstock valorisation in a cascading process, and contribute towards a sustainable circular bioeconomy.

Extractives-free halophyte biomass, consisting of relatively pure lignocellulose, was subject to hydrothermal pretreatment and enzymatic hydrolysis. The enzymatic convertibility was tested in order to estimate the pretreatment severity needed to break the lignocellulosic structure for potential biochemical or bioenergy production.

Overall, this PhD project provided a comprehensive general view of the potential processes and target products of halophyte-based green biorefinery, and the results can be used as a basis for further investigations. The interdisciplinary project also contributes to closing the gap between process engineering and other related scientific disciplines, which must be done to develop efficient and meaningful biomass processing systems and bring the green transition forward.

Resumé

I lyset af de udfordringer, der er forbundet med klimaforandringer, er det afgørende at foretage en omstilling fra en lineær, petroleum-baseret økonomi til en cirkulær økonomi baseret på biomasse. Dette kræver udvikling af avancerede teknologier, der kan udnytte verdens biomasseresourcer på en effektiv måde, samtidig med at affaldsproduktionen minimeres. En anden presserende problemstilling, der påvirker hele verden, er tilsaltning af jord og vand. Det er derfor nødvendigt at øge udnyttelsen af biomasse, samtidig med at dyrkningsjorden bliver tilsaltet og dermed uegnet til dyrkning af de fleste almindelige afgrøder. Desuden svinder kilderne til rent ferskvand ind med øgende hastighed, både på grund af tilsaltning og en stigning i forekomsten af tørke og hedebølger. Kun omkring 1 % af verdens flora består af halofytter, som er naturligt salttolerante planter, der trives i salte miljøer. Anvendelsen af disse planter som afgrøder er blevet foreslået som en del af løsningen på de fremtidige udfordringer.

I dette ph.d.-forskningsprojekt evalueres halofytarterne *Crithmum maritimum*, *Salicornia europaea*, *Salicornia ramosissima* og *Tripolium pannonicum* i tilsaltet landbrug med henblik på deres anvendelsesmuligheder i grønne bioraffinaderier. Grønne bioraffinaderier er designet til at producere proteinholdigt foder fra græsser og restprodukter fra landbruget. Biomassen opdeles i to fraktioner - juice og fibre - som processeres separat. I dette projekt blev kvaliteten af fraktioneringen og fordelingen af primære metabolitter undersøgt i biomasse dyrket under varierende grader af saltmættelse.

Efter fraktionering ved hjælp af skruepresse blev proteinudfældningen fra den salte, grønne halofytjuice undersøgt ved hjælp af metoder, der er etableret for græsbaserede bioraffinaderier, herunder varmekoagulering, forsuring og mælkesyrebakterie fermentering. Der blev foretaget yderligere undersøgelser af produktionen af proteinberiget koncentrat fra mælkesyrefermentering af *S. ramosissima* juice ved hjælp af en probiotisk bakteriestamme i en bioreaktor. Anvendelsen af halofyter til produktion af funktionelle fodertilskud kan potentielt bidrage til at diversificere proteinkilder og reducere behovet for importeret foder, idet halofyter kan dyrkes lokalt. Dette er særligt relevant i tørre og tørkeramte områder, hvor dyrkningsmulighederne for græsningsområder og konventionelle afgrøder er begrænsede.

Halofyter producerer høje koncentrationer af bioaktive sekundære metabolitter som respons på miljømæssige stressfaktorer såsom høj salinitet, UV-stråling, ekstreme temperaturer, tørke og vådlægning. Disse metabolitter, også kendt som fytokemikalier, har adskillige biologiske funktioner og sundhedsfordele, hvilket gør dem interessante som lægemidler, kosttilskud og tilsætningsstoffer i fødevarer, foder og kosmetik. De bioaktive stoffer i halofyter er derfor blevet undersøgt. Fytokemikalier kan ekstraheres fra overskydende fibre. Ekstrakter udvundet fra skruepressede halofytfibre blev analyseret for deres indhold af fenoler ved hjælp af forskellige absorptionsspektroskopiske assays og kromatografiske metoder. Der blev foretaget en søgen efter bioaktive højværdiprodukter ved at analysere ekstrakternes

in vitro antioxidantegenskaber, deres cellegiftighed og hæmning af enzymer relateret til neurodegenerative sygdomme, fordøjelseslidelser og hyperpigmentering. Målrettet ekstrahering af stoffer, der kan være til gavn for disse sektorer, kan øge det økonomiske incitament for integrerede bioraffinaderier, maksimere værdien af biomassen og bidrage til en bæredygtig cirkulær bioøkonomi.

Ekstraktiv-fri halofytbiomasse, bestående af relativt ren lignocellulose, blev udsat for hydrotermisk forbehandling og enzymatisk hydrolyse. Den nødvendige intensitet af forbehandlingen for at nedbryde lignocellulosestrukturen til potentiel anvendelse i produktionen af bioenergi eller biokemiske produkter blev estimeret ved hjælp af konverteringsgraden efter enzymatisk hydrolyse.

Overordnet set har dette PhD-projekt givet et dybdegående og generaliseret indblik i de potentielle processer og produkter, der kan produceres i det halofytbaserede bioraffinaderi, og resultaterne af projektet vil fungere som et springbræt for videre forskning på området. Dette interdisciplinære projekt bidrager også til at bygge bro mellem processteknik og andre relaterede videnskabelige discipliner, hvilket er en nødvendighed for at udvikle effektive og betydningsfulde systemer til biomasseudnyttelse og fremme af den grønne omstilling.

Thesis Details

The main body of this thesis is based on the following research articles:

I. Hulkko, L.S.S., Turcios, A., Kohnen, S. *et al.* Cultivation and characterisation of *Salicornia europaea*, *Tripolium pannonicum* and *Crithmum maritimum* biomass for green biorefinery applications. *Sci Rep* **12**, 20507 (2022).

II. Hulkko, L.S.S.; Chaturvedi, T.; Thomsen, M.H. Extraction and Quantification of Chlorophylls, Carotenoids, Phenolic Compounds, and Vitamins from Halophyte Biomasses. *Appl. Sci.* **2022**, *12*, 840.

III. Hulkko, L.S.S.; Rocha, R.M.; Trentin, R. *et al.* Bioactive Extracts from *Salicornia ramosissima* J. Woods Biorefinery as a Source of Ingredients for High-Value Industries. *Plants* **2023**, *12*, 1251.

IV. Hulkko, L.S.S.; Chaturvedi, T.; Custódio, L.; Thomsen, M.H. Harnessing the value of *Tripolium pannonicum* and *Crithmum maritimum* halophyte biomass through green biorefinery. *Mar. Drugs* **2023**, *21*(7), 380.

V. Hulkko, L.S.S.; Chaturvedi, T.; Thomsen, M.H. Valorisation of Residual Biomass Fractions from Biosaline Agriculture Through Green Biorefinery. *Submitted manuscript.*

The thesis based on the listed papers has been submitted for assessment for partial fulfilment of the PhD degree. The scientific papers are included directly or indirectly in the dissertation, and readers are referred to them for more details. As part of the assessment, co-author statements have been made available to the assessment committee and are also available to the faculty.

In addition, the following contributions in scientific papers and conferences were made during the PhD project period; however, they are not presented as a part of the dissertation:

- Giordano, R.; Saii, Z.; Fredsgaard, M.; Hulkko, L.S.S.; Poulsen, T.B.G.; Thomsen, M.E.; Henneberg, N.; Zucolotto, S.M.; Arendt-Nielsen, L.; Papenbrock, J.; Thomsen, M.H.; Stensballe, A. Pharmacological Insights into Halophyte Bioactive Extract Action on Anti-Inflammatory, Pain Relief and Antibiotics-Type Mechanisms. *Molecules* **2021**, *26*, 3140.

- Chaturvedi, T.; Hulkko, L.S.S.; Fredsgaard, M.; Thomsen, M.H. Extraction, Isolation, and Purification of Value-Added Chemicals from Lignocellulosic Biomass. *Processes* **2022**, *10*, 1752.

- Cybulska, I.; Brudecki, G. P.; Brown, J. J.; Hulkko, L. S. S.; Al Hosani, S.; Thomsen, M. H. Comparative study of chemical composition of the halophyte species native to the Persian (Arabian) gulf. *BioResources* **2021** *16(3)*, 5524-5537.

- Hulkko, L.S.S.; Chaturvedi, T.; Turcios, A.; Papenbrock, J.; Thomsen, M.H. Halophyte-based green biorefinery: potential biomass feedstocks and processing routes. Oral presentation at 30th European Biomass Conference and Exhibition. Online in 10 May 2022.

- Hulkko, L.S.S.; Chaturvedi, T.; Custódio, L.; Thomsen, M.H. Bioactive extracts from *Salicornia ramosissima*, *Tripolium pannonicum* and *Crithmum maritimum* as value-added products in halophyte-based biorefinery. Poster presentation at 18th International Conference on Renewable Resources & Biorefineries. 1-3 June 2022 in Bruges, Belgium.

- Hulkko, L.S.S.; Chaturvedi, T.; Thomsen, M.H. Valorisation of *Salicornia* waste biomass through green biorefinery. Poster presentation at 9th International Symposium on Energy from Biomass and Waste. 21-23 November 2022 in Venice, Italy.

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Chapter 1. General Introduction

1.1. Background and relevance of the project

1.1.1. Green transition and circular bioeconomy

Climate change and biodiversity loss are modern humankind's greatest challenges and threats. According to the Intergovernmental Panel on Climate Change (IPCC) report, to achieve the goals set by the Paris Agreement and limit global warming to 1.5 °C above pre-industrial levels, society has to aim for net-zero CO_2 emissions by 2050 [1,2]. This green transition requires significant changes in global production and consumption systems and actions, and to enforce the change, new legislations have been initiated, e.g., the European Green Deal in the European Union (EU) with several national policies, and the Green New Deal in the United States [3–5]. One of the key implementations is to transition from a linear fossil-based economy towards circular bioeconomies. Concerns about food security of the world's increasing population and the need for bio-based products and bioenergy are the key drivers leading to an increased demand for biomass, which consequently requires sustainable agricultural intensification to meet [6–8]. In order to ensure the efficient use of biomass resources, the development of circular bioeconomies should lay on the following key principles [6,9–12]:

- Sustainable resources (e.g., use of waste, residues and circular feedstocks)
- Integrated biorefineries and cascading processes
- Maximising feedstock valorisation and minimizing waste production
- Positive sustainability, environmental, and social aspects.

Circular bioeconomies should also avoid the depletion of resources, such as agricultural land and freshwater, and aim to apply regenerative practises, such as bioremediation [10]. According to the report by the International Energy Agency (IEA) from 2022, the highest number of biorefineries was found in the United States, followed by France, Germany, China, and the Netherlands [5]. Out of the analysed biorefineries, 50.3 % used primary biomass (e.g., starch crops, oil cops and lignocellulose from grasslands and forestry) in their processes, whereas secondary biomass (e.g., agricultural and forestry residues) was used by 27.7 %, and only 2.4 % of the biorefineries reported to use multiple feedstocks [5]. Besides, 61.4 % of the analysed biorefineries reported bioenergy as their main product type, followed by biomaterials (24.3 %) and biochemicals (10.6 %) [5]. Therefore, there is room for improvement for the biorefinery sector to meet the principles of sustainable bioeconomies regarding the source of biomass, conversion methods, and target products. According to Muscat et al. [10], the target products of focus need to be shifted from bioenergy production by encouraging the development of bioproducts. In integrated biorefinery systems, a multitude of products, as well as bioenergy, are produced from the used feedstock to maximise the material valorisation and minimise the waste stream. Even though optimisation is required to find the most suitable processing routes and target compounds for each type of biomass, these multi-product

systems often help to improve process economy and biorefinery competitiveness, which is often a challenge in single-product systems, and they are considered the most robust option for the future and an important factor for sustainable biomass use and circularity [9,11,13–15]. Oftentimes, products of high-value applications, such as pharmaceuticals, food and feed additives, and fine materials, are suggested as value-added products for biorefineries to increase process profitability [6,9,15,16], cascading based on the bioproduct value pyramid (**Figure 1**).

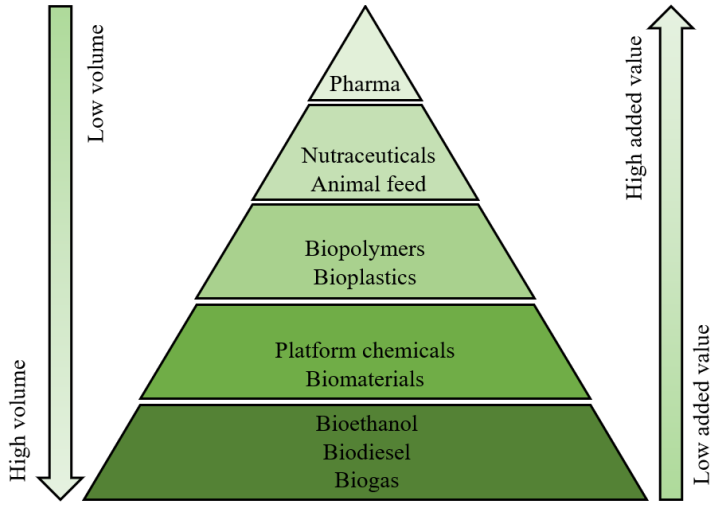

Figure 1 Value pyramid of bioproducts presented in Paper IV [17]. The original illustration was adapted from Stegmann et al. [9] and Zabaniotou [6].

High-value bioproducts have significant potential within circular bioeconomies [9]. Indeed, there is an urgent need to find alternatives to the variety of products and goods currently obtained from petrochemical sources. However, with the increased demand and limited biomass resources comes prioritisation. Muscat et al. [10] argued that the highest priority should be for bioproducts fulfilling basic human needs, such as nutrition and well-being, and sectors with limited sustainable alternatives, such as biochemicals, in order to use the limited biomass resources effectively. Hence, screening new biorefinery feedstocks for their potential for these applications could be seen as an inseparable part of integrated biorefinery design [17].

Bioprospecting, meaning the search for biological resources with economic potential, is becoming increasingly important as the green transition moves forward. The process can be driven by finding the best potential use for available feedstock, such as agro-residue, or by the demand for a certain type of product or application (**Figure 2**). Both approaches are of high importance in finding alternative sources for day-to-day goods. Currently, large amounts of valuable biomass, especially agro-residues and residues from food processing, end up in the production of bulk chemicals, bioenergy, compost, or even in landfills [18–21]. Manhongo et al. [21] reviewed the waste production from fruit processing and showed that up to 80 % of the fresh fruit

weight could en up to residue streams. Intensive research has been carried out in recent years to valorise these residual streams from agricultural and food processing industries for more high-value applications, such as the production of bioactive and functional compounds, in order to improve the sustainability of the sectors [18,21,22].

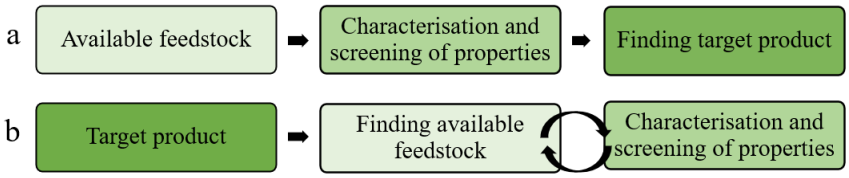

Figure 2 Bioprospecting driven by available feedstock (**a**) or desired target product or application (**b**).

1.1.2. Soil salinisation

Soil salinisation has been reported to be one of the main reasons for the degradation of arable land and a major threat to biodiversity and ecosystems, especially in arid and semi-arid regions [23–25]. According to the Food and Agriculture Organization of the United Nations (FAO) definition, the soil is considered saline when the electric conductivity of soil extracts is equal to or higher than 4 dS/m, corresponding to approximately 40 mM NaCl [26]. Degradation of agricultural land creates challenges in producing enough food to meet the demand of the world's increasing population and threatens food security, as most of the conventional and important food crops are salt-sensitive glycophytes. Soil salinities of 80 mM NaCl, 100 mM NaCl and 120 mM have been shown to cause a 50 % loss in yields of rice, durum wheat, and barley, respectively, and salinities less than 20 mM NaCl have shown a significant 12 % loss in yields of corn and potato [27,28].

Natural causes for soil salinisation (primary salinisation) are seawater intrusion, geological deposits, salt deposition by wind or rainfall, and weathering of the parent rock [26,29,30]. However, salinity is often caused or accelerated by human activity (secondary salinisation) via unsustainable agricultural actions, such as over-irrigation, insufficient draining and extensive use of fertilisers, as well as deforestation and poor water management [23,26,27,29,30]. Climate change also affects soils, as the higher annual temperatures, alteration in precipitation patterns, and sea level increase in coastal areas increase the risk of soil salinisation [29,31]. Irrigated areas, which cover 1.5 % of the total area of the EU, are at a higher risk for human-induced salinisation [31]. Globally, 20 – 50 % of irrigated soils have estimated to have increased salt content [25], creating a massive challenge for agriculture systems.

The issue is global and extensive, as 8.7 % of the world's soils are estimated to be salt-affected [25]. In 2015, the estimate of the total area of saline and sodic soils worldwide was 1 billion hectares, affecting more than 100 countries [26]. The total area of salinised soils in the EU is uncertain, and the estimates vary between 1 – 4 million hectares [31]. Soil salinisation causes significant limitations to plant growth potential (**Figure 3**), decreases water quality, and makes land areas prone to erosion [25]. Annually, soil salinisation takes 1.5 million hectares of agricultural land out of production and decreases the production potential by 46 million hectares [25]. This

comes with a high economical cost, as the annual loss in agricultural productivity caused by salinity is estimated to be 31 million USD, and on top of that, the report by European Commission [31] estimates the costs caused by overall soil degradation, including salinisation, to exceed 50 billion EUR per year only in the EU.

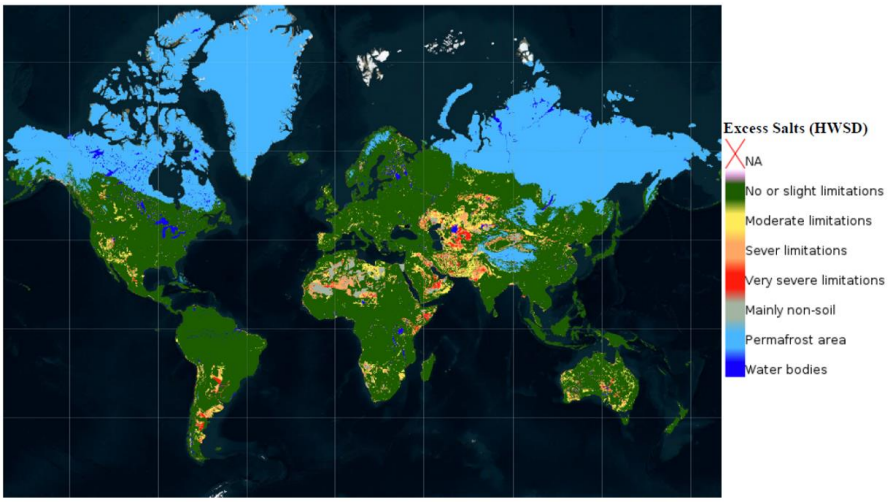

Figure 3 Saline and sodic soils and soil limitations to plant growth. Data from FAO Global Soil Information System and Excess Salts dataset provided by Harmonized World Soil Database (HWSD) [25,32]. No or slight limitations: 80 – 100 % growth potential, moderate limitations: 60 – 80 %, severe limitations: 40 – 60 %, very severe limitations: < 40 %.

1.2. Halophytes and their potential applications

1.2.1. Salt-loving plants

By definition by Flowers and Colmer [33], halophytes are plants that can complete their full lifecycle and reproduce under salinities of 200 mM or more, and these salt-loving plants constitute approximately 1 % of the known flora. In nature, halophytes can be found in marshlands (**Figure 4**), seashores, saline deserts, and other saline habitats, where the growth of salt-sensitive plants, glycophytes, is limited. Halophytes can be roughly divided into two categories: obligatory halophytes, which require salt for optimal growth, and facultative halophytes, which can tolerate salt but have their optimal growth in non-saline or low-salinity conditions. Thus, plants have developed various salt tolerance mechanisms to thrive in saline habitats, including but not limited to morphological adaptation such as succulence and specialised features for salt excretion, osmolyte accumulation, antioxidant regulation, and compartmentalisation and restricted uptake of sodium ions [34,35]. These adaptations allow halophytes to hold their osmotic pressure and maintain homeostasis even under extreme environmental conditions [36–38].

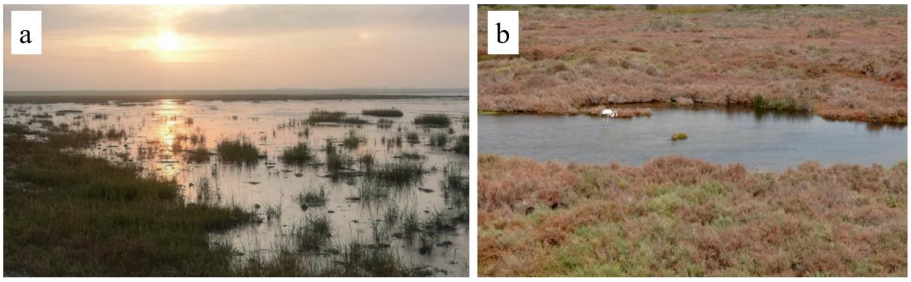

Figure 4 Marsh is a natural habitat of halophytes: Wadden Sea national park, Denmark (**a**) and Ria Formosa natural park, Portugal (**b**). Photos by Laura Sini Sofia Hulkko.

In their region of origin, fresh shoots of edible halophytes are used for culinary purposes [39–45]. Accogli et al. [45] reviewed edible halophytes in Southern Italy and their culinary use, highlighting their significance in traditional cuisine, the most important species being capers, sea beet, *Silene vulgaris*, *Allium monatum*, *Crithmum maritimum*, and *Salicornia* and *Sarcocornia* species. *Salicornia* and *Sarcocornia* species are sold as vegetables in the Mediterranean region and the Middle East, and they have increased interest due to their organoleptic properties, crunchy texture and salty, tangy taste, and they are typically consumed pickled in salads, stir-fries, and garnish [40,43,45–48]. Due to its resemblance with commonly used spinach, *Tripolium pannonicum* (sym. *Aster tripolium*) is also increased interest as an alternative vegetable and cash crop [27,49]. Several *Atriplex* species are used as herbs and garnish [27]. *C. maritimum* has also increased interest as a crop due to its wide use in ethnobotanical and ethnopharmacological use [44].

Indeed, various halophytes are traditionally used as medicinal herbs. Due to their pronounced exposure to abiotic stresses, such as salinity, extreme temperatures, strong UV radiation, drought or waterlogging, halophytes produce high concentrations of protective secondary metabolites, also called phytochemicals [50–56]. This is a structurally and functionally diverse group of chemicals; however, especially phenolic compounds have increased interest in previous years due to their wide use in high-value applications [57–62]. These compounds have various biological activities and functionalities, and they often contribute to the health benefits of plant extracts, such as antioxidant and anti-inflammatory effects, antimicrobial and anti-viral properties, and potential protective effects against cardiovascular diseases, neurogenerative diseases, and cancer. [41,53,64–72,54,56–61,63]. Bioactive compounds found in halophytes were reviewed in Paper II and discussed in detail in **Section 4.1**. In traditional ethnopharmacological medicine, species in *Salicornia* and *Sarcocornia* genera have been used to treat digestive issues, and they have been associated with diuretic effects, making them useful towards kidney-related issues [40,53]. *C. maritimum* has been used as a treatment for scurvy, whooping cough and cold, various digestive issues and as an anti-parasitic agent [44,53,73].

1.2.2. Halophyte cultivation

The development of biosaline agriculture is crucial in regions with saline soils or scarcity of freshwater, and domestication and cultivation of halophytes have a key

role in restoring the marginal land area to agricultural production [35,74]. Halophyte cultivation can yield as much as conventional crops; however, biomass production depends on the optimal salinity of cultivated species [75–77]. Especially dicotyledonous halophytes can grow at high rates, even though a susbtaintial amount of energy is used for salt-tolerance mechanisms and osmotic adjustments, such as ion accumulation and compartmentalisation [33]. Reported annual yields of halophytes cultivated in field conditions are 14 kg/m^2 for *T. pannonicum*, 15 kg/m^2 for *Salicornia persica*, and 28.0 kg/m^2 for *Sarcocornia fruticosa* [75,78]. Salt-accumulating halophytes have also been suggested for crop rotation and intercropping systems due to their potential to reduce soil salinity via salt uptake to aerial biomass [79]. Some halophytes with reported desalting potential in crop rotation are liquorice (*Glycyrrhiza glabra*), *Arthrocnemum macrostachyum*, and *Sesuvium portulacastrum*, whereas *Sueda salsa* has shown potential for intercropping in field trials [79]. Restoring and rehabilitating marginal land areas while increasing agricultural productivity prominently in arid and semi-arid regions could have significant socio-economic benefits regarding rural area development and local food and water security [35,79,80].

Besides salt, some halophyte plants are tolerant of other toxic compounds, such as heavy metals, which makes them interesting for bioremediation of polluted soils [35,81–83]. Halophytes can uptake heavy metals from the soil and accumulate them in aerial parts by excreting them through salt glands to the surface of the leaves or producing osmoprotectant and antioxidant compounds [81,82]. Indeed, the mechanisms for halophytes to tolerate heavy metals are the same as those used to protect plants from salinity and drought. Some species with phytoremediation potential are *T. pannonicum*, *Salicornia sinus-persica* (syn. *Salicornia iranica*), and *Salicornia ramosissima*, which have shown uptake and translocation of cadmium, lead, and nickel [82–84]. However, plants with accumulated toxins and contaminants cause a food safety risk if consumed [70,84]; thus, phytoremediation limits the potential use of cultivated biomass.

Many halophytes can also be efficiently grown in soilless systems, and hydroponic cultivation has increased its importance due to resource efficiency. Singh et al. [85] achieved slightly higher *Salicornia dolichostachya* yield in hydroponic systems compared to sand cultivation. For *Sueda glauca*, a 5.7 kg/ m^2 yield was reported from hydroponic cultivation [86]. Pairing saline agriculture with aquaculture and using halophytes to biofilter effluents has increased interest in recent years, and especially the potential of saline recirculating aquaculture systems (RAS), marine aquaponics, and constructed wetlands have been studied [87–90]. Using constructed wetlands, Shpigel et al. [88] reported *S. persica* plantation effectively uptake 53 – 71 % of total dissolved nitrogen from gilt-head sea bream aquaculture effluents under high nutrient loads. Vlahos et al. [91] combined brackish water gilt-head sea bream culture and *C. maritimum* aquaponic cultivation and reported good fish and plant performance and efficient nutrient removal from water. Brown et al. [92] also reported an average of 98.8 % total nitrogen and 99.7 % total phosphorus removal from water by *Suaeda esteroa*, *Salicornia bigelovii* and *Atriplex barclayana*. Besides nutrients, halophytes can also remove other compounds, such as antibiotic residues, from aquatic environments [93].

1.2.3. Halophytes as energy crops

Using halophytes for bioenergy has also been studied, and bioenergy production of biomass processing waste streams could help to close the loop and emphasise the circular approach for cascading systems. Using the residues of halophytes could help to establish environmentally sustainable production systems [94]. Considering liquid biofuels, Makkawi et al. [95] tested bio-oil production of *S. bigelovii* seeds via pyrolysis and recovered 35.2 % of the initial biomass to bio-oil fraction, organic fraction covering nearly 80 % of the pyrolysis liquid. A similar result, 31.4 % bio-oil production from *S. bigelovii* seeds, was also achieved in another study [96]. Folayan et al. [97] achieved 92.8 % and 81.3 % bio-oil conversion to biodiesel from *S. bigelovii* and *Salicornia brachiata* seed oils, respectively, which corresponded to 29.8 % and 21.6 % of the initial seed biomass, respectively. Bioethanol, which can be produced by fermenting the carbohydrates released from the lignocellulosic fraction with micro-organisms, is also a suggested fuel product from halophytes. Cybulska et al. [98] achieved 77 – 100 % and approximately 77 – 81 % of theoretical bioethanol yield from *S. bigelovii* pretreated fibres and pretreatment liquid, respectively. Similarly, Alassali et al. [99] reached approximately 70 % bioethanol yield from *S. sinus-persica* juice and up to 76.9 % from hydrothermally pretreated fibres. From *Atriplex crassifolia* enzymatically pretreated sample, 16.3 g ethanol/100 g was obtained [100]. Joshi et al. [101] also reviewed halophyte biofuel production and summarised several species from *Cyperus*, *Suaeda*, *Panicum*, and *Phagmites* genera to be considered for bioethanol production.

Turcios et al. [102] studied the effect of salinity on the anaerobic digestion of *T. pannonicum* and reported up to 554 mL/g volatile solids (VS) and 447 mL/g VS yields of biogas and biomethane, respectively. Kumar et al. [103] studied the biogas potential of wild herbs and reported 487.9 mL/g VS, 441.3 mL/g VS and 291.3 mL/g VS biogas yields for *Avicennia marina, Tamarix nilotica*, and *Zygophyllum album*, respectively, and suggested the potential use of residual digestate as fertilizer. Cayenne et al. [104] measured the biomethane potential of *Salicornia europaea* and *S. ramosissima* to be up to 250 mL CH_4/g VS and 300 mL CH_4/g VS, respectively. Nawaz et al. [105] reported de-lignification with deep eutectic solvent to increase biomass yield from *Atriplex crassifolia* by making it less recalcitrant and accessible for micro-organisms and measured biogas yield of 32.2 mL/g from the fresh solvent-treated sample. Akinshina et al. [106] studied the biogas potential of wild and cultivated halophytes and emphasised their importance for the development of marginal and salt-affected environments in Central Asia, *Karelinia caspia* being the most promising species with biogas potential of 310.6 mL/g VS.

1.3. Green biorefinery concept

Green biorefinery processes are typically developed for grass and fodder crops, such as alfalfa, clover, and perennial ryegrass, to provide a source of feed not only for ruminants but also monogastric animals, mainly pigs and poultry. In recent years, the use of green leafy agricultural residues with high moisture content, such as beet and cassava leaves, has been investigated in order to valorise fractions previously considered as waste [107–110]. Proteins are the main compounds to be valorised in

green biorefineries, whereas traditional lignocellulosic refineries often focus on processing carbohydrates [11]. There is a high demand for locally produced protein-rich feed products in the EU, as the number of livestock has been estimated to be 1.63 billion poultry birds and 142 million pigs, and the numbers are increasing [111,112]. Currently, farmers in the EU heavily depend on imported protein sources for their livestock, namely soybean or soybean meal [113]. Similarly, besides terrestrial livestock, aquaculture systems are developing fast to meet the increased demand for fish, and producers depend on fish meal for feeding [87,90,114]. Green biorefineries often aim for the multi-products approach for maximum feedstock valorisation, and some of the typical target products are presented in **Figure 5**.

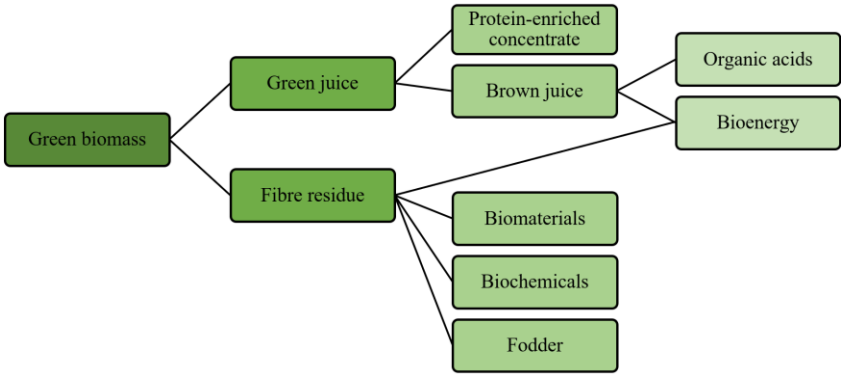

Figure 5 Simplified structure of the potential products from a typical green biorefinery system.

One of the key processes differentiating green biorefineries from others is the wet fractionation of green, fresh biomass, where the plant material is split into green juice and fibre residue. Various milling methods have been tested for fractionation over the years, but separation with a single- or twin-auger screw press has become predominant and is typically used in large-scale applications [115,116]. The use of a pneumatic press has also been recently studied [117]. Some studies suggest using alkali in the screw press to enhance cell disruption and adding water to better flush out the compounds of interest [115]; however, the disadvantages of such processes are chemical cost and increased freshwater usage.

The protein fraction is separated from the green juice after the screw press, and is typically obtained in the form of protein-enriched concentrate (PEC). Several methods have been developed to precipitate proteins from green juice, including but not limited to heat coagulation, acidification, the addition of flocculants, filtration, and fermentation with lactic acid bacteria (LAB), and these methods are described in detail in **Section 5.1.** The protein from grass or leafy biomass can be divided into green protein, which is chloroplastic lipoprotein typically used for feed application, and white protein, which is the cytoplasmic protein with higher digestibility and functionality, thus potential applications in food, cosmetics, and biomaterials [116,118]. These two fractions can be produced separately in a sequential system in order to target several different applications or together as a total protein concentrate for feed, and the latter approach was also chosen for this project.

The separation of chlorophyll and carotenoids from green protein has been studied; however, it may be hard to justify the use of organic solvents required for pigment removal, especially in large-scale systems targeting food and feed products [116,119]. After protein precipitation, the remaining nutrient-rich juice, often called brown juice, could be used as a fermentation medium to produce organic acids, such as lactic acid, caproic acid, and butyric acid, which are important platform chemicals [86,87,90,91]. An alternative approach is to use the juice for a process aiming for the production of single-cell protein [120].

The fibre fraction is typically used directly as fodder for ruminants. However, there has been an increasing interest in valorising this fraction for biomaterials, including biocomposites, packaging materials, insulation, and nanocellulose [74,118,121,122]. The fibre fraction can also be processed as in typical lignocellulosic biorefineries targeting biochemical and bioethanol production or digested into biogas, typically together with the juice residue [16,99,104,118,123,124].

1.4. Project overview and objectives

1.4.1. The AQUACOMBINE project

This PhD project was done as a part of the AQUACOMBINE project [125], which demonstrates combined aquaculture and halophyte farming with an integrated biorefinery of waste fractions, creating internal value and novel products according to the principles of a circular economy. Excess nutrients from on-land aquaponic systems, which can be placed in marginal land in rural regions, fertilise halophyte plants, and water is circulated back to aquaponics through a microbial water treatment system. The fresh shoots and leaves from the edible species can be harvested for culinary use; however, as plants mature, they undergo lignification [46,126,127], becoming unpalatable and unsuitable for culinary consumption. The halophyte biomass was harvested at two different non-food growth stages: partly lignified succulent or completely lignified shrubs after seed production (**Figure 6**). These biomasses were targeted for green biorefinery processing and extraction-based lignocellulose biorefinery, respectively.

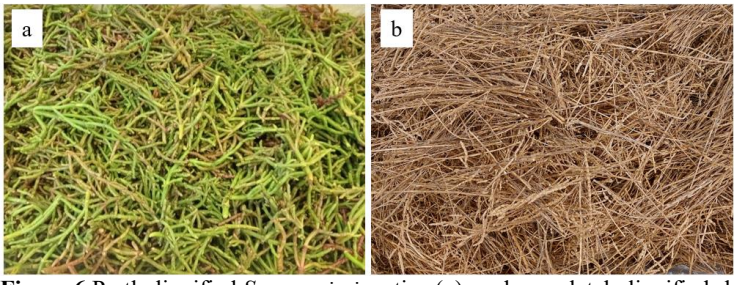

Figure 6 Partly lignified *S. ramosissima* tips (**a**) and completely lignified shrubs (**b**).

Using these two separate processing routes, the AQUACOMBINE project demonstrates the production of various value-added products: fine chemicals, aquaculture feed and feed supplements, dermo-cosmetics, functional food products

and ingredients, prebiotic polysaccharides, and biochar (**Figure 7**). Bioenergy production from the residual fractions is considered for maximum circularity.

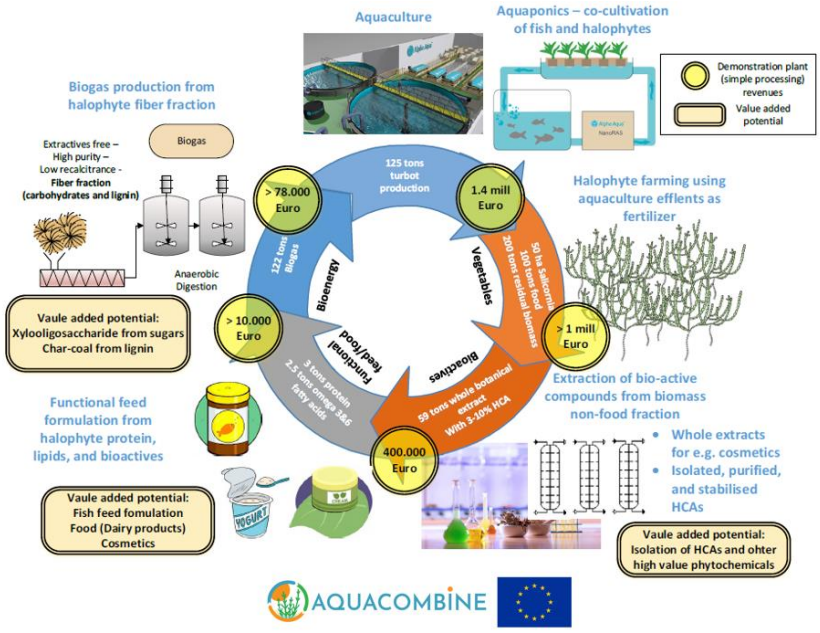

Figure 7 The AQUACOMBINE project is European Union Horizon 2020 funded research and innovation action. Original concept illustration by Mette Hedegaard Thomsen, 2019. The project consortium consisted of 17 partners, including eight academic partners, seven small and medium-sized enterprises, and two cluster organisations, and the project ran from October 2019 until December 2023.

1.4.2. Key project objectives

The project objectives were formatted to study the biorefinery potential of selected halophyte species, namely *Crithmum maritimum*, *Salicornia europaea*, *Salicornia ramosissima* and *Tripoium pannonicum*, and meet the overall goals of the AQUACOMBINE project targeting halophyte-based green biorefinery. The three key objectives for the PhD project are stated as follows:

- Determine the chemical composition of halophytes cultivated in different salinities to gain knowledge if modifying cultivation salinity could be used to enhance the concentration of desired compounds.
- Evaluate the green fractionated biomass in terms of value by analysing the biological activities and contents of polyphenolic compounds and pigments to help find the potential target products and applications.

- Investigate the green biorefinery approach and protein precipitation from halophyte biomass to learn if technologies well established to forage and grass biomass could be applied to halophytes.

1.4.3. Halophyte-based green biorefinery: potential future process?

The utilisation of halophytes in green biorefinery applications has not been widely studied, and only a handful of studies were found to consider the juice or screw-pressed fibres from *Salicornia* species [99,104,128,129]. Introducing halophytes to this type of processing could provide a novel biomass feedstock, increasing the biodiversity in agricultural production either in field cultivation in marginal land areas or marine aquaponics. When cultivated in this type of system, halophyte production does not compete for the arable land area with conventional food crops. Coastal halophytes could also contribute to developing sustainable blue-green bioeconomies [130]. By valorising the non-food fraction of plants, the biomass used for bioproducts is not taken from food resources (primary biomass).

After screw-press, the green juice covers a large fraction of the initial weight of fresh biomass, especially from succulent halophytes, and a significant fraction of the total crude protein (CP) can be found in the juice [34,128,131,132]. As halophytes are naturally rich in various compounds beneficial for animal health, and if these compounds could be transferred to the concentrate, the halophyte-based supplement could provide a novel feed source for aquacultures and terrestrial livestock and improve animal welfare [133–136]. Protein concentrates may have various functional properties [137], and due to the presence of bioactive secondary metabolites, these properties could be more pronounced when using halophytes. Besides producing green protein for monogastric animals, the fibre fraction after the screw press is typically used for fodder to ruminants in green biorefinery systems. However, due to high salt content and low animal acceptance, halophyte biomass can be only partially implemented with other feedstuff, typically with a 20 – 30 % blending fraction [128,138,139]. However, Ahmed et al. [140] showed that up to 50 % of berseem hay fodder could be replaced with *Atriplex nummularia* and *Acacia saligna* for the lamb diet. For aquaculture, Belal *et al.* [87] showed that up to 40 % of traditional fish meal can be replaced with *Salicornia bigelovii* meal without affecting the growth or composition of Nile tilapia, one of the most common species in fish farming. Studies disagree with the suitability of *Salicornia* for poultry feed: Jiao et al. [141] reported an enhanced growth performance and meat quality poultry chicken, whereas Attia et al. [142] report a significant depression in the growth rate and suggest additional cholesterol supplement of overcome the issue.

There is a need to find alternative applications for halophyte fibres. However, before the production of bulk materials or bioenergy, the halophyte fibres could be utilised for producing bioactive botanical extracts for biopharmaceuticals, nutraceuticals, and cosmetics applications; in other words, products on top of the bioproduct value pyramid (**Figure 1**). The potential of halophytes for these applications has been widely studied [53,56,63,65,143,144], but implementing the extraction step to an integrated green biorefinery system has been rather unexplored. Sustainable production of high-value ingredients could help to create added value and improve

the process economics, diversify the source of active molecules, and provide a way to maximum feedstock valorisation. The extraction process could also prepare the remaining lignocellulosic biomass for further processing, including potential bioenergy production [94,98,129]. Batog et al. [74] tested halophyte biomass for biocomposite production and discussed the potential of halophytes for the production of biomaterials. Dong et al. [145] tested biochar, a useful soil amendment, from different halophyte feedstocks and found biochar from *Tamarix chinensis* suitable to improve soil quality with the minimum adverse effect of increased soil salinity.

Considering this PhD project, the simplified schematic of the halophyte-based green biorefinery is visualised in **Figure 8**. Halophyte-based biorefineries could offer solutions for developing arid and semi-arid rural regions, where grass biomass or other lignocellulosic feedstock typically used for biorefineries, such as cereals straws and other agro-residues, cannot be produced sustainably [146]. Alassali et al. [99] also highlight the value of green juice as a water source for biomass processing in regions with freshwater sparsity. Halophytes can also provide a bioenergy source for rural arid regions, with low costs and no competition for land use and freshwater [94].

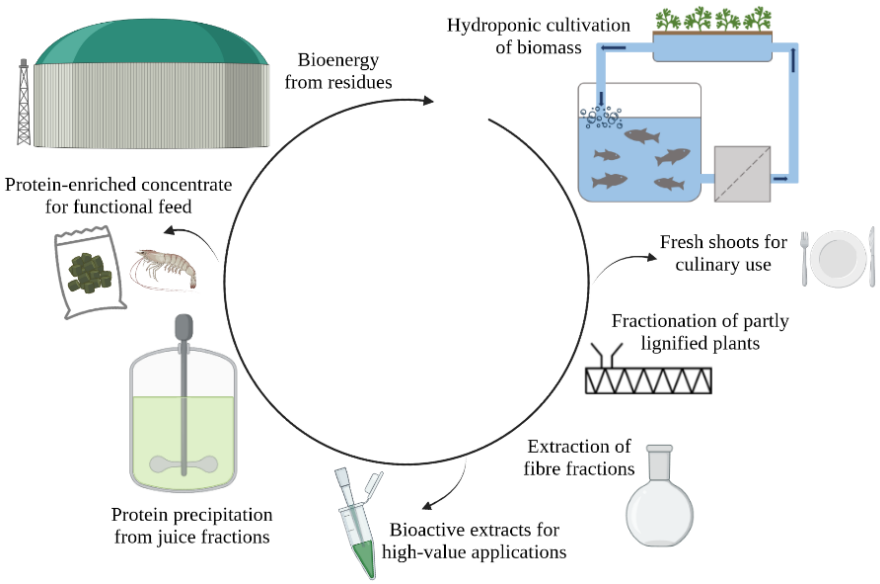

Figure 8 Halophyte-based green biorefinery with soilless biomass cultivation. Adapted from Paper V [132]. Figure created with BioRender.com.

1.4.4. Overview of included studies

Contributing to the project objectives listed in **Section 1.4.2**, five manuscripts in total were produced during the project period:

I. This study assessed the effect of cultivation salinity on the chemical composition of *S. europaea*, *T. pannonicum*, and *C. maritimum* with a focus

on macronutrients carbohydrates, fats, and protein and their distribution to fractions after screw pressing.

II. The review study incorporated the recently published literature reporting the concentration of phenolic compounds, pigments, and vitamins in halophytes, as well as used extraction and analysis methods, and combined the state-of-the-art knowledge in the field.

III. The first study considering the bioactivity of the selected species focused on different fractions and extracts obtained from *S. ramosissima* and determined the contents of different types of phenolic compounds and photosynthetic pigments in the extracts, antioxidant activity, and inhibition activity towards enzymes related to some emerging lifestyle diseases, such as diabetes and neurodegenerative diseases.

IV. In this study, the bioactivity of *T. pannonicum* and *C. maritimum* fractions and extracts was assessed by measuring the content of bioactive compounds and biological activities as described above. The role of plant extracts in circular bioeconomies and integrated biorefineries for maximum feedstock valorisation was discussed.

V. Protein is one of the target products of green biorefinery, and in this study, different methods for protein precipitation from green juice fractions of *S. ramosissim*a and *T. pannonicum* were tested.

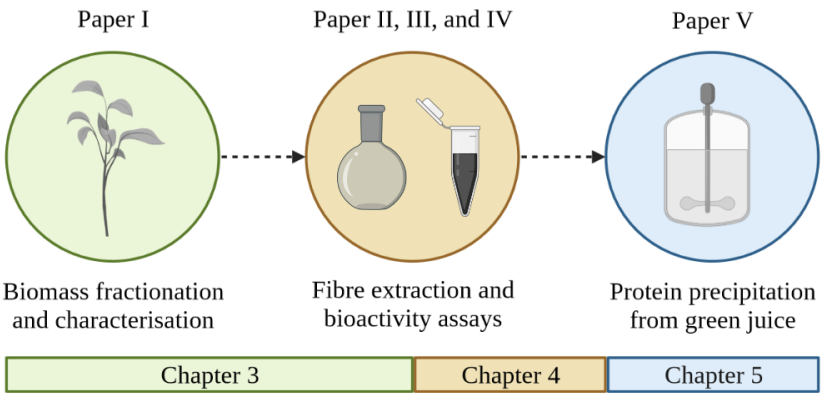

Figure 9 Overview of the studied themes, related papers, and outline of the dissertation. Figure created with BioRender.com.

In addition to the listed studies, the cytotoxicity of the extracts was tested using mammalian cell lines to learn about the extracts' safety and potential anti-cancer properties. Using an untargeted, explorative approach with liquid chromatography and mass spectrometry (LC-MS), the analysis of selected metabolites, such as flavonoids and phenolic acids, was carried out for extract samples.

Considering the refinery of extractives-free fibres, lignocellulose pretreatment and enzymatic convertibility were briefly explored. The protein precipitation from *S. ramosissima* green juice by lactic acid fermentation was further studied by conducting experiments in a bioreactor in a batch. These experiments were carried out to better understand the acidification process, its efficacy, and potential limitations.

1.4.5. Sustainable development goals

This project contributes, directly or indirectly, to several of the sustainable development goals (SDGs) defined by the United Nations [147]. "*Responsible Consumption and Production*", SDG no. 12, targets the sustainable use of natural resources, a decrease in the environmental footprint of produced goods, and waste reduction. This goal is one of the most directly linked to the PhD project, as this project studies the concept of halophyte-based biorefinery, which could be implemented in marginal land areas and provide multiple products through sustainable processing routes and maximum feedstock valorisation according to the principles of the circular economy.

Fertilising halophyte cultivation with aquaculture effluent and producing functional protein-enriched feed supplements could help reduce aquaculture's harmful effects, such as the released amount of excess nutrients and potential antibiotic residues, into blue ecosystems while improving animal health. Hence, protecting "*Life Below Water*" and contributing to SDG no. 14.

SDG no. 3, titled "*Good Health and Well-Being*", targets the development of effective and affordable medicines for non-communicable and infectious diseases. Bioprospecting plants to discover their potential medicinal properties contribute towards this goal. Finally, extractives-free fibres could be processed for bioenergy, such as bioethanol or biogas, allowing the project to contribute to SDG no. 7, "*Affordable and Clean Energy*".

Figure 10 Sustainable development goals [147] to which the research contributes.

Chapter 2. Methodology

2.1. Halophyte biomass

In total, four different halophyte species were used in the project for bioprospecting: *Crithmum maritimum* (Apicaceae), *Salicornia europaea* and *Salicornia ramosissima* (Amaranthaceae), and *Tripolium pannonicum* (Asteraceae). All samples were harvested non-food grade at a vegetative partly-lignified stage before seed production, except *C. maritimum* from seed plants, which was harvested at the flowering stage. *S. europaea* and *T. pannonicum* are widely distributed across the Nothern hemisphere, whereas *C. maritimum* and *S. ramosissima* are found mainly on the coasts of the Mediterranean Sea and Western Europe [148].

Table 1 Origin and cultivation of *Crithmum maritimum*, *Salicornia* spp. and *Tripolium pannonicum* biomass used in the project. Biomass producers are the Institute of Botany of Leibniz University Hannover, Germany (LUH), Riasearch Lda., Portugal (RSR), and Les Douceurs du Marais, France (LDM).

Species	Origin	Description	Harvest
C. maritimum	LUH	Hydroponic cultivation in a greenhouse with various cultivation salinities.	03-2021
		Seed plants cultivated in the soil in a greenhouse.	09-2021
S. europaea	LUH	Hydroponic cultivation in a greenhouse with various cultivation salinities.	10-2020
S. ramosissima	RSR	Cultivation in the soil in a greenhouse with irrigation and fertilisation with aquaculture effluent with a salinity of 250 mM NaCl.	09-2020 09-2021
	LDM	Organic open-field cultivation on marshland with seawater irrigation.	05-2021 10-2022
T. pannonicum	LUH	Hydroponic cultivation in a greenhouse with various cultivation salinities.	11-2020 06-2021

Plant biomass used in the projects was fractionated to green juice and fibre residue using a screw press coupled with a coarse filter, and both a laboratory-scale single-auger juicer and a pilot-scale twin-auger juicer were used. Biomass was not chopped prior to pressing due to the relatively small size of the received plant material. Separated fractions were weighed, and green juice was frozen immediately after the fractionation. The fibre residue fraction was dried at 60 °C, size-reduced, and stored at room temperature. Due to low biomass yields, *C. maritimum* and *T. pannonicum* grown in high salinities were not fractionated; instead, these plants were considered whole shrubs. For the studied halophyte biomasses, the green juice fraction constituted 67 – 90 % of the fresh weight (FW) of the biomass, the fraction being largest in *S. europaea* cultivated at 684 mM NaCl (40 g/L) salinity and smallest in *C. maritimum* cultivated at 171 mM NaCl (10 g/L) salinity [34]. The distribution of fractions in each biomass is described in papers [17,34,48,132].

The fractionation process was later successfully performed on a demonstration scale at the grass processing facility of Aarhus University in Foulum, Denmark, for *S. ramosissima* (batch from 10-2022). Separation performance comparable to small-scale processes was achieved, and from the fresh biomass, approximately 80 % was recovered in juice fraction and 20 % as wet fibres with approximately 38 % dry matter content. Therefore, a fractionation technology used in grass biorefinery systems could also be transferred to green halophyte-based biorefineries, paying attention to the material selections to tolerate the saline biomass.

Figure 11 Halophyte species: *Salicornia europaea* (**a**), *Salicornia ramosissima* (**b**), *Tripolium pannonicum* (**c**), and *Crithmum maritimum* (**d**). Photos by Laura Sini Sofia Hulkko.

2.2. Proximate composition analysis

All biomass samples were determined for their contents of ash, carbohydrates, Klason lignin, lipids, crude protein, and organic acids. For dry matter (DM) and ash content, structural carbohydrates and Klason lignin, and organic acids, methods described by National Renewable Energy Laboratory (NREL) were used [149–151]. Weak acid hydrolysis, sometimes called dilute acid hydrolysis, was performed for juice fractions to determine the total amount of carbohydrates in liquid fractions. For crude protein

(CP) content, the amount of total nitrogen was determined from dried samples using an elemental analyser and applying the Jones' conversion factor of 6.0 [152]. Lipids were extracted using a conventional Soxhlet extraction with n-hexane for fibre residues and liquid-liquid extraction for green juice fractions. All methods used to determine the composition of biomass fractions are described in detail in Paper I [34]. The transesterification and determination of the fatty acid (FA) profile were carried out as described in Paper III and Paper IV [17,48].

2.3. Extraction methods

The plant extracts were prepared with a conventional laboratory-scale Soxhlet system (**Figure 12**). Soxhlet extraction is a commonly used, reliable and replicable method for solid-liquid extraction, and the principle of continuous evaporation, simultaneous condensation, and cyclic siphoning of extract allows the production of concentrated extract without mass transfer limitations [16]. The extraction chamber has a volume of 100 mL, and 250 mL of solvent was used for the extraction. The extraction time was 8 h for water extracts, and 6 h for ethanol and n-hexane extracts, and the obtained amount of extractive material was determined by using a protocol by NREL [153].

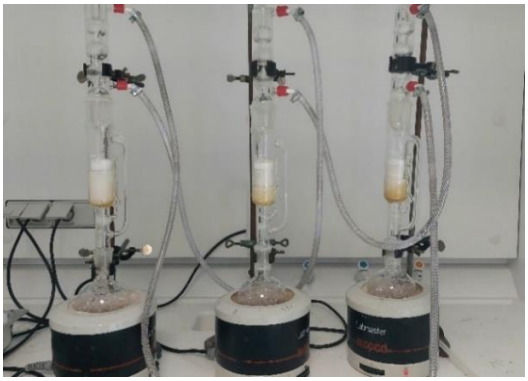

Figure 12 Soxhlet extraction setup with cooling water recirculation.

Two types of extraction methods were applied for solid biomass fractions (**Figure 13**). First, extractions with water, 70 % aqueous ethanol, and n-hexane were carried out as parallel experiments. This extraction method was used for fibre residues of *C. maritimum* (09-2021), *S. ramosissima* (05-2021) and *T. pannonicum* (06-2021), and the extracts were used in bioactivity studies included in Paper III and Paper IV [17,48]. In another approach, extractions with water and absolute ethanol were performed sequentially using the same initial 15 g sample. This method was applied for the same *T. pannonicum* and *S. ramosissima* biomass as the previously described method, as well as for another *S. ramosissima* batch (09-2021) and *C. maritimum* (09-2021) whole plant biomass. These extracts were used to test the cytotoxicity and run the untargeted analysis of selected metabolites.

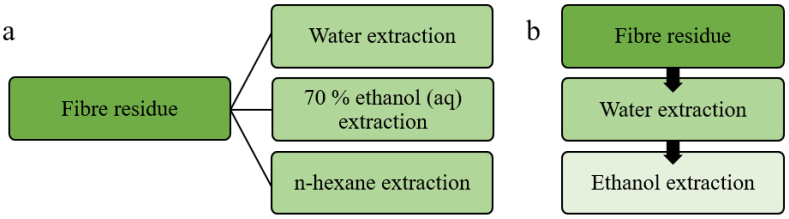

Figure 13 Parallel (**a**) and sequential (**b**) extraction methods

2.4. Pretreatment and enzymatic saccharification of fibres

After the sequential extractions, extractives-free fibres from *S. ramosissima* and *T. pannonicum* were used for hydrothermal pretreatment and enzymatic hydrolysis. Samples of 5 g were weighed into 250 mL flasks, 100 mL of demineralised water was added, and samples were mixed to ensure all fibres were submerged (5 w/v% DM loading). Samples were then treated in an autoclave under subcritical conditions at 121 °C for 30 min, corresponding to moderate severity factor 2.4, previously used as a hydrothermal pretreatment for lignocellulosic halophyte fibres from wet fractionation [131]. For comparison, *S. ramosissima* fibres were also pretreated at 190 °C for 10 min in a pilot-scale pretreatment reactor with active cooling and constant stirring, using 25 g extractives-free biomass and 500 mL water. This pretreatment condition corresponds to a higher severity factor of 3.7, previously tested for both wet fractionated and completely lignified *Salicornia* shrubs [98,128]. After cooling, the liquid was separated by vacuum filtration (MontaMil 0.45 µm PVDF membrane filter, Frisenette). However, due to membrane fouling, the *T. pannonicum* pretreatment liquid was filtered through a more coarse filter paper, with particle retention of 12 – 15 µm. The obtained pretreated fibres were dried at 60 °C overnight before their hydrolysation.

The pretreatment liquid was analysed for the contents of possible sugar degradation products. The concentrations of furfural and 5-hydroxymethylfurfural (HMF) were determined using an HPLC with a C18 column (InfinityLab Poroshell 120 EC-C18, Agilent Technologies), a mobile phase consisting of 80 % phosphoric acid (0.2 %) and 20 % acetonitrile, and diode array detector.

The convertibility of lignocellulosic residual fibres was tested using a 6 % enzymatic dosing (Cellic® CTec3 HS, Novozymes). Pretreated fibres were mixed with 0.05 M acetate buffer (pH 5.0) to 10 w/w% DM loading in 250 mL baffled flasks, and 0.18 g of enzyme solution was added. The enzymatic saccharification was carried out by incubating flasks at 50 °C with 150 rpm shaking for 24 h. Afterwards, the samples were centrifuged to separate the undigested fibres, and hydrolysate samples were diluted with ultrapure water with a 1:2 ratio and filtered through a 0.45 µm syringe filter in order to measure the free sugar monomers released in the saccharification.

2.5. Analysis of phytochemicals

2.5.1. Total contents of phenolic compounds

Samples were analysed for their contents of total phenolic compounds (TPC), total flavonoids (TFC), total condensed tannins (TCT), also called proanthocyanidins, and total anthocyanidins (TAC), using absorption spectroscopy [154–157]. The methods adapted to 96-well plates were carried out as described in detail in Paper III and Paper IV [17,48], and the results are given as a concentration of reference compounds equivalent, determined using a calibration curve (**Figure 14**). The reference compounds used for calibration were gallic acid for total phenolic compounds, quercetin for total flavonoids, catechin for total condensed tannins, and cyanidin chloride for total anthocyanidins (**Figure 15**).

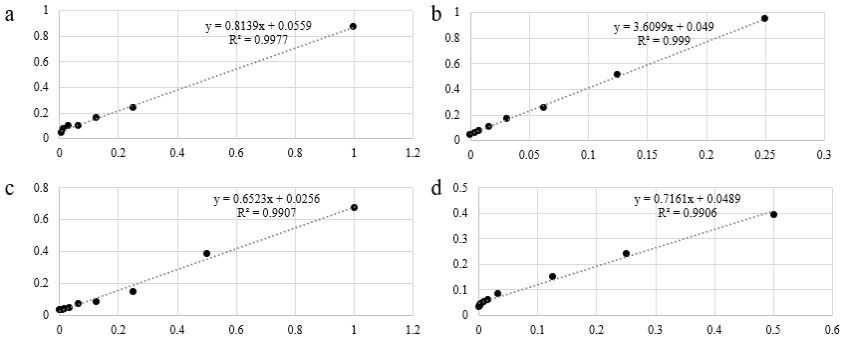

Figure 14 Calibration curves obtained for using gallic acid (**a**), quercetin (**b**), catechin (**c**), and cyanidin chloride (**d**). Calibration curves were used to calculate the total contents of phenolics, flavonoids, proanthocyanidins, and anthocyanidins, respectively.

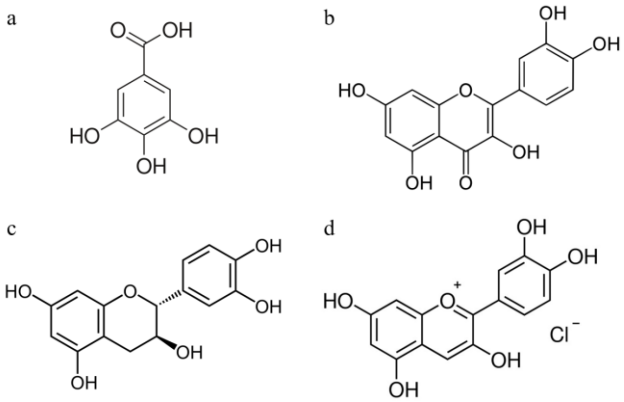

Figure 15 Chemical structures of phenolic compounds used as reference compounds in assays: gallic acid (**a**), quercetin (**b**), catechin (**c**), and cyanidin chloride (**d**).

2.5.2. Total contents of photosynthetic pigments

The approximated total amounts of photosynthetic pigments, chlorophylls (CHL) and carotenoids (TCA), were determined for juice fractions and ethanol extracts using a spectrophotometric method described by Lichtenthaler and Wellburn [158] using the following equations:

$$\text{CHL } a = 13.95 \times \text{A}_{665} - 6.88 \times \text{A}_{649}$$
$$\text{CHL } b = 24.96 \times \text{A}_{649} - 7.32 \times \text{A}_{665}$$
$$\text{TCA} = (1000 \times \text{A}_{470} - 2.05 \times \text{CHL } a - 114.8 \times \text{CHL } b)/245$$

2.5.3. Untargeted analysis of selected metabolites

Water and ethanol extracts dissolved to corresponding solvents with a 2 mg/mL concentration were analysed using HPLC coupled with high-resolution mass spectrometry. Separation was done using ultra-high performance liquid chromatography (UltiMate 3000, Thermo Scientific), RP-18 column (Accucore, 2.1 x 100 mm, 2.6 µm, Thermo Scientific) and mobile phase containing water (A) and acetonitrile (B), both supplemented with 0.1 % formic acid. The solvent gradient started with 100 v/v% A for 2 min, after which B linearly increased 30 v/v% in 30 min and v/v100 % in 16 min. In the end, the mobile phase was returned to 100 v/v% A for an additional 5 min. The flow rate inside the system was 0.3 mL/min, and the sample injection volume was 10 µL.

Mass analysis was carried out with a mass spectrometer (Orbitrap Elite, Thermo Scientific) with heated electrospray as an ionisation source. The following parameters were used: spray voltages of 3.7 kV (positive) and 4.0 kV (negative), 40 arbitrary units of sheath gas and 10 arbitrary units of auxiliary gas, and heater and capillary temperatures of 300 °C and 350 °C, respectively. Compounds were detected within a range of 100 – 1000 m/z, and fragmentation spectra were obtained using data-depended mode with dynamic exclusion. Obtained data were analysed using software (Compound Discoverer 3.3, Thermo Scientific) and compounds annotated using mzCloud, Arita Lab 6549 Flavonoid Structure Database, EFS HRAM Compound Database, Endogenous Metabolites database of 4400 compounds, LipidMaps Structure database 2021-09-13, and Natural Products Atlas 2020-06.

2.6. Biological activity of extracts

2.6.1. Antioxidant activity

In order to evaluate the *in vitro* antioxidant properties of the samples, three radical-based assays and three metal-based assays were carried out. The radical scavenging activity was tested against the following radicals: 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and nitric oxide (NO) [159–161]. For metal-based assays, iron and copper chelating activities (ICA and CCA, respectively), as well as ferric reducing antioxidant power (FRAP), were tested [162]. The assays are described in detail in Paper III and Paper IV [17,48].

Gallic acid, a known antioxidant, was used as a positive control and reference compound for DPPH and ABTS assays at 1 mg/mL concentration, and metal chelator ethylenediaminetetraacetic acid (EDTA) at 1 mg/mL was used as a positive control sample in ICA and CCA assays.

2.6.2. Enzyme inhibition activity

In vitro enzyme inhibition activity was tested against enzymes associated with type 2 diabetes mellitus (α-amylase and α-glucosidase), obesity and acne (lipase), hyperpigmentation (tyrosinase), and neurodegenerative diseases, such as Alzheimer's disease (acetylcholinesterase and butyrylcholinesterase) [163–167]. The protocols are described in detail in Paper III and Paper IV [17,48]. The results are expressed as percentages of inhibition at 10 mg/mL concentration based on the negative control. For comparison, the results were benchmarked against medicinal compounds currently in the market: galantamine (dementia drug), arbutin (used in cosmetics to reduce dark spots and hyperpigmentation), acarbose (diabetic drug), and orlistat (used to support weight loss in obese subjects).

2.6.3. Cytotoxicity

The cytotoxicity of the extracts to mammalian cells was tested using three cell lines provided by the Centre of Marine Sciences of the University of Algarve: S17 (healthy mice murine bone marrow stromal cells), HepG2 (human hepatocarcinoma), and RAW 264.7 (mice leukemic macrophage). Cells were maintained in a DMEM culture medium supplemented with 10 % inactivated fetal bovine serum, 1 % 2 mM L-glutamine, and 1 % 50 U/mL penicillin/50 µg/mL streptomycin at 37 °C humidified atmosphere with 5 % CO_2. Cells were passaged when 70 – 80 % confluence was reached, and a minimum of two cell division cycles with minimal observed morphological modifications were passaged before using the cells in assays.

Cytotoxicity assay was carried out for extracts obtained from fibre residue of *S. ramosissima* (09-2021) and *T. pannonicum* (06-2021), and whole *C. maritimum* plant biomass (09-2021). The cell viability was tested using a colourimetric assay with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as described by Rodrigues et al. [65]. Cells were seeded to 96-well plates with a density of $5x10^3$ cells/well (S17 and HepG2) and $10x10^3$ cells/well (RAW 264.7) and incubated for 24 h. When cells were attached to the bottom of the wells, extracts were applied with a concentration of 200 µg/mL, and plates were further incubated for 72 h. Afterwards, 20 µL of 5 mg/mL MTT in phosphate-buffered saline was added, and plates were incubated for 2 h. Viable cells converted MTT to purple crystals, which were then dissolved into 150 µL DMSO. Absorbance was read at 590 nm (Biotek Synergy 4, Biochrom), and results were expressed as a percentage of cell viability based on the negative control cells with no added extract. Extracts exhibiting cell viability below 75 % were considered cytotoxic at the tested concentration.

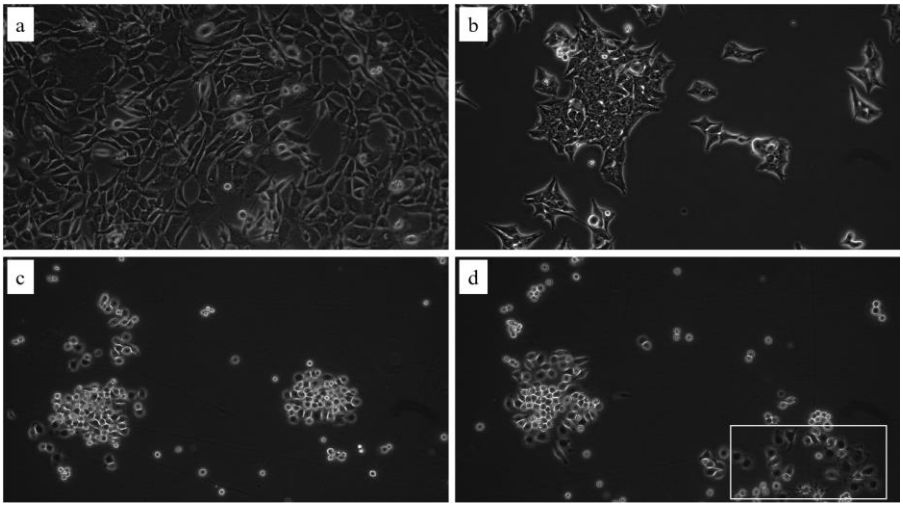

Figure 16 Mammalian cell lines used in the study: SP17 (**a**), HepG2 (**b**), and RAW 264.7 (**c**). Undesirable morphological modifications were observed in some RAW 264.7 cells (**d**). Cells are 200 times magnified and photographed with a camera linked to a Zeiss Axio microscope.

2.7. Protein precipitation methods

Different methods were tested to precipitate the protein from the *S. ramosissima* (09-2021) and *T. pannonicum* (06-2021, 120 mM NaCl salinity) juice fractions: heat coagulation, acidification with HCl, and fermentation with lactic acid bacteria (LAB). The heat coagulation was carried out as described by Christiansen et al. [128]. In brief, 200 mL green juice samples were heated in a water bath until 80 °C, kept at that temperature for an additional minute, and cooled down in an ice bath before separating the protein-enriched concentrate by centrifugation. Acidification to pH 3.5 was done by slowly adding < 1 mL of 7.7 M HCl at room temperature under constant stirring.

Protein was precipitated from juice also by using the LAB fermentation method adapted from one developed by Kiel et al. [168]. First fermentations were carried out in baffled shake flasks, where 180 mL of the green juice was inoculated with 10 v/v% overnight LAB pre-culture. Flasks were flushed with nitrogen, and fermentation was carried out at 37 °C for 48 h in a shaking incubator. A fairly large inoculate was used to ensure that the fermentation was taken over by the inoculated LAB and not an acidifier naturally present in the unsterilised juice medium.

Fermentation experiments for *S. ramosissima* juice were also carried out in a bioreactor (Biostream BioBench) with a 1 L working volume, nitrogen flushing, temperature control at 37 °C, and constant pH and dissolved oxygen measurements. Green juice (900 mL) was inoculated with 10 v/v% LAB pre-culture, and levels of available sugars and produced metabolites were determined by hourly sampling. Samples were cooled immediately to inhibit the LAB growth, filtered through 0.45 µm syringe filters, and sugars, acetic acid, lactic acid, and ethanol concentration were

measured using HPLC and protocol by NREL [149]. Fermentation was stopped when the target pH was reached.

The PEC was separated by centrifugation at 4000 rpm for 20 min. Brown juice was decanted and discarded. PEC was dried overnight in a 60 °C fan oven and homogenised. Recovery of DM and CP from juice to PEC were calculated as follows:

$$\text{DM recovery } [\%] = \text{DM in PEC } [\text{g}]/\text{DM in juice } [\text{g}] \times 100\ \%$$
$$\text{CP recovery } [\%] = \text{CP in PEC } [\text{g}]/\text{CP in juice } [\text{g}] \times 100\ \%$$

Chapter 3. Composition of Halophytes

3.1. Introduction to the nutritional composition of halophytes

Especially *Salicornia* species are known for their high phenotypic plasticity, meaning their ability to adapt to changing environmental conditions, which causes significant differences in the visual attributes of plants and the chemical composition of different ecotypes [85,169–172]. Indeed, one notable characteristic of halophyte biomass is the high ash content, the inorganic material in the plant matrix, which can constitute up to 30 – 50 % of the dry plant mass, especially in succulent species using sodium-ion accumulation and compartmentalisation strategies as their salt-tolerance mechanism [33]. Differences in chemical composition have also been observed in some halophytes of the Amaranthacaea family in different phenotypic stages, such as a decrease in crude protein (CP) content in the red-purple stage [173,174]. Succulent halophytes have been shown to produce these protective pigments in response to biotic and abiotic stresses, such as high salinity and high UV radiation intensity [173–176]. However, the occurrence of red-purple pigments tends to be in more mature plants, and the later harvest stage has been linked to decreasing protein content not only in halophyte *S. europaea* [177] but also in conventional forage biomass [178]. Intra-specific variations were also noted in this project: *S. ramosissima* biomass batch (05-2021) cultivated in an open field with high salinity exhibited succulent texture, high juice content (> 80 % of initial FW) and extremely high juice ash content (81.8 g/100g DM), whereas the batch cultivated in a greenhouse in brackish water (09-2020) was more lignified in texture, had lower juice content (66.7 % of initial FW) and lower ash content [48,132]. *S. ramosissima* from open-field cultivation was also partly red in colour, likely due to higher exposure to abiotic stresses.

The average CP content of herbaceous plants and green fodder commonly used as feed is 11.5 g/100g DM and 12.2 g/100g DM, respectively, and 9 – 19 g/100g DM CP content has been reported for crops typically used in green biorefineries [179,180]. Many edible halophytes have reported protein content within range. The lipid content of the vegetative fraction of halophytes, especially succulent shrubs, is typically low [181], and reviewed studies report lipids contents of 1.1 – 5.9 % for halophyte biomass, excluding *Arthrocnemum indicum* and *Suaeda fruticosa*. Agudelo et al. [144] also reported only up to 1.1 g/100g DM, 1.2 g/100g DM and 2.3 g/100g DM lipid content for *Atriplex halimus*, *Salicornia fruticosa*, and *Cakile maritima*. However, the halophyte seeds may be a notable source of lipids, and Joshi et al. [101] reviewed the reported oil concentration of seeds from different species, which varies between 6 – 44 %.

The lignocellulose from succulent halophytes is typically characterised by low lignin content. Abideen et al. [182] studied the lignocellulosic fraction of various halophyte plants and reported a lignin content of 2.3 – 8.3 % out of the lignocellulose fraction. For comparison, wood biomass has reported a lignin content of 15 – 4 %, depending on the species [183]. Similar low lignin contents were also reported in studies reviewed by Joshi et al. [101], and the same study shows how the fraction of cellulose

and hemicellulose varies greatly between different species. Trucios et al. [177] studied the changes in *S. europaea* composition over the growth period and showed the lignocellulose fraction to increase with age (lignification). Cybulska et al. [146] studied the composition of ten halophyte species and found the highest total carbohydrate concentrations in *Cornulaca aucheri* (34.3 g/100g DM) and *S. sinus-persica* (33.4 g/100g DM). In some studies, the carbohydrate fraction is determined as neutral detergent fibre (NDF), which includes most of the structural plant material, or total dietary fibre (TDF), which includes polysaccharides, lignin and other associated plant compounds but not potential sugars available as starch. Indeed, most available studies focus on the nutritional quality of edible plant fractions.

The nutritional composition of some edible halophytes is summarised in **Table 2**. For comparison, *C. maritimum* from non-saline hydroponic cultivation, *S. ramosissima* (09-2020), *S. europaea* cultivated in 342 mM NaCl salinity, and *T. pannonicum* from non-saline cultivation were added from the biomass batches considered in this study. However, the compositions are discussed in detail in **Section 3.2.1** and **Section 5.3.1**.

Table 2 Proximate (nutritional) composition of some edible halophytes. Dry matter is determined as [g/100g FW], whereas other constituents are determined as [g/100g DM]. FW: fresh weight, DM: dry matter, CP: crude protein, * determined as total dietary fibre, ** determined as neutral detergent fibre.

Biomass	DM	Carbohydrates	CP	Lipids	Ash	Ref.
Arthrocnemum indicum	19.9	50.3*	15.6	13.1	15.1	[174]
Atriplex lampa	n/a	35.5**	14.0	n/a	30.6	[184]
Crithmum maritimum	12.4	59.1	12.7	5.9	22.4	[44]
	11.1	**26.4**	**23.1**	**1.9**	**16.0**	[34]
Halminione portulacoides	22.0	40.5*	9.5	2.1	27.7	[185]
Inula crithmoides	11.9	15.5	23.2	3.1	35.8	[186]
Mesembryanthemum nodiflorum	11.9	12.2	16.4	5.6	39.1	[186]
Salicornia bigelovii	15.1	17.2	7.5	n/a	39.0	[128]
Salicornia europaea	**8.0**	**22.5**	**14.4**	**2.6**	**42.2**	[34]
Salicornia herbacea	26.1	51.3	7.7	1.1	23.4	[187]
Salicornia ramosissima	10.1	8.4*	20.9	4.8	47.9	[188]
	15.5	22.5**	5.2	1.9	29.2	[189]
	13.7	**37.2**	**9.6**	**2.7**	**26.4**	[132]
Sarcocornia fruticosa	9.0	9.3*	12.6	5.6	43.4	[190]
Sarcocornia perennis	14.2	34.1**	6.9	2.3	23.3	[189]
Suaeda fruticosa	25.6	39.5*	17.2	19.1	10.5	[174]
Suaeda maritima	10.4	12.8*	17.7	5.6	31.0	[190]
Triglochin maritima	9.9	49.8	27.8	2.5	27.8	[191]
Tripolium pannonicum	7.9	45.56	16.8	2.9	34.7	[192]
	8.1	**30.2**	**25.9**	**2.5**	**23.1**	[34]

3.2. Findings on the composition of halophyte biomass

3.2.1. Cultivation and characterisation of *Salicornia europaea*, *Tripolium pannonicum* and *Crithmum maritimum* biomass for green biorefinery applications

This section summarises the key findings and discussions of Paper I, titled "*Cultivation and characterisation of* Salicornia europaea, Tripolium pannonicum *and* Crithmum maritimum *biomass for green biorefinery applications*" and published in Scientific Reports [34], which studies the effect of cultivation salinity on the chemical composition of halophytes and the distribution of plant primary metabolites to the juice and fibre residue fractions. The results could aid in finding optimal cultivation conditions and potential process routes for halophyte biomass.

Biomass was cultivated in hydroponic systems under various salinities at the Institute of Botany, Leibniz University Hannover, Germany. Cultivation salinity significantly affected the biomass yield in all species, with *S. europaea* exhibiting the highest yield in 342 mM NaCl (corresponding to 20 g/L NaCl), whereas both *T. pannonicum* and *C. maritimum* had their highest biomass production in non-saline conditions. These results align with existing literature, as previous studies report the optimal salinity of obligatory halophyte *Salicornia* to be within 200 – 400 mM NaCl [193–196]. Similarly, significant growth inhibition of facultative halophytes *T. pannonicum* and *C. maritimum* under increased salinity has been observed previously [55,82,197–200]. Whereas both *S. europaea* and *T. pannonicum* are hydrohalophytes found in coastal marshlands, *C. maritimum* is a chasmophyte with a natural habitat in rocky seashores [201], which explains the observed lower salt tolerance and biomass production.

T. pannonicum and *C. maritimum* grown in salinities > 171 mM (corresponding to 10 g/L NaCl) exhibited too low biomass yields for the fractionation process, and they were considered whole shrubs. Other obtained biomass was screw-pressed, yielding 84.6 – 90.2 % green juice. The juice DM content was 4.7 – 5.8 % in *S. europaea*, 3.9 – 4.2 % in *T. pannonicum* and 5.4 – 7.4 % in *C. maritimum*. As the DM of the fibre fractions is highly dependent on the performance of the fractionation equipment, it was similar for all species, varying between 21.1. – 27.7 %. The total DM of plants increased as the salinity increased, except in *S. europaea*, which had no significant changes in the total DM content. In general, the cultivation salinity had a greater effect on the composition of *S. europaea*, whereas in *T. pannonicum* and *C. maritimum*, the increased salinity affected the biomass yield rather than the biomass composition. The tested compounds were ash, carbohydrates and Klason lignin, CP, lipids, and organic acids. The composition of *S. europaea*, *T. pannonicum*, and *C. maritimum* whole plant biomass from different salinities are presented in **Figure 17**. The fraction constituents, sugar profiles, and minerals present in the ash are reported in detail in Paper I [34].

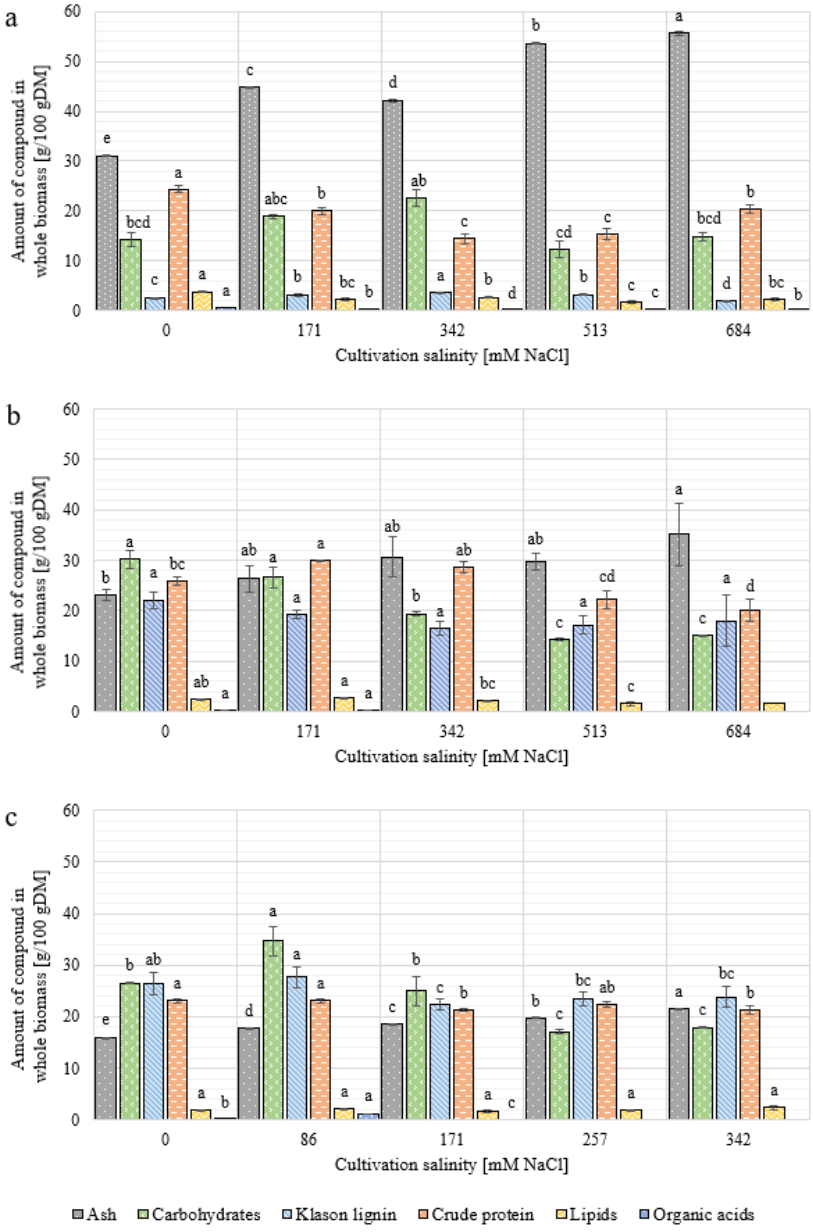

Figure 17 Effect of cultivation salinity on the composition of whole shrubs of partly lignified *Salicornia europaea* (**a**), *Tripolium pannonicum* (**b**), and *Crithmum maritimum* (**c**) [34], adapted and reproduced with permission from Springer Nature. The composition of fractionated biomass can be found in the original research paper.

Effect of salinity on the composition of *S. europaea*

In *S. europaea*, the cultivation salinity significantly affected all tested biomass constituents in whole plant biomass, as well as both green juice and fibre fraction. The protein content was highest in the plants cultivated in non-saline conditions, and besides plants cultivated in 513 mM NaCl (corresponding to 30 g/L NaCl), > 60 % f the initial CP was found in the juice fractions. High protein content in the vegetative part of a plant is typically linked to younger development stages [177,178], which could be the case in *S. europaea* cultivates at 0 and 684 mM NaCl (corresponding to 40 g/L NaCl) due to growth inhibition in unfavourable conditions (too low and high salinity). Similar CP concentrations have been reported for other *Salicornia* species [99,188,196]. High-succulence *Salicornia* species accumulate sodium ions in their tissues, and an increase in total ash content by salinity and the highest Klason lignin content close to the optimal cultivation salinity have been previously reported for *S. bigelovii* and *S. ramosissima* [188,202]. Considering juice fractions, *S. europaea* also had significantly lower concentrations of free sugar monomers compared to two other species, the highest concentrations being 3.7 g/100g DM, 26.1 g/100g DM, and 40.8 g/100g DM for *S. europaea*, *T. pannonicum*, and *C. maritimum*, respectively. Besides lignification by age, the largest lignocellulose fraction is linked to the larger plant size and highest biomass yield from optimal cultivation salinity [172,196], which was observed in both *S. europaea* and *T. pannonicum* samples.

Effect of salinity on the composition of *T. pannonicum*

In *T. pannonicum* samples, significant differences amongst all samples were observed only in total lipid, carbohydrate, and CP content. The ash content was significantly different in plants grown at 0 and 684 mM NaCl. According to Ludwiczak et al. [203], the first response of *T. pannonicum* to salinity stress is to produce high levels of antioxidant metabolites to balance the excess production of reactive oxygen species, whereas the accumulation of ions is a slower response. The used mechanism depends on the type and species of plant, growth stage, and intensity and duration of the stress, which leads to varying salt tolerance seen in nature [33,34,203]. *T. pannonicum* also had the highest CP concentration of all studied species, up to 29.97 ± 0.20 g/100g DM. Whereas differences in CP content of the whole plant biomass were significant between salinity conditions, no significant differences were observed when juice and fibre fractions from 0 and 171 mM NaCl (corresponding to 10 g/L NaCl) were considered separately. The CP content was higher than previously reported for Asteraceae species [102,204]; however, the presence of other nitrogen-containing compounds, such as nitrate, chlorophyll and non-protein amino acids, may cause an overestimation of protein content. A high content of xylose observed in both *T. pannonicum* makes it an interesting species for the production of hemicellulose-derived biochemicals, such as xylitol, furfural, or prebiotic oligosaccharides [16,34].

Effect of salinity on the composition of *C. maritimum*

Significant differences were observed in the contents of ash, CP, and lignocellulose constituent in *C. maritimum* from different cultivation salinities. Especially plants cultivated at 86 mM NaCl (corresponding to 5 g/L NaCl) were rich in total carbohydrates (34.71 ± 2.86 g/100g DM in whole plant) and available glucose (18.21 ± 0.47g/100g DM in juice). An inverse relationship between salinity and CP content of *C. maritimum* was also observed, whereas differences in CP content of fibre

fraction were non-significant. Ash from *C. maritimum* has a high calcium content, and calcium has a protective role in the salt tolerance of plants [205].

3.2.2. Processing of the fibre residue fraction

Extraction yields

From parallel extractions, the yield of different extracts is reported in **Table 3**. The highest concentration of both water and ethanol extractives was found in *S. ramosissima* fibres, with 33.7 g/100g DM and 31.5 g/100 gDM, respectively. On the contrary, the lowest content of water and ethanol extractives was found in *C. maritimum*, with 16.5 g/100g DM and 13.6 g/100g DM, respectively. Very low lipid content (n-hexane extractives), which is typical for succulent halophytes in the vegetative stage, was observed in all species. Similar low lipid contents, 1.6 – 4.8 g/100g DM, were also observed in other studied halophyte fibre fractions [34].

Table 3 Content of extractive material [g/100g DM] obtained from parallel extractions with solvents with increasing polarity.

Species	Water extractives	Ethanol extractives	Hexane extractives	Ref.
C. maritimum	16.54 ±0.33	13.56	2.20 ± 0.10	[17]
S. ramosissima	33.68 ±2.31	31.45 ± 0.59	1.13 ± 0.06	[48]
T. pannonicum	18.73 ± 0.50	25.88 ± 2.47	2.87 ± 0.04	[17]

Besides biological and environmental factors (discussed in detail in studies presented in **Chapter 4)**, the extraction yield highly depends on the used extraction methods, biomass pre-processing and storage, and used solvent and its purity [16,206–210]. Commonly used solvent extraction methods are maceration, infusion, decoction, and Soxhlet extraction. The benefit of Soxhlet extraction is that the extraction is not limited by mass transfer constraint, as the solvent is gradually introduced to the biomass through extraction cycles [16]. However, some compounds of interest in plant extracts are sensitive to elevated temperatures or are unstable in neutral or alkaline pH [16,211–213]. Therefore, the benefits of prolonged extractions in these conditions should be evaluated for each biomass and compound of interest. Ultrasoun-assisted and microwave-assisted extractions, pressurised-liquid extraction, and other unconventional methods have been tested in recent years to improve extraction yields and reduce the extraction time in potentially undesired conditions [209,211,214,215].

In subsequential extractions (**Figure 18**), the highest content was extractives was found in *C. maritimum*, likely due to the use of whole, non-fractionated plant biomass in these extractions. Considering the total water and ethanol extractive material, the results are comparable to those of ash-free extracts reported by Cybulska et al. [146] for ten different halophyte species. This may indicate that when water-soluble salts, which the halophyte ash mainly constitutes, end up with the juice fraction after the screw press, while the majority of the potentially valuable extractives remain in the fibre fraction. The amount of water extractives was highest in *C. maritimum*, 27.00 ± 0.54 %, followed by *S. ramosissima* and *T. pannonicum*, with 23.61 ± 0.49 % and 10.75 ± 1.16 %, respectively. The highest content of ethanol extractives, 5.38 ± 0.22,

was found in *T. pannonicum*, and low (< 5 %) ethanol extractive content was also previously found in most of the different halophytes tested in a previous study [146]. The hydrothermal pretreatment with higher severity subritical conditions allowed the better extraction of compounds from lignocellulose matrix, the amount of released increasing from 3.77 ± 0.14 % to 19.51 ± 3.76 % when changing the pretreatment conditions from SF 2.4 to SF 3.7.

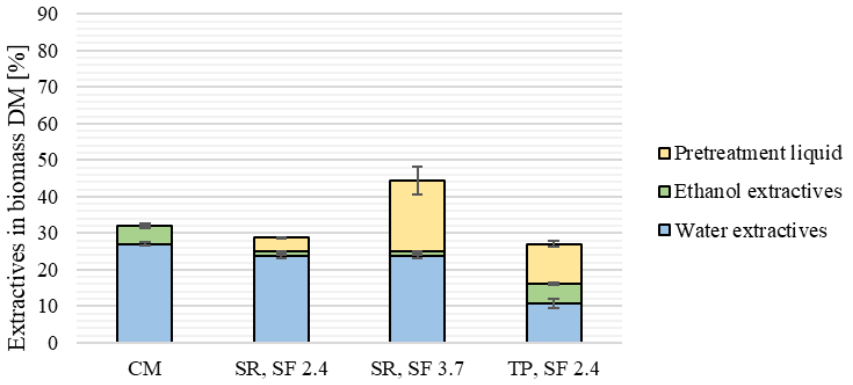

Figure 18 Amount of extractives after subsequential Soxhlet extractions of halophyte dry matter. CM: *Crithmum maritimum*, SR: *Salicornia ramosissima*, TP: *Tripolium pannonicum* (120 mM NaCl), SF: severity factor. CM fibres were not pretreated. SF 2.4: 121 °C 30 min, SF 3.7: 190 °C 10 min.

Convertibility of fibres

The recoveries of sugars from the pretreated lignocellulosic fibres to hydrolysate after enzymatic saccharification are shown in **Figure 19**. In a previous study carried out for *S. sinus-persica*, Alassali et al. [131] reached the highest cellulose recovery to pretreated de-juiced fibres with SF 2.4 and also achieved > 60 % ethanol yields with simultaneous enzymatic saccharification and fermentation of pretreated fibres with *Saccharomyces cerevisiae*, showing good sugar convertibility. However, this was not the case with extractives-free *S. ramosissima*, as the convertibility of fibres treated with SF 2.4 was close to those without hydrothermal pretreatment and recovery of all the sugars to hydrolysate was < 10 %. For *S. ramosissima*, 70 – 80 % sugar recoveries were achieved using pretreatment with a higher severity factor. Christiansen et al. [128] also tested SF 3.7 pretreatment for de-juices *S. bigelovii* fibres and reached > 80 % glucose and xylose and > 40 % arabinose recovery to hydrolysate based on the content of carbohydrates in untreated fibres. These high pentose recoveries indicate only low levels of sugar degradation during pretreatment. For completely lignified *S. bigelovii*, pretreatment at SF 3.7 has also shown high cellulose recovery to fibres, efficient xylose release to pretreatment liquids with minimum furfural production, and high fibre convertibility and ethanol yields [98,216]. Differences in the results may be due to variations between closely related species and differences in the biomass growth stage and processing methods. On the other hand, the pretreatment with SF 2.4 significantly enhanced the glucose recovery from *T. pannonicum* fibres to hydrolysate, reaching 73.0 %, whereas the convertibility of pentose sugars was not

significantly affected. Overall, the results highlight the importance of pretreatment optimisation for each specific type of biomass.

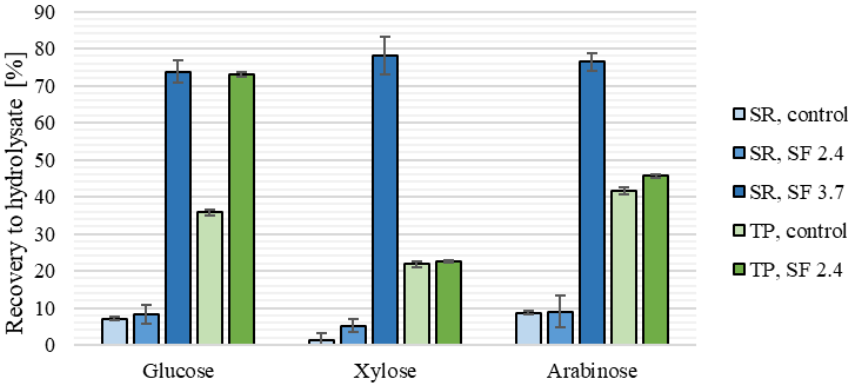

Figure 19 Sugar recovery from pretreated fibres to hydrolysate after enzymatic saccharification. SR: *Salicornia ramosissima*, TP: *Tripolium pannonicum* (120 mM NaCl), SF: severity factor.. SF 2.4: 121 °C 30 min, SF 3.7: 190 °C 10 min.

In pretreated *S. ramosissima* fibres, the fraction of available glucose in the fibres increased from 39.0 g/100g DM to 70.9 g/100 g DM, moving from lower to higher severity, and only < 0.5 g/100g DM glucose monomers were found in SF 3.7 pretreatment liquid, showing high cellulose recovery to fibres. However, the xylose fraction in the fibres decreased from 26.9 g/100g DM to 10.4 g/100g DM and arabinose from 3.3 g/100g DM to 0.7 g/100g DM due to pentose sugar release to a liquid fraction. Therefore, the high recoveries of xylose and arabinose to the hydrolysate obtained in this study have been from significantly lower initial sugar content in pretreated fibres. The pretreatment liquid from SF 3.7 was rich in free pentose sugars, with xylose and arabinose concentrations of 10.07 ± 0.53 g/100g DM and 12.83 ± 0.32 g/100g DM, respectively. However, whereas nearly all of the total glucose and arabinose were recovered in pretreatment fractions, > 55 % of the xylose was lost due to degradation. Heating and cooling rates of the used equipment can have a significant role in the pretreatment performance by affecting the time the biomass has been exposed to elevated temperatures, and the effect may be more pronounced when moving from laboratory-scale vessels to larger-scale systems.

Sugar degradation products

Considering the sugar degradation products, < 0.5 g/100g DM of HMF was measured from both *S. ramosissima* pretreatment liquids (**Figure 20**). On the contrary, 41.95 ± 5.35 g/100g DM of furfural was measured from *S. ramosissima* pretreatment liquid from SF 3.7, whereas no furfural was detected from SF 2.4 pretreatment liquid. Indeed, more than 40 % of the SF 3.7 pretreatment liquid DM was furfural and pretreatment at SF 3.7 could be considered too severe and harsh for *S. ramosissima* fibres. From the *T. pannonicum* pretreatment liquid, neither furfural nor HMF was measured. A pretreatment optimisation study is needed to find the conditions to achieve high sugar release and recoveries with the minimum production of toxic degradation products, which in high can concentrations can be inhibitory agents in the

biorefinery targeting sugar fermentation to bioethanol [131]. On the other hand, furfural can also be a desired compound of interest, as it is already commercially produced and used as a platform chemical in, for example, furfuryl alcohol and 2-furoic acid production [16].

Figure 20 *Salicornia ramosissima* fibres and pretreatment liquid obtained from hydrothermal treatment of extractives-free fibres at 190 °C for 10 min (SF 3.7).

3.2.3. Fatty acid profiles

Fatty acid profiles were determined from the n-hexane extract from fibre residues. In *C. maritimum*, the lipids constituted 49.9% polyunsaturated fatty acids (PUFA), 36.7 % saturated fatty acids (SFA), and 13.4 % monounsaturated fatty acids (MUFA), the main FA being linoleic acid (34.4%) [17]. In *S. ramosissima*, the corresponding values were 58.2 % PUFA, 41.0 % SFA, and 34.5 %, with the same predominant FA linoleic acid (34.5 %) [48]. In *T. pannonicum*, the amount of PUFA was significantly higher, 78.2 %, followed by 20.6 % SFA and 1.2 % MUFA, and the main FA was α-linolenic acid with 53.2 % relative concentration [17]. Very-long chain fatty acids arachidic acid, behenic acid, and lignoceric acid were only found in *S. ramosissima.* Obtained results are aligned with the existing literature reporting the FA profiles from different fractions for the species of interest [192,217–220].

All samples exhibited the ω-6 and ω-3 ratio < 5 reported to contribute to the anti-inflammatory properties of PUFA and reduced risk of cardiovascular diseases and cancer [221,222]. The ω-6 and ω-3 ratios were 2.2, 1.5, and 0.5 for *C. maritimum*, *S. ramosissima*, and *T. pannonicum*, respectively [17,48]. FA profiles are presented in **Table 4**. Even if the FA profile may be important for some nutraceutical or feed applications, due to the very low total content of lipids described in the previous section, the role of health-beneficial PUFA for biorefinery added value creation may not be significant.

Table 4 Relative concentrations of fatty acids (FA) in obtained lipids [% FA/total FA] from *S. ramosissima* [48], and *C. maritimum* and *T. pannonicum* [17]. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

Fatty acid	*C. maritimum*	*S. ramosissima*	*T. pannonicum*
Myristic acid	1.5 ± 2.2	0.4 ± 0.4	n.d.
Palmitic acid	28.9 ± 1.8	30.9 ± 2.3	19.0 ± 0.2
Palmitoleic acid	1.9 ± 2.7	n.d.	n.d.
Stearic acid	6.3 ± 0.3	2.7 ± 0.1	1.6 ± 0.0

Oleic acid	11.5 ± 0.2	1.3 ± 0.1	1.2 ± 0.5
Linoleic acid	34.4 ± 1.8	34.5 ± 0.7	24.9 ± 0.2
α-Linolenic acid	15.5 ± 0.7	23.7 ± 1.4	53.2 ± 0.3
Arachidic acid	n.d.	1.0 ± 0.9	n.d.
Behenic acid	n.d.	3.9 ± 0.1	n.d.
Lignoceric acid	n.d.	2.1 ± 1.8	n.d.
Σ SFA	36.7 ± 4.3	41.0 ± 0.9	20.6 ± 0.2
Σ MUFA	13.4 ± 2.9	1.3 ± 0.1	1.2 ± 0.5
Σ PUFA	49.9 ± 1.2	58.2 ± 2.0	78.2 ± 0.5
ω-6 / ω-3	2.2 ± 0.2	1.5 ± 0.1	0.5 ± 0.0

Chapter 4. Bioactivity of Plant Extracts

4.1. Review on bioactive compounds in halophyte extracts

This section summarises Paper II titled "*Extraction and Quantification of Chlorophylls, Carotenoids, Phenolic Compounds, and Vitamins from Halophyte Biomasses*" and published in MDPI Applied Sciences [223], which considers the phenolic compounds, proanthocyanidins, pigments and vitamins in halophytes and the related extraction and analytical methods. This study aimed to gather the existing knowledge of the concentration of compounds of interest found in different halophyte species and the protocols used in their extraction and determination. For this section, the review was extended to cover the reported concentrations of total flavonoids and consider the main specific polyphenols found from the halophyte species of interest.

Polyphenolic compounds in halophytes

Phenolic compounds are a diverse group of phytochemicals, including flavonoids, phenolic acids, lignans, and their various derivatives. These compounds can be effectively extracted from biomass using methanol or acetone as a solvent, and the majority of the reviewed studies used either of these as solvents to achieve the highest phenolic yields [223]. Aqueous extracts have also been studied [143,206,224–226], key drivers being chemical safety and a wide range of extract applications. The total contents of different phenolic groups can be determined using established absorption spectroscopy assays, also used in this PhD project for Paper III and Paper IV, and different chromatographic methods are developed to detect and quantify the concentration of specific phenolic compounds in the extracts.

Polyphenols are the phytochemicals linked to the biological activities and health benefits of plants, including antioxidant and anti-inflammatory properties, prevention of cardiovascular and metabolic diseases, and antimicrobial, anti-viral and anti-cancer effects, amongst others [50,62,66,71]. The group of phenolic acids constitute hydroxybenzoic and hydroxycinnamic acids and their derivates. Flavonoids can be divided into various sub-classes, including flavanones, flavanols, flavonols, isoflavones, flavones, and anthocyanidins [61]. Both phenolic acids and flavonoids are compounds of interest due to their various potential applications in biomedicines, nutraceuticals, and cosmetics [61,62,66,211,227]. Proanthocyanidins are polymerised flavonoids found in various plant fractions, giving them astringent, bitter and sour taste [228,229]. These compounds yield anthocyanidins, responsible for the red, purple and blue colour of plants under acidic oxidative conditions [227]. In nature, proanthocyanidins protect plants from insects, parasitic nematodes, fungal infections and other diseases, and they have recently increased interest due to their potential pharmacological use [228–231].

Considering the species of interest for this PhD project, the main phenolic acids quantified and identified from *Salicornia* species extracts are *p*-coumaric acid, gallic acid, protocatechuic acid, chlorogenic and ferulic acid, and main flavonoids catechin, myricetin, rutin, isorhamnetin, and some quercetin derivatives [206,208,232–234].

Extracts from *T. pannonicum* are less studied for the concentration of specific compounds; however, quercetin, luteolin, apigenin, and several quercetin and kaempferol derivates have been identified in this species [235]. *C. maritimum* has been characterised to be especially rich in phenolic acids, mainly chlorogenic acid and its isomers neochlorogenic acid and cryptochlorogenic acid, but also *trans*-ferulic acid and other phenolic acid derivatives [44,143,236–238]. Various other polyphenols were also reported in lower concentrations from extracts of the studied halophytes and reported concentrations vary between studies. There are thousands of naturally occurring polyphenols in plants, and their diverse structures and complex derivates make them difficult to identify and quantify [66,239].

The concentration of TPC, TFC, and TCT depends on biological factors, such as genotype and the plant's growth stage, but also on the specific plant organ used for the extraction. Extracts from *Limonum algarvense* and *Tamarix gallica* flowers have been shown to contain significantly higher concentrations of phenolics compared to leaves and other plant organs [240,241]. Similarly, from *Limonium delicatulum, S. europaea*, and *C. maritimum*, higher content of phenols was obtained in plants in the flowering growth stage compared to the vegetative stage [238,242]. High polyphenolic contents were also found in other species with distinctive flowers (**Figure 21**), such as *T. pannonicum* (syn. *Aster tripolium*), *Retama raetam*, and *Mesembryanthemum edule* (syn. *Carpobrotus edulis*) [63,226,243,244]. High polyphenolic content in later growth stages has also been reported for *Salicornia* species [177,245]. On the contrary, the opposite was observed in *Salsola kali*, which had a higher phenolic content in leaves compared to flowers and the highest polyphenolic content at the end of the vegetative stage before flowering [246,247]. As many polyphenols are produced to adapt to environmental stressors, their concentrations are also highly dependent on temperature, water and soil salinity, drought or flooding conditions, light exposure and the strength of UV radiation [66,82,188,190,247]. Due to multiple factors affecting the production of polyphenols in plants, a large variation in concentrations of TPC, TFC, and TCT between studies using the same species can be observed. The total contents of different types of polyphenols and pigments in halophytes are collected in **Table 5**.

Figure 21 Blooming halophytes: Hottentot Fig (*Carpobrotus edulis*) (**a**) and Tamarisk (*Tamarix gallica*) (**b**), both by Phil Sellens, are licensed under CC BY 2.0 [248] and *Retama raetam* (**c**) by Mitch Van Dyke and *Limonium algarvense* (**d**) by Lies Van Rompaey are licensed under CC BY 4.0 [249].

Table 5 Contents of total phenolic compounds (TPC), total flavonoids (TFC), total condensed tannins (TCT), chlorophyll (CHL), and total carotenoids (TCA) in halophyte extracts. Studies were first reviewed in Paper II [223]. The extraction methods, solvents, and analytical methods vary between studies. GAE: gallic acid equivalent, QE: quercitin equivalent, CE: catechin equivalent, n.d.: not detected, n/a: not available, * unit in fresh weight (FW) basis, ** unit as rutin equivalent, *** unit as catechin equivalent.

Species	TPC [mg GAE/g DM]	TFC [mg QE/g DM]	TCT [mg CE/g DM]	CHL [mg/100g DM]	TCA [mg/100g DM]	Ref.
Artemisia santonicum	212.7	57.3**	n/a	n/a	n/a	[54]
Arthrocnemum macrostachyum	10.2 – 49.2	1.8; 4.6 – 19.5**	7.5	28.3	210	[189,224,233]
Aster tripolium	31 – 223	3.7 – 55.4**	n.d.	8.5; 51 mg/cm^2	2.5; 8 mg/cm^2	[49,54,63,82,250]
Cakile maritima	43.7	37.1	14.9	n/a	n/a	[251,252]
Cladium mariscus	254	13.8	38.7	n/a	n/a	[63]
Crithmum maritimum	7.9 – 35.3	6.1 – 6.6	0.2 – 1.1	n/a	n/a	[237,238,253,254]
Frankenia laevis	253	12.7**	n.d.	n/a	n/a	[63]
Halimone portulacoides	14.6	6.2**	n/a	9.5*	5.1*	[233,255]
Inula crithmoides	14 – 141	1.3**	1.0	n/a	n/a	[63,254]
Ipomoea pes-caprae	54.2	23.7	19.7	n/a	61	[256]
Limoniastrum monopetalum	16 – 278	13.7**	1.4 – 6.6	n/a	n/a	[63,257]
Limonium algarvense	228	236**	145	n/a	n/a	[241]
Limonium delicatulum	92.9	15.1***	38.4	n/a	n/a	[242]
Limonium densiflorum	56.2	n/a	n/a	n/a	n/a	[258]
Lycium shawii	52.7	13.0	n/a	n/a	n/a	[72]
Mesembryanthemum edule	70.1 – 272.8	48.1 – 66.0***	7.2 – 20.3	n/a	n/a	[225,243,244,247]
Mesembryanthemum nodiflorum	6.8	5.0	2.0	280	21	[190]
Pluchea lanceolata	42.3	12.3	20.5	n/a	7	[256]
Reaumuria vermiculata	117.1	29.9***	28.0	n/a	n/a	[259]

Retama raetam	137.0	10.4***	10.2	n/a	n/a	[226]
Rumex vesicarius	28.5	12.6	n/a	n/a	n/a	[72]
Salicornia bigelovii	n/a	n/a	n/a	56.9*	15.9*	[260]
Salicornia brachiata	n/a	n/a	n/a	74.7	43.4	[173]
Salicornia europaea	11 – 58	18.3 – 46.9**	n/a	125*	43*	[54,233,261,262]
Salicornia neei	15.7	0.8	n/a	23.3	2.9	[263]
Salicornia persica	n/a	n/a	n/a	33 – 212*	5 – 44*	[171,262]
Salicornia prostata	n/a	n/a	n/a	14*	25*	[264]
Salicornia ramosissima	12.9 – 74.1	4.5**; 0.5 – 8.4***	n.d.; 32.5	21.6	290; 3.5*	[39,63,188,206]
Salsola kali	10.7 – 17.3	3.5 – 15.3	1.9 – 2.0	n/a	n/a	[246,247]
Salsola vermiculata	19 – 133	2.9; 13.3**	n.d.	n/a	n/a	[63,265]
Salvadora persica	5.4 – 58.2	3.2	22.4	n/a	11.4 µg	[256,266]
Sarcocornia fruticosa	5.7	3.0	0.5	290; 10.2 – 35*	16; 3.5 – 5.7*	[171,190,255]
Sarcocornia perennis	20.5 – 75.7	4.6**; 19.9***	n/a	14.8*	280*	[39,63]
Suaeda aegyptiaca	15.9	64.2**	n/a	n/a	n/a	[267]
Suaeda fruticosa	10.4 – 47.7	1.6 – 21.4; 32.9**	1.5 – 15.8	n/a	56	[174,224,256,267–269]
Suaeda maritima	16.6 – 62.9	5.9; 73.5**	1.2	280	12	[54,190]
Suaeda prostata	n/a	n/a	n/a	8	19	[264]
Suaeda vera	n/a	n/a	n/a	n/a	11.5*	[41]
Tamarix africana	61.1	1.9	118.4	n/a	n/a	[224]
Tamarix gallica	70.6 – 135.4	12.3***	17.3	180 mg	70 mg	[240,247,270]
Thespesia populnea	64 – 270	37 - 140	20.1	n/a	72 - 234	[256,271]
Zaleya pentandra	22.6	31.5	n/a	n/a	n/a	[272]

Photosynthetic pigments in halophytes

Chlorophylls and carotenoids are considered photosynthetic pigments. Chlorophylls *a* and *b* are pigments responsible for the green colour of plants, which in human nutrition not only make products more desirable for consumption but have antioxidant and potential chemopreventive properties [39,171,273,274]. Carotenoids are yellow and orange pigments protecting plant photosystems, and they can be divided into carotene and xanthophylls [126,275]. Carotenoids are linked to many health benefits, including pro-vitamin A activity, immunomodulation, improvement of cognitive functions, and prevention of cardiovascular and degenerative disorders [275–277]. *Sarcocornia fruticosa* has shoen to be rich in β-carotene, as well as lutein [190,255]. Cultivation salinity has been shown to affect the pigment concentration of plants. Some studies show an inverse relationship between salinity and pigment content [126,255,262,264], but increased pigment content or no significant change has also been reported for halophytes [126,171,188]. Pigment content has also been shown to be highest in plants at the younger growth stage [173].

Vitamins in halophytes

Vitamins are essential nutrients required for the average growth and health of humans. Thus they are highly commercialised for functional food additives and nutraceuticals, as the key sources of vitamins, such as vegetables, fruits, unrefined cereals and nuts, are often lacking, especially in the Western pattern diet [276,278]. Different chromatography methods are typically used to determine the concentration of vitamins in plant extracts [223]. Phenotypic stage and cultivation conditions, such as salinity, and storing conditions, have been shown to affect the nutritional composition of halophytes [174,188,260]. Considering water-soluble vitamins, ascorbic acid (vitamin C) is a potent antioxidant, having various roles, such as preventing cellular damage and oxidative stress, improving skin health, improving the immune system, and preventing cardiovascular diseases [266,271,279]. The shoots from *S. fruticosa* and *Suaeda maritima* have been shown to be rich in vitamin C [190], and leaves of *Thespesia populnea* could fulfil the daily vitamin C intake requirements of a healthy adult [271]. Vitamins in B complex group are essential co-enzymes and antioxidants supporting various processes, such as carbohydrate and protein metabolism, nucleic acid synthesis, and enhancing the cell growth, bone health, and function of neurons [188,276,280]. *Mesembryanthemum nodiflorum, S. fruticosa* and *S. maritima* have exhibited higher pyridoxine (vitamin B6) content than conventional green vegetables, asparagus and watercress [190]. Cobalamin (vitamin B12) is scarce in plants, and supplementation is often necessary for individuals following a strictly plant-based diet to ensure sufficient intake. Interestingly, *Suaeda aegyptiaca* has exhibited higher cobalamin content than sea buckthorn, one of the richest known sources of vitamin B12 among plants [280,281].

Tocopherols (vitamin E) and retinyl acetate (vitamin A) are lipid-soluble vitamins. Tocopherols have been reported to prevent cardiovascular diseases, different types of degenerative disorders and cancer, and their potent antioxidant activity protects cell membranes and lipoprotein from lipid peroxidation [39,276,277]. *Arthrocnemum macrostachyum* has shown vitamin E content comparable to conventional green vegetables, kale and broccoli [39]. The content of lipids is highest in halophyte seeds, and *Salicornia* and *Teucrium* seed oils have been shown to have high concentrations

of tocopherols [282–284]. Few studies report the concentration of retinyl acetate (vitamin A) in plant extracts, likely due to humans' ability to convert β-carotene, an important plant-based nutrient, to vitamin A. The concentrations of vitamins found in halophytes are summarised in **Table 6**.

Table 6 Vitamin content [mg/100g DM] reported for halophytes. Studies were first reviewed in Paper II [223]. The considered water-soluble vitamins were thiamine (B1), pyridoxine (B6), folate (B9), cobalamin (B12), and ascorbic acid (C), and lipid-soluble vitamins retinyl acetate (A) α-tocopherol (E). * Unit in fresh weight (FW) basis, n.d.: not detected, n/a: not available. For *Teucrium* spp., the seed oil fraction was considered [282].

Species	Vitamin A	Vitamin B	Vitamin C	Vitamin E	Ref.
Arthrocnemum indicum	n/a	n/a	19.2*	2.1	[174]
Arthrocnemum macrostachyum	n/a	n/a	n/a	8.7	[39]
Aster tripolium	n/a	n/a	13.7*	n/a	[49]
Cakile maritima	n/a	n/a	n/a	20	[252]
Halocnemum strobilaceum	n/a	n/a	7.4*	3.4*	[174]
Mesembryanthemum nodiflorum	4.5	24 (B6)	500	n.d.	[190]
Salicornia bigelovii	n/a	n/a	5.8*	n/a	[260]
Salicornia ramosissima	n/a	0.03* (B1); 0.003* (B6)	n/a	0.2*	[188]
Salvadora persica	n/a	n/a	68.0	n/a	[266]
Sarcocornia fruticosa	n/a	10 (B6)	1000	18	[190]
Sarcocornia perennis	n/a	n/a	n/a	1.1	[39]
Suaeda aegyptiaca	n/a	18.1 (B6); 11.8 (B9); 46.6 (B12)	n/a	n/a	[280]
Suaeda fruticosa	n/a	n/a	2.5*	11.4	[174,190]
Suaeda maritima	5.4	9.5	3000	12.5	[190]
Suaeda vera	n/a	10.2 (B1); 11.8 (B6); 27.1 (B12)	n/a	n/a	[280]
Thespesia populnea	n/a	n/a	44.3	n/a	[271]
Teucrium alopecurus	n/a	n/a	n/a	31.6	[282]
Teucrium polium	n/a	n/a	n/a	27.7	[282]
Teucrium nabli	n/a	n/a	n/a	29.6	[282]

4.2. Findings on the bioactive properties of halophytes

4.2.1. Annotated bioactive compounds from extracts

Various interesting compounds were annotated after the untargeted LC-MS analysis; however, an analysis using standard compounds would be needed to identify and

quantify the metabolites. For the annotated compounds, the deviation of the measured and theoretical mass (Δmass) was $-5.0 \leq \Delta mass \leq 5.0$, the mzCloud Best Match score was ≥ 85 % and had matches in one or more of the used compound databases. The bioactive compounds found and annotated from water extracts are summarised in **Table 7**. Not all annotated metabolites were reported, and besides the reported compounds, several types of compounds were found in water extracts, including benzoic acid derivatives, nucleic acid derivatives, peptides, compounds related to amino acid metabolism, and some unknown flavonoids. Phenolic compounds often linked to biological activities of extracts were found from all species: flavonoids, phenolic acids, coumarins, and their derivatives. Similarly, vitamin B2, riboflavin, and vitamin B5, pantothenic acid, were detected in all water extracts. Vitamin B9 was found in *C. maritimum* extracts in the form of folinic acid, one of the natural forms of folate. A previous study by Lima et al. [188] reported vitamins B1 and B5 found in *S. ramosissima* methanol-ammonium acetate extract.

Table 7 Some of the bioactive metabolites annotated in water extracts after LC-MS analysis, organised by m/z values. RT: retention time, CM: *Crithmum maritimum*, SR: *Salicornia ramosissima*, TP: *Tripolium pannonicum*, +: presence in extract, -: absence.

Compound	m/z $[M-H]^{+1}$	m/z $[M-H]^{-1}$	RT [min]	CM	SR	TP
4-Hydroxybenzoic acid	n/a	137.02439	13.87	+	+	+
5-Methoxysalicylic acid	n/a	167.0348	9.77	-	+	-
Isovanillic acid	n/a	167.0349	7.81	-	+	-
Isoferulic acid	177.0544	n/a	11.03	+	+	-
Caffeic acid	n/a	179.0347	12.79	-	-	+
Azelaic acid	n/a	187.0972	14.66	+	+	+
Quinic acid	n/a	191.05558	10.63	+	-	+
Scopoletin	193.0496	n/a	13.16	+	+	+
Ferulic acid	n/a	193.0502	13.35	+	+	-
Pantothenic acid	220.1181	n/a	6.09	+	+	+
Dodecanedioic acid	n/a	229.1441	20.43	-	+	+
Apigenin	n/a	269.0452	19.54	-	+	-
Xanthosine	n/a	283.0680	5.56	-	+	-
Luteolin	n/a	285.0399	16.78	-	+	-
Isokaempferide	301.0702	n/a	19.87	+	+	-
Quercetin	303.0496	n/a	14.23	-	+	+
Isorhamnetin	317.0655	n/a	15.27	-	+	+
Aesculin	n/a	339.0711	9.60	+	+	+
Chlorogenic acid	n/a	353.08658	10.65	+	-	+
Neochlorogenic acid	n/a	353.0867	8.64	+	-	+
3-O-Feruloylquinic acid	n/a	367.1022	12.43	+	-	-
Riboflavin	377.1452	n/a	11.88	+	+	+
Nobiletin	403.1383	n/a	22.34	+	+	+
Syringaresinol	n/a	417.1543	14.47	-	+	-
Kaempferol-3-O-rhamnoside	n/a	431.09705	16.19	+	-	-
Apigenin 7-glucuronide	447.0916	n/a	15.39	-	+	-
Astragalin	n/a	447.0921	15.08	-	+	+
Kaempferol-3-galactoside	449.1077	n/a	15.09	-	-	+

Kaempferol 3-glucuronide	463.0869	n/a	14.31	-	+	-
Quercetin-3β-D-glucoside	465.1022	n/a	14.24	-	+	+
Folinic acid	474.1731	n/a	9.56	+	-	-
Miquelianin	n/a	477.0661	14.21	-	-	+
4,5-Dicaffeoylquinic acid	n/a	515.1177	14.59	+	-	+
Rutin	n/a	609.1442	13.92	+	-	+
Hesperidin	n/a	609.1802	14.88	+	-	-
Diosmin	609.1812	n/a	15.31	+	-	-

Some fatty acids, fatty acid conjugates and their derivates, such as fatty acid amides (not reported), were found in ethanol extracts. The role of different fatty acids in non-polar extracts of studied species is discussed in published papers [17,48]. Many of the annotated flavonoids and other bioactive compounds were present in both water and ethanol extracts; however, some interesting flavonoid derivatives were found only in ethanol-based samples, likely due to their lower solubility in water. In addition, some unknown flavonoid compounds were also found. Interestingly, some flavonoids were present in both water and ethanol extracts from *S. ramosissima* but were found only in ethanol extracts of *C. maritimum* and *T. pannonicum*. Some potential explanations are low compound concentrations below the detection limit, or longer extraction time and solvent required to access potentially conjugated compounds from biomass with higher lignin content [214,285]. The annotated compounds from ethanol extracts are summarised in **Table 8**.

Table 8 Metabolites annotated in ethanol extracts after LC-MS analysis, organised by m/z values. RT: retention time, CM: *Crithmum maritimum*, SR: *Salicornia ramosissima*, TP: *Tripolium pannonicum*, +: presence in extract, -: absence.

Compound	**m/z $[M-H]^{+1}$**	**m/z $[M-H]^{-1}$**	**RT [min]**	**CM**	**SR**	**TP**
4-Hydroxybenzoic acid	n/a	137.02438	7.97	+	+	+
Methyl salicylate	n/a	151.04	9.75	+	+	+
Suberic acid	n/a	173.0818	12.43	+	+	+
D-Mannitol	n/a	181.0716	1.96	+	-	-
Azelaic acid	n/a	187.0971	14.63	+	+	+
Isoferulic acid	n/a	193.0501	12.29	-	+	-
Ferulic acid	n/a	193.0502	10.99	+	-	-
Dodecanedioic acid	n/a	229.1439	20.41	+	+	+
Palmitic Acid	n/a	255.23232	26.37	+	+	+
Apigenin	n/a	269.0447	19.54	+	+	+
Galangin	271.0602	n/a	19.55	+	+	-
Naringenin	n/a	271.0603	18.82	-	+	-
Cardamomin	271.09637	n/a	23.67	-	+	-
γ-Linolenic acid	n/a	277.2166	24.44	+	+	+
α-Linolenic acid	279.23136	n/a	28.99	+	-	+
Pinolenic acid	279.23209	n/a	26.44	-	+	-
Linoleic acid	n/a	279.2322	25.41	+	+	+
Oleic acid	283.26303	n/a	31.88	-	+	-
Stearic acid	n/a	283.2634	28.58	+	+	+

Luteolin	n/a	285.0397	17.94	+	+	+
Sakuranetin	287.09113	n/a	18.91	-	+	-
Isokaempferide	301.07074	n/a	19.83	-	+	+
Hesperetin	n/a	301.07104	19.35	+	-	-
Quercetin	303.04963	n/a	14.22	-	+	+
N-Feruloyloctopamine	312.12265	n/a	14.08	-	+	-
Isorhamnetin	317.06542	n/a	15.24	-	+	-
Neochlorogenic acid	n/a	353.0868	9.67	+	-	-
Chlorogenic acid	n/a	353.087	10.65	+	+	+
3-O-Feruloylquinic acid	n/a	367.1025	12.51	+	-	-
Riboflavin	377.14523	n/a	11.85	-	-	+
Nobiletin	403.13812	n/a	22.29	+	+	+
Kaempferol-3-O-rhamnoside	n/a	431.09708	16.15	+	-	-
Astragalin	n/a	447.09225	15.07	-	+	+
Kaempferol-3-galactoside	449.1073	n/a	15.06	-	-	+
Quercetin-3β-D-glucoside	465.1021	n/a	14.23	-	+	+
18-β-Glycyrrhetinic acid	471.34549	n/a	25.99	-	+	-
Miquelianin	n/a	477.0657	14.19	-	-	+
4,5-Dicaffeoylquinic acid	n/a	515.11829	14.94	+	-	+
Diosmin	609.1804	n/a	15.30	+	-	-

Discussion on found phytochemicals

As described previously in **Section 4.1**, flavonoids have long been known to be beneficial for human health, and their antioxidant and anti-inflammatory properties are highlighted [286]. These compounds are also potent anti-diabetic agents, and from the annotated compounds, apigenin, isorhamnetin, quercetin, quercetin-3β-D-glucoside, rutin, and sakuranetin have been reported to contribute to the anti-diabetic properties of plant extracts by inhibiting α-glucosidase, improving insulin sensitivity, and reducing oxidative stress [287]. Flavanone naringenin and its derivate sakuranetin, annotated only from *S. ramosissima* ethanol extract, have been described as anti-cancer and anti-viral agents [288,289]. Commonly found in citrus fruits, flavanone hesperetin, its derivate hesperidin, and flavone glycoside diosmin have also previously detected form halophytes, including *C. maritimum* [290–294]. Diosmin and hesperidin are used in the medical industry to treat chronic venous disorders, including reticular and varicose veins, swelling, stasis dermatitis, and ulcers [293,295,296]. Flavonol galangin, annotated from *C. maritimum* and *S. ramosissima* extracts, and flavone luteolin, found in all species, have been compounds of interest in recent years due to their therapeutic properties towards various types of cancer [297,298]. Folinic acid, also found in *C. maritimum*, is already used in the medical industry to improve the effects of chemotherapy on cancer patients [299]. Flavonoids kaempferol-3-galactoside, and miquelianin were detected only in *T. pannonicum* extracts, of which the latter has been recently studied for its potential as an antidepressant and inhibition of allergic responses [300,301]. Miquelianin also has been shown to inhibit the formation of β-amyloid peptides, which have been linked to the development of Alzheimer's disease together with oxidative stress and a decrease in the level of neurotransmitters [301,302]. Galanging and apigenin have been

reported to be strong BuChE inhibitors *in vitro*, whereas quercetin has a high inhibition activity towards AChE [303].

Besides flavonoids, phenolic acids are an abundant group of polyphenols known for their antioxidant and potential therapeutic properties [50,60,227,276,304]. Chlorogenic acid, one of the dominant compounds in *C. maritimum* extracts [143,305–307], is especially known for its anti-diabetic properties by regulating both sugar and lipid metabolism [308–310]. Its isomer, neochlorogenic acid, has been shown to have strong anti-inflammatory activity [311,312]. Caffeic acid, found in *T. pannonicum* water extract, is used in cosmetics due to its antioxidant and anti-ageing properties and has shown potential for treating dermal diseases [304]. Recently, caffeic acid has increased interest due to its activity against hepatocarcinoma [313]. Ferulic acid is commonly found in plants and was detected from *C. maritimum* and *S. ramosissima* extracts. It is commercially used in cosmetics due to its antioxidant properties, but it has shown other medicinal properties, such as protection against cardiovascular diseases [314,315]. Caffeic acid, chlorogenic acid, and ferulic acid have all also shown anti-viral properties [71].

Quinic acid derivative 4,5-dicaffeoylquinic acid, found in *C. maritimum* and *T. pannonicum* extracts, has previously been shown to be a strong a-glucosidase inhibitor and contributing to the anti-hyperglycemic potential of plant extract [316]. Found only in water extracts, 5-methoxy salicylic acid has previously been shown to contribute to the inhibition of tyrosinase activity [317], and lignan syringaresinol has shown significant anti-inflammatory properties both *in vitro* and *in vivo* [318]. Scopoletin is a coumarin found in all tested water extracts, which has exhibited anti-cancer properties, amelioration of metabolic and inflammatory diseases, and potent effects against neurological diseases *in vitro* and *in vivo* [319]. Found in *S. ramosissima* ethanol extract, triterpenoid saponin glycoside 18-β-glycyrrhetinic acid has also exhibited tyrosinase inhibition, but also antioxidant and anti-inflammatory properties and inhibition activity against an enzyme connected to acne [320]. D-mannitol, a polyalcohol with antioxidant and diuretic properties, have previously been found to be a predominant compound in *C. maritimum* [191].

Indeed, various interesting secondary metabolites were found in the studied extracts. Technologies like bioguided fractionation [321] could be utilised to find compounds responsible for biological activities of interest. Overall, residual fibres from screw-press halophytes have shown to be a potential source of valuable secondary metabolites; hence, extraction processes should be considered as a part of green biorefineries.

4.2.2. Bioactive Extracts from *Salicornia ramosissima* J. Woods Biorefinery as a Source of Ingredients for High-Value Industries

In this section, the results and discussion from Paper III, titled "*Bioactive Extracts from* Salicornia ramosissima *J. Woods Biorefinery as a Source of Ingredients for High-Value Industries*" and published in MDPI Plants, are summarised [48]. The paper considers the different extracts and fractions from *S. ramosissima*, their contents of phenolic compounds and pigments, their biological activities, and how these

bioactive extracts could be used in high-value applications. Besides juice fractions (05-2021 and 09-2021) and extracts from *S. ramosissima* fibre (05-2021) residue, a water extract obtained from completely lignified plants is also included in the study. This section focuses on the result considering the phenolic compounds, pigments, and bioactivity assays, as the amount of extractives and the fatty acid profiles have been presented in **Section 3.2.2** and **Section 3.2.3**, respectively.

Total bioactive compounds in *S. ramosissima* extracts

The ethanol extract from fibres had the highest TPC content, 41.1 mg GAE/g DM, followed by the extract from completely lignified plants with 30.1 mg GAE/g DM. All extracts had similar TFC content, 3.2 – 3.9 mg QE/g DM, whereas flavonoids were not detected from juice fractions. Proanthocyanidins were not detected from any of the *S. ramosissima* samples, and despite the bright red colour of the juice from French phenotype (05-2021), anthocyanidins were detected only in water extracts with low concentration < 1.5 mg CCE/g DM. This may indicate that the red colour of the juice comes from betalains, also known as betacyanins, previously detected from other *Salicornia* and *Suaeda* species [173,175]. The total chlorophyll and carotenoid content of ethanol extract were 1446 µg/g DM and 262 µg/g DM, respectively. In the juice from the French phenotype, only 15 µg/g DM chlorophyll was found, whereas the carotenoid content of juice was 73 µg/g DM. The juice from the Portuguese phenotype (09-2021) had a higher concentration of chlorophyll, 39 µg/g DM, but a lower amount of carotenoids, 52 µg/g DM.

The phytochemical content of *S. ramosissima* and many other halophytes is strongly influenced by cultivation conditions, specifically, the biotic and abiotic stresses the plants have been exposed to [52,61,190,263,264,322–325]. Potential halophyte-based biorefinery processes need to be designed to be robust enough to withstand a certain degree of intraspecific variation. The growth stage [245] and processing methods, such as extraction, drying and storage conditions [206,208,260], also play a role in the nutritional value of plants and extract composition. Therefore, the TPC concentration reported for *S. ramosissima* extracts varies between studies, 6 – 74 mg GAE/g DM [48,134,188,189,206,208,223,326]. This variation can also be seen in the contents of TFC and proanthocyanidins [56,63,188].

Antioxidant activity of *S. ramosissima*

Plants produce phytochemicals to protect tissues from cellular damage caused by the excessive production of reactive oxygen species triggered by environmental stressors; hence, these compounds hold potent antioxidant properties [144,277]. The antioxidant properties of *S. ramosissima* juice and extracts are presented in **Figure 22**. The aqueous extract from screw-pressed fibres had the highest DPPH and ABTS radical scavenging activities at 10 mg/mL, 47.7 % and 60.1 %, respectively, and the EC_{50} value of ABTS activity of 2.5 mg/mL is lower than previously reported to acetone extract from *S. ramosissima* grown in similar salinity [188]. Antioxidant activity was also high on the metal-based assays, and aqueous extracts also showed lower EC_{50} values in FRAP and ICA assays than those previously reported for *S. ramosissima* ethanol extract [39]. Achieving high antioxidant activity of extract without organic solvents is desirable for biorefinery applications to avoid high costs related to purchasing and handling hazardous material. Samples also exhibited NO scavenging

activity, which is linked to potential anti-inflammatory properties [161], which, together with antioxidant activity, have made *Salicornia* an interesting ingredient for dermo-cosmetics in recent years [68,73,326,327].

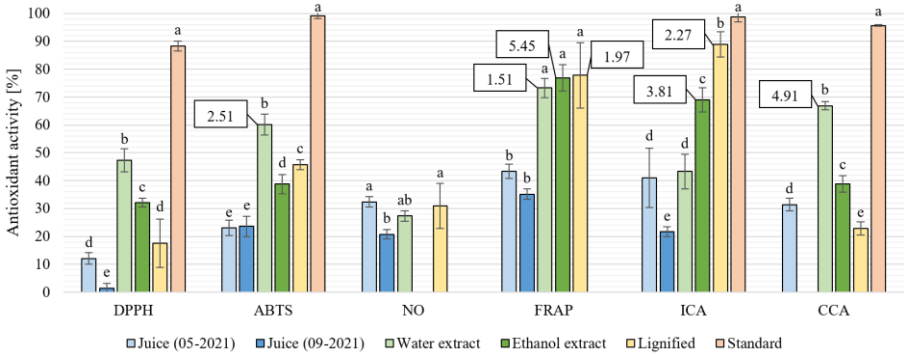

Figure 22 Antioxidant activity of *S. ramosissima* juice and extracts at 10 mg/mL concentration. EC_{50} values [mg/mL] are marked in call-out boxes for samples exhibiting activity > 50 %. Figure combined and adapted from ones in Paper III [48] licenced with CC BY 4.0 [249]. DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), NO: nitric oxide, FRAP: ferric reducing antioxidant power, ICA: iron chelating activity, CCA: copper chelating activity. Standard compounds are gallic acid at 1 mg/mL for DPPH and ABTS and ethylenediaminetetraacetic acid at 1 mg/mL for ICA and CCA.

Enzyme inhibition activity of *S. ramosissima*

Juice fractions and extracts were tested for their enzyme-inhibitory activities. One type of treatment for type II diabetes mellitus is to prevent hyperglycemia by regulating the activity of enzymes responsible for the digestion of complex carbohydrates to available glucose: α-glucosidase and α-amylase [328,329]. The ethanol extract exhibited a 68.6 % α-glucosidase inhibition at 10 mg/mL, whereas the benchmark compound acarbose had an inhibition activity of 85.6 % at the same concentration. There was no significant difference between α-glucosidase inhibition of aqueous extracts, with measured activities of 34.1 % and 38.9 % of extract from screw-pressed fibres and lignified plants, respectively. Purifying the raw extract and increasing the content of phytochemicals could yield a halophyte-based α-glucosidase inhibitor competitive to the commercial drug. However, acarbose also has inhibition activity towards α-amylase (61.2 %), whereas aqueous samples had very low inhibition (< 5 %). Measuring the inhibition activity of ethanol extract was not possible due to sample precipitation, even if some colour change in the assay due to α-amylase inhibition was visually observed.

The increased prevalence of obesity, and its effects on metabolic health and risk of developing various diseases, has led to a search for natural compounds regulating lipid absorption [330]. Lipase inhibitors also have applications in dermo-cosmetics for treating acne, as skin inflammation may be partly caused by bacterial lipase breaking the sebum fats to free fatty acids [331]. The ethanol extract had a moderate lipase inhibition activity of 41.7 % at 10 mg/mL concentration. Interestingly, the water extract from de-juiced fibres had no detected activity, whereas juice fractions and aqueous extract from lignified plants all had similar activities between 19.2 – 21.4 %.

Ethanol extract from *S. ramosissima* exhibited high tyrosinase inhibition activity, 71.9 % at 10 mg/mL, compared to the benchmark compound arbutin, with 41.5 % inhibition activity at 1 mg/mL. Tyrosinase inhibition activity of *Salicornia* has also been previously studied: *S. bigelovii* ethyl acetate extract has shown > 50 % inhibition activity at 60 mg/mL, *S. europaea* ethanol extract 21 % at 1 mg/mL, and aqueous S. herbacea extract > 50 % at 0.1 mg/mL [324,332,333]. Tyrosinase inhibitors are compounds of interest in the food, cosmetics, and pharmaceutical sectors. In food, they are used to prevent undesired browning in cut fresh plant products caused by polyphenol oxidases, including tyrosinase [334,335]. In cosmetics, tyrosinase inhibitors are used as skin-whitening agents, and their potential to treat hyperpigmentation and skin cancer melanoma has been studied [64,336–338].

Increasing the levels of neurotransmitters by inhibiting the enzymes responsible for their degradation is one of the potential therapeutic approaches to target symptoms of dementia and neurodegenerative diseases, such as Alzheimer's and Parkinson's disease [339,340]. Due to the ageing population and the late-onset nature of neurodegenerative diseases, the number of people suffering from these conditions has been projected to increase, presenting major health concerns and financial burdens to healthcare systems [341]. Therefore, the inhibition activity of *S. ramosissima* samples towards acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) was tested. The highest AChE inhibition activity, 68.4 %, was observed in the ethanol extract, whereas the juice fraction from the Portuguese phenotype had the highest BuChE inhibition activity of 46.3 % at 10 mg/mL. For reference, the dementia drug used as a benchmark had AChE and BuChE inhibition activities of 88.9 % and 41.4 % at 1 mg/mL, respectively. The aqueous extracts from screw-pressed and lignified plants showed no inhibition activity towards AChE or BuChE. The neuroprotective properties of *Salicornia* have also been previously studied *in vitro* [342,343], and *S. europaea* has shown positive results also in animal models and a small clinical trial [343,344]. Interspecific and intraspecific variations could explain the varying activity between studies, but different processing methods can also play a role in extracting and preserving the compounds responsible for the activity.

Previous studies considering the bioactivity and phytochemical composition of *Salicornia* have focused mainly on fresh edible fractions and their nutritional qualities. This study showcases the potential of partly and completely lignified plant fractions, often considered agricultural waste, for the production of ingredients for high-value industries, such as cosmetics, nutraceuticals, and even biopharmaceuticals. Residual fractions of *S. ramosissima* could be considered in future studies related to the therapeutic properties of this medicinal plant. Utilising the residues could help maximise the valorisation of the available feedstock and potentially bring additional value to the *Salicornia* farmers in the future.

4.2.3. Harnessing the value of *Tripolium pannonicum* and *Crithmum maritimum* halophyte biomass through integrated green biorefinery

This section summarises the key points and results of Paper IV, titled "*Harnessing the value of* Tripolium pannonicum *and* Crithmum maritimum *halophyte biomass*

through integrated green biorefinery" published in MDPI Marine Drugs [17]. The article considers the bioactivity of juice fraction and extracts from species mentioned above and discusses the role of halophyte-based biorefinery targeting high-value compounds in future sustainable circular economies. Similar to Paper III summarised in **Section 4.2.2** [48], juice and extract samples were analysed for their contents on different types of phenolic compounds and pigments, as well as *in vitro* antioxidant and enzyme-inhibitory properties. Existing literature regarding the bioactivity of *C. maritimum* is heavily focused on the edible fraction and the essential oils of the plant, and the bioactivity of *T. pannonicum* is still rather unexplored for bioprospecting. Similar to **Section 4.2.2**, this section focuses on phytochemical contents and bioactivity assay results.

Total bioactive compounds in *C. maritimum* and *T. pannonicum* extracts

The highest concentration of TPC, 64.7 mg GAE/g DM, was observed in *C. maritimum* ethanol extract, followed by 45.2 mg GAE/g DM in *T. pannonicum* ethanol extract. Water extract from both species had nearly the same concentration of TPC, slightly above 30 mg GAE/g DM, and a low concentration of TPC was also found in *C. maritimum* juice. The highest concentration of TFC was found in *T. pannonicum* ethanol and water extract, with concentrations of 6.6 mg QE/g DM and 5.4 mg QE/g DM, respectively. Previous studies have also shown that phenolic acids, such as chlorogenic acid and neochlorogenic acid, are the predominant phenolics in *C. maritimum* [345], which may explain the lower TFC content of *C. maritimum* water extract, 4.9 mg QE/g DM. Anthocyanidins were found in concentrations of 1.9 mg CCE/g DM and 4.4 mg CCE/g DM from *C. maritimum* and *T. pannonicum* water extracts, respectively. Photosynthetic pigments, namely chlorophylls *a* and *b* and carotenoids, were mainly present in the ethanol extracts. Especially *T. pannonicum* was rich in total chlorophylls and carotenoids, with concentrations in ethanol extract being 3632 µg/g DM and 299 µg/g DM, respectively, and > 0.1 mg/g DM of carotenoids were also found in the juice fraction. In *C. maritimum* ethanol extract, the total chlorophyll and carotenoid contents were approximately 1439 µg/g DM and 262 µg/g DM, respectively.

The phenolic content of *C. maritimum* varies greatly between studies, and extracts from plant leaves have shown a significantly higher content of bioactive compounds compared to extracts from other plant organs, such as stems [143,237,306,346]. As our *C. maritimum* extracts have been prepared from biomass containing both leaves and stems, as well as some flowers, the difference in the phenolic content compared to edible leaves is expected. Besides intraspecific variation, the effect of cultivation conditions and stress on the content of phenolics and pigments has been highlighted in various studies [49,76,82,188,190,217,237]. Different biomass processing conditions may also have a role in the concentration of bioactive compounds, as extraction and drying in elevated temperatures could cause degradation and instability of the sensitive compounds [211,347].

Antioxidant activity of *C. maritimum* and *T. pannonicum*

Different antioxidant activity mechanisms were tested for juice fractions at the initial concentration of 10 mg/mL. *C. maritimum* water extract had the highest DPPH scavenging activity (71.2 %), followed by the corresponding ethanol extract (68.9 %)

and *T. pannonicum* water extract (67.2 %). ABTS scavenging activity was similar in all tested extracts, varying between 72.0 – 79.4 %, and high ABTS scavenging activity was also observed in *C. maritimum* juice (60.8 %). Juice and water extract from *C. maritimum* exhibited NO scavenging activity of 22.5 % and 24.6 %, respectively, indicating potential anti-inflammatory properties. On the contrary, *T. pannonicum* samples exhibited more pronounced activity in metal-based antioxidant activity assays, showing lower EC_{50} values in ICA and CCA assays (**Table 9**).

Table 9 Antioxidant properties of *C. maritimum* and *T. pannonicum* juice and extracts [17], given as half-maximum effective concentrations (EC_{50}) [mg/mL]. DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), NO: nitric oxide, FRAP: ferric reducing antioxidant power, ICA: iron chelating activity, CCA: copper chelating activity, n.d.: no activity detected, n/a: not available.

Species	Extract	DPPH	ABTS	NO	FRAP	ICA	CCA
C. maritimum	Juice	> 10	4.59	> 10	3.46	> 10	6.79
	Water	3.53	4.36	> 10	1.13	> 10	7.03
	Ethanol	2.84	3.95	n/a	< 10	3.10	3.32
T. pannonicum	Juice	n.d.	> 10	> 10	> 10	> 10	3.18
	Water	< 10	2.24	> 10	1.91	< 10	3.40
	Ethanol	7.67	4.86	n/a	1.66	1.15	2.37

For both *C. maritimum* and *T. pannonicum*, antioxidant activities with lower EC_{50} values have been reported in previous studies [54,210,307]. As mentioned before, the studies on *C. maritimum* have focused on the edible leaves rich in bioactive compounds, which may explain some of the differences together with growth stage, biotic and abiotic stresses, and whether used plant material has been cultivated or harvested from the wild. As many phytochemicals are produced to help plants to withstand different stressors, plants cultivated in controlled conditions have been shown to have lower concentrations of these secondary metabolites [190,348]. Different antioxidant properties of *T. pannonicum* have not been widely studied, but different caffeoyl esters and flavonoids, quercetin, apigenin, and luteolin, have been shown to be pronounced in extract fractions with radical scavenging activity [235]. These flavonoids were also later found in our *T. pannonicum* ethanol extract. Technologies such as bioguided fractionation could help to identify and separate compounds responsible for certain bioactivities, which may be desired when targeting products for biomedicines and cosmetics. However, the interactions and potential synergistic effects of complex matrices of bioactive compounds and other health-beneficial factors, such as probiotics, have been acknowledged but little understood and hereby should not be overlooked [349–352].

Enzyme inhibition activity of *C. maritimum* and *T. pannonicum*

Enzyme inhibition activities of *C. maritimum* and *T. pannonicum* samples are presented in **Figure 23**. Considering the inhibition of AChE, an enzyme responsible for the degradation of the neurotransmitter acetylcholine and linked to the pathogenesis of neurodegenerative diseases, *C. maritimum* ethanol extract at 10 mg/mL concentration exhibited an inhibition activity of 86.7 %, which is nearly the same as benchmark compounds galantamine at 1 mg/mL (88.9 %). AChE inhibition activity of *T. pannonicum* ethanol extract was also high, 71.5 %. Interestingly, both

water extracts had AChE inhibition activity < 10 %, whereas juice fractions had significantly higher activity > 40 %, indicating that the compounds responsible for the AChE inhibition activity of the species are likely mostly ethanol-soluble, or compounds that can be flushed to the juice fraction in the initial screw press. Drug development to treat the symptoms of neurodegenerative diseases targets the inhibition of AChE but also BuChE, an enzyme whose activity has been shown to correlate with abnormal β-amyloid peptide deposition, which is another characteristic of Alzheimer's disease [353]. The highest BuChE inhibition activity of the tested samples was observed with *T. pannonicum* ethanol extract at 10 mg/mL (34.3 %).

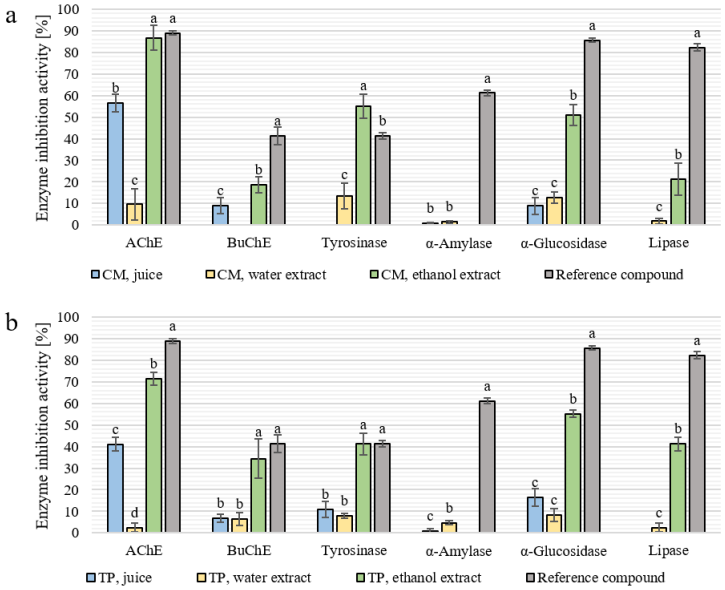

Figure 23 Enzyme inhibition activity of *C. maritimum* (CM, **a**) and *T. pannonicum* (TP, **b**) at 10 mg/mL. Data obtained and figure adapted from Paper IV [17] licenced with CC BY 4.0 [249]. Reference compounds were galantamine at 1 mg/mL for AChE and BuChE, arbutin at 1 mg/mL for tyrosinase, acarbose at 10 mg/mL for glucosidase and amylase, and orlistat at 1 mg/mL for lipase.

Potential anti-diabetic and anti-obesity properties were tested by measuring the inhibition of key enzymes related to sugar (α-glucosidase and α-amylase) and fat (lipase) metabolism. Ethanol extracts from *C. maritimum* and *T. pannonicum* had moderate α-glucosidase inhibition activity of 51.0 % and 55.2 %, respectively, whereas juice fractions and water extracts showed low α-glucosidase and α-amylase inhibition activities. Many plant phytochemicals, including flavonoids, phenols, alkaloids, curcuminoids, and terpenoids, have shown potent α-glucosidase inhibition activity; however, the inhibition activity of specific compounds may vary whether the enzyme is from the fungal, bacterial or mammalian origin [328]. The lipase inhibition activity of ethanol extracts was 21.4 % and 41.3 % for *C. maritimum* and *T. pannonicum*, respectively, and low activity was observed using water extracts. The benchmark compound, orlistat, had an inhibition activity of 82.3 % at 1 mg/mL. Orlistat is the only anti-obesity drug currently in clinical use that does not enter the

bloodstream or act on the central nervous system, and the interest in finding lipase inhibitors from secondary metabolites, such as polyphenols, saponins, alkaloids and terpenoids, has increased in recent years due to their low toxicity and diversity [330,354].

Both ethanol extracts at 10 mg/mL exhibited tyrosinase inhibition activity the same or higher than the benchmark compound arbutin at 1 mg/mL (41.5 %): for *T. pannonicum* extract, the activity was 41.2 %, whereas *C. maritimum* reached 55.1 % tyrosinase inhibition. The tyrosinase inhibition activity of other samples was low at the tested concentration, 8 – 14 %. Both *C. maritimum* and *T. pannonicum* 1:1 water-ethanol extracts have shown 30 – 40 % tyrosinase inhibition at 10 mg/mL [355], which is aligned with the results obtained in this study.

As value-added products for human nutrition and health are highly important in future sustainable bioeconomies [2,98], and using plant-derived bioactive compounds for medicinal purposes against chronic conditions has increased interest in recent years [73,211,328,354,356,357], bioprospecting novel feedstock for these purposes could be seen as an inseparable part of tomorrow's biorefinery design. Extracting compounds with antioxidant and other biological properties from sustainable sources could be beneficial not only to process economics but also to the overall socioeconomic impact of biorefinery.

4.2.4. Cytotoxicity of plant extracts

The severe effects and impact of cancer on society are apparent, being a major health problem and one of the leading causes of death globally [57,58,358,359]. Despite the significant advances in understanding and treating this group of diseases, there is a constant demand for new research and more effective drugs, as the number of cases is forecast to increase, ageing population and risk factors of unhealthy lifestyles being some of the drivers in developed countries [58,358,360,361]. Chemotherapy is one of the most common approaches for metastasised tumour therapy; however, many chemotherapeutic agents have high costs, limited effectiveness due to drug resistance, and cause severe adverse drug reactions and immune deficiency [58,359,361–363]. Therefore, there is an urgent demand for anti-cancer agents with fewer and less-harmful side effects. Safety is a key concern in drug discoveries, and potential anti-cancer agents should exhibit high selectivity towards malignant cells, meaning that the compound is more toxic to cancerous cells and non-toxic to normal, healthy cells [364,365]. This is measured with the selectivity index, which compares the cell viability IC_{50} values of cancerous cells to their healthy counterpart [365].

Cell viability below 75 % is considered cytotoxic; therefore, extracts exhibiting lower viability were also tested with a lower concentration. None of the extracts showed clear selectivity between healthy S17 cells and tumoral HepG2 cells with the tested concentration; however, half maximal inhibitory concentration (IC_{50}) values were not determined to extracts that did not exhibit cytotoxicity at tested concentrations. On the contrary, the ethanol extracts showed significantly lower RAW 264.7 cell viability at 200 µg/mL. Each cell line and tissue has different susceptibilities to the compounds present in the extract, which translates to the differences in cell viability between cell

lines; hence, the results should be considered only as preliminary screening. Cell viabilities after extract inclusion are presented in **Table 10**.

Table 10 Cell viability [%] after incorporating extracts with 200 μg/mL concentration. * Cell viability at 100 μg/mL, ** cell viability at 50 μg/mL. S17: healthy mice murine bone marrow stromal cells, HepG2: human hepatocarcinoma, RAW 264.7: mice leukemic macrophage.

Species	Extract	S17	HepG2	RAW 264.7
C. maritimum	Water	94.49 ± 4.43	84.07 ± 8.35	70.26 ± 6.02 98.36 ± 6.83*
	Ethanol	60.82 ± 8.40 79.88 ± 5.88 *	78.49 ± 5.90	17.31 ± 5.63 65.00 ± 4.41* 89.86 ±6.47**
S. ramosissima	Water	91.67 ± 9.54	96.87 ± 3.75	90.55 ± 6.94
	Ethanol	83.39 ± 4.50	83.54 ± 7.72	13.95 ± 1.71 79.52 ± 6.46*
T. pannonicum	Water	102.84 ± 5.16	96.27 ± 5.68	76.74 ± 1.88
	Ethanol	78.93 ± 14.25	88.24 ± 3.90	18.10 ± 4.51 99.84 ± 7.81*

According to the American National Cancer Institute, raw extracts should exhibit IC_{50} values below 30 μg/mL for them to be relevant for use in anti-tumoral applications, and this criterion is commonly used in studies related to cancer drug discovery [58]. None of the tested extracts filled this criterion, as all samples had IC_{50} values > 100 μg/mL. Lopes et al. [63] tested 80 % aqueous acetone extracts from *S. ramosissima* and *T. pannonicum* for their cytotoxicity at 125 μg/mL against S17, HepG2, and THP1 (human leukemic monocyte) and showed cell viability results comparable to those obtained in this study. Gnocchi et al. [366] also showed in a preclinical study that ethyl acetate extract from *C. maritimum* combined with a reduced dose of traditional chemotherapeutic drug effectively inhibited the growth of HepG2 cells while reducing the toxic effects. Methanolic extracts from *S. brachiata* and *S. europaea* have exhibited IC_{50} > 250 μg/mL towards HepG2 and IC_{50} > 100 μg/mL towards MCF7 (breast carcinoma), respectively [367,368].

The research on plant-derived compounds has led to the discovery of phytochemical cancer drugs currently in clinical use, including alkaloids from *Catharanthus roseus* used to treat leukaemias and lymphomas, and terpenes from *Taxus brevifolia* used for solid tumours [57,58,361]. Pronounced biosynthesis of antioxidant and other bioactive compounds has made halophytes an interesting and valuable source of potential medicinal phytochemicals [58,358]. Plant metabolites with reported cytotoxic properties include alkaloids, flavonoids and other phenolics, terpenes and terpenoids, lignans and carotenoids [57,227,359,362]. The potential of flavonoids and polyphenols as adjuvants for immunotherapy has also been briefly explored [358]. Regardless of the potential of plant extracts shown *in vitro*, animal models, and early clinical trials, there is no approved polyphenol medicine in the market due to regulatory issues related to the complex nature of plant extracts [227,362]. Extensive studies are still needed to understand which compounds in novel plant extracts are responsible for the cytotoxic effect and the potential interactions between plant metabolites and drugs [362].

Chapter 5. Protein from Green Juice

5.1. Introduction to protein precipitation methods

Heat coagulation is one of the most well-established methods for high-efficacy protein precipitation, which is demonstrated and used in large-scale applications [122,369,370]. The green juice is heated to 60 – 90 ° C, typically with heat exchangers or direct stem injection, which triggers the opening of the hydrophobic sites in the protein surface and protein denaturation [115,116,371–374]. As different types of protein have different denaturation temperatures, step-by-step heat coagulation allows the separation of green protein, which denature in temperatures 50 – 60 °C, and more water-soluble white protein when increasing the temperature up to 75 – 90 °C [115,370,373,375]. One of the main disadvantages of heat coagulation is the high energy input required to heat a large volume of juice to high temperatures [116,376]. A Maillard reaction between amino acids and sugars may occur, formatting undesired reaction products if a temperature in the system gets too high locally [377]. Also, even if high protein recoveries to concentrate have been achieved with heat coagulation, irreversible changes due to denaturation may affect protein functionality, solubility, and digestibility [115,373].

Acidification, sometimes called isoelectric precipitation, has been suggested to be a gentler method to preserve protein quality and yield concentrate with the highest content of essential amino acids [116,371]. The pH level of the green juice is decreased using acid to an isoelectric point, which is the pH where proteins have an equal negative and positive charge and minimum solubility to water [115]. For green leafy biomass protein, this level is typically between pH 3.5 – 4.5 [115,116,375,378]. Existing literature shows divergent results considering the effect of final acidification pH on protein yields. Damborg et al. [375] reported non-significant differences in protein yields from white clover, alfalfa, and perennial ryegrass juices within the pH range of 3.0 – 5.0, whereas protein yield from red clover juice was dependent on the final pH. On the contrary, Miller et al. [379] showed a significant increase in protein yield when acidification pH was decreased from 4.5 to 3.5. Acidification can also be combined with heat treatment to enhance the precipitation or to separate the green and white protein fractions [373,380]. Decreasing the pH below 4.0 can also improve the juice stability and preserve it from contamination, thus lengthening the potential storage time [381]. However, chemical costs, as well as storing and handling hazardous materials, can limit the potential of acidification for scale-up applications.

Flocculants are chemicals that promote the agglomeration of suspended particles in the liquid to larger clusters, and their use in protein precipitation has been studied for easier and more efficient separation of aggregated proteins [371,374,382,383]. Flocculants are usually combined with acidification, heat coagulation, or both, but Knuckles et al. [383] reported cationic flocculants to improve green protein separation from alfalfa juice even without elevated temperature. Baraniak [371] also tested anionic and cationic flocculants as separate methods to precipitate protein from alfalfa juice and obtained concentrates with slightly lower protein content than those from

heat coagulation and acidification. The use of calcium salt has also been shown to allow lower temperatures for heat coagulation without compromising the protein yield [382]. La Cour et al. [374] were able to promote the total nitrogen recovery from grass and clover juices to concentrate by using lignosulfonates; however, a high dosage was needed for significant improvement. As the addition of chemicals increases the operational costs of the biorefinery, the use of flocculants on a large scale may not be economically feasible and justified without significant process development.

Fermentation with lactic acid bacteria (LAB) uses the same principle as acidification, but the pH decrease is achieved with lactic acid produced by bacterial metabolism instead of adding concentrated acid. In this method, developed by Kiel et al. [180], the green juice is inoculated with LAB, which consumes the available carbohydrates in the juice and produces lactic acid, which acidifies the juice to the protein isoelectric point. Fermentation has been shown to have high efficacy in separating protein from various forages [178,180], and it has been tested on a demonstration scale for the mixture of grass and clover [384]. Plant enzymes present in the juice may also convert the complex carbohydrates to sugar monomers usable by bacteria [377]. Also, produced lactic acid, an important platform chemical with various applications, could provide additional value for the processing [385,386], whereas bacterial biomass in the concentrate could increase the final product's functionality. Potential challenges could be microbial consumption of protein, as well as extended residence time in slightly elevated temperatures [116].

Ultrafiltration has also been tested for protein separation, especially to separate the more water-soluble white protein [372,373]. However, due to the long process time and costs related to membrane fouling, the potential of filtration in large-scale processes is still to be evaluated [116]. The separation of protein using solvents, such as acetone, butanol and isopropyl alcohol, has been tested [119,387,388]; however, these methods are not viable as they require large quantities of toxic and costly organic solvents. Precipitated proteins are typically separated from juice using centrifugation and decantation, but different filtration techniques can also be applied. The crude protein (CP) content of protein-enriched concentrate (PEC) and CP recoveries achieved with different methods are summarised in **Table 11**.

Table 11 Crude protein content of dried concentrates and recovery of crude protein (or total nitrogen) from green juice to concentrate of different biomass. CP: crude protein, PEC: protein-enriched concentrate.

Biomass	Method	CP in PEC [%]	CP recovery [%]	Ref.
Alfalfa	*L. salivarius* fermentation	~ 42	38.6	[180]
	Ultrafiltration	n/a	~ 51	[372]
	Heat coagulation	n/a	~ 52	
		n/a	54.9	[375]
		53.0	n/a	[371]
	Flocculents	42.7 – 45.0	n/a	
	Acidification pH 3.5	42.9	n/a	
	Acidification pH 4.0	38.8	n/a	[378]
Cassava leaf	Acidification pH 4.0	n/a	48.7	[108]

		45.1	n/a	[107]
	Heat coagulation	42.2	n/a	
	Spontaneous fermentation	40.4	n/a	
Chicory	*L. salivarius* fermentation	28.7 – 32.8	72 – 86	[178]
Clover grass	*L. salivarius* fermentation	~ 40	51.7	[180]
Oilseed radish	*L. salivarius* fermentation	~ 45	43.7	
Orchardgrass	Heat coagulation + Acidification pH 3.3	n/a	~ 30	[380]
Perennial ryegrass	Ultrafiltration	n/a	~ 59	[372]
	Heat coagulation	n/a	~ 45	
		33.9	n/a	[389]
		n/a	58.5	[375]
		31.6	~ 35	[374]
	Flocculents	25.7	~ 44	
	Acidification pH 4.0 – 4.5	30.4	~ 37	
	Acidification pH 4.0	24.4	n/a	[378]
Red clover	*L. salivarius* fermentation	35.0 – 41.5	72 – 80	[178]
		~ 39	66.7	[180]
	Heat coagulation	n/a	60.6	[375]
		43.6	~ 45	[374]
	Acidification pH 4.0 – 4.5	46.8	~ 46	
	Flocculents	39.0	~ 51	
S. bigelovii	Heat coagulation	n/a	46 – 57	[128]
Sugar beet leaves	Heat coagulation	29.9	n/a	[110]
Switchgrass	Heat coagulation + Acidification pH 3.3	n/a	~ 23	[380]
Timothy	*L. salivarius* fermentation	19.3 – 23.2	76 – 86	[178]
White clover	Acidification pH 4.0	34.7	n/a	[378]
	Heat coagulation	n/a	59.4	[375]

5.2. Selection of lactic acid bacteria for fermentation

This section elaborates on the process of selecting the LAB for the experiments. Lactic acid can be produced using micro-organisms through anaerobic bacterial fermentation or aerobic fungal fermentation [390]. In this study, fermentation using LAB is considered, and the final product is targeted for feed production, which sets limitations for the used strain; however, the majority of LAB are generally recognised as safe (GRAS) organisms [391]. From a technical point of view, Morlon-Guyot et al. [392] determined the criterion for bacteria used for silage fermentation, which is partly applicable to choosing the acidifying organism for green biorefinery [377]. According to the aforementioned criterion, the used LAB strain should have the following properties [377,392]:

- Rapidly growing strain able to outcompete other micro-organisms.
- Lactic acid is the main metabolic product (homofermentative).
- Tolerant to acidic conditions and able to decrease the medium pH $\leq$ 4.0.

- Capable of using glucose, fructose, sucrose, and preferably other types of sugars, such as pentoses, as substrate.
- Not using or having any action on organic acids.

Unlike in silage fermentation, thermophilic strain with tolerance to high temperatures is not necessary for protein precipitation, and mesophilic bacteria thriving in moderate temperatures can be used. Using lower process temperatures is also beneficial due to its lower energy consumption. Homofermentative strains are preferred for commercial use and applications aiming at maximum lactic acid production [390,393] as they produce lactic acid as their main product of glucose metabolism, whereas heterofermentative strains produce lactic acid together with acetic acid or ethanol. The homofermentative Embden-Meyerhof metabolic pathway is the most common in LAB, producing two lactic acid molecules from one glucose molecule under anaerobic conditions [390]. LAB are commonly anaerobes, but they can tolerate oxygen (microaerophilic) and some strains, such as *L. plantarum*, have shown to be capable of aerobic growth; however, exposure to oxygen may affect the used metabolic pathways of the micro-organism [375,377,394].

The key parameters considering the growth of LAB are the possible presence of oxygen, sufficient availability of essential nutrients, temperature and pH level [393,395,396]. Additionally, considering the fermentation of halophyte juice, the strain tolerance to NaCl and saline conditions is of great importance. The use of halophilic LAB *Tetragenococcus halophilus*, commonly used in the food industry to produce high salt content condiments, with optimal salinity of 7.5 – 12. 5 w/v% was considered. However, it was omitted due to a longer lag phase and slower growth, heterofermentative nature, and limited literature considering its probiotic properties; in other words, it did not fill the set criterion [397,398]. Studies report *L. plantarum* and closely related *Lactiplantibacillus pentosus* to tolerate 6 – 10 % NaCl in culture media and being able to grow at minimum pH of 3.0 – 4.5 [395,399–401]. Robust *L. salivarius* has been shown to tolerate the approximate media salinity of 5 % [402,403], and it has also been previously used for fermentation in green biorefineries [178,180]. Reddy et al. [404] showed that both *L. plantarum* and *L. salivarius* could tolerate acidic conditions to pH as low s 2.5 and had high survival rates through spray-drying, which could be a desired quality for industrial applications. *Lactococcus lactis*, which produces the antimicrobial peptide nisin, has been shown to tolerate up to 4 % NaCl culture media salinity and grow at minimum pH of 3.0 – 4.0 [405–407].

In order to improve the potential functionality of the separated PEC, the search for LAB strains was focused on those with reported probiotic properties. Probiotics are micro-organisms which provide several health benefits for the host by either colonising the host's gastrointestinal tract or creating unfavourable conditions for pathogens, for example, by producing antimicrobial bacteriocins [408]. Indeed, probiotic LAB could be used as a more safe and sustainable replacement for antibiotic supplements in animal feed for aquacultures and terrestrial livestock, and several studies with animal models have been carried out reporting the probiotic effects of novel LAB supplementation, which are summarised in **Table 12**. The focus of this study is on novel probiotics, as some of the most commonly used and known probiotic LAB, e.g., *Lactobacillus acidophilus* and *Lacticaseibacillus casei*, are sensitive to saline conditions [399,409]; hence, they are not suitable for fermenting the halophyte

juice. Supplementation is commonly done using live bacteria, but some studies show probiotic potential and protective effects against diseases with dead bacteria [410–412] or using only some specific protein of bacterial cells [413]. In aquacultures, probiotic LAB supplementation has shown a protective effect against several pathogens [410,414–420], attenuation of the effect of some toxins, e.g. pesticides [421], decrease the accumulation of heavy metals in fish tissues [422], and improved stress capacity in acute exposure to changing salinity [411,423]. In addition, several studies reported enhanced growth rate and feed utilisation in probiotic-fed subjects compared to subjects with basal diets under challenged conditions [410,414,415,417,420,424]. For mono-gastric terrestrial livestock, the LAB supplement has shown a protective effect against various pathogens, including common *Salmonella* and *Escherichia coli* [425–429], as well as enhancing the gut microflora and immune system of healthy animals [430–433].

Screening experiments

Based on the studies existing literature, the selected strains for experiments were *L. salivarius*, *L. plantarum*, *L. pentosus*, and *L. lactis*. Initial tests in flasks and bioreactor were run to show their acidification potential and viability in saline halophyte juice. In the first test, the LAB was grown in MRS broth, an optimised media for LAB, in a batch system where the optical density (OD) at 600 nm and the pH of the culture were measured hourly (**Figure 24**). All tested strains, except *L. lactis*, were able to acidify the juice to final pH < 4.0 within 14 – 20 h before inhibition. In the second initial test, the LAB viability in juice samples was tested by fermenting fresh and pasteurised juice samples; pasteurised samples were used as a control, as the acidification could also be enhanced by micro-organisms naturally present in the juice. All selected strains were able to ferment the juice from the Portuguese phenotype of *S. ramosissima*. However, no acidification was observed in the juice from the French phenotype of *S. ramosissima*, likely due to extremely high salt content, as the ash content of the juice was 81.83 g/100g DM [48]. Finally, *L. salivarius* and *L. plantarum* were selected to be used for further experiments based on the screening and reported probiotic properties.

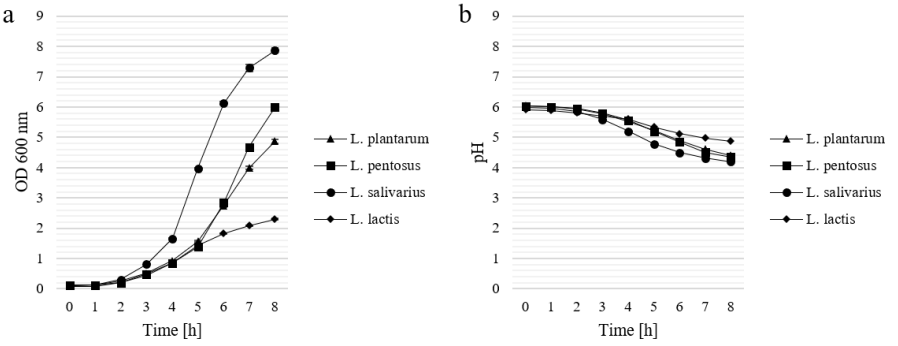

Figure 24 Optical density (OD) at 600 nm (**a**) and pH (**b**) of lactic acid bacteria culture in MRS media for the first 8 hours of the fermentation.

Table 12 Lactic acid bacteria (LAB) feeding trials performed for aquacultures and terrestrial livestock. The listed effects of probiotic supplemented diet are an overview of results reported in each study based on research-specific supplement administration, biochemical, immunological, and haematological parameters, histopathological changes, and levels of several gene expressions. ↓: decrease, ↑: increase or improvement, n/a: no challenge.

Model	Strain	Challenge	Probiotic effects on subjects	Ref.
Abalone	*L. pentosus*	*Vibrio parahaemolyticus*	↓ mortality; ↑ immunological activity; ↑ antioxidant levels; ↓ toxin levels	[419]
Caspian trout	*L. plantarum*	n/a	↑ immunological activity; ↑ anti-inflammatory expression	[434]
Chicken	*L. salivarius* *L. agilis*	n/a	↑ LAB colonisation; ↑ weight gain (10.7 %)	[430]
	L. salivarius	*Campylobacter jejuni* *Salmonella enteritidis* *Clostridium perfringens*	↑ LAB colonisation; ↑ inhibition of pathogens.	[426]
		Campylobacter jejuni	↑ LAB colonisation; ↓ pathogen colonisation; ↑ immunological activity	[427]
	L. plantarum	n/a	↑ intestinal barrier; ↑ antioxidative properties; ↑ immunological activity; ↑ anti-inflammatory expression.	[431]
		Escherichia coli	↑ LAB colonisation, ↓ pathogen colonisation (similar to antibiotic supplement); ↑ intestinal immunity	[428]
	L. pentosus *L. acidipiscis*	*Escherichia coli*	↑ LAB colonisation; ↓ pathogen colonisation	[429]
	L. lactis	n/a	↓ mortality due to digestive issues; ↑ blood profile	[435]
Sea bass	*L. delbrueckii*	n/a	↑ gut microflora; ↑ juvenile survival rate	[436]
Giant freshwater prawn	*L. plantarum*	*Aeromonas hydrophila*	↑ gut microflora; ↑ immunological activity; ↑ growth performance	[424]
Goat	*L. plantarum*	n/a	↑ gut microflora; ↑ polyunsaturated fatty acids in milk	[437]
Japanese eel	*L. pentosus*	*Edwardsiella tarda*	↑ blood profile; ↑ immunological activity; ↑ growth performance	[417]
Nile tilapia	*L. plantarum*	Deltamethrin	↑ blood profile; ↑ liver function; ↑ immunological activity; ↑ antioxidant levels	[421]
		Ammonium chloride *Streptococcus agalactiae*	↑ growth performance; ↑ immunological activity; ↓ mortality	[410]

		Aeromonas hydrophila	↑ LAB colonisation; ↑ immunological activity; ↑ growth performance	[414]
		Aluminium	↓ mortality; ↑ leukocyte recovery; ↓ aluminium accumulation; ↑ antioxidant levels	[422]
	L. lactis	*Streptococcus agalactiae*	↑ survival rate; ↑ immunological activity; ↑ growth performance	[438]
Olive flounder	*L. lactis*	*Streptococcus parauberis*	↑ protection against the pathogen, ↑ immunological activity; ↑ growth performance	[405]
		Streptococcus iniae	↑ survival rate, ↑ immunological activity; ↑ growth performance	[439]
Orange-spotted grouper	*L. plantarum*	*Streptococcus* sp.	↑ survival rate, ↑ immunological activity; ↑ growth performance	[415]
Piglet	*L. casei*	n/a	↑ gut microflora; no change in growth performance	[432]
	L. delbrueckii	n/a	↑ immunological activity (long-lasting); ↑ antioxidant capacity	[433]
	L. lactis	n/a	↑ gut microflora; no change in growth performance	[432]
	L. gasseri	n/a	no change in growth performance	[440]
Rainbow trout	*L. rhamnosus*	*Aeromonas salmonicida*	↓ mortality; ↑ protection against the disease	[416]
Whiteleg shrimp	*L. pentosus*	*Vibrio parahaemolyticus*	↑ immunological activity; ↑ antioxidant levels; ↑ LAB colonisation	[413]
	L. plantarum	*Vibrio alginolyticus*	↑ immunological activity; ↓ mortality	[418]
		Vibrio harveyi	↑ LAB colonisation; ↑ hemocyte amount; ↑ survival rate; ↑ growth performance.	[420]
		Changing salinity	↑ anti-stress capacity; ↑ growth performance	[411]
	L. lactis	n/a	↑ body protein content; ↑ digestive enzyme activity; ↑ gut microflora	[441]
		Changing salinity	↑ anti-stress capacity	[423]

5.3. Findings on the protein precipitation from halophyte juice

5.3.1. Precipitation and separation of protein-enriched concentrate from *S. ramosissima* and *T. pannonicum* green juice

This section summarises the concept, results, and discussion of Paper V, a submitted manuscript titled "*Valorisation of Residual Biomass Fractions from Biosaline Agriculture Through Green Biorefinery*" [132]. Green biorefinery targeting the production of protein-enriched concentrate (PEC) from juice fractions for animal feed applications has been well-established for grasses and other leafy biomass, and the process is currently run on a demonstration scale [370,384,389,442,443]. However, there is a gap in knowledge regarding the potential and suitability of succulent halophyte biomass for this type of processing, and only one study conducted with *Salicornia bigelovii* was found in the literature [128]. In this study, protein precipitation from *S. ramosissima* and *T. pannonicum* juices was tested using different precipitation methods: heat coagulation, acidification with HCl, and fermentations with *Lactiplantibacillus plantarum* and *Ligilactobacillus salivarius* to shed light on the potential of halophytes for green protein production.

Screw-press has shown to separate 55 – 60 % of the inherent liquid in biomass like alfalfa, clover, and grass [444], and green juice fractions of 40 – 71 % have been reported for different biomass commonly used in green biorefineries [178,180,384]. However, halophytes often hold more water leading to a larger green juice fraction (67 – 90 %) with lower DM content (4 – 14 %) [34,128,131,132]. The DM contents of tested *S. ramosissima* and *T. pannonicum* juices were 6.47 ± 0.21 % and 4.01 ± 0.04 %, respectively, and the constituents are presented in **Figure 25** [132].

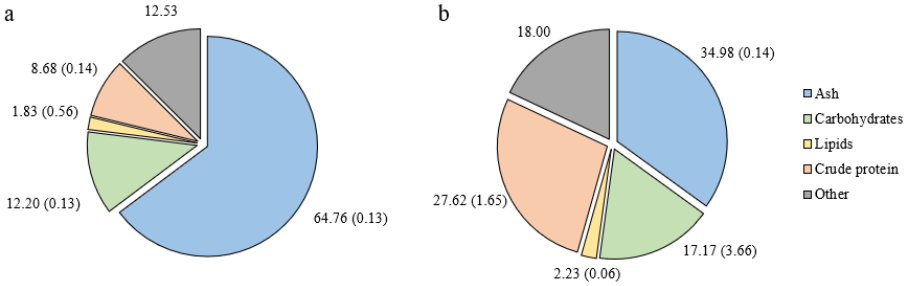

Figure 25 Composition of the green juice dry matter (DM) from *S. ramosissima* (**a**) and *T. pannonicum* (**b**). Values are given as [g/100g DM], and standard deviations are marked in brackets. Data from Paper V, in which the composition of fibre fraction is also available [132].

The different methods were tested on a small scale in flasks, and the protocols were described earlier and in Paper V [132]. Both LAB species were able to acidify the juice media to average final pH of 3.5, consuming 83.4 – 99.9 % of the available glucose in juice samples. The final pH is within the pH range of 3.2 – 4.5, in which proteins from plant leaves typically have minimal solubility in water (isoelectric point) [116,178]. From *S. ramosissima* samples, 7.3 – 12.8 % of the initial juice weight

was recovered to wet PEC, with DM contents in the 18.0 – 21.6 % range. The largest PEC fraction was achieved with acidification with HCl, whereas the smallest fraction was obtained with *L. plantarum* fermentation. On the contrary, fermentation with *L. plantarum* yielded the PEC with the highest DM content, 21.6 %, followed by the sample fermented with *L. salivarius.* In the *T. pannonicum* samples, the wet PEC constituted 10.7 – 29.4 % of the initial juice mass; however, the centrifuge cake crumbled during the decantation of some samples, leading to PEC with low DM content, 4.6 – 9.4 %.

Considering DM and CP recoveries from juice to PEC, differences in the results between precipitation methods and control samples were non-significant, excluding the acidification of *S. ramosissima* juice. In *S. ramosissima*, DM recoveries varied from 24.2 %, obtained using *L. salivarius* fermentation, to 35.3 % from acidification. In *T. pannonicum* samples, the DM recovery was 12.2 – 34.4 %. Average mass balances (**Table 13**) were calculated from the data presented in the paper [132]. Besides the selected protein precipitation methods, the role of pre-processing, like efficient fractionation and potential filtering steps, should not be overlooked. Previous studies show that adding water to the screw press could help the compounds to flush out and improve the protein recovery to the liquid fraction [445,446]. However, adding freshwater to the processing would counter-effect the benefits of using the saline biomass, as the key argument for halophyte processing is to reduce the amount of freshwater needed. Also, fibrous solid particles suspended in the juice were not removed by filtration, as some of the phytochemicals present in the fresh biomass [48] could potentially enhance the functionality of the PEC product in feed applications.

Table 13 Fresh weight, dry matter, and crude protein mass balances, averaged from the data from all tested precipitation methods. PEC: protein-enriched concentrate. *Calculated using fractionation and dry matter data from 171 mM NaCl cultivated *T. pannonicum* [34].

Species	Whole	Fibres	Green juice	PEC	Juice residue
Fresh weight [w/w%]					
S. ramosissima	100	33.3	66.7	6.2	60.5
T. pannonicum	100	17.5 *	82.5 *	14.4	68.1
Dry matter [w/w%]					
S. ramosissima	100	69.7	30.3	8.6	21.7
T. pannonicum	100	58.2 *	41.4	10.7	31.1
Crude protein [w/w%]					
S. ramosissima	100	72.6	27.4	18.4	9.0
T. pannonicum	100	42.5	57.5	16.5	41.0

The obtained results for the CP recoveries and CP contents (**Table 14**) in PEC were compared to those reported in the existing literature on forage-based green biorefineries (**Section 5.1**). For *S. ramosissima*, the achieved CP recoveries, 61.7 – 81.8 %, are comparable to some of the biomasses with the highest reported CP recoveries, such as chicory, red clover, timothy, and white clover [178,180,375]. However, the challenge regarding the process feasibility lies in the low initial CP content of the plant, which can be seen in the lower CP content of the PEC, 19.0 – 22.1 %. As the protein content of the plant may vary significantly depending on the

cultivation conditions, growth stage, and harvest time [34,108,178,447], optimisation in this regard could be beneficial. In *T. pannonicum* juice, the initial CP content was higher, which was reflected in the higher CP content of the PEC, 27.4 – 33.9 %, which are similar to those reported to perennial ryegrass, chicory, and sugar beet leaves [110,178,374]. On the other hand, the CP recovery from juice to PEC was low, only 12.8 – 36.8 %. Similar low results have been previously reported for switchgrass and orchardgrass [380]. Low CP recovery could indicate that the juice is rich in other non-protein nitrogen-containing compounds, such as nitrate, which can not be precipitated but affect the total nitrogen measurement. Overall, based on this screening study, *S. ramosissima* and *T. pannonicum* could be seen as interesting species for green protein production; however, significant process development and optimisation are needed.

Table 14 Crude protein content of dried concentrates and recovery of crude protein from green juice to concentrate of *S. ramosissima* and *T. pannonicum*. CP: crude protein, PEC: protein-enriched concentrate. Data from Paper V [132].

Biomass	Method	CP in PEC [%]	CP recovery [%]
S. ramosissima	Heat coagulation	19.0 ± 1.0	62.1 ± 2.5
	Acidification pH 3.5	20.1 ±1.3	81.8 ± 7.8
	L. plantarum fermentation	21.6 ±1.5	63.3 ±3.0
	L. salivarius fermentation	22.1 ± 0.9	61.7 ± 0.0
T. pannonicum	Heat coagulation	27.4 ±1.5	36.8 ±12.1
	Acidification pH 3.5	29.0 ± 0.7	34.1 ± 5.5
	L. plantarum fermentation	31.4 ± 1.9	12.8 ±0.8
	L. salivarius fermentation	33.9 ±1.9	29.8 ±12.4

5.3.2. Fermentation experiments in bioreactor

S. ramosissima juice was fermented in a bioreactor to study the consumption of sugars and production of different metabolites during the process and to see if there is a difference in DM and CP recoveries when the juice is acidified to pH 4.0 and pH 3.5. In previous studies, a target pH within the range of 3.3 – 4.5 has been used for direct acidification [107,371,374,378,380], as well as LAB fermentations [178,180]. With the used 10 v/v% *L. plantarum* inoculate (average OD 600 nm of 1.1206), the final pH of 3.5 was reached within 9 h 30 min ± 40 min, whereas acidification to pH 4.0 was achieved in an average time of 5 h 30 min ± 15 min. However, the starting pH was slightly lower in the experiment targeting pH 4.0, likely due to a higher concentration of lactic acid injected into the media with the pre-culture. The lower inoculate volume (4 - 5 v/v%) used for forage biomass [178,180,385] should also be investigated for halophyte juice in order to reduce costs related to the purchase, processing, and handling of pre-culture media, especially in scale-up applications. During the fermentation to target pH of 3.5, on average, 58.7 % of the available glucose was used, whereas only 11.6 % of available sugar was used to each pH of 4.0. The concentrations of glucose and metabolites in the media throughout the fermentation are presented in **Figure 26**. Free xylose was not detected from the juice.

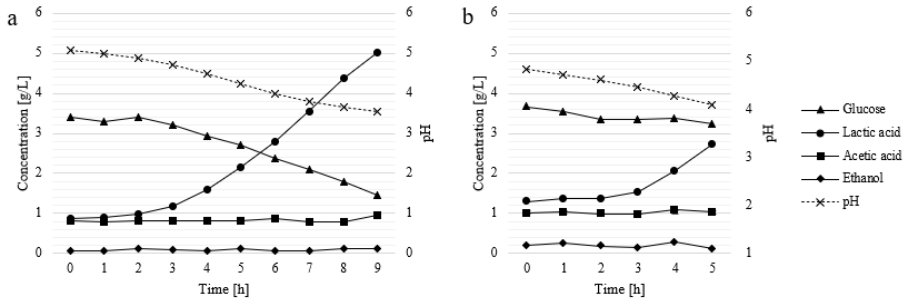

Figure 26 Sugars and metabolites in *S. ramosissima* juice fermented to final pH of 3.5 (**a**) and 4.0 (**b**) with 10 v/v% *L. plantarum* inoculate. The reported values are averages of two runs.

After fermentation, the wet PEC constituted 5.2 ± 0.3 % and 6.1 ± 0.8 % of the initial fresh juice weight with target pH of 3.5 and 4.0, respectively, and results are slightly lower than those previously obtained in flask fermentations presented in Paper V [132]. However, similar CP contents of PEC were obtained in a bioreactor, values being 22.07 % and 20.9 % for target pH 3.5 and 4.0, respectively, and there were no significant differences between the results. Both DM and CP recoveries from juice PEC were significantly lower in bioreactor experiments than in flask fermentation trials [132]. DM recoveries were 14.7 ± 0.5 % and 15.3 ± 1.0 % for target pH 3.5 and 4.0, respectively. Finally, CP recoveries from juice to PEC were 35.8 %and 36.9 %, and the differences between results were non-significant. Potential explanations for lower recoveries could be partial hydrolysis of some suspended solid particles in the acidic conditions in the bioreactor, smaller amount of bacterial biomass, and losses due to biomass handling. These experiments provide an interesting first sight to PEC production from *S. ramosissima*, but significant process optimisation would be needed to reach higher CP recoveries and make the protein precipitation by fermentation of halophyte juice a viable process option for integrated biorefineries.

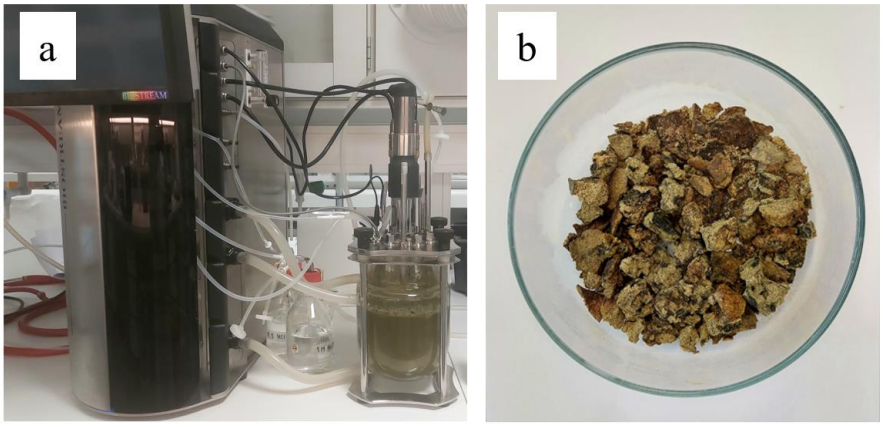

Figure 27 Fermentation setup with a 2 L bioreactor vessel (**a**) and an example of protein-enriched concentrate obtained after precipitation, centrifugation and oven drying (**b**).

Chapter 6. Conclusions and Future Work

In this PhD project, the potential of selected halophyte species for green biorefinery processing was studied in an exploratory manner. The overview of the studied process and general mass balance of *S. ramosissima* is provided in **Figure 28**. The research carried out during the PhD project period led to the development of process technologies, which are currently in the patent application filing process or under investigation for their patentability.

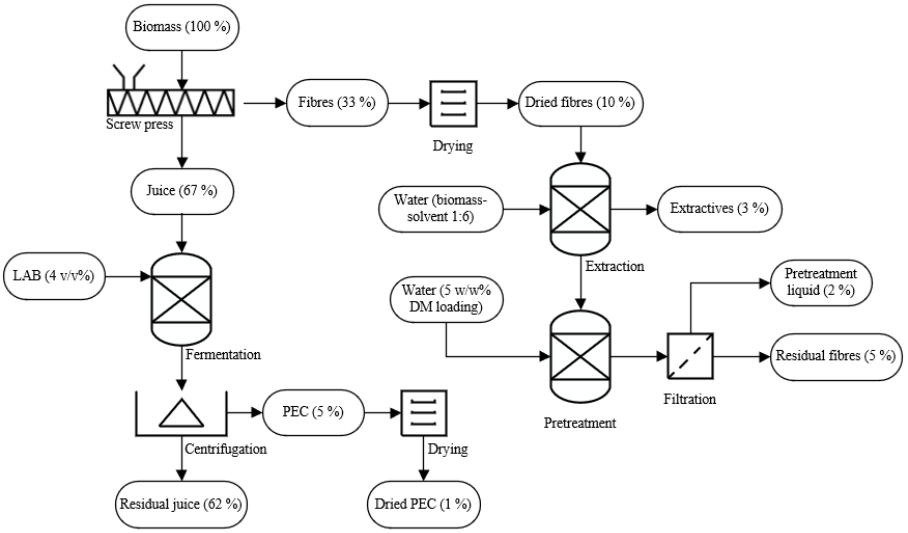

Figure 28 Overall mass balance of halophyte green biorefinery, the case of *S. ramosissima* (09-2021), combined from the data obtained during the PhD project period. DM: dry matter, LAB: lactic acid bacteria, PEC: protein-enriched concentrate.

The key research objectives, introduced in **Section 1.4.2**, were met as studies contributing to these goals were performed. Firstly, the biomass of unedible aerial parts of *C. maritimum*, *S. europaea*, and *T. pannonicum* was characterised, and the effect of cultivation salinity on the chemical composition of selected species was investigated. This study showed selected species having different responses to the increasing salinity, and cultivation salinity affected especially the composition of *S. europaea*. The study highlighted the importance of optimising cultivation conditions and processes for each type of biomass, especially when using plant species known for their phenotypic plasticity, such as *Salicornia* and *Sarcocornia* genera.

Halophytes are super-producers of bioactive secondary metabolites with increased interest in high-value industries, such as biopharmaceuticals, nutraceuticals, food and feed additives, and cosmetics. The concentration of phenolic compounds, pigments and vitamins found in halophyte plants was reviewed. In order to evaluate the potential value of fractionated halophyte biomass, juice fractions and the extractive material from *C. maritimum*, *S. ramosissima*, and *T. pannonicum* were used for bioprospecting,

where their potential for high-value application was assessed by testing antioxidant activity, enzyme inhibition activity and cytotoxicity *in vitro*. Also, the total concentrations of polyphenolic compounds and pigments were tested with absorption spectroscopy assays and from the extracts, specific bioactive compounds were annotated using untargeted chromatographic methods.

Indeed, the results showed interesting properties of *C. maritimum*, *S. ramosissima*, and *T. pannonicum.* Plant extracts exhibited high antioxidant activities and possible anti-diabetic, neuroprotective, and tyrosinase-inhibitory properties. This brings new insights into the potential of these species as feedstock for cascading, multi-product biorefinery, and using screw-pressed fibres to extract and produce bioactive compounds could be seen as an intriguing processing option in halophyte-based green biorefinery. Using bio-guided fractionation technologies, finding the fractions and specific compounds responsible for the bioactivities of interest could be a study of interest in the future.

The extractives-free, relatively pure lignocellulose fibres were pretreated and tested for their enzymatic convertibility, which is essential if there is a desire to use lignocellulose-derived sugars for biofuels or biochemical production. Neither of the tested pretreatment conditions gave the desired results, as only low sugar recoveries were achieved at milder severity, and a high concentration of toxic furfural was produced at higher severity. Therefore, optimising hydrothermal pretreatment conditions and the simultaneous enzymatic saccharification and fermentation to bioethanol could also be interesting, considering the biorefinery process design.

Finally, the protein precipitation from *S. ramosissima* and *T. pannonicum* juice fraction was tested with methods well-established for green biorefineries using grass as feedstock. Heat coagulation, acidification with hydrochloric acid, and fermentation with lactic acid bacteria were tested. Out of these two species, *S. ramosissima* exhibited higher crude protein recovery from juice to the protein-enriched concentrate and better suitability for such a process; however, challenges related to the low initial protein content of the plant are something to overcome. As the PEC obtained from green juice processing is often targeted to be used in animal feed supplements, determining the true protein content and amino acid profile of the PEC product would be necessary to evaluate its nutritional qualities. Testing potential juice pre-processing options, and optimising the fermentation process, complemented by feeding trials with animal models to evaluate the product's functionality and bioavailability, would also be essential. The juice fraction from halophytes also exhibited biological activities, and possible further valorisation of the residual juice fraction after protein separation could be investigated.

Considering future work, process simulation and techno-economical assessment of the process are of high importance in order to evaluate the economic feasibility of the suggested halophyte-based biorefinery. The challenges regarding the assessment lie in determining the price for halophyte extracts, as the market value is highly dependent on extract purity, potency, and targeted use. Identifying and quantifying the bioactive compounds using mass spectrometry with analytical standards would provide important information regarding the target markets of different extracts.

Halophytes can have a significant role in the development of biosaline agriculture and valorising marginal lands in arid and semi-arid rural regions. They can provide a novel source of food and biomass feedstock for cascading integrated biorefineries. This project provided a comprehensive general view of utilising selected species in green biorefinery applications and studied species can be seen as potential raw materials for further investigations and process development towards establishing said biorefineries. Overall, one of the aims of the interdisciplinary PhD project was also to narrow the gap between process engineering, botany, phytochemistry, pharmacology, and other associated research fields. Communication and collaboration between scientific disciplines are crucial for the development of sustainable, efficient and effective biomass use, new production systems for essential goods, and in the end, a circular bioeconomy.

References

1. Masson-Delmotte, V.P.Z.; Pirani, A.; Connors, S.L.; Péan, C.; Berger, S.; Caud, N.; Chen, Y.; Goldfarb, L.; Gomis, M.I.; Huang, M.; et al. *IPCC, 2021: Summary for Policymakers. In: Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*; 2021;

2. *United Nations Framework Convention on Climate Change (UNFCC): Adoption of the Paris Agreement - Paris Agreement text English*; 2016;

3. Green New Deal - www.gp.org Available online: https://www.gp.org/green_new_deal (accessed on Jul 2, 2023).

4. A European Green Deal Available online: https://commission.europa.eu/strategy-and-policy/priorities-2019-2024/european-green-deal_en (accessed on Aug 2, 2023).

5. *IEA Bioenergy: Global biorefinery status report 2022*; 2022;

6. Zabaniotou, A. Redesigning a bioenergy sector in EU in the transition to circular waste-based Bioeconomy-A multidisciplinary review. *J. Clean. Prod.* **2018**, *177*, 197–206, doi:10.1016/j.jclepro.2017.12.172.

7. Mauser, W.; Klepper, G.; Zabel, F.; Delzeit, R.; Hank, T.; Putzenlechner, B.; Calzadilla, A. Global biomass production potentials exceed expected future demand without the need for cropland expansion. *Nat. Commun.* **2015**, *6*, 1–11, doi:10.1038/ncomms9946.

8. Ahmadzai, H.; Tutundjian, S.; Elouafi, I. Policies for Sustainable Agriculture and Livelihood in Marginal Lands: A Review. *Sustainability* **2021**, *13*, 8692, doi:10.3390/su13168692.

9. Stegmann, P.; Londo, M.; Junginger, M. The circular bioeconomy: Its elements and role in European bioeconomy clusters. *Resour. Conserv. Recycl. X* 2020, *6*, 100029.

10. Muscat, A.; de Olde, E.M.; Ripoll-Bosch, R.; Van Zanten, H.H.E.; Metze, T.A.P.; Termeer, C.J.A.M.; van Ittersum, M.K.; de Boer, I.J.M. Principles, drivers and opportunities of a circular bioeconomy. *Nat. Food* 2021, *2*, 561–566.

11. Chaturvedi, T.; Torres, A.I.; Stephanopoulos, G.; Thomsen, M.H.; Schmidt, J.E. Developing process designs for biorefineries-definitions, categories, and unit operations. *Energies* **2020**, *13*, 1493, doi:10.3390/en13061493.

12. Velenturf, A.P.M.; Purnell, P. Principles for a sustainable circular economy. *Sustain. Prod. Consum.* 2021, *27*, 1437–1457.

13. Torres, A.I.; Ashraf, M.T.; Chaturvedi, T.; Schmidt, J.E.; Stephanopoulos, G. Hydrothermal pretreatment: Process modeling and economic assessment within the framework of biorefinery processes. In *Hydrothermal Processing in Biorefineries: Production of Bioethanol and High Added-Value Compounds of Second and Third Generation Biomass*; Springer International Publishing, 2017; pp. 207–235 ISBN 9783319564579.

14. Severo, I.A.; Siqueira, S.F.; Deprá, M.C.; Maroneze, M.M.; Zepka, L.Q.; Jacob-Lopes, E. Biodiesel facilities: What can we address to make biorefineries commercially competitive? *Renew. Sustain. Energy Rev.* **2019**, *112*, 686–705, doi:10.1016/j.rser.2019.06.020.

15. Budzianowski, W.M. High-value low-volume bioproducts coupled to bioenergies with potential to enhance business development of sustainable biorefineries. *Renew. Sustain. Energy Rev.* 2017, *70*, 793–804.

16. Chaturvedi, T.; Hulkko, L.S.S.; Fredsgaard, M.; Thomsen, M.H. Extraction, Isolation, and Purification of Value-Added Chemicals from Lignocellulosic Biomass. *Process. 2022, Vol. 10,*

Page 1752 **2022**, *10*, 1752, doi:10.3390/pr10091752.

17. Hulkko, L.S.S.; Chaturvedi, T.; Custódio, L.; Thomsen, M.H. Harnessing the Value of Tripolium pannonicum and Crithmum maritimum Halophyte Biomass through Integrated Green Biorefinery. *Mar. Drugs* **2023**, *21*, 380.

18. Jimenez-Lopez, C.; Fraga-Corral, M.; Carpena, M.; García-Oliveira, P.; Echave, J.; Pereira, A.G.; Lourenço-Lopes, C.; Prieto, M.A.; Simal-Gandara, J. Agriculture waste valorisation as a source of antioxidant phenolic compounds within a circular and sustainable bioeconomy. *Food Funct.* 2020, *11*, 4853–4877.

19. Yaashikaa, P.R.; Senthil Kumar, P.; Varjani, S. Valorization of agro-industrial wastes for biorefinery process and circular bioeconomy: A critical review. *Bioresour. Technol.* **2022**, *343*, 126126, doi:10.1016/j.biortech.2021.126126.

20. Fermoso, F.G.; Serrano, A.; Alonso-Fariñas, B.; Fernández-Bolaños, J.; Borja, R.; Rodríguez-Gutiérrez, G. Valuable Compound Extraction, Anaerobic Digestion, and Composting: A Leading Biorefinery Approach for Agricultural Wastes. *J. Agric. Food Chem.* 2018, *66*, 8451–8468.

21. Manhongo, T.T.; Chimphango, A.F.A.; Thornley, P.; Röder, M. Current status and opportunities for fruit processing waste biorefineries. *Renew. Sustain. Energy Rev.* 2022, *155*, 111823.

22. Caldeira, C.; Vlysidis, A.; Fiore, G.; De Laurentiis, V.; Vignali, G.; Sala, S. Sustainability of food waste biorefinery: A review on valorisation pathways, techno-economic constraints, and environmental assessment. *Bioresour. Technol.* **2020**, *312*, 123575, doi:10.1016/j.biortech.2020.123575.

23. Hrynkiewicz, K.; Patz, S.; Ruppel, S. Salicornia europaea L. as an underutilized saline-tolerant plant inhabited by endophytic diazotrophs. *J. Adv. Res.* **2019**, *19*, 49–56, doi:10.1016/j.jare.2019.05.002.

24. EIP-AGRI Focus Group Soil Salinisation: Final report Available online: https://ec.europa.eu/eip/agriculture/en/publications/eip-agri-focus-group-soil-salinisation-final (accessed on Jul 11, 2023).

25. Soil salinity | Global Soil Partnership | Food and Agriculture Organization of the United Nations Available online: https://www.fao.org/global-soil-partnership/areas-of-work/soil-salinity/en/ (accessed on Jul 11, 2023).

26. FAO *Intergovernmental Technical Panel on Soils: Status of the World's Soil Resources*; 2015;

27. Ventura, Y.; Eshel, A.; Pasternak, D.; Sagi, M. The development of halophyte-based agriculture: past and present. *Ann. Bot.* **2014**, *115*, 529–540, doi:10.1093/aob/mcu173.

28. Munns, R.; Tester, M. Mechanisms of Salinity Tolerance. *Annu. Rev. Plant Biol.* **2008**, *59*, 651–681, doi:10.1146/annurev.arplant.59.032607.092911.

29. Hassani, A.; Azapagic, A.; Shokri, N. Global predictions of primary soil salinization under changing climate in the 21st century. *Nat. Commun.* **2021**, *12*, 1–17, doi:10.1038/s41467-021-26907-3.

30. Daliakopoulos, I.N.; Tsanis, I.K.; Koutroulis, A.; Kourgialas, N.N.; Varouchakis, A.E.; Karatzas, G.P.; Ritsema, C.J. The threat of soil salinity: A European scale review. *Sci. Total Environ.* 2016, *573*, 727–739.

31. Veerman, C.; Pinto Correia, T.; Bastioli, C.; Biro, B.; Bouma, J.; Cienciala, E.; Emmett, B.; Frison, E.A.; Grand, A.; Filchew, L.H.; et al. *Caring for soil is caring for life*; 2020;

32. Fischer, G.; Nachtergaele, F.; Prieler, S.; Velthuizen, H.T. van; Verelst, L.; Wiberg, D. *Global Agroecological Zones Assessment for Agriculture (GAEZ 2008)*; 2008;

33. Flowers, T.J.; Colmer, T.D. Salinity tolerance in halophytes. *New Phytol.* **2008**, *179*, 945–963, doi:10.1111/j.1469-8137.2008.02531.x.

34. Hulkko, L.S.S.; Turcios, A.E.; Kohnen, S.; Chaturvedi, T.; Papenbrock, J.; Thomsen, M.H. Cultivation and characterisation of Salicornia europaea, Tripolium pannonicum and Crithmum maritimum biomass for green biorefinery applications. *Sci. Rep.* **2022**, *12*, 20507, doi:10.1038/s41598-022-24865-4.

35. Nikalje, G.C.; Srivastava, A.K.; Pandey, G.K.; Suprasanna, P. Halophytes in biosaline agriculture: Mechanism, utilization, and value addition. *L. Degrad. Dev.* **2018**, *29*, 1081–1095, doi:10.1002/ldr.2819.

36. Glenn, E.P.; Brown, J.J.; Blumwald, E. Salt Tolerance and Crop Potential of Halophytes. *CRC. Crit. Rev. Plant Sci.* **1999**, *18*, 227–255, doi:10.1080/07352689991309207.

37. Antlfinger, A.E.; Dunn, E.L. Water use and salt balance in three salt marsh succulents. *Am. J. Bot.* **1983**, *70*, 561–567, doi:10.2307/2443167.

38. Bose, J.; Rodrigo-Moreno, A.; Shabala, S. ROS homeostasis in halophytes in the context of salinity stress tolerance. *J. Exp. Bot.* 2014, *65*, 1241–1257.

39. Barreira, L.; Resek, E.; Rodrigues, M.J.; Rocha, M.I.; Pereira, H.; Bandarra, N.; da Silva, M.M.; Varela, J.; Custódio, L. Halophytes: Gourmet food with nutritional health benefits? *J. Food Compos. Anal.* **2017**, *59*, 35–42, doi:10.1016/j.jfca.2017.02.003.

40. Custódio, L.; Rodrigues, M.J.; Pereira, C.G.; Castañeda-Loaiza, V.; Fernandes, E.; Standing, D.; Neori, A.; Shpigel, M.; Sagi, M. A review on sarcocornia species: Ethnopharmacology, nutritional properties, phytochemistry, biological activities and propagation. *Foods* 2021, *10*, 2778.

41. Duarte, B.; Feijão, E.; Pinto, M.V.; Matos, A.R.; Silva, A.; Figueiredo, A.; Fonseca, V.F.; Reis-Santos, P.; Caçador, I. Nutritional valuation and food safety of endemic mediterranean halophytes species cultivated in abandoned salt pans under a natural irrigation scheme. *Estuar. Coast. Shelf Sci.* **2022**, *265*, 107733, doi:10.1016/j.ecss.2021.107733.

42. Sudjaroen, Y. Evaluation of ethnobotanical vegetables and herbs in Samut Songkram province. In Proceedings of the Procedia Engineering; Elsevier Ltd, 2012; Vol. 32, pp. 160–165.

43. Biscotti, N.; Pieroni, A.; Luczaj, L. The hidden Mediterranean diet: Wild vegetables traditionally gathered and consumed in the Gargano area, Apulia, SE Italy. *Acta Soc. Bot. Pol.* **2015**, *84*, 327–338, doi:10.5586/asbp.2015.031.

44. Renna, M. Reviewing the prospects of sea fennel (Crithmum maritimum L.) as emerging vegetable crop. *Plants* 2018, *7*.

45. Accogli, R.; Tomaselli, V.; Direnzo, P.; Perrino, E.V.; Albanese, G.; Urbano, M.; Laghetti, G. Edible Halophytes and Halo-Tolerant Species in Apulia Region (Southeastern Italy): Biogeography, Traditional Food Use and Potential Sustainable Crops. *Plants* **2023**, *12*, 549, doi:10.3390/plants12030549.

46. Cárdenas-Pérez, S.; Piernik, A.; Chanona-Pérez, J.J.; Grigore, M.N.; Perea-Flores, M.J. An overview of the emerging trends of the Salicornia L. genus as a sustainable crop. *Environ. Exp. Bot.* 2021, *191*.

47. Petropoulos, S.A.; Karkanis, A.; Martins, N.; Ferreira, I.C.F.R. Edible halophytes of the Mediterranean basin: Potential candidates for novel food products. *Trends Food Sci. Technol.* 2018, *74*, 69–84.

48. Hulkko, L.S.S.; Rocha, R.M.; Trentin, R.; Fredsgaard, M.; Chaturvedi, T.; Custódio, L.; Thomsen, M.H. Bioactive Extracts from Salicornia ramosissima J. Woods Biorefinery as a Source of Ingredients for High-Value Industries. *Plants* **2023**, *12*, 1251, doi:10.3390/plants12061251.

49. Ventura, Y.; Myrzabayeva, M.; Alikulov, Z.; Cohen, S.; Shemer, Z.; Sagi, M. The importance of iron supply during repetitive harvesting of Aster tripolium. In Proceedings of the Functional Plant Biology; 2013; Vol. 40, pp. 968–976.

50. Limongelli, F.; Crupi, P.; Clodoveo, M.L.; Corbo, F.; Muraglia, M. Overview of the Polyphenols in Salicornia: From Recovery to Health-Promoting Effect. *Molecules* 2022, *27*, 7954.

51. Mohammed, H.A. The Valuable Impacts of Halophytic Genus Suaeda; Nutritional, Chemical, and Biological Values. *Med. Chem. (Los. Angeles).* **2020**, *16*, 1044–1057, doi:10.2174/1573406416666200224115004.

52. Cybulska, I.; Brudecki, G.P.; Alassali, A.; Thomsen, M.H.; Brown, J.J. Phytochemical composition of some common coastal halophytes of the United Arab Emirates. *Emirates J. Food Agric.* **2014**, *26*, 1046–1056, doi:10.9755/ejfa.v26i12.19104.

53. Ksouri, R.; Ksouri, W.M.; Jallali, I.; Debez, A.; Magné, C.; Hiroko, I.; Abdelly, C. Medicinal halophytes: potent source of health promoting biomolecules with medical, nutraceutical and food applications. *Crit. Rev. Biotechnol.* **2012**, *32*, 289–326, doi:10.3109/07388551.2011.630647.

54. Stanković, M.S.; Petrović, M.; Godjevac, D.; Stevanović, Z.D. Screening inland halophytes from the central balkan for their antioxidant activity in relation to total phenolic compounds and flavonoids: Are there any prospective medicinal plants? *J. Arid Environ.* **2015**, *120*, 26–32, doi:10.1016/j.jaridenv.2015.04.008.

55. Martins-Noguerol, R.; Matías, L.; Pérez-Ramos, I.M.; Moreira, X.; Muñoz-Vallés, S.; Mancilla-Leytón, J.M.; Francisco, M.; García-González, A.; DeAndrés-Gil, C.; Martínez-Force, E.; et al. Differences in nutrient composition of sea fennel (Crithmum maritimum) grown in different habitats and optimally controlled growing conditions. *J. Food Compos. Anal.* **2022**, *106*, 104266, doi:10.1016/j.jfca.2021.104266.

56. Lopes, M.; Sanches-Silva, A.; Castilho, M.; Cavaleiro, C.; Ramos, F. Halophytes as source of bioactive phenolic compounds and their potential applications. *Crit. Rev. Food Sci. Nutr.* **2021**, doi:10.1080/10408398.2021.1959295.

57. Greenwell, M.; Rahman, P.K.S.M. Medicinal Plants: Their Use in Anticancer Treatment. *Int. J. Pharm. Sci. Res.* **2015**, *6*, 4103–4112, doi:10.13040/IJPSR.0975-8232.6(10).4103-12.

58. Custodio, L.; Garcia-Caparros, P.; Pereira, C.G.; Castelo-Branco, P. Halophyte Plants as Potential Sources of Anticancer Agents: A Comprehensive Review. *Pharmaceutics* **2022**, *14*, 2406, doi:10.3390/pharmaceutics14112406.

59. Limongelli, F.; Crupi, P.; Clodoveo, M.L.; Corbo, F.; Muraglia, M. Overview of the Polyphenols in Salicornia: From Recovery to Health-Promoting Effect. *Molecules* 2022, *27*.

60. Kumar, N.; Goel, N. Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnol. Reports* 2019, *24*, e00370.

61. Falcone Ferreyra, M.L.; Rius, S.P.; Casati, P. Flavonoids: Biosynthesis, biological functions, and biotechnological applications. *Front. Plant Sci.* 2012, *3*, 222.

62. Lin, D.; Xiao, M.; Zhao, J.; Li, Z.; Xing, B.; Li, X.; Kong, M.; Li, L.; Zhang, Q.; Liu, Y.; et al. An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. *Molecules* 2016, *21*, 1374.

63. Lopes, A.; Rodrigues, M.J.; Pereira, C.; Oliveira, M.; Barreira, L.; Varela, J.; Trampetti, F.; Custódio, L. Natural products from extreme marine environments: Searching for potential industrial uses within extremophile plants. *Ind. Crops Prod.* **2016**, *94*, 299–307, doi:10.1016/j.indcrop.2016.08.040.

64. Jdey, A.; Falleh, H.; Ben Jannet, S.; Mkadmini Hammi, K.; Dauvergne, X.; Ksouri, R.; Magné, C. Phytochemical investigation and antioxidant, antibacterial and anti-tyrosinase performances of six medicinal halophytes. *South African J. Bot.* **2017**, *112*, 508–514, doi:10.1016/j.sajb.2017.05.016.

65. Rodrigues, M.; Gangadhar, K.; Vizetto-Duarte, C.; Wubshet, S.; Nyberg, N.; Barreira, L.; Varela, J.; Custódio, L. Maritime Halophyte Species from Southern Portugal as Sources of Bioactive Molecules. *Mar. Drugs* **2014**, *12*, 2228–2244, doi:10.3390/md12042228.

66. Abbas, M.; Saeed, F.; Anjum, F.M.; Afzaal, M.; Tufail, T.; Bashir, M.S.; Ishtiaq, A.; Hussain, S.; Suleria, H.A.R. Natural polyphenols: An overview. *Int. J. Food Prop.* **2017**, *20*, 1689–1699, doi:10.1080/10942912.2016.1220393.

67. Chai, W.; Chen, L.; Lian, X.Y.; Zhang, Z. Anti-glioma Efficacy and Mechanism of Action of Tripolinolate A from Tripolium pannonicum. *Planta Med.* **2018**, *84*, 786–794, doi:10.1055/s-0044-101038.

68. Doi, N.; Togari, H.; Minagi, K.; Nakaoji, K.; Hamada, K.; Tatsuka, M. Protective Effects of Salicornia europaea on UVB-Induced Misoriented Cell Divisions in Skin Epithelium. *Cosmetics* **2020**, *7*, 44, doi:10.3390/cosmetics7020044.

69. Hymery, N.; Dauvergne, X.; Boussaden, H.; Cérantola, S.; Faugère, D.; Magné, C. Evaluation of the Antioxidant, Anti-Inflammatory and Cytoprotective Activities of Halophyte Extracts against Mycotoxin Intoxication. *Toxins (Basel).* **2021**, *13*, 312, doi:10.3390/toxins13050312.

70. Oliveira-Alves, S.C.; Andrade, F.; Sousa, J.; Bento-Silva, A.; Duarte, B.; Caçador, I.; Salazar, M.; Mecha, E.; Serra, A.T.; Bronze, M.R. Soilless Cultivated Halophyte Plants: Volatile, Nutritional, Phytochemical, and Biological Differences. *Antioxidants* **2023**, *12*, 1161, doi:10.3390/antiox12061161.

71. Fredsgaard, Malthe; Kabemba Kaniki, S.E.; Antonopoulou, I.; Chaturvedi, T.; Thomsen, M.H. Phenolic Compounds in Salicornia spp. and Their Potential Therapeutic Effects on H1N1, HBV, HCV, and HIV: A Review. *Molecules* **2023**, *28*, 5312, doi:doi.org/10.3390/molecules28145312.

72. Mohammed, H.A.; Ali, H.M.; Qureshi, K.A.; Alsharidah, M.; Kandil, Y.I.; Said, R.; Mohammed, S.A.A.; Al-Omar, M.S.; Rugaie, O. Al; Abdellatif, A.A.H.; et al. Comparative Phytochemical Profile and Biological Activity of Four Major Medicinal Halophytes from Qassim Flora. *Plants* **2021**, *10*, 2208, doi:10.3390/plants10102208.

73. Giordano, R.; Saii, Z.; Fredsgaard, M.; Hulkko, L.S.S.; Poulsen, T.B.G.; Thomsen, M.E.; Henneberg, N.; Zucolotto, S.M.; Arendt-Nielsen, L.; Papenbrock, J.; et al. Pharmacological insights into halophyte bioactive extract action on anti-inflammatory, pain relief and antibiotics-type mechanisms. *Molecules* 2021, *26*, 3140.

74. Batog, J.; Bujnowicz, K.; Gieparda, W.; Wawro, A.; Rojewski, S. Effective Utilisation of Halophyte Biomass from Saline Soils for Biorefinering Processes. *Molecules* **2021**, *26*, 5393, doi:10.3390/molecules26175393.

75. Ventura, Y.; Sagi, M. Halophyte crop cultivation: The case for Salicornia and Sarcocornia. *Environ. Exp. Bot.* **2013**, *92*, 144–153, doi:10.1016/j.envexpbot.2012.07.010.

76. Boestfleisch, C.; Wagenseil, N.B.; Buhmann, A.K.; Seal, C.E.; Wade, E.M.; Muscolo, A.; Papenbrock, J. Manipulating the antioxidant capacity of halophytes to increase their cultural and economic value through saline cultivation. *AoB Plants* **2014**, *6*, plu046–plu046,

doi:10.1093/aobpla/plu046.

77. Agudelo, A.; Carvajal, M.; Martinez-Ballesta, M. del C. Halophytes of the Mediterranean Basin—Underutilized Species with the Potential to Be Nutritious Crops in the Scenario of the Climate Change. *Foods* **2021**, *10*, 119, doi:10.3390/foods10010119.

78. Gunning, D. Cultivating Salicornia europaea (Marsh Samphire). **2016**, 92.

79. Ben Hamed, K.; Castagna, A.; Ranieri, A.; García-Caparrós, P.; Santin, M.; Hernandez, J.A.; Espin, G.B. Halophyte based Mediterranean agriculture in the contexts of food insecurity and global climate change. *Environ. Exp. Bot.* **2021**, *191*, 104601, doi:10.1016/j.envexpbot.2021.104601.

80. Duarte, B.; Caçador, I. Iberian Halophytes as Agroecological Solutions for Degraded Lands and Biosaline Agriculture. *Sustainability* **2021**, *13*, 1005, doi:10.3390/su13021005.

81. Manousaki, E.; Kalogerakis, N. Halophytes-An Emerging Trend in Phytoremediation. *Int. J. Phytoremediation* **2011**, *13*, 959–969, doi:10.1080/15226514.2010.532241.

82. Wiszniewska, A.; Koźmińska, A.; Hanus-Fajerska, E.; Dziurka, M.; Dziurka, K. Insight into mechanisms of multiple stresses tolerance in a halophyte Aster tripolium subjected to salinity and heavy metal stress. *Ecotoxicol. Environ. Saf.* **2019**, *180*, 12–22, doi:10.1016/j.ecoenv.2019.04.059.

83. Kaviani, E.; Niazi, A.; Moghadam, A.; Taherishirazi, M.; Heydarian, Z. Phytoremediation of Ni-contaminated soil by Salicornia iranica. *Environ. Technol. (United Kingdom)* **2019**, *40*, 270–281, doi:10.1080/09593330.2017.1387179.

84. Sanjosé, I.; Navarro-Roldán, F.; Montero, Y.; Ramírez-Acosta, S.; Jiménez-Nieva, F.J.; Infante-Izquierdo, M.D.; Polo-ávila, A.; Muñoz-Rodríguez, A.F. The Bioconcentration and the Translocation of Heavy Metals in Recently Consumed Salicornia ramosissima J. Woods in Highly Contaminated Estuary Marshes and Its Food Risk. *Diversity* **2022**, *14*, 452, doi:10.3390/d14060452.

85. Singh, D.; Buhmann, A.K.; Flowers, T.J.; Seal, C.E.; Papenbrock, J. Salicornia as a crop plant in temperate regions: Selection of genetically characterized ecotypes and optimization of their cultivation conditions. *AoB Plants* **2014**, *6*, doi:10.1093/aobpla/plu071.

86. Kong, Y.; Zheng, Y. Suaeda glauca can be produced hydroponically at moderate NACL salinity. *HortScience* **2015**, *50*, 847–850, doi:10.21273/hortsci.50.6.847.

87. Belal, I.E.H.; Al-Dosari, M. Replacement of Fish Meal with Salicornia Meal in Feeds for Nile Tilapia Oreochromis niloticus. *J. World Aquac. Soc.* **1999**, *30*, 285–289, doi:10.1111/j.1749-7345.1999.tb00877.x.

88. Shpigel, M.; Ben-Ezra, D.; Shauli, L.; Sagi, M.; Ventura, Y.; Samocha, T.; Lee, J.. Constructed wetland with Salicornia as a biofilter for mariculture effluents. *Aquaculture* **2013**, *412–413*, 52–63, doi:10.1016/j.aquaculture.2013.06.038.

89. Gunning, D.; Maguire, J.; Burnell, G. The development of sustainable saltwater-based food production systems: A review of established and novel concepts. *Water (Switzerland)* 2016, *8*, 598.

90. Custódio, M.; Villasante, S.; Cremades, J.; Calado, R.; Lillebø, A. Unravelling the potential of halophytes for marine integrated multi-trophic aquaculture (IMTA)— a perspective on performance, opportunities and challenges. *Aquac. Environ. Interact.* **2017**, *9*, 445–460, doi:10.3354/aei00244.

91. Vlahos, N.; Levizou, E.; Stathopoulou, P.; Berillis, P.; Antonopoulou, E.; Bekiari, V.; Krigas, N.; Kormas, K.; Mente, E. An Experimental Brackish Aquaponic System Using Juvenile

Gilthead Sea Bream (Sparus aurata) and Rock Samphire (Crithmum maritimum). *Sustainability* **2019**, *11*, 4820, doi:10.3390/su11184820.

92. Brown, J.J.; Glenn, E.P.; Fitzsimmons, K.M.; Smith, S.E. Halophytes for the treatment of saline aquaculture effluent. *Aquaculture* **1999**, *175*, 255–268, doi:10.1016/S0044-8486(99)00084-8.

93. Turcios, A.E.; Weichgrebe, D.; Papenbrock, J. Uptake and biodegradation of the antimicrobial sulfadimidine by the species Tripolium pannonicum acting as biofilter and its further biodegradation by anaerobic digestion and concomitant biogas production. *Bioresour. Technol.* **2016**, *219*, 687–693, doi:10.1016/j.biortech.2016.08.047.

94. Turcios, A.E.; Cayenne, A.; Uellendahl, H.; Papenbrock, J. Halophyte Plants and Their Residues as Feedstock for Biogas Production—Chances and Challenges. *Appl. Sci.* **2021**, *11*, 2746, doi:10.3390/app11062746.

95. Makkawi, Y.; El Sayed, Y.; Lyra, D.A.; Pour, F.H.; Khan, M.; Badrelzaman, M. Assessment of the pyrolysis products from halophyte Salicornia bigelovii cultivated in a desert environment. *Fuel* **2021**, *290*, 119518, doi:10.1016/j.fuel.2020.119518.

96. Iaccarino, A.; Gautam, R.; Sarathy, S.M. Bio-oil and biochar production from halophyte biomass: effects of pre-treatment and temperature onSalicornia bigeloviipyrolysis. *Sustain. Energy Fuels* **2021**, *5*, 2234–2248, doi:10.1039/d0se01664k.

97. Folayan, A.J.; Anawe, P.A.L.; Ayeni, A.O.; Arellano-Garcia, H. Synthesis and characterization of *Salicornia bigelovii* and *Salicornia brachiata* halophytic plants oil extracted by supercritical CO_2 modified with ethanol for biodiesel production via enzymatic transesterification reaction using immobilized *Candida antarctica* lipase catalyst in tert-butyl alcohol (TBA) solvent. *Cogent Eng.* **2019**, *6*, 1625847, doi:10.1080/23311916.2019.1625847.

98. Cybulska, I.; Chaturvedi, T.; Brudecki, G.P.; Kádár, Z.; Meyer, A.S.; Baldwin, R.M.; Thomsen, M.H. Chemical characterization and hydrothermal pretreatment of Salicornia bigelovii straw for enhanced enzymatic hydrolysis and bioethanol potential. *Bioresour. Technol.* **2014**, *153*, 165–172, doi:10.1016/j.biortech.2013.11.071.

99. Alassali, A.; Cybulska, I.; Galvan, A.; Thomsen, M. Wet fractionation of the succulent halophyte Salicornia sinus-persica, with the aim of low input (water saving) biorefining into bioethanol. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 1769–1779, doi:10.1007/s00253-016-8049-8.

100. Nawaz, A.; Qadoos, K.; Haq, I.U.; Feng, Y.; Mukhtar, H.; Huang, R.; Jiang, K. Effect of pretreatment strategies on halophyte Atriplex crassifolia to improve saccharification using thermostable cellulases. *Front. Bioeng. Biotechnol.* **2023**, *11*, 1135424, doi:10.3389/fbioe.2023.1135424.

101. Joshi, A.; Kanthaliya, B.; Arora, J. Halophytes: The Nonconventional Crops as Source of Biofuel Production. *Handb. Halophytes* **2020**, 1–28, doi:10.1007/978-3-030-17854-3_126-1.

102. Turcios, A.E.; Weichgrebe, D.; Papenbrock, J. Effect of salt and sodium concentration on the anaerobic methanisation of the halophyte Tripolium pannonicum. *Biomass and Bioenergy* **2016**, *87*, 69–77, doi:10.1016/j.biombioe.2016.01.013.

103. Kamel, M.; Hammad, S.; Khalaphallah, R.; Abd Elazeem, M. Halophytes and salt tolerant wild plants as a feedstock for biogas production. *J. Biosci. Biotechnol.* **2019**, *8*, 151–159.

104. Cayenne, A.; Turcios, A.E.; Thomsen, M.H.; Rocha, R.M.; Papenbrock, J.; Uellendahl, H. Halophytes as Feedstock for Biogas Production: Composition Analysis and Biomethane Potential of Salicornia spp. Plant Material from Hydroponic and Seawater Irrigation Systems. *Fermentation* **2022**, *8*, 189, doi:10.3390/fermentation8040189.

105. Nawaz, A.; Chaudhary, R.; Haq, I.U.; Fu, X.; Huang, R.; Mukhtar, H.; Jiang, K. Delignification

of Halophyte Atriplex crassifolia by Green Recyclable Deep Eutectic Solvents for Enhanced Production of Biogas. *Fermentation* **2023**, *9*, 314, doi:10.3390/fermentation9030314.

106. Akinshina, N.; Azizov, A.; Karasyova, T.; Klose, E. On the issue of halophytes as energy plants in saline environment. *Biomass and Bioenergy* **2016**, *91*, 306–311, doi:10.1016/j.biombioe.2016.05.034.

107. Gundersen, E.; Christiansen, A.H.C.; Jørgensen, K.; Lübeck, M. Production of leaf protein concentrates from cassava: Protein distribution and anti-nutritional factors in biorefining fractions. *J. Clean. Prod.* **2022**, *379*, 134730, doi:10.1016/j.jclepro.2022.134730.

108. Coldebella, P.F.; Gomes, S.D.; Evarini, J.A.; Cereda, M.P.; Coelho, S.R.M.; Coldebella, A. Evaluation of protein extraction methods to obtain protein concentrate from cassava leaf. *Eng. Agric.* **2013**, *33*, 1223–1233, doi:10.1590/S0100-69162013000600015.

109. Martin, A.H.; Castellani, O.; de Jong, G.A.; Bovetto, L.; Schmitt, C. Comparison of the functional properties of RuBisCO protein isolate extracted from sugar beet leaves with commercial whey protein and soy protein isolates. *J. Sci. Food Agric.* **2019**, *99*, 1568–1576, doi:10.1002/jsfa.9335.

110. Tamayo Tenorio, A.; Gieteling, J.; De Jong, G.A.H.; Boom, R.M.; Van Der Goot, A.J. Recovery of protein from green leaves: Overview of crucial steps for utilisation. *Food Chem.* **2016**, *203*, 402–408, doi:10.1016/j.foodchem.2016.02.092.

111. Poultry statistics - Statistics Explained Available online: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Poultry_statistics#Poultry_production (accessed on Jun 22, 2023).

112. Livestock population in numbers - Products Eurostat News - Eurostat Available online: https://ec.europa.eu/eurostat/web/products-eurostat-news/-/ddn-20220517-2 (accessed on Jun 22, 2023).

113. Santamaría-Fernández, M.; Lübeck, M. Production of leaf protein concentrates in green biorefineries as alternative feed for monogastric animals. *Anim. Feed Sci. Technol.* **2020**, *268*, 114605, doi:10.1016/j.anifeedsci.2020.114605.

114. Li, M.; Wei, D.; Huang, S.; Huang, L.; Xu, F.; Yu, Q.; Liu, M.; Li, P. Medicinal herbs and phytochemicals to combat pathogens in aquaculture. *Aquac. Int.* 2022, *30*, 1239–1259.

115. Santamaría-Fernández, M.; Lübeck, M. Production of leaf protein concentrates in green biorefineries as alternative feed for monogastric animals. *Anim. Feed Sci. Technol.* 2020, *268*, 114605.

116. Møller, A.H.; Hammershøj, M.; Dos Passos, N.H.M.; Tanambell, H.; Stødkilde, L.; Ambye-Jensen, M.; Danielsen, M.; Jensen, S.K.; Dalsgaard, T.K. Biorefinery of Green Biomass-How to Extract and Evaluate High Quality Leaf Protein for Food? *J. Agric. Food Chem.* 2021, *69*, 14341–14357.

117. Ayanfe, N.; Franco, M.; Stefański, T.; Pap, N.; Rinne, M. The effects of grass biomass preservation methods, organic acid treatment and press type on the separation efficiency in the green biorefinery. *Bioresour. Technol. Reports* **2023**, *21*, 101356, doi:10.1016/j.biteb.2023.101356.

118. Gaffey, J.; Rajauria, G.; McMahon, H.; Ravindran, R.; Dominguez, C.; Ambye-Jensen, M.; Souza, M.F.; Meers, E.; Aragonés, M.M.; Skunca, D.; et al. Green Biorefinery systems for the production of climate-smart sustainable products from grasses, legumes and green crop residues. *Biotechnol. Adv.* 2023, *66*, 108168.

119. Huang, K.H.; Tao, M.C.; Boulet, M.; Riel, R.R.; Julien, J.P.; Brisson, G.J. A Process for the Preparation of Leaf Protein Concentrates Based on the Treatment of Leaf Juices with Polar Solvents. *Can. Inst. Food Technol. J.* **1971**, *4*, 85–90, doi:10.1016/s0008-3860(71)74188-9.

120. Deepchand, K. System for the production of electricity, leaf protein and single cell protein from sugar cane tops and leaves. *Sol. Energy* **1985**, *35*, 477–482, doi:10.1016/0038-092X(85)90115-X.

121. Jebali, Z.; Nabili, A.; Majdoub, H.; Boufi, S. Cellulose nanofibrils (CNFs) from Ammophila arenaria, a natural and a fast growing grass plant. *Int. J. Biol. Macromol.* **2018**, *107*, 530–536, doi:10.1016/j.ijbiomac.2017.09.024.

122. Unlocking the full potential of grass | Grassa Available online: https://grassa.nl/ (accessed on Jun 10, 2023).

123. Kamm, B.; Schönicke, P.; Hille, C. Green biorefinery - Industrial implementation. *Food Chem.* **2016**, *197*, 1341–1345, doi:10.1016/j.foodchem.2015.11.088.

124. Santamaría-Fernández, M.; Molinuevo-Salces, B.; Lübeck, M.; Uellendahl, H. Biogas potential of green biomass after protein extraction in an organic biorefinery concept for feed, fuel and fertilizer production. *Renew. Energy* **2018**, *129*, 769–775, doi:10.1016/j.renene.2017.03.012.

125. The AQUACOMINE Project Available online: https://www.aquacombine.eu/ (accessed on Jun 9, 2023).

126. de Souza, M.; Mendes, C.; Doncato, K.; Badiale-Furlong, E.; Costa, C. Growth, Phenolics, Photosynthetic Pigments, and Antioxidant Response of Two New Genotypes of Sea Asparagus (Salicornia neei Lag.) to Salinity under Greenhouse and Field Conditions. *Agriculture* **2018**, *8*, doi:10.3390/agriculture8070115.

127. Myrzabayeva, M.T.; Omarov, R.T.; Alikulov, Z.A. Physiological adaptation mechanism of aster tripolium. l under salinity stress. **1995**, 247–251.

128. Christiansen, A.H.C.; Lyra, D.A.; Jørgensen, H. Increasing the value of Salicornia bigelovii green biomass grown in a desert environment through biorefining. *Ind. Crops Prod.* **2020**, 113105, doi:10.1016/j.indcrop.2020.113105.

129. Monção, M.; Wretborn, T.; Rova, U.; Matsakas, L.; Christakopoulos, P. Salicornia dolichostachya organosolv fractionation: towards establishing a halophyte biorefinery. *RSC Adv.* **2022**, *12*, 28599–28607, doi:10.1039/d2ra04432c.

130. Thomas, J.E.; Sinha, R.; Strand, Å.; Söderqvist, T.; Stadmark, J.; Franzén, F.; Ingmansson, I.; Gröndahl, F.; Hasselström, L. Marine biomass for a circular blue-green bioeconomy? A life cycle perspective on closing nitrogen and phosphorus land-marine loops. *J. Ind. Ecol.* **2022**, *26*, 2136–2153, doi:10.1111/jiec.13177.

131. Alassali, A.; Cybulska, I.; Galvan, A.R.; Thomsen, M.H. Wet fractionation of the succulent halophyte Salicornia sinus-persica, with the aim of low input (water saving) biorefining into bioethanol. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 1769–1779, doi:10.1007/s00253-016-8049-8.

132. Hulkko, L.S.S.; Chaturvedi, T.; Thomsen, M.H. *Valorisation of Residual Biomass Fractions from Biosaline Agriculture Through Green Biorefinery*; 2023;

133. Harikrishnan, R.; Kim, J.S.; Kim, M.C.; Dharaneedharan, S.; Kim, D.H.; Hong, S.H.; Song, C.Y.; Balasundaram, C.; Heo, M.S. Effect of dietary supplementation with Suaeda maritima on blood physiology, innate immune response, and disease resistance in olive flounder against Miamiensis avidus. *Exp. Parasitol.* **2012**, *131*, 195–203, doi:10.1016/j.exppara.2012.03.024.

134. García-Rodríguez, P.; Ma, F.; Río, C. del; Romero-Bernal, M.; Najar, A.M.; Cádiz-Gurrea, M. de la L.; Leyva-Jimenez, F.J.; Ramiro, L.; Menéndez-Valladares, P.; Pérez-Sánchez, S.; et al. Diet Supplementation with Polyphenol-Rich Salicornia ramosissima Extracts Protects against Tissue Damage in Experimental Models of Cerebral Ischemia. *Nutrients* **2022**, *14*, 5077, doi:10.3390/nu14235077.

135. Abd El-Hack, M.E.; Samak, D.H.; Noreldin, A.E.; Arif, M.; Yaqoob, H.S.; Swelum, A.A. Towards saving freshwater: halophytes as unconventional feedstuffs in livestock feed: a review. *Environ. Sci. Pollut. Res.* 2018, *25*, 14397–14406.

136. Isyakaeva, R.R.; Lagutkina, L.Y.; Akhmedzhanova, A.B.; Golubkina, E. V.; Kaplanov, M.T.; Khazova, N.A. Alternative sources of aquaculture feed in the context of organic production priorities. In Proceedings of the IOP Conference Series: Earth and Environmental Science; IOP Publishing Ltd, 2021; Vol. 640, p. 062024.

137. Hadidi, M.; Hossienpour, Y.; Nooshkam, M.; Mahfouzi, M.; Gharagozlou, M.; Aliakbari, F.S.; Aghababaei, F.; McClement, D.J. Green leaf proteins: a sustainable source of edible plant-based proteins. *Crit. Rev. Food Sci. Nutr.* **2023**, *0*, 1–18, doi:10.1080/10408398.2023.2229436.

138. Abdal, M.S. Salicornia production in Kuwait. *World Appl. Sci. J.* **2009**, *6*, 1033–1038.

139. Norman, H.C.; Masters, D.G.; Barrett-Lennard, E.G. Halophytes as forages in saline landscapes: Interactions between plant genotype and environment change their feeding value to ruminants. *Environ. Exp. Bot.* **2013**, *92*, 96–109, doi:10.1016/j.envexpbot.2012.07.003.

140. Ahmed, M.H.; Salem, A.Z.M.; Zeweil, H.S.; Sun, X.Z.; Kholif, A.E.; Elghandour, M.M.Y.; Bahar, M.S.I. Growth performance and carcass characteristics of lambs fed halophytes as a partial or whole replacement of berseem hay. *Small Rumin. Res.* **2015**, *128*, 1–9, doi:10.1016/j.smallrumres.2015.05.004.

141. Jiao, Y.; Hosseindoust, A.; Zhang, W.-L.; Kim, I.-H. Effects of Salicornia herbacea on Growth Performance, Meat Quality, Excreta Microbial Populations, and Noxious Gas Emissions in Broiler Chicks. *J. Poult. Sci.* **2019**, *56*, 44–51, doi:10.2141/jpsa.0170210.

142. Attia, F.M.; Alsobayel, A.A.; Kriadees, M.S.; Al-Saiady, M.Y.; Bayoumi, M.S. Nutrient composition and feeding value of Salicornia bigelovii torr meal in broiler diets. *Anim. Feed Sci. Technol.* **1997**, *65*, 257–263, doi:10.1016/S0377-8401(96)01074-7.

143. Pereira, C.G.; Barreira, L.; da Rosa Neng, N.; Nogueira, J.M.F.; Marques, C.; Santos, T.F.; Varela, J.; Custódio, L. Searching for new sources of innovative products for the food industry within halophyte aromatic plants: In vitro antioxidant activity and phenolic and mineral contents of infusions and decoctions of Crithmum maritimum L. *Food Chem. Toxicol.* **2017**, *107*, 581–589, doi:10.1016/j.fct.2017.04.018.

144. Agudelo, A.; Carvajal, M.; Martinez-Ballesta, M. del C. Halophytes of the Mediterranean Basin—Underutilized Species with the Potential to Be Nutritious Crops in the Scenario of the Climate Change. *Foods* **2021**, *10*, 119, doi:10.3390/foods10010119.

145. Dong, X.; Wang, J.; Liu, X.; Singh, B.P.; Sun, H. Characterization of halophyte biochar and its effects on water and salt contents in saline soil. *Environ. Sci. Pollut. Res.* **2022**, *29*, 11831–11842, doi:10.1007/s11356-021-16526-2.

146. Cybulska, I.; Brudecki, G.P.; Brown, J.J.; Hulkko, L.S.S.; Al Hosani, S.; Hedegaard Thomsen, M. Comparative study of chemical composition of the halophyte species native to the Persian (Arabian) gulf. *BioResources* **2021**, *16*, 5524–5537.

147. Home | Sustainable Development Available online: https://sdgs.un.org/ (accessed on Jun 22, 2023).

148. The Global Biodiversity Information Facility (GBIF) Available online: https://www.gbif.org/ (accessed on Jun 23, 2023).

149. Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. Laboratory Analytical Procedure (LAP) Issue Date : 12/08/2006 Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples. **2008**.

150. Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. *NREL/TP-510-42622 analytical procedure - Determination of Ash in Biomass*; 2005;

151. Sluiter, A.; Hames, B.; Hyman, D.; Payne, C.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Wolfe, J. NREL/TP-510-42621 analytical procedure - Determination of total solids in biomass and total dissolved solids in liquid process samples. **2008**, 3–5.

152. Jones, D. Factors for Converting Percentages of Nitrogen in Foods and Feeds into Percentages of Proteins. *United States Dep. Agric.* **1931**, 16–21.

153. Sluiter, A.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. NREL/TP-510-42619 analytical procedure - Determination fo Extractives in Biomass. **2005**.

154. Mazza, G.; Fukumoto, L.; Delaquis, P.; Girard, B.; Ewert, B. Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia. *J. Agric. Food Chem.* **1999**, *47*, 4009–4017, doi:10.1021/jf990449f.

155. Li, Y.G.; Tanner, G.; Larkin, P. The DMACA-HCl protocol and the threshold proanthocyanidin content for bloat safety in forage legumes. *J. Sci. Food Agric.* **1996**, *70*, 89–101, doi:10.1002/(SICI)1097-0010(199601)70:1<89::AID-JSFA470>3.0.CO;2-N.

156. Pirbalouti, A.G.; Siahpoosh, A.; Setayesh, M.; Craker, L. Antioxidant activity, total phenolic and flavonoid contents of some medicinal and aromatic plants used as herbal teas and condiments in Iran. *J. Med. Food* **2014**, *17*, 1151–1157, doi:10.1089/jmf.2013.0057.

157. Velioglu, Y.S.; Mazza, G.; Gao, L.; Oomah, B.D. Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products. *J. Agric. Food Chem.* **1998**, *46*, 4113–4117, doi:10.1021/jf9801973.

158. Lichtenthaler, H.K.; Wellburn, A.R. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* **1983**, *11*, 591–592, doi:10.1042/bst0110591.

159. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Sci. Technol.* 1995, *28*, 25–30.

160. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237, doi:10.1016/S0891-5849(98)00315-3.

161. Baliga, M.S.; Jagetia, G.C.; Rao, S.K.; Babu S, K. Evaluation of nitric oxide scavenging activity of certain spicesin vitro: A preliminary study. *Nahrung/Food* **2003**, *47*, 261–264, doi:10.1002/food.200390061.

162. Megías, C.; Pastor-Cavada, E.; Torres-Fuentes, C.; Girón-Calle, J.; Alaiz, M.; Juan, R.; Pastor, J.; Vioque, J. Chelating, antioxidant and antiproliferative activity of Vicia sativa polyphenol extracts. *Eur. Food Res. Technol.* **2009**, *230*, 353–359, doi:10.1007/s00217-009-1178-x.

163. Xiao, Z.; Storms, R.; Tsang, A. A quantitative starch-iodine method for measuring alpha-amylase and glucoamylase activities. *Anal. Biochem.* **2006**, *351*, 146–148, doi:10.1016/j.ab.2006.01.036.

164. Custódio, L.; Patarra, J.; Alberício, F.; Neng, N. da R.; Nogueira, J.M.F.; Romano, A. Phenolic composition, antioxidant potential and in vitro inhibitory activity of leaves and acorns of Quercus suber on key enzymes relevant for hyperglycemia and Alzheimer's disease. *Ind. Crops Prod.* **2015**, *64*, 45–51, doi:10.1016/j.indcrop.2014.11.001.

165. McDougall, G.J.; Kulkarni, N.N.; Stewart, D. Berry polyphenols inhibit pancreatic lipase activity in vitro. *Food Chem.* **2009**, *115*, 193–199, doi:10.1016/j.foodchem.2008.11.093.

166. Trentin, R.; Custódio, L.; Rodrigues, M.J.; Moschin, E.; Sciuto, K.; da Silva, J.P.; Moro, I. Exploring Ulva australis Areschoug for possible biotechnological applications: In vitro antioxidant and enzymatic inhibitory properties, and fatty acids contents. *Algal Res.* **2020**, *50*, 101980, doi:10.1016/j.algal.2020.101980.

167. Ellman, G.L.; Courtney, K.D.; Andres, V.; Featherstone, R.M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **1961**, *7*, 88–95, doi:10.1016/0006-2952(61)90145-9.

168. Kiel, P.; Anderson, M.; Lübeck, M. A method of providing functional proteins from a plant material 2015.

169. Chaturvedi, T.; Christiansen, A.H.C.; Gołębiewska, I.; Thomsen, M.H. Salicornia Species: Current Status and Future Potential. In *Future of Sustainable Agriculture in Saline Environments*; CRC Press, 2021; pp. 461–482.

170. Kadereit, G.; Ball, P.; Beer, S.; Mucina, L.; Sokoloff, D.; Teege, P.; Yaprak, A.E.; Freitag, H. A taxonomic nightmare comes true: phylogeny and biogeography of glassworts (*Salicornia* L., Chenopodiaceae). *Taxon* **2007**, *56*, 1143–1170, doi:10.2307/25065909.

171. Ventura, Y.; Wuddineh, W.A.; Myrzabayeva, M.; Alikulov, Z.; Khozin-Goldberg, I.; Shpigel, M.; Samocha, T.M.; Sagi, M. Effect of seawater concentration on the productivity and nutritional value of annual Salicornia and perennial Sarcocornia halophytes as leafy vegetable crops. *Sci. Hortic. (Amsterdam).* **2011**, *128*, 189–196, doi:10.1016/j.scienta.2011.02.001.

172. Araus, J.L.; Rezzouk, F.Z.; Thushar, S.; Shahid, M.; Elouafi, I.A.; Bort, J.; Serret, M.D. Effect of irrigation salinity and ecotype on the growth, physiological indicators and seed yield and quality of Salicornia europaea. *Plant Sci.* **2021**, *304*, 110819, doi:10.1016/j.plantsci.2021.110819.

173. Parida, A.K.; Kumari, A.; Panda, A.; Rangani, J.; Agarwal, P.K. Photosynthetic pigments, betalains, proteins, sugars, and minerals during Salicornia brachiata senescence. *Biol. Plant.* **2018**, *62*, 343–352, doi:10.1007/s10535-017-0764-1.

174. Maatallah Zaier, M.; Ciudad-Mulero, M.; Cámara, M.; Pereira, C.; Ferreira, I.C.F.R.; Achour, L.; Kacem, A.; Morales, P. Revalorization of Tunisian wild Amaranthaceae halophytes: Nutritional composition variation at two different phenotypes stages. *J. Food Compos. Anal.* **2020**, *89*, 103463, doi:10.1016/j.jfca.2020.103463.

175. Zaier, M.M.; Heleno, S.A.; Mandim, F.; Calhelha, R.C.; Ferreira, I.C.F.R.; Achour, L.; Kacem, A.; Dias, M.I.; Barros, L. Effects of the seasonal variation in the phytochemical composition and bioactivities of the wild halophyte Suaeda fruticosa. *Food Biosci.* **2022**, *50*, 102131, doi:10.1016/j.fbio.2022.102131.

176. Ibdah, M.; Krins, A.; Seidlitz, H.K.; Heller, W.; Strack, D.; Vogt, T. Spectral dependence of flavonol and betacyanin accumulation in *Mesembryanthemum crystallinum* under enhanced ultraviolet radiation. *Plant. Cell Environ.* **2002**, *25*, 1145–1154, doi:10.1046/j.1365-3040.2002.00895.x.

177. Turcios, A.E.; Braem, L.; Jonard, C.; Lemans, T.; Cybulska, I.; Papenbrock, J. Compositional Changes in Hydroponically Cultivated Salicornia europaea at Different Growth Stages. *Plants* **2023**, *12*, 2472, doi:10.3390/plants12132472.

178. Santamaría-Fernández, M.; Karkov Ytting, N.; Lübeck, M. Influence of the development stage of perennial forage crops for the recovery yields of extractable proteins using lactic acid fermentation. *J. Clean. Prod.* **2019**, *218*, 1055–1064, doi:10.1016/j.jclepro.2019.01.292.

179. Das, L.K.; Kundu, S.S.; Kumar, D.; Datt, C. Fractionation of carbohydrate and protein content of some forage feeds of ruminants for nutritive evaluation. *Vet. World* **2015**, *8*, 197–202, doi:10.14202/vetworld.2015.197-202.

180. Santamaría-Fernández, M.; Molinuevo-Salces, B.; Kiel, P.; Steenfeldt, S.; Uellendahl, H.; Lübeck, M. Lactic acid fermentation for refining proteins from green crops and obtaining a high quality feed product for monogastric animals. *J. Clean. Prod.* **2017**, *162*, 875–881, doi:10.1016/j.jclepro.2017.06.115.

181. Patel, M.K.; Pandey, S.; Brahmbhatt, H.R.; Mishra, A.; Jha, B. Lipid content and fatty acid profile of selected halophytic plants reveal a promising source of renewable energy. *Biomass and Bioenergy* **2019**, *124*, 25–32, doi:10.1016/j.biombioe.2019.03.007.

182. Abideen, Z.; Ansari, R.; Khan, M.A. Halophytes: Potential source of ligno-cellulosic biomass for ethanol production. *Biomass and Bioenergy* **2011**, *35*, 1818–1822, doi:10.1016/j.biombioe.2011.01.023.

183. Lourenço, A.; Pereira, H. Compositional Variability of Lignin in Biomass. In *Lignin - Trends and Applications*; InTech, 2018.

184. Colomer, J.S.; Passera, C.B. The nutritional value of Atriplex spp. as fodder for arid regions. *J. Arid Environ.* **1990**, *19*, 289–295, doi:10.1016/s0140-1963(18)30793-6.

185. Pires, A.; Agreira, S.; Ressurreição, S.; Marques, J.; Guiné, R.; Barroca, M.J.; Moreira da Silva, A. Sea Purslane as an Emerging Food Crop: Nutritional and Biological Studies. *Appl. Sci.* **2021**, *11*, 7860, doi:10.3390/app11177860.

186. Lima, A.R.; Gama, F.; Castañeda-Loaiza, V.; Costa, C.; Schüler, L.M.; Santos, T.; Salazar, M.; Nunes, C.; Cruz, R.M.S.; Varela, J.; et al. Nutritional and Functional Evaluation of Inula crithmoides and Mesembryanthemum nodiflorum Grown in Different Salinities for Human Consumption. *Molecules* **2021**, *26*, 4543, doi:10.3390/molecules26154543.

187. Jin-Gi Min; Doo-Seog Lee; Tae-Jin Kim; Jeong-Heum Park; Tae-Yong Cho; Dong-In Park Chemical Composition of Salicornia Herbacea L. *Prev. Nutr. food Sci.* **2002**, *7*, 105–107.

188. Lima, A.R.; Castañeda-Loaiza, V.; Salazar, M.; Nunes, C.; Quintas, C.; Gama, F.; Pestana, M.; Correia, P.J.; Santos, T.; Varela, J.; et al. Influence of cultivation salinity in the nutritional composition, antioxidant capacity and microbial quality of Salicornia ramosissima commercially produced in soilless systems. *Food Chem.* **2020**, *333*, 127525, doi:10.1016/j.foodchem.2020.127525.

189. Barreira, L.; Resek, E.; Rodrigues, M.J.; Rocha, M.I.; Pereira, H.; Bandarra, N.; Da Silva, M.M.; Varela, J.; Custódio, L. Halophytes: Gourmet food with nutritional health benefits? *J. Food Compos. Anal.* **2017**, *59*, 35–42, doi:10.1016/j.jfca.2017.02.003.

190. Castañeda-Loaiza, V.; Oliveira, M.; Santos, T.; Schüler, L.; Lima, A.R.; Gama, F.; Salazar, M.; Neng, N.R.; Nogueira, J.M.F.; Varela, J.; et al. Wild vs cultivated halophytes: Nutritional and functional differences. *Food Chem.* **2020**, *333*, 127536, doi:10.1016/j.foodchem.2020.127536.

191. Sánchez-Faure, A.; Calvo, M.M.; Pérez-Jiménez, J.; Martín-Diana, A.B.; Rico, D.; Montero, M.P.; Gómez-Guillén, M. del C.; López-Caballero, M.E.; Martínez-Alvarez, O. Exploring the potential of common iceplant, seaside arrowgrass and sea fennel as edible halophytic plants. *Food Res. Int.* **2020**, *137*, 109613, doi:10.1016/j.foodres.2020.109613.

192. Montero, M.P.; Sánchez-Faure, A.; Rico, D.; Martín-Diana, A.B.; Palencia, P.F. de; Sánchez-Guisado, M.J.; Gómez-Guillén, C.; López-Caballero, M.E.; Martínez-Alvarez, O. Sea Aster (Aster Tripolium) As a Source of Bioactive Molecules.; 49th WEFTA Conference: Tórshavn, Faroe Islands, 2019.

193. van Andel, J. Vegetation Between Land and Sea: Structure and Processes . A. H. L. Huiskes , C. W. P. M. Blom, J. Rozema. *Q. Rev. Biol.* **1989**, *64*, 73–74, doi:10.1086/416161.

194. Lv, S.; Jiang, P.; Chen, X.; Fan, P.; Wang, X.; Li, Y. Multiple compartmentalization of sodium conferred salt tolerance in Salicornia europaea. *Plant Physiol. Biochem.* **2012**, *51*, 47–52,

doi:10.1016/j.plaphy.2011.10.015.

195. Katschnig, D.; Broekman, R.; Rozema, J. Salt tolerance in the halophyte Salicornia dolichostachya Moss: Growth, morphology and physiology. *Environ. Exp. Bot.* **2013**, *92*, 32–42, doi:10.1016/j.envexpbot.2012.04.002.

196. Cárdenas-Pérez, S.; Rajabi Dehnavi, A.; Leszczyński, K.; Lubińska-Mielińska, S.; Ludwiczak, A.; Piernik, A. Salicornia europaea L. Functional Traits Indicate Its Optimum Growth. *Plants* **2022**, *11*, 1051, doi:10.3390/plants11081051.

197. Uno, Y.; Kanechi, M.; Inagaki, N.; Sugimoto, M.; Maekawa, S. The Evaluation of Salt Tolerance during Germination and Vegetative Growth of Asparagus, Table Beet and Sea Aster. *Engei Gakkai zasshi* **1996**, *65*, 579–585, doi:10.2503/jjshs.65.579.

198. Ueda, A.; Kanechi, M.; Uno, Y.; Inagaki, N. Photosynthetic limitations of a halophyte sea aster (Aster tripolium L) under water stress and NaCl stress. *J. Plant Res.* **2003**, *116*, 65–70, doi:10.1007/s10265-002-0070-6.

199. Ben Amor, N.; Ben Hamed, K.; Debez, A.; Grignon, C.; Abdelly, C. Physiological and antioxidant responses of the perennial halophyte Crithmum maritimum to salinity. *Plant Sci.* **2005**, *168*, 889–899, doi:10.1016/j.plantsci.2004.11.002.

200. Ben Hamed, K.; Castagna, A.; Salem, E.; Ranieri, A.; Abdelly, C. Sea fennel (Crithmum maritimum L.) under salinity conditions: A comparison of leaf and root antioxidant responses. *Plant Growth Regul.* **2007**, *53*, 185–194, doi:10.1007/s10725-007-9217-8.

201. Santos, J.; Al-Azzawi, M.; Aronson, J.; Flowers, T.J. eHALOPH a Database of Salt-Tolerant Plants: Helping put Halophytes to Work. *Plant Cell Physiol.* **2016**, *57*, e10–e10, doi:10.1093/pcp/pcv155.

202. Cybulska, I.; Chaturvedi, T.; Alassali, A.; Brudecki, G.P.; Brown, J.J.; Sgouridis, S.; Thomsen, M.H. Characterization of the Chemical Composition of the Halophyte Salicornia bigelovii under Cultivation. *Energy and Fuels* **2014**, *28*, 3873–3883, doi:10.1021/ef500478b.

203. Ludwiczak, A.; Ciarkowska, A.; Rajabi Dehnavi, A.; Cárdenas-Pérez, S.; Piernik, A. Growth Stage-, Organ- and Time-Dependent Salt Tolerance of Halophyte Tripolium pannonicum (Jacq.) Dobrocz. *Life* **2023**, *13*, 462, doi:10.3390/life13020462.

204. García-Herrera, P.; Sánchez-Mata, M.C.; Cámara, M.; Fernández-Ruiz, V.; Díez-Marqués, C.; Molina, M.; Tardío, J. Nutrient composition of six wild edible Mediterranean Asteraceae plants of dietary interest. *J. Food Compos. Anal.* **2014**, *34*, 163–170, doi:10.1016/j.jfca.2014.02.009.

205. Hadi, M.R.; Karimi, N. The Role of Calcium in Plants' Salt Tolerance. *J. Plant Nutr.* **2012**, *35*, 2037–2054, doi:10.1080/01904167.2012.717158.

206. Silva, A.M.; Lago, J.P.; Pinto, D.; Moreira, M.M.; Grosso, C.; Cruz Fernandes, V.; Delerue-Matos, C.; Rodrigues, F. Salicornia ramosissima Bioactive Composition and Safety: Eco-Friendly Extractions Approach (Microwave-Assisted Extraction vs. Conventional Maceration). *Appl. Sci.* **2021**, *11*, 4744, doi:10.3390/app11114744.

207. Barroca, M.J.; Guiné, R.P.F.; Amado, A.M.; Ressurreição, S.; da Silva, A.M.; Marques, M.P.M.; de Carvalho, L.A.E.B. The drying process of Sarcocornia perennis: impact on nutritional and physico-chemical properties. *J. Food Sci. Technol.* **2020**, *57*, 4443–4458, doi:10.1007/s13197-020-04482-7.

208. Oliveira-Alves, S.C.; Andrade, F.; Prazeres, I.; Silva, A.B.; Capelo, J.; Duarte, B.; Caçador, I.; Coelho, J.; Serra, A.T.; Bronze, M.R. Impact of drying processes on the nutritional composition, volatile profile, phytochemical content and bioactivity of salicornia ramosissima j. Woods. *Antioxidants* **2021**, *10*, 1312, doi:10.3390/antiox10081312.

209. Cristina, C.; Lucia, P.; Sara, S.; Francesco, S.; Nobile Matteo Alessandro, D.; Amalia, C. Study of the Efficacy of Two Extraction Techniques from *Crithmum maritimum* and *Salicornia europaea*. *J. Food Nutr. Res.* **2018**, *6*, 456–463, doi:10.12691/jfnr-6-7-6.

210. Pedreiro, S.; Figueirinha, A.; Cavaleiro, C.; Cardoso, O.; Donato, M.M.; Salgueiro, L.; Ramos, F. Exploiting the Crithmum maritimum L. Aqueous Extracts and Essential Oil as Potential Preservatives in Food, Feed, Pharmaceutical and Cosmetic Industries. *Antioxidants* **2023**, *12*, 252, doi:10.3390/antiox12020252.

211. Kapadia, P.; Newell, A.S.; Cunningham, J.; Roberts, M.R.; Hardy, J.G. Extraction of High-Value Chemicals from Plants for Technical and Medical Applications. *Int. J. Mol. Sci.* 2022, *23*, 10334.

212. Khoo, H.E.; Azlan, A.; Tang, S.T.; Lim, S.M. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food Nutr. Res.* 2017, *61*.

213. Friedman, M.; Jürgens, H.S. Effect of pH on the stability of plant phenolic compounds. *J. Agric. Food Chem.* **2000**, *48*, 2101–2110, doi:10.1021/jf990489j.

214. Rodríguez De Luna, S.L.; Ramírez-Garza, R.E.; Serna Saldívar, S.O. Environmentally Friendly Methods for Flavonoid Extraction from Plant Material: Impact of Their Operating Conditions on Yield and Antioxidant Properties. *Sci. World J.* 2020, *2020*.

215. Manzano Durán, R.; Sánchez, J.E.F.; Velardo-Micharet, B.; Gómez, M.J.R. Multivariate optimization of ultrasound-assisted extraction for the determination of phenolic compounds in plums (Prunus salicina Lindl.) by high-performance liquid chromatography (HPLC). *Instrum. Sci. Technol.* **2020**, *48*, 113–127, doi:10.1080/10739149.2019.1662438.

216. Bañuelos, J.A.; Velázquez-Hernández, I.; Guerra-Balcázar, M.; Arjona, N. Production, characterization and evaluation of the energetic capability of bioethanol from Salicornia Bigelovii as a renewable energy source. *Renew. Energy* **2018**, *123*, 125–134, doi:10.1016/j.renene.2018.02.031.

217. Duarte, B.; Cabrita, M.T.; Gameiro, C.; Matos, A.R.; Godinho, R.; Marques, J.C.; Caçador, I. Disentangling the photochemical salinity tolerance in *Aster tripolium* L.: connecting biophysical traits with changes in fatty acid composition. *Plant Biol.* **2017**, *19*, 239–248, doi:10.1111/plb.12517.

218. Ben Hamed, K.; Ben Youssef, N.; Ranieri, A.; Zarrouk, M.; Abdelly, C. Changes in content and fatty acid profiles of total lipids and sulfolipids in the halophyte Crithmum maritimum under salt stress. *J. Plant Physiol.* **2005**, *162*, 599–602, doi:10.1016/j.jplph.2004.11.010.

219. Guil-Guerrero, J.L.; Rodríguez-García, I. Lipids classes, fatty acids and carotenes of the leaves of six edible wild plants. *Eur. Food Res. Technol.* **1999**, *209*, 313–316, doi:10.1007/s002170050501.

220. Maciel, E.; Lillebø, A.; Domingues, P.; Da Costa, E.; Calado, R.; Domingues, M.R.M. Polar lipidome profiling of Salicornia ramosissima and Halimione portulacoides and the relevance of lipidomics for the valorization of halophytes. *Phytochemistry* **2018**, *153*, 94–101, doi:10.1016/j.phytochem.2018.05.015.

221. Simopoulos, A.P. Omega-6/Omega-3 Essential Fatty Acid Ratio and Chronic Diseases. *Food Rev. Int.* **2004**, *20*, 77–90, doi:10.1081/FRI-120028831.

222. Isca, V.M.S.; Seca, A.M.L.; Pinto, D.C.G.A.; Silva, H.; Silva, A.M.S. Lipophilic profile of the edible halophyte Salicornia ramosissima. *Food Chem.* **2014**, *165*, 330.

223. Hulkko, L.S.S.; Chaturvedi, T.; Thomsen, M.H. Extraction and Quantification of Chlorophylls, Carotenoids, Phenolic Compounds, and Vitamins from Halophyte Biomasses. *Appl. Sci.* **2022**,

12, 840, doi:10.3390/app12020840.

224. Chekroun-Bechlaghem, N.; Belyagoubi-Benhammou, N.; Belyagoubi, L.; Gismondi, A.; Nanni, V.; Di Marco, G.; Canuti, L.; Canini, A.; El Haci, I.A.; Atik Bekkara, F. Phytochemical analysis and antioxidant activity of *Tamarix africana, Arthrocnemum macrostachyum* and *Suaeda fruticosa,* three halophyte species from Algeria. *Plant Biosyst. - An Int. J. Deal. with all Asp. Plant Biol.* **2019**, *153*, 843–852, doi:10.1080/11263504.2018.1555191.

225. Falleh, H.; Ksouri, R.; Boulaaba, M.; Guyot, S.; Abdelly, C.; Magne, C. Phenolic nature, occurrence and polymerization degree as marker of environmental adaptation in the edible halophyte Mesembryanthemum edule. *South African J. Bot.* **2012**, *79*, 117.

226. Mariem, S.; Hanen, F.; Inès, J.; Mejdi, S.; Riadh, K. Phenolic profile, biological activities and fraction analysis of the medicinal halophyte Retama raetam. *South African J. Bot.* **2014**, *94*, 114–121, doi:10.1016/j.sajb.2014.06.010.

227. Dai, J.; Mumper, R.J. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules* 2010, *15*, 7313–7352.

228. Rauf, A.; Imran, M.; Abu-Izneid, T.; Iahtisham-Ul-Haq; Patel, S.; Pan, X.; Naz, S.; Sanches Silva, A.; Saeed, F.; Rasul Suleria, H.A. Proanthocyanidins: A comprehensive review. *Biomed. Pharmacother.* 2019, *116*, 108999.

229. Qi, Q.; Chu, M.; Yu, X.; Xie, Y.; Li, Y.; Du, Y.; Liu, X.; Zhang, Z.; Shi, J.; Yan, N. Anthocyanins and Proanthocyanidins: Chemical Structures, Food Sources, Bioactivities, and Product Development. *Food Rev. Int.* 2022.

230. Smeriglio, A.; Barreca, D.; Bellocco, E.; Trombetta, D. Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects. *Br. J. Pharmacol.* **2017**, *174*, 1244–1262, doi:10.1111/bph.13630.

231. Kruger, M.J.; Davies, N.; Myburgh, K.H.; Lecour, S. Proanthocyanidins, anthocyanins and cardiovascular diseases. *Food Res. Int.* **2014**, *59*, 41–52, doi:10.1016/j.foodres.2014.01.046.

232. Guerreiro, A.; Rassal, C.; Afonso, C.M.; Galego, L.; Serra, M.; Rodrigues, M.A. Healthy, Tasty and Sustainable Mediterranean Food. UMAMI Taste and Polyphenols of Twiggy Glasswort (Salicornia ramosissima). In *INCREaSE*; Springer International Publishing, 2018; pp. 191–198.

233. Zengin, G.; Aumeeruddy-Elalfi, Z.; Mollica, A.; Yilmaz, M.A.; Mahomoodally, M.F. In vitro and in silico perspectives on biological and phytochemical profile of three halophyte species—A source of innovative phytopharmaceuticals from nature. *Phytomedicine* **2018**, *38*, 35–44, doi:10.1016/j.phymed.2017.10.017.

234. Nájar, A.M.; Romero-Bernal, M.; del Río, C.; Montaner, J. A Review on Polyphenols in Salicornia ramosissima with Special Emphasis on Their Beneficial Effects on Brain Ischemia. *Nutrients* 2023, *15*, 793.

235. Wubshet, S.G.; Schmidt, J.S.; Wiese, S.; Staerk, D. High-resolution screening combined with HPLC-HRMS-SPE-NMR for identification of potential health-promoting constituents in sea aster and searocket - New nordic food ingredients. *J. Agric. Food Chem.* **2013**, *61*, 8616–8623, doi:10.1021/jf402949y.

236. Souid, A.; Della Croce, C.M.; Frassinetti, S.; Gabriele, M.; Pozzo, L.; Ciardi, M.; Abdelly, C.; Hamed, K. Ben; Magné, C.; Longo, V. Nutraceutical Potential of Leaf Hydro-Ethanolic Extract of the Edible Halophyte Crithmum maritimum L. *Molecules* **2021**, *26*, 5380, doi:10.3390/molecules26175380.

237. Meot-Duros, L.; Magné, C. Antioxidant activity and phenol content of Crithmum maritimum L. leaves. *Plant Physiol. Biochem.* **2009**, *47*, 37–41, doi:10.1016/j.plaphy.2008.09.006.

238. Jallali, I.; Megdiche, W.; M'Hamdi, B.; Oueslati, S.; Smaoui, A.; Abdelly, C.; Ksouri, R. Changes in phenolic composition and antioxidant activities of the edible halophyte Crithmum maritimum L. With physiological stage and extraction method. *Acta Physiol. Plant.* **2012**, *34*, 1451–1459, doi:10.1007/s11738-012-0943-9.

239. Kumar, S.; Pandey, A.K. Chemistry and biological activities of flavonoids: An overview. *Sci. World J.* 2013, *2013*.

240. Ksouri, R.; Falleh, H.; Megdiche, W.; Trabelsi, N.; Mhamdi, B.; Chaieb, K.; Bakrouf, A.; Magné, C.; Abdelly, C. Antioxidant and antimicrobial activities of the edible medicinal halophyte Tamarix gallica L. and related polyphenolic constituents. *Food Chem. Toxicol.* **2009**, *47*, 2083–2091, doi:10.1016/j.fct.2009.05.040.

241. Rodrigues, M.J.; Soszynski, A.; Martins, A.; Rauter, A.P.; Neng, N.R.; Nogueira, J.M.F.; Varela, J.; Barreira, L.; Custódio, L. Unravelling the antioxidant potential and the phenolic composition of different anatomical organs of the marine halophyte Limonium algarvense. *Ind. Crops Prod.* **2015**, *77*, 315–322, doi:10.1016/j.indcrop.2015.08.061.

242. Medini, F.; Fellah, H.; Ksouri, R.; Abdelly, C. Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant Limonium delicatulum. *J. Taibah Univ. Sci.* **2014**, *8*, 216–224, doi:10.1016/j.jtusci.2014.01.003.

243. Castañeda-Loaiza, V.; Placines, C.; Rodrigues, M.J.; Pereira, C.; Zengin, G.; Uysal, A.; Jeko, J.; Cziáky, Z.; Reis, C.P.; Gaspar, M.M.; et al. If you cannot beat them, join them: Exploring the fruits of the invasive species Carpobrotus edulis (L.) N.E. Br as a source of bioactive products. *Ind. Crops Prod.* **2020**, *144*, 112005, doi:10.1016/j.indcrop.2019.112005.

244. Falleh, H.; Ksouri, R.; Medini, F.; Guyot, S.; Abdelly, C.; Magné, C. Antioxidant activity and phenolic composition of the medicinal and edible halophyte Mesembryanthemum edule L. *Ind. Crops Prod.* **2011**, *34*, 1066–1071, doi:10.1016/j.indcrop.2011.03.018.

245. Kim, J.; Cho, J.-Y.; Moon, J.-H.; Choi, G.-C.; Lee, K.-D.; Ham, K.-S.; Kim, S.-J. Change of phenylpropanoic acid and flavonol contents at different growth stage of glasswort (Salicornia herbacea L.). *Food Sci. Biotechnol.* **2014**, *23*, 685–691, doi:10.1007/s10068-014-0093-5.

246. Boulaaba, M.; Medini, F.; Hajlaoui, H.; Mkadmini, K.; Falleh, H.; Ksouri, R.; Isoda, H.; Smaoui, A.; Abdelly, C. Biological activities and phytochemical analysis of phenolic extracts from Salsola kali L. Role of endogenous factors in the selection of the best plant extracts. *South African J. Bot.* **2019**, *123*, 193–199, doi:10.1016/j.sajb.2019.03.003.

247. Ksouri, R.; Megdiche, W.; Falleh, H.; Trabelsi, N.; Boulaaba, M.; Smaoui, A.; Abdelly, C. Influence of biological, environmental and technical factors on phenolic content and antioxidant activities of Tunisian halophytes. *Comptes Rendus - Biol.* **2008**, *331*, 865–873, doi:10.1016/j.crvi.2008.07.024.

248. Creative Commons — Attribution 2.0 Generic — CC BY 2.0 Available online: https://creativecommons.org/licenses/by/2.0/?ref=openverse. (accessed on Jul 4, 2023).

249. Creative Commons — Attribution 4.0 International — CC BY 4.0 Available online: https://creativecommons.org/licenses/by/4.0/?ref=openverse (accessed on Jul 4, 2023).

250. Geissler, N.; Hussin, S.; Koyro, H.-W. Elevated atmospheric CO2 concentration ameliorates effects of NaCl salinity on photosynthesis and leaf structure of Aster tripolium L. *J. Exp. Bot.* **2008**, *60*, 137–151, doi:10.1093/jxb/ern271.

251. Houta, O.; Neffati, M.; Amri, H. Phenolic Contents and Antioxidant Potential of Cakile maritimum : *J. Biol. Act. Prod. from Nat.* **2012**, *2*, 387–391, doi:10.1080/22311866.2012.10719148.

252. Ellouzi, H.; Ben Hamed, K.; Cela, J.; Munné-Bosch, S.; Abdelly, C. Early effects of salt stress

on the physiological and oxidative status of Cakile maritima (halophyte) and Arabidopsis thaliana (glycophyte). *Physiol. Plant.* **2011**, *142*, 128–143, doi:10.1111/j.1399-3054.2011.01450.x.

253. Houta, O.; Akrout, A.; Amri, H. Phenolic amounts, antioxidant and antimicrobial potential of Crithmum maritimum cultivated in Tunisian arid zones. *Planta Med.* **2011**, *77*, 1416–1416, doi:10.1055/s-0031-1282826.

254. Jallali, I.; Zaouali, Y.; Missaoui, I.; Smeoui, A.; Abdelly, C.; Ksouri, R. Variability of antioxidant and antibacterial effects of essential oils and acetonic extracts of two edible halophytes: Crithmum maritimum L. and Inula crithmoïdes L. *Food Chem.* **2014**, *145*, 1031–1038, doi:10.1016/j.foodchem.2013.09.034.

255. Duarte, B.; Santos, D.; Marques, J.C.; Caçador, I. Ecophysiological adaptations of two halophytes to salt stress: Photosynthesis, PS II photochemistry and anti-oxidant feedback - Implications for resilience in climate change. *Plant Physiol. Biochem.* **2013**, *67*, 178–188, doi:10.1016/j.plaphy.2013.03.004.

256. Qasim, M.; Abideen, Z.; Adnan, M.Y.; Gulzar, S.; Gul, B.; Rasheed, M.; Khan, M.A. Antioxidant properties, phenolic composition, bioactive compounds and nutritive value of medicinal halophytes commonly used as herbal teas. *South African J. Bot.* **2017**, *110*, 240–250, doi:10.1016/j.sajb.2016.10.005.

257. Trabelsi, N.; Megdiche, W.; Ksouri, R.; Falleh, H.; Oueslati, S.; Soumaya, B.; Hajlaoui, H.; Abdelly, C. Solvent effects on phenolic contents and biological activities of the halophyte Limoniastrum monopetalum leaves. *LWT - Food Sci. Technol.* **2010**, *43*, 632–639, doi:10.1016/j.lwt.2009.11.003.

258. Medini, F.; Bourgou, S.; Lalancette, K.G.; Snoussi, M.; Mkadmini, K.; Coté, I.; Abdelly, C.; Legault, J.; Ksouri, R. Phytochemical analysis, antioxidant, anti-inflammatory, and anticancer activities of the halophyte Limonium densiflorum extracts on human cell lines and murine macrophages. *South African J. Bot.* **2015**, *99*, 158–164, doi:10.1016/j.sajb.2015.04.007.

259. Karker, M.; Falleh, H.; Msaada, K.; Smaoui, A.; Abdelly, C.; Legault, J.; Ksouri, R. Antioxidant, Anti-inflammatory and anticancer activities of the medicinal halophyte Reaumuria vermiculata. *EXCLI J.* **2016**, *15*, 297–307, doi:10.17179/excli2016-187.

260. Lu, D.; Zhang, M.; Wang, S.; Cai, J.; Zhou, X.; Zhu, C. Nutritional characterization and changes in quality of Salicornia bigelovii Torr. during storage. *LWT - Food Sci. Technol.* **2010**, *43*, 519–524, doi:10.1016/j.lwt.2009.09.021.

261. Padalino, L.; Costa, C.; Del Nobile, M.A.; Conte, A. Extract of Salicornia europaea in fresh pasta to enhance phenolic compounds and antioxidant activity. *Int. J. Food Sci. Technol.* **2019**, *54*, 3051–3057, doi:10.1111/ijfs.14218.

262. Aghaleh, M.; Niknam, V.; Ebrahimzadeh, H.; Razavi, K. Salt stress effects on growth, pigments, proteins and lipid peroxidation in Salicornia persica and S. europaea. *Biol. Plant.* **2009**, *53*, 243–248, doi:10.1007/s10535-009-0046-7.

263. de Souza, M.; Mendes, C.; Doncato, K.; Badiale-Furlong, E.; Costa, C. Growth, Phenolics, Photosynthetic Pigments, and Antioxidant Response of Two New Genotypes of Sea Asparagus (Salicornia neei Lag.) to Salinity under Greenhouse and Field Conditions. *Agriculture* **2018**, *8*, 115, doi:10.3390/agriculture8070115.

264. Akcin, A.; Yalcin, E. Effect of salinity stress on chlorophyll, carotenoid content, and proline in Salicornia prostrata Pall. and Suaeda prostrata Pall. subsp. prostrata (Amaranthaceae). *Rev. Bras. Bot.* **2016**, *39*, 101–106, doi:10.1007/s40415-015-0218-y.

265. Al-Tohamy, R.; Ali, S.S.; Saad-Allah, K.; Fareed, M.; Ali, A.; El-Badry, A.; El-Zawawy, N.A.; Wu, J.; Sun, J.; Mao, G.H.; et al. Phytochemical analysis and assessment of antioxidant and

antimicrobial activities of some medicinal plant species from Egyptian flora. *J. Appl. Biomed.* **2018**, *16*, 289–300, doi:10.1016/j.jab.2018.08.001.

266. Kumari, A.; Parida, A.K.; Rangani, J.; Panda, A. Antioxidant Activities, Metabolic Profiling, Proximate Analysis, Mineral Nutrient Composition of Salvadora persica Fruit Unravel a Potential Functional Food and a Natural Source of Pharmaceuticals. *Front. Pharmacol.* **2017**, *08*, 61, doi:10.3389/fphar.2017.00061.

267. Amin, E.; Musa, A. Evaluation of the Antioxidant, Total Phenolics and Total Flavonoids of Suaeda Species Collected in Al Jouf Area. *European J. Med. Plants* **2016**, *17*, 1–6, doi:10.9734/ejmp/2016/30748.

268. Saleem, H.; Khurshid, U.; Sarfraz, M.; Tousif, M.I.; Alamri, A.; Anwar, S.; Alamri, A.; Ahmad, I.; Abdallah, H.H.; Mahomoodally, F.M.; et al. A comprehensive phytochemical, biological, toxicological and molecular docking evaluation of Suaeda fruticosa (L.) Forssk.: An edible halophyte medicinal plant. *Food Chem. Toxicol.* **2021**, *154*, 112348, doi:10.1016/j.fct.2021.112348.

269. Oueslati, S.; Ksouri, R.; Falleh, H.; Pichette, A.; Abdelly, C.; Legault, J. Phenolic content, antioxidant, anti-inflammatory and anticancer activities of the edible halophyte Suaeda fruticosa Forssk. *Food Chem.* **2012**, *132*, 943–947, doi:10.1016/j.foodchem.2011.11.072.

270. Sghaier, D.B.; Duarte, B.; Bankaji, I.; Caçador, I.; Sleimi, N. Growth, chlorophyll fluorescence and mineral nutrition in the halophyte Tamarix gallica cultivated in combined stress conditions: Arsenic and NaCl. *J. Photochem. Photobiol. B Biol.* **2015**, *149*, 204–214, doi:10.1016/j.jphotobiol.2015.06.003.

271. Rangani, J.; Kumari, A.; Patel, M.; Brahmbhatt, H.; Parida, A.K. Phytochemical profiling, polyphenol composition, and antioxidant activity of the leaf extract from the medicinal halophyte *Thespesia populnea* reveal a potential source of bioactive compounds and nutraceuticals. *J. Food Biochem.* **2019**, *43*, e12731, doi:10.1111/jfbc.12731.

272. Saleem, H.; Zengin, G.; Locatelli, M.; Ahmad, I.; Khaliq, S.; Mahomoodally, M.F.; Hussain, R.; Rengasamy, K.R.R.; Mollica, A.; Zainal Abidin, S.A.; et al. Pharmacological, phytochemical and in-vivo toxicological perspectives of a xero-halophyte medicinal plant: Zaleya pentandra (L.) Jeffrey. *Food Chem. Toxicol.* **2019**, *131*, 110535, doi:10.1016/j.fct.2019.05.043.

273. Pangestuti, R.; Kim, S.K. Biological activities and health benefit effects of natural pigments derived from marine algae. *J. Funct. Foods* 2011, *3*, 255–266.

274. Egner, P.A.; Muñoz, A.; Kensler, T.W. Chemoprevention with chlorophyllin in individuals exposed to dietary aflatoxin. *Mutat. Res. - Fundam. Mol. Mech. Mutagen.* **2003**, *523–524*, 209–216, doi:10.1016/S0027-5107(02)00337-8.

275. Eggersdorfer, M.; Wyss, A. Carotenoids in human nutrition and health. *Arch. Biochem. Biophys.* 2018, *652*, 18–26.

276. Bernal, J.; Mendiola, J.A.; Ibáñez, E.; Cifuentes, A. Advanced analysis of nutraceuticals. *J. Pharm. Biomed. Anal.* 2011, *55*, 758–774.

277. Ksouri, R.; Ksouri, W.M.; Jallali, I.; Debez, A.; Magné, C.; Hiroko, I.; Abdelly, C. Medicinal halophytes: potent source of health promoting biomolecules with medical, nutraceutical and food applications. *Crit. Rev. Biotechnol.* **2012**, *32*, 289–326, doi:10.3109/07388551.2011.630647.

278. Vitamin and Mineral Requirements in Human Nutrition - World Health Organization, FAO - Google Books Available online: https://books.google.dk/books?hl=en&lr=&id=NjdkYRkHla0C&oi=fnd&pg=PR1&dq=vitamin+source+human+nutrition&ots=I9UYVSFFzB&sig=BFJ4bRMC2iIqlkmb8wenIOQRQks&redi

r_esc=y#v=onepage&q=vitamin source human nutrition&f=false (accessed on Aug 18, 2021).

279. Berretta, M.; Quagliariello, V.; Maurea, N.; Di Francia, R.; Sharifi, S.; Facchini, G.; Rinaldi, L.; Piezzo, M.; Ceccarelli, M.; Nunnari, G.; et al. Multiple effects of ascorbic acid against chronic diseases: Updated evidence from preclinical and clinical studies. *Antioxidants* 2020, *9*, 1–27.

280. Chamkouri, N.; Khodadoust, S.; Ghalavandi, F. Solid-Phase Extraction Coupled with HPLC-DAD for Determination of B Vitamin Concentrations in Halophytes. *J. Chromatogr. Sci.* **2015**, *53*, bmv080, doi:10.1093/chromsci/bmv080.

281. Nakos, M.; Pepelanova, I.; Beutel, S.; Krings, U.; Berger, R.G.; Scheper, T. Isolation and analysis of vitamin B12 from plant samples. *Food Chem.* **2017**, *216*, 301–308, doi:10.1016/j.foodchem.2016.08.037.

282. Hachicha, S.F.; Barrek, S.; Skanji, T.; Zarrouk, H.; Ghrabi, Z.G. Fatty acid, tocopherol, and sterol content of three Teucrium species from Tunisia. *Chem. Nat. Compd.* **2009**, *45*, 304–308, doi:10.1007/s10600-009-9355-5.

283. Anwar, F.; Bhanger, M.I.; Nasir, M.K.A.; Ismail, S. Analytical characterization of Salicornia bigelovii seed oil cultivated in Pakistan. *J. Agric. Food Chem.* **2002**, *50*, 4210–4214, doi:10.1021/jf0114132.

284. Choi, D.; Lim, G.S.; Piao, Y.L.; Choi, O.Y.; Cho, K.A.; Park, C.B.; Chang, Y.C.; Song, Y. Il; Lee, M.K.; Cho, H. Characterization, stability, and antioxidant activity of Salicornia herbaciea seed oil. *Korean J. Chem. Eng.* **2014**, *31*, 2221–2228, doi:10.1007/s11814-014-0163-7.

285. Cocuron, J.C.; Casas, M.I.; Yang, F.; Grotewold, E.; Alonso, A.P. Beyond the wall: High-throughput quantification of plant soluble and cell-wall bound phenolics by liquid chromatography tandem mass spectrometry. *J. Chromatogr. A* **2019**, *1589*, 93–104, doi:10.1016/j.chroma.2018.12.059.

286. Dias, M.C.; Pinto, D.C.G.A.; Silva, A.M.S. Plant flavonoids: Chemical characteristics and biological activity. *Molecules* 2021, *26*, 5377.

287. COMAN, C.; RUGINA, O.D.; SOCACIU, C. Plants and Natural Compounds with Antidiabetic Action. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2012**, *40*, 314, doi:10.15835/nbha4017205.

288. Salehi, B.; Fokou, P.; Sharifi-Rad, M.; Zucca, P.; Pezzani, R.; Martins, N.; Sharifi-Rad, J. The Therapeutic Potential of Naringenin: A Review of Clinical Trials. *Pharmaceuticals* **2019**, *12*, 11, doi:10.3390/ph12010011.

289. Stompor, M. A review on sources and pharmacological aspects of sakuranetin. *Nutrients* 2020, *12*, 513.

290. Pungin, A.; Lartseva, L.; Loskutnikova, V.; Shakhov, V.; Krol, O.; Popova, E.; Kolomiets, A.; Nikolaeva, N.; Volodina, A. The Content of Certain Groups of Phenolic Compounds and the Biological Activity of Extracts of Various Halophyte Parts of Spergularia marina (L.) Griseb. and Glaux maritima L. at Different Levels of Soil Salinization. *Plants* **2022**, *11*, 1738, doi:10.3390/plants11131738.

291. Li, Q.; Song, J. Analysis of widely targeted metabolites of the euhalophyte Suaeda salsa under saline conditions provides new insights into salt tolerance and nutritional value in halophytic species. *BMC Plant Biol.* **2019**, *19*, 388, doi:10.1186/s12870-019-2006-5.

292. Essaidi, I.; Brahmi, Z.; Snoussi, A.; Ben Haj Koubaier, H.; Casabianca, H.; Abe, N.; El Omri, A.; Chaabouni, M.M.; Bouzouita, N. Phytochemical investigation of Tunisian Salicornia herbacea L., antioxidant, antimicrobial and cytochrome P450 (CYPs) inhibitory activities of its methanol extract. *Food Control* **2013**, *32*, 125–133, doi:10.1016/j.foodcont.2012.11.006.

293. Cornara, L.; D'Arrigo, C.; Pioli, F.; Borghesi, B.; Bottino, C.; Patrone, E.; Mariotti, M.G.

Micromorphological investigation on the leaves of the rock samphire (*Crithmum maritimum* L.): Occurrence of hesperidin and diosmin crystals. *Plant Biosyst. - An Int. J. Deal. with all Asp. Plant Biol.* **2009**, *143*, 283–292, doi:10.1080/11263500902722527.

294. Atia, A.; Barhoumi, Z.; Mokded, R.; Abdelly, C.; Smaoui, A. Environmental eco-physiology and economical potential of the halophyte Crithmum maritimum L. (Apiaceae). *J. Med. Plants Res.* **2011**, *5*, 3564–3571.

295. Steinbruch, M.; Nunes, C.; Gama, R.; Kaufman, R.; Gama, G.; Suchmacher Neto, M.; Nigri, R.; Cytrynbaum, N.; Brauer Oliveira, L.; Bertaina, I.; et al. Is Nonmicronized Diosmin 600 mg as Effective as Micronized Diosmin 900 mg plus Hesperidin 100 mg on Chronic Venous Disease Symptoms? Results of a Noninferiority Study. *Int. J. Vasc. Med.* **2020**, *2020*, doi:10.1155/2020/4237204.

296. Cazaubon, M.; Benigni, J.-P.; Steinbruch, M.; Jabbour, V.; Gouhier-Kodas, C. Is There a Difference in the Clinical Efficacy of Diosmin and Micronized Purified Flavonoid Fraction for the Treatment of Chronic Venous Disorders? Review of Available Evidence. *Vasc. Health Risk Manag.* **2021**, *Volume 17*, 591–600, doi:10.2147/VHRM.S324112.

297. Rampogu, S.; Gajula, R.G.; Lee, K.W. A comprehensive review on chemotherapeutic potential of galangin. *Biomed. Pharmacother.* 2021, *141*, 111808.

298. Imran, M.; Rauf, A.; Abu-Izneid, T.; Nadeem, M.; Shariati, M.A.; Khan, I.A.; Imran, A.; Orhan, I.E.; Rizwan, M.; Atif, M.; et al. Luteolin, a flavonoid, as an anticancer agent: A review. *Biomed. Pharmacother.* 2019, *112*, 108612.

299. Barone, C.; Nuzzo, G.; Cassano, A.; Basso, M.; Schinzari, G.; Giuliante, F.; D'Argento, E.; Trigila, N.; Astone, A.; Pozzo, C. Final analysis of colorectal cancer patients treated with irinotecan and 5-fluorouracil plus folinic acid neoadjuvant chemotherapy for unresectable liver metastases. *Br. J. Cancer* **2007**, *97*, 1035–1039, doi:10.1038/sj.bjc.6603988.

300. Verjee, S.; Weston, A.; Kolb, C.; Kalbhenn-Aziz, H.; Butterweck, V. Hyperforin and Miquelianin from St Johns Wort Attenuate Gene Expression in Neuronal Cells after Dexamethasone-Induced Stress. *Planta Med.* **2018**, *84*, 696–703, doi:10.1055/a-0581-5286.

301. Choi, D.W.; Jung, S.Y.; Kim, G.D.; Lee, S.Y.; Shin, H.S. Miquelianin inhibits allergic responses in mice by suppressing cd4+ t cell proliferation. *Antioxidants* **2021**, *10*, 1120, doi:10.3390/antiox10071120.

302. Ho, L.; Ferruzzi, M.G.; Janle, E.M.; Wang, J.; Gong, B.; Chen, T.Y.; Lobo, J.; Cooper, B.; Wu, Q.L.; Talcott, S.T.; et al. Identification of brain-targeted bioactive dietary quercetin-3-O-glucuronide as a novel intervention for Alzheimer's disease. *FASEB J.* **2013**, *27*, 769–781, doi:10.1096/fj.12-212118.

303. Katalinić, M.; Rusak, G.; Domaćinović Barović, J.; Šinko, G.; Jelić, D.; Antolović, R.; Kovarik, Z. Structural aspects of flavonoids as inhibitors of human butyrylcholinesterase. *Eur. J. Med. Chem.* **2010**, *45*, 186–192, doi:10.1016/j.ejmech.2009.09.041.

304. Kiokias, S.; Proestos, C.; Oreopoulou, V. Phenolic acids of plant origin-a review on their antioxidant activity in vitro (O/W emulsion systems) along with their in vivo health biochemical properties. *Foods* 2020, *9*, 534.

305. Generalić Mekinić, I.; Blažević, I.; Mudnić, I.; Burčul, F.; Grga, M.; Skroza, D.; Jerčić, I.; Ljubenkov, I.; Boban, M.; Miloš, M.; et al. Sea fennel (Crithmum maritimum L.): phytochemical profile, antioxidative, cholinesterase inhibitory and vasodilatory activity. *J. Food Sci. Technol.* **2016**, *53*, 3104–3112, doi:10.1007/s13197-016-2283-z.

306. Politeo, O.; Popović, M.; Veršić Bratinčević, M.; Kovačević, K.; Urlić, B.; Generalić Mekinić, I. Chemical Profiling of Sea Fennel (Crithmum maritimum L., Apiaceae) Essential Oils and Their Isolation Residual Waste-Waters. *Plants* **2023**, *12*, 214, doi:10.3390/plants12010214.

307. Siracusa, L.; Kulisic-Bilusic, T.; Politeo, O.; Krause, I.; Dejanovic, B.; Ruberto, G. Phenolic composition and antioxidant activity of aqueous infusions from Capparis spinosa L. and Crithmum maritimum L. before and after submission to a two-step in vitro digestion model. *J. Agric. Food Chem.* **2011**, *59*, 12453–12459, doi:10.1021/jf203096q.

308. Ma, C.M.; Hattori, M.; Daneshtalab, M.; Wang, L. Chlorogenic acid derivatives with alkyl chains of different lengths and orientations: Potent α-glucosidase inhibitors. *J. Med. Chem.* **2008**, *51*, 6188–6194, doi:10.1021/jm800621x.

309. Meng, S.; Cao, J.; Feng, Q.; Peng, J.; Hu, Y. Roles of chlorogenic acid on regulating glucose and lipids metabolism: A review. *Evidence-based Complement. Altern. Med.* **2013**, *2013*, doi:10.1155/2013/801457.

310. Wang, S.; Li, Y.; Huang, D.; Chen, S.; Xia, Y.; Zhu, S. The inhibitory mechanism of chlorogenic acid and its acylated derivatives on α-amylase and α-glucosidase. *Food Chem.* **2022**, *372*, 131334, doi:10.1016/j.foodchem.2021.131334.

311. Park, S.Y.; Jin, M.L.; Yi, E.H.; Kim, Y.; Park, G. Neochlorogenic acid inhibits against LPS-activated inflammatory responses through up-regulation of Nrf2/HO-1 and involving AMPK pathway. *Environ. Toxicol. Pharmacol.* **2018**, *62*, 1–10, doi:10.1016/j.etap.2018.06.001.

312. Gao, X.; Zhang, S.; Wang, L.; Yu, L.; Zhao, X.; Ni, H.; Wang, Y.; Wang, J.; Shan, C.; Fu, Y. Anti-Inflammatory Effects of Neochlorogenic Acid Extract from Mulberry Leaf (Morus alba L.) Against LPS-Stimulated Inflammatory Response through Mediating the AMPK/Nrf2 Signaling Pathway in A549 Cells. *Molecules* **2020**, *25*, 1385, doi:10.3390/molecules25061385.

313. Espíndola, K.M.M.; Ferreira, R.G.; Narvaez, L.E.M.; Silva Rosario, A.C.R.; da Silva, A.H.M.; Silva, A.G.B.; Vieira, A.P.O.; Monteiro, M.C. Chemical and Pharmacological Aspects of Caffeic Acid and Its Activity in Hepatocarcinoma. *Front. Oncol.* **2019**, *9*, 467241, doi:10.3389/fonc.2019.00541.

314. Kumar, N.; Pruthi, V. Potential applications of ferulic acid from natural sources. *Biotechnol. Reports* 2014, *4*, 86–93.

315. Mancuso, C.; Santangelo, R. Ferulic acid: Pharmacological and toxicological aspects. *Food Chem. Toxicol.* 2014, *65*, 185–195.

316. Gao, H.; Huang, Y.N.; Gao, B.; Xu, P.Y.; Inagaki, C.; Kawabata, J. α-Glucosidase inhibitory effect by the flower buds of Tussilago farfara L. *Food Chem.* **2008**, *106*, 1195–1201, doi:10.1016/j.foodchem.2007.07.064.

317. Zhang, J.P.; Chen, Q.X.; Song, K.K.; Xie, J.J. Inhibitory effects of salicylic acid family compounds on the diphenolase activity of mushroom tyrosinase. *Food Chem.* **2006**, *95*, 579–584, doi:10.1016/j.foodchem.2005.01.042.

318. Bajpai, V.K.; Alam, M.B.; Quan, K.T.; Ju, M.K.; Majumder, R.; Shukla, S.; Huh, Y.S.; Na, M.K.; Lee, S.H.; Han, Y.K. Attenuation of inflammatory responses by (+)-syringaresinol via MAP-Kinase-mediated suppression of NF-κB signaling in vitro and in vivo. *Sci. Rep.* **2018**, *8*, 1–10, doi:10.1038/s41598-018-27585-w.

319. Parama, D.; Girisa, S.; Khatoon, E.; Kumar, A.; Alqahtani, M.S.; Abbas, M.; Sethi, G.; Kunnumakkara, A.B. An overview of the pharmacological activities of scopoletin against different chronic diseases. *Pharmacol. Res.* 2022, *179*, 106202.

320. Kowalska, A.; Kalinowska-Lis, U. 18 *β* -Glycyrrhetinic acid: its core biological properties and dermatological applications. *Int. J. Cosmet. Sci.* **2019**, *41*, ics.12548, doi:10.1111/ics.12548.

321. Weller, M.G. A unifying review of bioassay-guided fractionation, effect-directed analysis and related techniques. *Sensors (Switzerland)* 2012, *12*, 9181–9209.

322. Magni, N.N.; Veríssimo, A.C.S.; Silva, H.; Pinto, D.C.G.A. Metabolomic Profile of Salicornia perennis Plant's Organs under Diverse In Situ Stress: The Ria de Aveiro Salt Marshes Case. *Metabolites* **2023**, *13*, 280, doi:10.3390/metabo13020280.

323. Duan, H.; Tiika, R.J.; Tian, F.; Lu, Y.; Zhang, Q.; Hu, Y.; Cui, G.; Yang, H. Metabolomics analysis unveils important changes involved in the salt tolerance of Salicornia europaea. *Front. Plant Sci.* **2023**, *13*, 5651, doi:10.3389/fpls.2022.1097076.

324. Sung, J.H.; Park, S.H.; Seo, D.H.; Lee, J.H.; Hong, S.W.; Hong, S.S. Antioxidative and Skin-Whitening Effect of an Aqueous Extract of Salicornia herbacea. *Biosci. Biotechnol. Biochem.* **2009**, *73*, 552–556, doi:10.1271/bbb.80601.

325. Ghanem, A.-M.F.M.; Mohamed, E.; Kasem, A.M.M.A.; El-Ghamery, A.A. Differential Salt Tolerance Strategies in Three Halophytes from the Same Ecological Habitat: Augmentation of Antioxidant Enzymes and Compounds. *Plants* **2021**, *10*, 1100, doi:10.3390/plants10061100.

326. Correia, A.; Silva, A.M.; Moreira, M.M.; Salazar, M.; Švarc-Gajić, J.; Brezo-Borjan, T.; Cádiz-Gurrea, M. de la L.; Carretero, A.S.; Loschi, F.; Dall'Acqua, S.; et al. Salicornia ramosissima: A New Green Cosmetic Ingredient with Promising Skin Effects. *Antioxidants* **2022**, *11*, 2449, doi:10.3390/antiox11122449.

327. Giordano, R.; Aliotta, G.E.; Johannesen, A.S.; Voetmann-Jensen, D.; Laustsen, F.H.; Andersen, L.A.; Rezai, A.; Fredsgaard, M.; Vecchio, S. Lo; Arendt-Nielsen, L.; et al. Effects of Salicornia-Based Skin Cream Application on Healthy Humans' Experimental Model of Pain and Itching. *Pharmaceuticals* **2022**, *15*, 150, doi:10.3390/ph15020150.

328. Kumar, S.; Narwal, S.; Kumar, V.; Prakash, O. α-glucosidase inhibitors from plants: A natural approach to treat diabetes. *Pharmacogn. Rev.* 2011, *5*, 19–29.

329. Khadayat, K.; Marasini, B.P.; Gautam, H.; Ghaju, S.; Parajuli, N. Evaluation of the alpha-amylase inhibitory activity of Nepalese medicinal plants used in the treatment of diabetes mellitus. *Clin. Phytoscience* **2020**, *6*, 34, doi:10.1186/s40816-020-00179-8.

330. Rajan, L.; Palaniswamy, D.; Mohankumar, S.K. Targeting obesity with plant-derived pancreatic lipase inhibitors: A comprehensive review. *Pharmacol. Res.* 2020, *155*, 104681.

331. Soleymani, S.; Farzaei, M.H.; Zargaran, A.; Niknam, S.; Rahimi, R. Promising plant-derived secondary metabolites for treatment of acne vulgaris: a mechanistic review. *Arch. Dermatol. Res.* 2020, *312*, 5–23.

332. Ahn, B.K.; Kim, R.; Choi, D.; Kim, Y.S. Effect of Salicornia bigelovii extract on the activities of whitening and anti-wrinkle. *Appl. Chem. Eng.* **2011**, *22*, 56–60.

333. Jiratchayamaethasakul, C.; Ding, Y.; Hwang, O.; Im, S.T.; Jang, Y.; Myung, S.W.; Lee, J.M.; Kim, H.S.; Ko, S.C.; Lee, S.H. In vitro screening of elastase, collagenase, hyaluronidase, and tyrosinase inhibitory and antioxidant activities of 22 halophyte plant extracts for novel cosmeceuticals. *Fish. Aquat. Sci.* **2020**, *23*, 1–9, doi:10.1186/s41240-020-00149-8.

334. Kubo, I.; Chen, Q.X.; Nihei, K.I. Molecular design of antibrowning agents: Antioxidative tyrosinase inhibitors. *Food Chem.* **2003**, *81*, 241–247, doi:10.1016/S0308-8146(02)00418-1.

335. Zolghadri, S.; Bahrami, A.; Hassan Khan, M.T.; Munoz-Munoz, J.; Garcia-Molina, F.; Garcia-Canovas, F.; Saboury, A.A. A comprehensive review on tyrosinase inhibitors. *J. Enzyme Inhib. Med. Chem.* **2019**, *34*, 279–309, doi:10.1080/14756366.2018.1545767.

336. Guillerme, J.B.; Couteau, C.; Coiffard, L. Applications for marine resources in cosmetics. *Cosmetics* 2017, *4*, 35.

337. Pillaiyar, T.; Manickam, M.; Namasivayam, V. Skin whitening agents: medicinal chemistry perspective of tyrosinase inhibitors. *J. Enzyme Inhib. Med. Chem.* **2017**, *32*, 403–425,

doi:10.1080/14756366.2016.1256882.

338. Li, J.; Feng, L.; Liu, L.; Wang, F.; Ouyang, L.; Zhang, L.; Hu, X.; Wang, G. Recent advances in the design and discovery of synthetic tyrosinase inhibitors. *Eur. J. Med. Chem.* **2021**, *224*, 113744, doi:10.1016/j.ejmech.2021.113744.

339. Pagano, G.; Rengo, G.; Pasqualetti, G.; Femminella, G.D.; Monzani, F.; Ferrara, N.; Tagliati, M. Cholinesterase inhibitors for Parkinson's disease: A systematic review and meta-analysis. *J. Neurol. Neurosurg. Psychiatry* **2015**, *86*, 767–773, doi:10.1136/jnnp-2014-308764.

340. Hassan, M.; Raza, H.; Abbasi, M.A.; Moustafa, A.A.; Seo, S.Y. The exploration of novel Alzheimer's therapeutic agents from the pool of FDA approved medicines using drug repositioning, enzyme inhibition and kinetic mechanism approaches. *Biomed. Pharmacother.* **2019**, *109*, 2513–2526, doi:10.1016/j.biopha.2018.11.115.

341. Pohl, F.; Lin, P.K.T. The potential use of plant natural products and plant extracts with antioxidant properties for the prevention/treatment of neurodegenerative diseases: In vitro, in vivo and clinical trials. *Molecules* **2018**, *23*, 3283, doi:10.3390/molecules23123283.

342. Pinto, D.; Reis, J.; Silva, A.M.; Salazar, M.; Dall'Acqua, S.; Delerue-Matos, C.; Rodrigues, F. Valorisation of Salicornia ramosissima biowaste by a green approach – An optimizing study using response surface methodology. *Sustain. Chem. Pharm.* **2021**, *24*, 100548, doi:10.1016/j.scp.2021.100548.

343. Karthivashan, G.; Park, S.Y.; Kweon, M.H.; Kim, J.; Haque, M.E.; Cho, D.Y.; Kim, I.S.; Cho, E.A.; Ganesan, P.; Choi, D.K. Ameliorative potential of desalted Salicornia europaea L. extract in multifaceted Alzheimer's-like scopolamine-induced amnesic mice model. *Sci. Rep.* **2018**, *8*, 8826, doi:10.1038/s41598-018-25381-0.

344. Lee, W.J.; Shin, Y.W.; Kim, D.E.; Kweon, M.H.; Kim, M. Effect of desalted Salicornia europaea L. ethanol extract (PM-EE) on the subjects complaining memory dysfunction without dementia: a 12 week, randomized, double-blind, placebo-controlled clinical trial. *Sci. Rep.* **2020**, *10*, 1–12, doi:10.1038/s41598-020-76938-x.

345. Souid, A.; Della Croce, C.M.; Frassinetti, S.; Gabriele, M.; Pozzo, L.; Ciardi, M.; Abdelly, C.; Hamed, K. Ben; Magné, C.; Longo, V. Nutraceutical Potential of Leaf Hydro-Ethanolic Extract of the Edible Halophyte Crithmum maritimum L. *Molecules* **2021**, *26*, 5380, doi:10.3390/molecules26175380.

346. Generalić Mekinić, I.; Blažević, I.; Mudnić, I.; Burčul, F.; Grga, M.; Skroza, D.; Jerčić, I.; Ljubenkov, I.; Boban, M.; Miloš, M.; et al. Sea fennel (Crithmum maritimum L.): phytochemical profile, antioxidative, cholinesterase inhibitory and vasodilatory activity. *J. Food Sci. Technol.* **2016**, *53*, 3104–3112, doi:10.1007/s13197-016-2283-z.

347. Renna, M.; Gonnella, M.; Caretto, S.; Mita, G.; Serio, F. Sea fennel (Crithmum maritimum L.): from underutilized crop to new dried product for food use. *Genet. Resour. Crop Evol.* **2017**, *64*, 205–216, doi:10.1007/s10722-016-0472-2.

348. Zafeiropoulou, V.; Tomou, E.-M.; Karioti, A.; Skaltsa, H.; Ioannidou, O. Sea fennel: Phytochemical analysis of Greek wild and cultivated Crithmum maritimum L. populations, based on HPLC-PDA-MS and NMR methods. ~ *998* ~ *J. Pharmacogn. Phytochem.* **2020**, *9*, 998–1004.

349. Hajimehdipoor, H.; Shahrestani, R.; Shekarchi, M. Investigating the synergistic antioxidant effects of some flavonoid and phenolic compounds. *Res. J. Pharmacogn.* **2014**, *1*, 35–40.

350. Skroza, D.; Generalić Mekinić, I.; Svilović, S.; Šimat, V.; Katalinić, V. Investigation of the potential synergistic effect of resveratrol with other phenolic compounds: A case of binary phenolic mixtures. *J. Food Compos. Anal.* **2015**, *38*, 13–18, doi:10.1016/j.jfca.2014.06.013.

351. Holkem, A.T.; Silva, M.P. da; Favaro-Trindade, C.S. Probiotics and plant extracts: a promising synergy and delivery systems. *Crit. Rev. Food Sci. Nutr.* 2022.

352. Mundy, L.; Pendry, B.; Rahman, M. Antimicrobial resistance and synergy in herbal medicine. *J. Herb. Med.* 2016, *6*, 53–58.

353. Zhou, S.; Huang, G. The biological activities of butyrylcholinesterase inhibitors. *Biomed. Pharmacother.* 2022, *146*, 112556.

354. Liu, T.T.; Liu, X.T.; Chen, Q.X.; Shi, Y. Lipase Inhibitors for Obesity: A Review. *Biomed. Pharmacother.* 2020, *128*, 110314.

355. Calvo, M.M.; Tzamourani, A.; Martínez-Alvarez, O. Halophytes as a potential source of melanosis-inhibiting compounds. Mechanism of inhibition of a characterized polyphenol extract of purslane (Portulaca oleracea). *Food Chem.* **2021**, *355*, 129649, doi:10.1016/j.foodchem.2021.129649.

356. Sharifi-Rad, M.; Anil Kumar, N. V.; Zucca, P.; Varoni, E.M.; Dini, L.; Panzarini, E.; Rajkovic, J.; Tsouh Fokou, P.V.; Azzini, E.; Peluso, I.; et al. Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. *Front. Physiol.* 2020, *11*, 694.

357. Zhang, Y.J.; Gan, R.Y.; Li, S.; Zhou, Y.; Li, A.N.; Xu, D.P.; Li, H. Bin; Kitts, D.D. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. *Molecules* 2015, *20*, 21138–21156.

358. Mohammed, H.A.; Emwas, A.-H.; Khan, R.A. Salt-Tolerant Plants, Halophytes, as Renewable Natural Resources for Cancer Prevention and Treatment: Roles of Phenolics and Flavonoids in Immunomodulation and Suppression of Oxidative Stress towards Cancer Management. *Int. J. Mol. Sci.* **2023**, *24*, 5171, doi:10.3390/ijms24065171.

359. Tariq, A.; Sadia, S.; Pan, K.; Ullah, I.; Mussarat, S.; Sun, F.; Abiodun, O.O.; Batbaatar, A.; Li, Z.; Song, D.; et al. A systematic review on ethnomedicines of anti-cancer plants. *Phyther. Res.* **2017**, *31*, 202–264, doi:10.1002/ptr.5751.

360. Nigam, M.; Suleria, H.A.R.; Farzaei, M.H.; Mishra, A.P. Marine anticancer drugs and their relevant targets: a treasure from the ocean. *DARU, J. Pharm. Sci.* 2019, *27*, 491–515.

361. Solowey, E.; Lichtenstein, M.; Sallon, S.; Paavilainen, H.; Solowey, E.; Lorberboum-Galski, H. Evaluating Medicinal Plants for Anticancer Activity. *Sci. World J.* **2014**, *2014*, 1–12, doi:10.1155/2014/721402.

362. Oyenihi, A.B.; Smith, C. Are polyphenol antioxidants at the root of medicinal plant anti-cancer success? *J. Ethnopharmacol.* 2019, *229*, 54–72.

363. Wu, Q.; Yang, Z.; Nie, Y.; Shi, Y.; Fan, D. Multi-drug resistance in cancer chemotherapeutics: Mechanisms and lab approaches. *Cancer Lett.* 2014, *347*, 159–166.

364. Rodrigues, M.J.; Jekő, J.; Cziáky, Z.; Pereira, C.G.; Custódio, L. The Medicinal Halophyte Frankenia laevis L. (Sea Heath) Has In Vitro Antioxidant Activity, α-Glucosidase Inhibition, and Cytotoxicity towards Hepatocarcinoma Cells. *Plants* **2022**, *11*, 1353, doi:10.3390/plants11101353.

365. Chang, M.C.; Wu, J.Y.; Liao, H.F.; Chen, Y.J.; Kuo, C.D. Comparative assessment of therapeutic safety of norcantharidin, N -farnesyloxy-norcantharimide, and N -farnesyl-norcantharimide against Jurkat T cells relative to human normal lymphoblast: A quantitative pilot study. *Med. (United States)* **2016**, *95*, doi:10.1097/MD.0000000000004467.

366. Gnocchi, D.; Castellaneta, F.; Cesari, G.; Fiore, G.; Sabbà, C.; Mazzocca, A. Treatment of liver cancer cells with ethyl acetate extract of *Crithmum maritimum* permits reducing sorafenib dose

and toxicity maintaining its efficacy. *J. Pharm. Pharmacol.* **2021**, *73*, 1369–1376, doi:10.1093/jpp/rgab070.

367. Santhanakrishnan, D.; Perumal, R.K.; Kanth, S.V.; Rao, J.R. Antioxidant and cytotoxic effects of methanolic extract of Salicornia brachiata L . in HepG2 cells. **2013**, *4*, 512–517.

368. Samuel, P.; Kumar, J.V. Bioprospecting of marine halophyte Salicornia Europaea L . and evaluation of its biological potential with special reference to anticancer activity Bioprospecting of marine halophyte Salicornia europaea L . and evaluation of its biological potential with . **2018**, doi:10.29011/2574-7711.100038.

369. Fra frø til græs til grønt protein - BioRefine Available online: https://biorefine.dk/ (accessed on Jun 10, 2023).

370. Kamm, B.; Hille, C.; Schönicke, P.; Dautzenberg, G. Green biorefinery demonstration plant in Havelland (Germany). *Biofuels, Bioprod. Biorefining* **2010**, *4*, 253–262, doi:10.1002/bbb.218.

371. Baraniak, B. The effect of flocculant applied in the process of fractionating alfalfa juice on the chemical composition of the obtained protein concentrates. *Anim. Feed Sci. Technol.* **1990**, *31*, 305–311, doi:10.1016/0377-8401(90)90135-U.

372. Koschuh, W.; Povoden, G.; Hong, V.; Kromus, S.; Dieter, K.; Novalin, S.; Krotscheck, C. Production of leaf protein concentrate from ryegrass (Lolium perenne x multiflorum) and alfalfa (Medicago sativa subsp , sativa). Comparison between heat coagulation / centrifugation and ultrafiltration. **2004**, *163*, 253–259.

373. Lamsal, B.P.; Koegel, R.G.; Gunasekaran, S. Some physicochemical and functional properties of alfalfa soluble leaf proteins. *LWT* **2007**, *40*, 1520–1526, doi:10.1016/j.lwt.2006.11.010.

374. la Cour, R.; Schjoerring, J.K.; Jørgensen, H. Enhancing Protein Recovery in Green Biorefineries by Lignosulfonate-Assisted Precipitation. *Front. Sustain. Food Syst.* **2019**, *3*, 112, doi:10.3389/fsufs.2019.00112.

375. Damborg, V.K.; Jensen, S.K.; Weisbjerg, M.R.; Adamsen, A.P.; Stødkilde, L. Screw-pressed fractions from green forages as animal feed: Chemical composition and mass balances. *Anim. Feed Sci. Technol.* **2020**, *261*, 114401, doi:10.1016/j.anifeedsci.2020.114401.

376. Kamm, B.; Schönicke, P.; Kamm, M. Biorefining of green biomass - Technical and energetic considerations. *Clean - Soil, Air, Water* **2009**, *37*, 27–30, doi:10.1002/clen.200800122.

377. Thomsen, M.H.; Alassali, A.; Cybulska, I.; Yousef, A.F.; Brown, J.J.; Andersen, M.; Ratkov, A.; Kiel, P. Microorganisms for Biorefining of Green Biomass. In; Springer, Berlin, Heidelberg, 2015; pp. 157–181.

378. Stodkilde, L.; Damborg, V.K.; Jorgensen, H.; Lærke, H.N.; Jensen, S.K. Digestibility of fractionated green biomass as protein source for monogastric animals. *Animal* **2019**, *13*, 1817–1825, doi:10.1017/S1751731119000156.

379. Miller, R.E.; de Fremery, D.; Bickoff, E.M.; Kohler, G.O. Soluble Protein Concentrate from Alfalfa by Low-Temperature Acid Precipitation. *J. Agric. Food Chem.* **1975**, *23*, 1177–1179, doi:10.1021/jf60202a037.

380. Kammes, K.L.; Bals, B.D.; Dale, B.E.; Allen, M.S. Grass leaf protein, a coproduct of cellulosic ethanol production, as a source of protein for livestock. *Anim. Feed Sci. Technol.* **2011**, *164*, 79–88, doi:10.1016/j.anifeedsci.2010.12.006.

381. *Microorganisms in biorefineries*; Kamm, B., Ed.; Microbiology monographs, volume 26; Springer-Verlag Berlin Heidelberg, 2015; Vol. 26; ISBN 9783662452080.

382. Gwiazda, S.; Saio, K. Preparation of White Leaf Protein Concentrates Using Calcium Salts.

Agric. Biol. Chem. **1981**, *45*, 2659–2660, doi:10.1271/bbb1961.45.2659.

383. Knuckles, B.E.; Edwards, R.H.; Kohler, G.O.; Whitney, L.F. Flocculants in the Separation of Green and Soluble White Protein Fractions from Alfalfa. *J. Agric. Food Chem.* **1980**, *28*, 32–36, doi:10.1021/jf60227a032.

384. Santamaria-Fernandez, M.; Ambye-Jensen, M.; Damborg, V.K.; Lübeck, M. Demonstration-scale protein recovery by lactic acid fermentation from grass clover – a single case of the production of protein concentrate and press cake silage for animal feeding trials. *Biofuels, Bioprod. Biorefining* **2019**, *13*, 502–513, doi:10.1002/bbb.1957.

385. Santamaría-Fernández, M.; Schneider, R.; Lübeck, M.; Venus, J. Combining the production of L-lactic acid with the production of feed protein concentrates from alfalfa. *J. Biotechnol.* **2020**, *323*, 180–188, doi:10.1016/j.jbiotec.2020.08.010.

386. Sakarika, M.; Delmoitié, B.; Ntagia, E.; Chatzigiannidou, I.; Gabet, X.; Ganigué, R.; Rabaey, K. Production of microbial protein from fermented grass. *Chem. Eng. J.* **2022**, *433*, 133631, doi:10.1016/j.cej.2021.133631.

387. Hanczakowski, P.; Skraba, B. The effect of different precipitating agents on quality of leaf protein concentrate from lucerne. *Anim. Feed Sci. Technol.* **1984**, *12*, 11–17, doi:10.1016/0377-8401(84)90032-4.

388. Hernández, T.; Martínez, C.; Hernández, A. Influence of solvent extraction on the dietary fibre fractions of alfalfa protein concentrates. *J. Sci. Food Agric.* **1995**, *68*, 187–190, doi:10.1002/jsfa.2740680208.

389. Ravindran, R.; Koopmans, S.; Sanders, J.P.M.; McMahon, H.; Gaffey, J. Production of Green Biorefinery Protein Concentrate Derived from Perennial Ryegrass as an Alternative Feed for Pigs. *Clean Technol.* **2021**, *3*, 656–669, doi:10.3390/cleantechnol3030039.

390. Wee, Y.; Kim, J.; Ryu, H. Biotechnological Production of Lactic Acid and Its Recent Applications. *Food Technol. Biotechnol.* **2006**, *44*, 163–172.

391. Florou-Paneri, P.; Christaki, E.; Bonos, E. Lactic Acid Bacteria as Source of Functional Ingredients. In *Lactic Acid Bacteria - R & D for Food, Health and Livestock Purposes*; Kongo, M., Ed.; 2013.

392. Morlon-Guyot, J.; Guyot, J.P.; Pot, B.; Jacobe De Haut, I.; Raimbault, M. Lactobacillus manihotivorans sp. nov., a new starch-hydrolysing lactic acid bacterium isolated during cassava sour starch fermentation. *Int. J. Syst. Bacteriol.* **1998**, *48*, 1101–1109, doi:10.1099/00207713-48-4-1101.

393. Taskila, S.; Ojamo, H. The Current Status and Future Expectations in Industrial Production of Lactic Acid by Lactic Acid Bacteria. In *Lactic Acid Bacteria - R & D for Food, Health and Livestock Purposes*; Kongo, M., Ed.; 2013.

394. Zotta, T.; Parente, E.; Ricciardi, A. Aerobic metabolism in the genus Lactobacillus: impact on stress response and potential applications in the food industry. *J. Appl. Microbiol.* **2017**, *122*, 857–869, doi:10.1111/jam.13399.

395. Rao, M..; Pintado, J.; Stevens, W..; Guyot, J.. Kinetic growth parameters of different amylolytic and non-amylolytic Lactobacillus strains under various salt and pH conditions. *Bioresour. Technol. 94*, 331–337, doi:10.1016/j.biortech.2003.11.028.

396. Burgos-Rubio, C.N.; Okos, M.R.; Wankat, P.C. Kinetic Study of the Conversion of Different Substrates to Lactic Acid Using Lactobacillus bulgaricus. *Biotechnol. Prog.* **2000**, *16*, 305–314, doi:10.1021/bp000022p.

397. Wakinaka, T.; Iwata, S.; Takeishi, Y.; Watanabe, J.; Mogi, Y.; Tsukioka, Y.; Shibata, Y.

Isolation of halophilic lactic acid bacteria possessing aspartate decarboxylase and application to fish sauce fermentation starter. *Int. J. Food Microbiol.* **2019**, *292*, 137–143, doi:10.1016/j.ijfoodmicro.2018.12.013.

398. Gürtler, M.; Gänzle, M.G.; Wolf, G.; Hammes, W.P. Physiological Diversity Among Strains of Tetragenococcus halophilus. *Syst. Appl. Microbiol.* **1998**, *21*, 107–112, doi:10.1016/S0723-2020(98)80013-1.

399. Ye, K.; Li, P.; Gu, Q. Complete genome sequence analysis of a strain Lactobacillus pentosus ZFM94 and its probiotic characteristics. *Genomics (San Diego, Calif.) 112*, 3142–3149, doi:10.1016/j.ygeno.2020.05.015.

400. Parente, E.; Ciocia, F.; Ricciardi, A.; Zotta, T.; Felis, G.E.; Torriani, S. Diversity of stress tolerance in Lactobacillus plantarum, Lactobacillus pentosus and Lactobacillus paraplantarum: A multivariate screening study. *Int. J. Food Microbiol. 144*, 270–279, doi:10.1016/j.ijfoodmicro.2010.10.005.

401. Cai, Y.; Ohmomo, S.; Ogawa, M.; Kumai, S. Effect of NaCl-tolerant lactic acid bacteria and NaCl on the fermentation characteristics and aerobic stability of silage. *J. Appl. Microbiol. 83*, 307–313.

402. Vasala, A.; Panula, J.; Neubauer, P. Efficient lactic acid production from high salt containing dairy by-products by Lactobacillus salivarius ssp. salicinius with pre-treatment by proteolytic microorganisms. *J. Biotechnol. 117*, 421–431, doi:10.1016/j.jbiotec.2005.02.010.

403. Gong, X.; Yu, H.; Chen, J.; Han, B. Cell surface properties of Lactobacillus salivarius under osmotic stress. *Eur. Food Res. Technol.* **2012**, *234*, 671–678, doi:10.1007/s00217-012-1677-z.

404. Reddy, K.; Madhu, A.; Prapulla, S. Comparative survival and evaluation of functional probiotic properties of spray-dried lactic acid bacteria. *Int. J. Dairy Technol.* **2009**, *62*, 240–248, doi:10.1111/j.1471-0307.2009.00480.x.

405. Nguyen, T.L.; Park, C. Il; Kim, D.H. Improved growth rate and disease resistance in olive flounder, Paralichthys olivaceus, by probiotic Lactococcus lactis WFLU12 isolated from wild marine fish. *Aquaculture* **2017**, *471*, 113–120, doi:10.1016/j.aquaculture.2017.01.008.

406. Sanders, J.W.; Venema, G.; Kok, J. Environmental stress responses in *Lactococcus lactis*. *FEMS Microbiol. Rev.* **1999**, *23*, 483–501, doi:10.1111/j.1574-6976.1999.tb00409.x.

407. Guillot, A.; Obis, D.; Mistou, M.Y. Fatty acid membrane composition and activation of glycine-betaine transport in Lactococcus lactis subjected to osmotic stress. In Proceedings of the International Journal of Food Microbiology; Elsevier, 2000; Vol. 55, pp. 47–51.

408. Khelissa, S.; Chihib, N.E.; Gharsallaoui, A. Conditions of nisin production by Lactococcus lactis subsp. lactis and its main uses as a food preservative. *Arch. Microbiol.* 2021, *203*, 465–480.

409. Zamfir, M.; Grosu-Tudor, S. Impact of stress conditions on the growth of Lactobacillus acidophilus IBB 801 and production of acidophilin 801. *J. Gen. Appl. Microbiol.* **2009**, *55*, 277–282, doi:10.2323/jgam.55.277.

410. Van Nguyen, N.; Onoda, S.; Van Khanh, T.; Hai, P.D.; Trung, N.T.; Hoang, L.; Koshio, S. Evaluation of dietary Heat-killed Lactobacillus plantarum strain L-137 supplementation on growth performance, immunity and stress resistance of Nile tilapia (Oreochromis niloticus). *Aquaculture* **2019**, *498*, 371–379, doi:10.1016/j.aquaculture.2018.08.081.

411. Zheng, X.; Duan, Y.; Dong, H.; Zhang, J. Effects of dietary Lactobacillus plantarum in different treatments on growth performance and immune gene expression of white shrimp Litopenaeus vannamei under normal condition and stress of acute low salinity. *Fish Shellfish Immunol.* **2017**, *62*, 195–201, doi:10.1016/j.fsi.2017.01.015.

412. Dash, G.; Raman, R.P.; Pani Prasad, K.; Makesh, M.; Pradeep, M.A.; Sen, S. Evaluation of paraprobiotic applicability of Lactobacillus plantarum in improving the immune response and disease protection in giant freshwater prawn, Macrobrachium rosenbergii (de Man, 1879). *Fish Shellfish Immunol.* **2015**, *43*, 167–174, doi:10.1016/j.fsi.2014.12.007.

413. Du, Y.; Wang, M.; Wang, B.; Liu, M.; Jiang, K.; Wang, L. The influence of surface proteins on the probiotic effects of Lactobacillus pentosus HC-2 in the Litopenaeus vannamei hepatopancreas. *Fish Shellfish Immunol.* **2019**, *92*, 119–124, doi:10.1016/j.fsi.2019.06.003.

414. Hamdan, A.M.; El-Sayed, A.F.M.; Mahmoud, M.M. Effects of a novel marine probiotic, Lactobacillus plantarum AH 78, on growth performance and immune response of Nile tilapia (Oreochromis niloticus). *J. Appl. Microbiol.* **2016**, *120*, 1061–1073, doi:10.1111/jam.13081.

415. Son, V.M.; Chang, C.C.; Wu, M.C.; Guu, Y.K.; Chiu, C.H.; Cheng, W. Dietary administration of the probiotic, Lactobacillus plantarum, enhanced the growth, innate immune responses, and disease resistance of the grouper Epinephelus coioides. *Fish Shellfish Immunol.* **2009**, *26*, 691–698, doi:10.1016/j.fsi.2009.02.018.

416. Nikoskelainen, S.; Ouwehand, A.; Salminen, S.; Bylund, G. Protection of rainbow trout (Oncorhynchus mykiss) from furunculosis by Lactobacillus rhamnosus. *Aquaculture* **2001**, *198*, 229–236, doi:10.1016/S0044-8486(01)00593-2.

417. Lee, J.S.; Cheng, H.; Damte, D.; Lee, S.J.; Kim, J.C.; Rhee, M.H.; Suh, J.W.; Park, S.C. Effects of dietary supplementation of Lactobacillus pentosus PL11 on the growth performance, immune and antioxidant systems of Japanese eel Anguilla japonica challenged with Edwardsiella tarda. *Fish Shellfish Immunol.* **2013**, *34*, 756–761, doi:10.1016/j.fsi.2012.11.028.

418. Chiu, C.H.; Guu, Y.K.; Liu, C.H.; Pan, T.M.; Cheng, W. Immune responses and gene expression in white shrimp, Litopenaeus vannamei, induced by Lactobacillus plantarum. *Fish Shellfish Immunol.* **2007**, *23*, 364–377, doi:10.1016/j.fsi.2006.11.010.

419. Gao, X.; Zhang, M.; Li, X.; Han, Y.; Wu, F.; Liu, Y. The effects of feeding Lactobacillus pentosus on growth, immunity, and disease resistance in Haliotis discus hannai Ino. *Fish Shellfish Immunol.* **2018**, *78*, 42–51, doi:10.1016/j.fsi.2018.04.010.

420. Kongnum, K.; Hongpattarakere, T. Effect of Lactobacillus plantarum isolated from digestive tract of wild shrimp on growth and survival of white shrimp (Litopenaeus vannamei) challenged with Vibrio harveyi. *Fish Shellfish Immunol.* **2012**, *32*, 170–177, doi:10.1016/j.fsi.2011.11.008.

421. Dawood, M.A.O.; Moustafa, E.M.; Gewaily, M.S.; Abdo, S.E.; AbdEl-kader, M.F.; SaadAllah, M.S.; Hamouda, A.H. Ameliorative effects of Lactobacillus plantarum L-137 on Nile tilapia (Oreochromis niloticus) exposed to deltamethrin toxicity in rearing water. *Aquat. Toxicol.* **2020**, *219*, 105377, doi:10.1016/j.aquatox.2019.105377.

422. Yu, L.; Zhai, Q.; Zhu, J.; Zhang, C.; Li, T.; Liu, X.; Zhao, J.; Zhang, H.; Tian, F.; Chen, W. Dietary Lactobacillus plantarum supplementation enhances growth performance and alleviates aluminum toxicity in tilapia. *Ecotoxicol. Environ. Saf.* **2017**, *143*, 307–314, doi:10.1016/j.ecoenv.2017.05.023.

423. Hashemi Panah, A.; Rafiee, G.; Rezaei Tavabe, K.; Bozorgi, S.; Mirvaghefi, A. Effects of utilization of *Lactococcus lactis* and *Pediococcus pentosaseus* as probiotic to improve quality of west white leg shrimp (*Litopenaeus vannamei*) postlarvae. *Aquac. Res.* **2021**, are.15028, doi:10.1111/are.15028.

424. Dash, G.; Raman, R.P.; Pani Prasad, K.; Makesh, M.; Pradeep, M.A.; Sen, S. Evaluation of Lactobacillus plantarum as feed supplement on host associated microflora, growth, feed efficiency, carcass biochemical composition and immune response of giant freshwater prawn, Macrobrachium rosenbergii (de Man, 1879). *Aquaculture* **2014**, *432*, 225–236, doi:10.1016/j.aquaculture.2014.05.011.

425. Pascual, M.; Hugas, M.; Badiola, J.I.; Monfort, J.M.; Garriga, M. Lactobacillus salivarius CTC2197 Prevents Salmonella enteritidis Colonization in Chickens. *Appl. Environ. Microbiol.* **1999**, *65*, 4981–4986.

426. Kizerwetter-Świda, M.; Binek, M. Protective effect of potentially probiotic Lactobacillus strain on infection with pathogenic bacteria in chickens. *Pol. J. Vet. Sci.* **2009**, *12*, 15–20.

427. Saint-Cyr, M.J.; Haddad, N.; Taminiau, B.; Poezevara, T.; Quesne, S.; Amelot, M.; Daube, G.; Chemaly, M.; Dousset, X.; Guyard-Nicodème, M. Use of the potential probiotic strain Lactobacillus salivarius SMXD51 to control Campylobacter jejuni in broilers. *Int. J. Food Microbiol.* **2017**, *247*, 9–17, doi:10.1016/j.ijfoodmicro.2016.07.003.

428. Wang, S.; Peng, Q.; Jia, H.M.; Zeng, X.F.; Zhu, J.L.; Hou, C.L.; Liu, X.T.; Yang, F.J.; Qiao, S.Y. Prevention of Escherichia coli infection in broiler chickens with Lactobacillus plantarum B1. *Poult. Sci.* **2017**, *96*, 2576–2586, doi:10.3382/ps/pex061.

429. Altaher, Y.W.; Jahromi, M.F.; Ebrahim, R.; Zulkifli, I.; Liang, J.B. Lactobacillus pentosus Ita23 and L. Acidipiscis Ita44 enhance feed conversion efficiency and beneficial gut microbiota in broiler chickens. *Rev. Bras. Cienc. Avic.* **2015**, *17*, 159–164, doi:10.1590/1516-635x1702159-164.

430. Lan, P.T.N.; Binh, L.T.; Benno, Y. Impact of two probiotic Lactobacillus strains feeding on fecal lactobacilli and weight gains in chicken. *J. Gen. Appl. Microbiol.* 2003, *49*, 29–36.

431. Wu, Y.; Wang, B.; Zeng, Z.; Liu, R.; Tang, L.; Gong, L.; Li, W. Effects of probiotics Lactobacillus plantarum 16 and Paenibacillus polymyxa 10 on intestinal barrier function, antioxidative capacity, apoptosis, immune response, and biochemical parameters in broilers. *Poult. Sci.* **2019**, *98*, 5028–5039, doi:10.3382/ps/pez226.

432. Guerra, N.P.; Bernárdez, P.F.; Méndez, J.; Cachaldora, P.; Pastrana Castro, L. Production of four potentially probiotic lactic acid bacteria and their evaluation as feed additives for weaned piglets. *Anim. Feed Sci. Technol.* **2007**, *134*, 89–107, doi:10.1016/j.anifeedsci.2006.05.010.

433. Li, Y.; Hou, S.; Peng, W.; Lin, Q.; Chen, F.; Yang, L.; Li, F.; Huang, X. Oral Administration of Lactobacillus delbrueckii during the Suckling Phase Improves Antioxidant Activities and Immune Responses after the Weaning Event in a Piglet Model. *Oxid. Med. Cell. Longev.* **2019**, *2019*, doi:10.1155/2019/6919803.

434. Jami, M.J.; Abedian Kenari, A.; Paknejad, H.; Mohseni, M. Effects of dietary b-glucan, mannan oligosaccharide, Lactobacillus plantarum and their combinations on growth performance, immunity and immune related gene expression of Caspian trout, Salmo trutta caspius (Kessler, 1877). *Fish Shellfish Immunol.* **2019**, *91*, 202–208, doi:10.1016/j.fsi.2019.05.024.

435. Brzóska, F.; Śliwiński, B.; Stecka, K. Effect of Lactococcus lactis vs. Lactobacillus spp. bacteria on chicken body weight, mortality, feed conversion and carcass quality. *Ann. Anim. Sci.* **2012**, *12*, 549–559, doi:10.2478/v10220-012-0046-y.

436. Silvi, S.; Nardi, M.; Sulpizio, R.; Orpianesi, C.; Caggiano, M.; Carnevali, O.; Cresci, A. Effect of the addition of *Lactobacillus delbrueckii* subsp. *delbrueckii* on the gut microbiota composition and contribution to the well-being of European sea bass (*Dicentrarchus labrax* , L.). *Microb. Ecol. Health Dis.* **2008**, *20*, 53–59, doi:10.1080/08910600701876020.

437. Maragkoudakis, P.A.; Mountzouris, K.C.; Rosu, C.; Zoumpopoulou, G.; Papadimitriou, K.; Dalaka, E.; Hadjipetrou, A.; Theofanous, G.; Strozzi, G.P.; Carlini, N.; et al. Feed supplementation of Lactobacillus plantarum PCA 236 modulates gut microbiota and milk fatty acid composition in dairy goats - a preliminary study. *Int. J. Food Microbiol.* **2010**, *141*, 109–116, doi:10.1016/j.ijfoodmicro.2010.03.007.

438. Xia, Y.; Lu, M.; Chen, G.; Cao, J.; Gao, F.; Wang, M.; Liu, Z.; Zhang, D.; Zhu, H.; Yi, M. Effects of dietary Lactobacillus rhamnosus JCM1136 and Lactococcus lactis subsp. lactis

JCM5805 on the growth, intestinal microbiota, morphology, immune response and disease resistance of juvenile Nile tilapia, Oreochromis niloticus. *Fish Shellfish Immunol.* **2018**, *76*, 368–379, doi:10.1016/j.fsi.2018.03.020.

439. Heo, W.S.; Kim, Y.R.; Kim, E.Y.; Bai, S.C.; Kong, I.S. Effects of dietary probiotic, Lactococcus lactis subsp. lactis I2, supplementation on the growth and immune response of olive flounder (Paralichthys olivaceus). *Aquaculture* **2013**, *376–379*, 20–24, doi:10.1016/j.aquaculture.2012.11.009.

440. Bogovic Matijasi?, B.; Stojkovi?, S.; Salobir, J.; Malovrh, S.; Rogelj, I. Evaluation of the Lactobacillus gasseri K7 and LF221 strains in weaned piglets for their possible probiotic use and their detection in the faeces. *Anim. Res.* **2004**, *53*, 35–44, doi:10.1051/animres:2003046.

441. Adel, M.; El-Sayed, A.F.M.; Yeganeh, S.; Dadar, M.; Giri, S.S. Effect of Potential Probiotic Lactococcus lactis Subsp. lactis on Growth Performance, Intestinal Microbiota, Digestive Enzyme Activities, and Disease Resistance of Litopenaeus vannamei. *Probiotics Antimicrob. Proteins* **2017**, *9*, 150–156, doi:10.1007/s12602-016-9235-9.

442. Jørgensen, U.; Jensen, S.K.; Ambye-Jensen, M. Coupling the benefits of grassland crops and green biorefining to produce protein, materials and services for the green transition. *Grass Forage Sci.* **2022**, *77*, 295–306, doi:10.1111/gfs.12594.

443. Höltinger, S.; Schmidt, J.; Schönhart, M.; Schmid, E. A spatially explicit techno-economic assessment of green biorefinery concepts. *Biofuels, Bioprod. Biorefining* **2014**, *8*, 325–341, doi:10.1002/bbb.1461.

444. Arlabosse, P.; Blanc, M.; Kerfaï, S.; Fernandez, A. Production of green juice with an intensive thermo-mechanical fractionation process. Part I: Effects of processing conditions on the dewatering kinetics. *Chem. Eng. J.* **2011**, *168*, 586–592, doi:10.1016/j.cej.2011.01.027.

445. Knuckles, B.E.; Bickoff, E.M.; Kohler, G.O. Pro-Xan Process: Methods for Increasing Protein Recovery from Alfalfa. *J. Agric. Food Chem.* **1972**, *20*, 1055–1057, doi:10.1021/jf60183a020.

446. Colas, D.; Doumeng, C.; Pontalier, P.Y.; Rigal, L. Twin-screw extrusion technology, an original solution for the extraction of proteins from alfalfa (Medicago sativa). *Food Bioprod. Process.* **2013**, *91*, 175–182, doi:10.1016/j.fbp.2013.01.002.

447. Solati, Z.; Jørgensen, U.; Eriksen, J.; Søegaard, K. Dry matter yield, chemical composition and estimated extractable protein of legume and grass species during the spring growth. *J. Sci. Food Agric.* **2017**, *97*, 3958–3966, doi:10.1002/jsfa.8258.

List of Papers

Papers I – V are published scientific papers and manuscripts on which the PhD thesis is based, whereas papers VI – VIII are co-authored papers on relevant topics produced during the PhD project period but not included in the assessment.

Paper I

Hulkko, L.S.S., Turcios, A., Kohnen, S. et al. Cultivation and characterisation of *Salicornia europaea*, *Tripolium pannonicum* and *Crithmum maritimum* biomass for green biorefinery applications. *Sci Rep* 12, 20507 (2022).

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Cultivation and characterisation of *Salicornia europaea*, *Tripolium pannonicum* and *Crithmum maritimum* biomass for green biorefinery applications

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Salt-tolerant halophytes have shown potential for biorefinery and agricultural use in salt-affected soils, increasing the value of marginal lands. They could provide a bio-based source for compounds obtained from the petrochemical industry or an alternative for biomass currently imported overseas. *Salicornia europaea*, *Tripolium pannonicum* and *Crithmum maritimum* were cultivated in hydroponic systems under various salinity conditions, harvested green but not food-grade, and fractionated to green juice and fibre residue. Obtained fractions were characterised for contents of carbohydrates, Klason lignin, crude protein, organic acids, lipids, and minerals to evaluate the biomass' suitability for biorefinery. Significant differences were observed in the biomass yield and the composition of the biomass fractions from different cultivation salinities. High concentrations of crude protein were found. Thus, these species could have the potential for green protein production. Fractions rich in carbohydrates could be used for lignocellulose processing and processes utilising micro-organisms.

Soil salinisation has been reported as one of the major factors to the degradation of agricultural land[1]. The Food and Agriculture Organization of the United Nations (FAO) estimates that 79 million hectares are considered either salt-affected or sodic in Europe, covering 3.9% of the total land area[2,3]. Worldwide, salt-affected soils consider more than 100 countries, and total area of saline and sodic soils is estimated to be more than 1 billion hectares[2]. According to The United States Department of Agriculture, 10 million hectares of farmland is lost every year due to over-irrigation and poor water management[4]. Most conventional crops are glycophytes, meaning that their growth is inhibited in the presence of salt, and a 50% decrease in biomass yields have been reported for rice, durum wheat and barley at salinities of 80 mM NaCl, 100 mM NaCl and 120 mM NaCl, respectively[4]. FAO defines saline soils as areas, where the electric conductivity of soil extract is 4 dS/m or higher[2], which corresponds to approximately 40 mM NaCl. Besides economic losses, soil salinisation is a threat to food security, as it creates challenges to meet the demand for food for the world's increasing population.

The natural habitat of halophytes are seashores, marshes and salt deserts, and utilisation of these naturally salt-tolerant plants in agricultural applications is a key to re-valorise these salt-affected marginal lands, which are not suitable for conventional farming[3,4]. Flowers and Colmer[5] define halophytes as plants that can complete their full life cycle and reproduce under the salinity of 200 mM or more, and these type of plants cover approximately 1% of known plant species. Some halophytes can yield as much biomass as traditional crops under full seawater irrigation[6]. Several cultivation practises for saline agriculture has been set for halophytes, including field or greenhouse cultivation with brackish or seawater irrigation, constructed wetlands, or saline hydroponic systems[6,7]. Hence, the irrigation water from valuable freshwater resources may not be needed for cultivation. Due to their capability to grow in hydroponics and constructed wetlands, plants can also be used to bio-filter excessive nutrients[8] or residues of antimicrobial compounds[8] from aquaculture effluents. Combining aquaculture with *Salicornia* cultivation has also been evaluated for its potential to reduce halophyte production costs[9,10]. The most suitable cultivation system depends on the species, salinity of the irrigation water, and available soil type[7].

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Halophytes have previously been used as medicinal plants, and nowadays, fresh tips of many edible species are sold for culinary use[4,11]. After harvesting for food, the remaining fraction of halophyte is often seen as waste as the plant lignifies, trapping high concentrations of salt within the plant structure. Due to this high salt concentration, halophytes are suitable for animal feed only when incorporated with other feed sources. The halophyte-supplemented feed has been tested for aquacultures[12], chicken[13,14] and ruminants[15,16].

According to the IPCC 2021 report[17], humans as a society have to aim for net-zero carbon dioxide emission by 2050 in order to limit global warming to 1.5 °C. As a part of this green transition, it is necessary to find bio-based alternatives to the variety of products currently obtained from the petrochemical industry. Considering bioenergy, *Salicornia* spp. have been tested for their potential for bioethanol[18,19] and biodiesel[20,21] production, and *Tripolium pannonicum* has been tested for biogas production[8,22]. As the production of only biofuels is rarely feasible, value-added products can be introduced to the biorefinery process. These multi-product systems are seen as the most robust option for the future[23,24]. The utilisation of residual fractions for bioenergy production would also lead to a zero-waste biorefinery.

Green biorefinery, where fresh but non-food grade biomass is fractionated to green juice and fibre residue, could provide an opportunity to produce protein-rich feed supplements[25] and nutraceutical compounds, such as polyunsaturated fatty acids and carotenoids[26,27]. This approach has been tested previously for *Salicornia sinuspersica* and *Salicornia bigelovii*[19,28]. One of the key compounds to be valorised in green biorefinery is protein and halophytes could provide a source for locally produced feed. The demand for high-quality plant-derived protein is increasing and farmers are currently strongly dependent on imported sources, such as soybean, for their livestock[25]. High-value bioactive compounds suitable for cosmetics and pharmaceutical ingredients can also be found from *Salicornia* spp.[27,29–32], *Tripolium pannonicum*[33,34] and *Crithmum maritimum*[35,36] extracts, and these compounds have exhibited antioxidant activity, anti-inflammatory and antimicrobial effects, anti-obesity properties, and even cancer-preventing capabilities.

Halophytes could provide a valuable feedstock for multi-product green biorefinery. Regardless of their potential, halophytes are currently underutilised in agriculture and industrial applications. In this study, three halophyte plants native to European seashores are cultivated and characterised: *S. europaea* (glasswort, marsh samphire, sea asparagus, pickleweed or sea beans), *T. pannonicum* (sea aster, previously defined in taxonomy as *Aster tripolium*) and *C. maritimum* (sea fennel or rock samphire). These species represent different plant families (Amarathaceae, Asteraceae, and Apiaceae, respectively) and they have all suggested as potential crops for halophyte-based agriculture by Ventura et al.[4]. The focus is to study the effect of cultivation salinity on biomass yields and the chemical composition of biomass fractions. The plant material was characterised for the contents of carbohydrates, Klason lignin, organic acids, crude protein, lipids, and minerals, for the further evaluation of the species' suitability for green biorefinery and different types of processes.

Methods

Biomass cultivation. The plants were cultivated in a greenhouse at the Institute of Botany, Leibniz University Hannover, Germany (52°23′42″N; 9°42′13″E), with temperatures varying between 14 °C (minimum temperature during the night) and 35 °C (maximum temperature during the day). The seeds of *T. pannonicum* (Jacq.) Dobrocz. were collected with official permission at the North Sea, Germany (53°29′13″N; 8°03′16″E). The formal identification of the species was carried out at the Institute of Botany, Leibniz University Hannover, and the voucher specimen was deposited in the herbarium with specimen number TP20191001. Seeds of *S. europaea* L. var. Aprica were obtained from Serra Maris bvba, Belgium, and the seeds of *C. maritimum* L. were obtained from mother plants grown at the Institute of Botany, Leibniz University Hannover. The agronomic handling from sowing through transplanting was carried out as described by Buhmann et al.[37]. *S. europaea* and *T. pannonicum* were cultivated with different NaCl concentrations in hydroponic systems: 0, 10, 20, 30, and 40 g/l NaCl (corresponding to 0, 171, 342, and 685 mM NaCl, respectively). It was noted that *C. maritimum* did not survive under the highest salinities. Hence, it was grown with lower salinities: 0, 5, 10, 15, and 20 g/l NaCl (corresponding to 0, 86, 171, 257, and 342 mM NaCl, respectively). All plants were cultivated in polypropylene containers (400 mm × 300 mm × 175 mm) with a capacity of 16 l, and each container had 13 l of Hoagland solution containing: 606 mg/l KNO_3, 944 mg/l $Ca(NO_3)_2{\cdot}4H_2O$, 230 mg/l $NH_4H_2PO_4$, 246 mg/l $MgSO_4{\cdot}7H_2O$, 3.73 mg/l KCl, 1.55 mg/l H_3BO_3, 0.34 mg/l $MnSO_4{\cdot}H_2O$, 0.58 mg/l $ZnSO_4{\cdot}7H_2O$, 0.12 mg/l $CuSO_4{\cdot}5H_2O$, 0.12 mg/l $MoNa_2O_4{\cdot}2H_2O$, and 9.16 mg/l Fe-EDDHA (0.56 mg/l Fe). Small compressors constantly aerated the water, and one air stone was placed in the middle of each tank. The hypocotyl was fixed with soft foam in 35 mm holes. The water level was adjusted constantly in each tank with tap water to compensate for evapotranspiration. Each experimental unit consisted of eight plants per container and three replicates (separate containers) per treatment. Plants were exposed to 14 h of artificial light from sodium vapour lamps (SON-T Agro 400, Philips), and the light intensity ranged from 65 to 850 µmol m^{-2} s^{-1} depending on the time of the year, the time of the day, and the weather conditions. The cultivation time in hydroponic systems was 5 weeks for *T. pannonicum* and *S. europaea* and 11 weeks for *C. maritimum*. Plants were harvested partly lignified, weighed, immediately frozen in liquid nitrogen to inhibit metabolism, and then kept at − 80 °C.

Biomass fractionation and processing. Biomass processing and characterisation were performed in AAU Energy, Aalborg University, Denmark. Harvested aerial parts of biomass were thawed and fractionated to green juice and fibre residue by using a horizontal single-auger juicer. Both fractions were recovered to pre-weighed containers. The contents of dry matter (DM) and ash in green juice and fibre residue fractions were determined using the analytical protocols by National Renewable Energy Laboratory (NREL)[38,39]. The juice was analysed unfiltered and contained small suspended solid particles, which passed through the particle retention of the juicer. For storage, the fibre residue was dried overnight at 60 °C in a fan oven, knife-milled to particle

size < 2 mm and kept at room temperature in dry conditions protected from light. Green juice was frozen after fractionation and kept at − 40 °C before composition analysis.

Characterisation methods. *Crude protein determination.* The crude protein content of the biomass was determined from homogenised DM by measuring the total nitrogen content using an elemental analyser and applying the Jones conversion factor of 6.00[40].

Extraction of lipids. The lipid content was defined as a lipid-enriched non-polar fraction in the biomass. For solid biomasses, a dried sample (3–5 g) was weighed onto a cellulose thimble. Lipids were extracted with 250 ml n-hexane using the Soxhlet apparatus with a 100 ml extraction chamber. After extraction, the solvent was recovered using a rotary evaporator, and extracted non-polar compounds were weighed.

In order to extract non-polar compounds from green juice, liquid–liquid extraction was performed by mixing a juice sample (10 ml) with n-hexane (20 ml) in Falcon tubes. Tubes were kept in a nutation mixer for 1 h at room temperature, and juice solids and liquid phases were separated afterwards by centrifuging for 15 min with 4000 rpm (SL16, Thermo Scientific). The non-polar fraction was recovered, the solvent evaporated in the fume hood, and the lipid-enriched fraction was weighed.

Total carbohydrates and organic acids. To analyse the lignocellulosic fraction of solid samples, subsequential 10 h water and 8 h ethanol extractions were performed to remove non-structural compounds. Extraction was performed in a similar setup as used for lipid extraction, and one sample (5 g) was used from each biomass batch. The extracted material was recovered and weighed. Strong acid hydrolysis and determination of structural carbohydrates and Klason lignin in the extractive-free biomass were carried out in duplicate using a protocol by NREL[41]. Carbohydrates from juice fractions were measured after weak acid hydrolysis, where the juice sample (10 g) was mixed with H_2SO_4 (10 ml, 8%) in Pyrex tubes and autoclaved at 121 °C for 10 min. Hydrolysis and analytics were run as duplicates for samples and recovery standards. Free sugar monomers and the concentration of organic acids were also determined directly from fresh, untreated juice samples using a protocol by NREL[42].

All hydrolysates and fresh juice samples were filtered through 0.22 µm syringe filters. Sugars and organic acids were analysed with high-performance liquid chromatography (*1260 Infinity II, Agilent Technologies*), using H_2SO_4 mobile phase (0.005 M), organic acid column (*Aminex HPX-87H, Rio-Rad Laboratories Inc.*), and refractive index detector. Separated sugars were glucose, xylose and arabinose, and the calculations for the concentrations of sugars were performed as described by Alassali et al.[19]. Analysed organic acids were lactic acid, acetic acid, malic acid, succinic acid and glycolic acid.

Mineral analysis. The minerals present in the ash fraction were analysed at the scientific service centre CELABOR, Belgium. Ash samples were digested in acidic conditions under pressure (40 bar) at 240 °C using a microwave system in compliance with EN 13805:2014 standard. Concentrations of the following minerals were measured using inductively coupled plasma atomic emission spectrometry *(8300 DV ICP-AES, Perkin Elmer)* with a method adapted from EN 11885 standard: aluminium, antimony, arsenic, barium, cadmium, cobalt, chromium, copper, iron, potassium, magnesium, manganese, molybdenum, sodium, nickel, lead, silver, selenium, titanium, zinc, phosphorus, strontium, vanadium, thallium and calcium. Inductively coupled plasma with mass spectrometry *(820 ICP-MS CRI, Varian)* with a method adapted from EN 15763 standard was used to determine the concentrations of rubidium, scandium and yttrium. Detected minerals with a concentration higher than 100 ppm in DM were reported. Analysis was run only once to each ash sample; thus, results are presented in Supplementary Information.

Statistical methods. All analyses were carried out as triplicates unless stated otherwise. All results, excluding fractionation yields and results from the mineral analysis, are given as mean values with standard deviation. One-way analysis of variance (ANOVA) coupled with Tukey honest significance test was used to test the statistical significance of differences between results from biomass batches cultivated in different salinity conditions.

Ethics. The study complies with local and national guidelines.

Results

Biomass yields. In all plant species, significant differences were observed between biomass yields from different cultivation salinities ($p < 0.001$). The lowest total yield of *S. europaea* biomass (1586 g) was gained from the non-saline cultivation conditions, whereas the highest total biomass yield (4365 g) was achieved with a cultivation salinity of 342 mM NaCl.

On the other hand, there was an inverse relationship between cultivation salinity and *T. pannonicum* yield. The total amounts decreased from 1641 g of fresh biomass (0 mM NaCl) to 44 g (684 mM NaCl). Similarly, the total *C. maritimum* yield decreased from 1440 g (0 mM NaCl) to 126 g (324 mM NaCl). *C. maritimum* also exhibited lower biomass production compared to two other species. Obtained biomass yields are presented in Fig. 1.

Fractionation and dry matter determination. After fractionation, green juice covered 84.6–90.2 w/w% out of the total biomass. Biomass yields of *T. pannonicum* and *C. maritimum* cultivated in high salinities (> 171 mM NaCl) were too low to perform the fractionation process. Hence, these plants were considered only as whole shrubs. In *S. europaea* and *T. pannonicum*, the juice fraction increased when cultivation salinity increased, whereas, in *C. maritimum*, the juice fraction decreased.

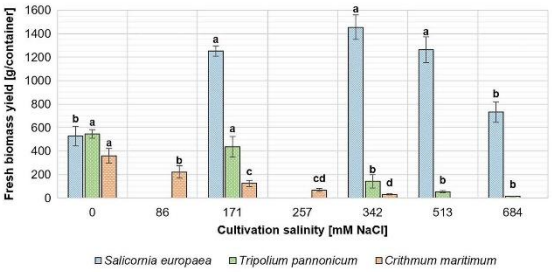

Figure 1. Yield of fresh halophyte biomass cultivated under different salinities. Different letters above the bars stand for significantly different ($p < 0.05$) results calculated individually for each plant species.

Species	Salinity [mM NaCl]	Juice [w/w%]	Fibres [w/w%]	DM_{juice} [w/w%]	DM_{fibres} [w/w%]	DM_{whole} [w/w%]
S. europaea	0	84.61	15.39	4.71 ± 0.03^{c}	27.24 ± 1.18^{a}	9.15 ± 0.65^{c}
	171	85.71	14.28	4.71 ± 0.04^{c}	23.00 ± 0.28^{b}	n/a
	342	85.29	14.71	5.10 ± 0.00^{bc}	21.80 ± 1.16^{b}	8.02 ± 0.38^{b}
	513	86.42	13.58	$5.26 \pm 0.39^{b,c}$	21.11 ± 0.54^{b}	7.85 ± 0.74^{d}
	684	90.23	9.77	5.82 ± 0.12^{a}	21.54 ± 1.32^{b}	8.05 ± 0.04^{a}
T. pannonicum	0	74.97	25.03	3.87 ± 0.03^{b}	23.78 ± 0.20^{b}	8.07 ± 0.19^{c}
	171	82.51	17.49	4.17 ± 0.04^{a}	26.30 ± 1.23^{a}	7.96 ± 0.67^{c}
	342	n/a	n/a	n/a	n/a	11.45 ± 0.39^{b}
	513	n/a	n/a	n/a	n/a	11.47 ± 0.47^{b}
	684	n/a	n/a	n/a	n/a	14.65 ± 0.22^{a}
C. maritimum	0	70.35	29.65	5.58 ± 0.02^{b}	27.22 ± 0.68^{a}	11.06 ± 0.22^{b}
	86	70.46	29.54	5.39 ± 0.00^{c}	27.15 ± 0.35^{a}	11.77 ± 0.53^{b}
	171	66.97	33.03	7.44 ± 0.00^{a}	28.06 ± 0.61^{a}	13.56 ± 0.54^{a}
	257	n/a	n/a	n/a	n/a	13.40 ± 0.14^{a}
	342	n/a	n/a	n/a	n/a	14.79 ± 0.98^{a}

Table 1. Green juice and fibre residue fractions obtained from halophyte biomass and their respective dry matter (DM) contents, and the total DM content of the biomass. Due to small biomass yields, batches of *T. pannonicum* and *C. maritimum* cultivated in high salinity conditions were not fractionated. Results are expressed as [w/w%] on a fresh weight basis. Different letters denote significantly different ($p < 0.05$) results calculated individually for each plant species and its biomass fractions.

There was a positive correlation between *S. europaea* juice DM content and cultivation salinity, as water-soluble salt is mainly present in the juice fraction. The total DM content of *T. pannonicum* and *C. maritimum* biomasses increased as cultivation salinity increased, and significant changes were observed between samples ($p < 0.001$). In contrast, the changes in the total DM content of *S. europaea* were non-significant ($p < 0.058$). Halophyte fractions and their respective DM contents are summarised in Table 1. The characterisation results for compounds in juice and fibre residue fractions are given on the basis of their respective DM and the total DM calculated from these two fractions (whole biomass).

Composition of halophyte biomasses. *Salicornia europaea.* The composition of *S. europaea* biomass fractions are shown in Fig. 2. The crude protein content of *S. europaea* was relatively high, and considering the biomass fractions separately, the changes in the crude protein content were more significant in the juice fraction ($p < 0.001$) than in the pulp fraction ($p < 0.011$). Out of the total crude protein, > 60% was present in the juice fractions, except for the *S. europaea* cultivated at 513 mM NaCl, where most of the protein was found in the fibre residue fraction. The lipid content of green *S. europaea* was low, 1.7–3.7 g/100 g DM. Based on the results, no clear relationship between cultivation salinity and the lipid content of the halophyte biomass was observed.

The analysis showed the content of carbohydrates in the biomass being highest when the biomass was cultivated in more optimal conditions in terms of biomass yield (342 mM NaCl). Significant differences were

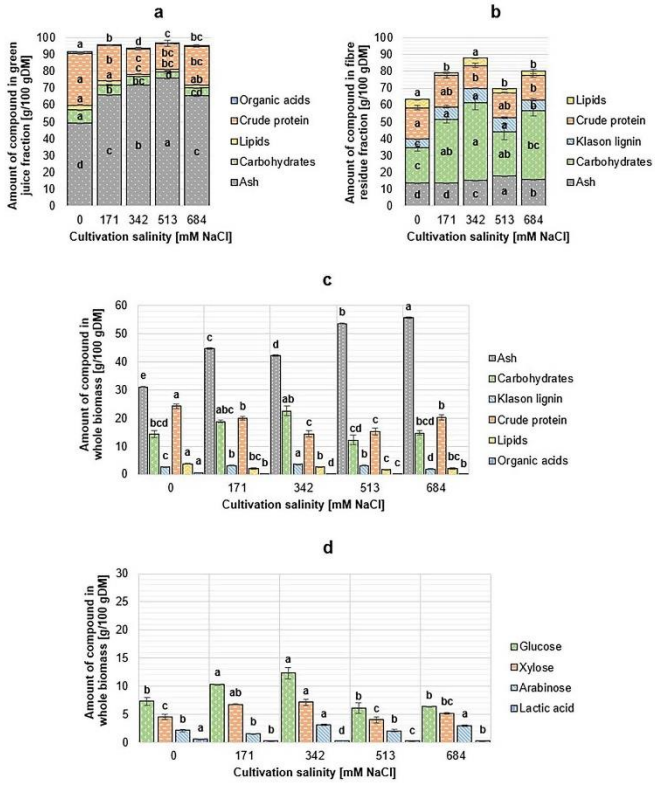

Figure 2. Chemical composition of *Salicornia europaea* green juice (**a**), fibre residue (**b**) and whole biomass (**c**), and total sugar profile (**d**). DM: dry matter. The content of a compound in whole biomass was calculated from juice and fibre residue fractions. Different letters above the bars denote significantly different ($p < 0.05$) results calculated individually for each biochemical group.

observed for all types of sugars measured in the hydrolysates from juice and fibre residue ($p < 0.001$). Contrary to the total carbohydrate content, in the green juice fractions, the concentration of carbohydrates determined from hydrolysates had an inverse relationship to cultivation salinity, the concentration of sugars being highest in the juice obtained from 0 mM NaCl cultivated plants. Therefore, the carbohydrates from the fibre fraction are more pronounced in the total carbohydrate content of the biomass. Overall, the Klason lignin content was very low in all *S. europaea* fibre residues. Similarly to the total carbohydrates, the lignin content correlated with the obtained biomass yields (more lignocellulose from more optimal cultivation salinity). In *S. europaea* plants, even the highest share of lignocellulose was only 26.1% of the total biomass composition (342 mM NaCl). The average composition of lignocellulose was 43.5% cellulose (glucose), 41.3% hemicellulose (xylose and arabinose) and 15.2% Klason lignin.

In the fresh juice (non-hydrolysed sample), low concentrations of free sugar monomers were detected. The glucose concentration was the highest in the juice from biomass grown in 0 mM NaCl (2.15 ± 0.01 g/100 g DM) and the lowest in the juice from 513 mM NaCl cultivated biomass (0.91 ± 0.79 g/100 g DM). Arabinose was not present in any fresh juice samples, and xylose was detected only from juice samples from 171 mM NaCl (2.06 ± 0.02 g/100 g DM) and 684 mM NaCl (1.29 ± 0.00 g/100 g DM) cultivated biomass. The results of the organic acid analysis showed only low amounts of lactic acid in the fresh juice samples (< 1.00 g/100 g DM in total DM), whereas other acids were not detected in the analysis.

The ash content of *S. europaea* increased as the cultivation salinity increased due to the high amount of water-soluble salts in the juice fraction. This can be observed when fractions are considered separately: ash content of fibre residue fraction varied between 13.7 and 18.2 g/100 g DM, but the ash content of the juice increased from 49.31 ± 0.19 g/100 g DM (0 mM NaCl) up to 76.02 ± 0.11 g/100 g DM (513 mM NaCl).

Tripolium pannonicum. Overall, increased cultivation salinity affected the biomass yield and size of fractions rather than the chemical composition of *T. pannonicum* plants. An inverse relationship was observed between cultivation salinity and the total crude protein content, and the differences between samples were significant ($p < 0.001$). However, considering the green juice and fibre residue fractions from lower salinities (0 mM and 171 mM NaCl) individually, the crude protein content was stable, and changes were non-significant ($p = 0.259$ and $p = 0.063$, respectively). The total lipid content of *T. pannonicum* varied between 1.59 ± 0.46 g/100 g DM (513 mM NaCl) and 2.81 ± 0.04 g/100 g DM (171 mM NaCl). Despite the significant differences in the total lipid content of biomass samples ($p = 0.002$), it was not possible to observe a clear relationship between cultivation salinity and lipid content based on the obtained results. Due to the small amount of biomass available, it was not possible to run the lipid extraction in triplicate for 684 mM NaCl salinity grown biomass. The changes in the lipid content were more pronounced in the fibre residue fraction, as in the juice fractions, the lipid content stayed nearly constant and changes were non-significant ($p = 0.488$).

Significant differences were observed in the total carbohydrate content of samples from different cultivation conditions ($p < 0.001$). As sugars were determined from the extractive-free fraction of the solid samples, only the amount of structural carbohydrates was determined for the samples considered whole (not fractionated). An inverse relationship between cultivation salinity and the content of structural carbohydrates was observed, but only samples cultivated in the two highest salinities were significantly different to the others. Considering the types of sugars separately, the increased cultivation salinity caused significant changes in total glucose and xylose contents of total DM ($p < 0.001$), but there were no significant differences in the arabinose content of biomass ($p = 0.171$). The Klason lignin content of *T. pannonicum* was up to 21.97 ± 1.70 g/100 g DM (171 mM NaCl), and the changes between biomass samples were non-significant ($p = 0.072$).

High concentrations of free sugar monomers were detected from fresh *T. pannonicum* juice, xylose being the most abundant sugar monomer with concentrations of 16.43 ± 0.32 g/100 g DM and 7.99 ± 0.08 g/100 g DM in green juice from 0 mM NaCl and 171 mM NaCl cultivated biomasses, respectively. Fresh green juice was also rich in glucose, but arabinose was not detected in the samples. Only a low amount of lactic acid was measured from fresh juice; other acids were not detected.

The significant difference in the total ash content was only observed in the samples cultivated in the lowest and the highest salinity ($p = 0.020$), whereas changes between other samples were non-significant. When considered separately, the green juice fractions showed no significant change in the ash content ($p = 0.829$). During the composition analysis, the cumulative mass balance of *T. pannonicum* samples exceeded 100%, which is suggested to be caused by the overestimated amount of crude protein and large standard deviations in the ash content results. The composition of *T. pannonicum* biomass fractions are shown in Fig. 3.

Crithmum maritimum. The composition of *C. maritimum* biomass fractions is shown in Fig. 4. Cultivation salinity affected mainly the biomass yield and less the chemical composition of *C. maritimum*. The cumulative mass balance exceeded 100% during the composition analysis of all fibre residue fractions and the juice fraction from biomass grown with 0 mM NaCl. Significant changes ($p = 0.002$) were observed in plants' total crude protein content, which varied between 21.3 and 23.1 g/100 g DM. When fractions were considered separately, differences were statistically significant in green juice samples ($p < 0.001$) and an inverse relationship between crude protein content and cultivation salinity was observed, whereas no significant differences were observed in the crude protein content of fibre residue fractions ($p = 0.070$). The total lipid content of *C. maritimum* was low (< 2.5 g/100 g DM) in all samples, with changes between biomass batches being non-significant ($p = 0.045$). Most of the total lipids (> 60%) were present in the fibre residue fraction after the screw press. In the green juice fractions, the lipid content was < 2.2 g/100 g DM, and the differences between juice samples were also non-significant ($p = 0.475$).

Significant changes were observed in the total carbohydrate content of the biomass samples ($p < 0.001$). The content of carbohydrates was highest in the plants cultivated in 86 mM NaCl, the total amount of sugars being 34.71 ± 2.86 g/100 g DM. Significant changes were observed in the total contents of glucose ($p < 0.001$), xylose ($p < 0.001$), and arabinose ($p = 0.002$) in total DM of *C. maritimum*. However, when hydrolysed juice samples were considered separately, only non-significant changes were observed in the concentrations of glucose ($p = 0.510$) and xylose ($p = 0.051$), and arabinose was detected only in the sample from 171 mM NaCl cultivated plants in very low concentration (< 0.1 g/100 g DM). Considering the amounts of structural carbohydrates in solid samples, only the pulp fraction from 86 mM NaCl cultivation salinity was significantly different, whereas the carbohydrate content in other solid samples remained nearly constant. Klason lignin content of total DM varied from 22.37 ± 1.09 g/100 g DM (171 mM NaCl) to 27.68 ± 1.99 g/100 g DM (86 mM NaCl) with the p-value of 0.004.

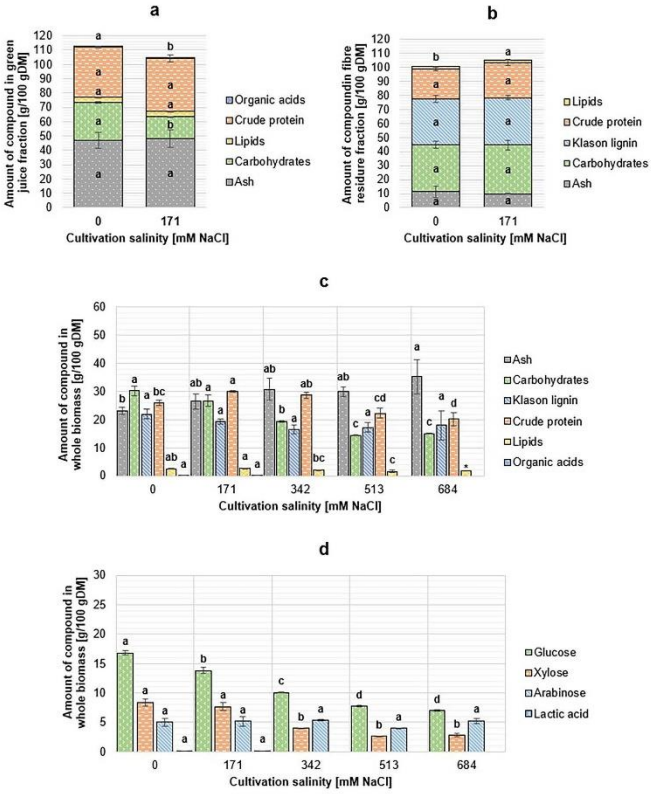

Figure 3. Chemical composition of *Tripolium pannonicum* green juice (**a**), fibre residue (**b**) and whole biomass (**c**), and total sugar profile (**d**). DM: dry matter. For 0 and 171 mM NaCl salinity cultivated samples, the content of a compound in whole biomass was calculated from juice and fibre residue fractions. Only structural carbohydrates were analysed for samples cultivated in 342, 513, and 684 mM NaCl. Biomass samples which were not fractionated were not analysed for their contents of organic acids. Different letters above the bars denote significantly different ($p < 0.05$) results calculated individually for each biochemical group. *Not possible to give the standard deviation for the sample.

Fresh *C. maritimum* juice was rich in free sugar monomers, and concentration varied between samples ($p < 0.001$). In these samples, glucose was the most abundant sugar, and the concentration reached up to 22.14 ± 0.20 g/100 g DM (0 mM NaCl). The amount of xylose was also found to be high, being 18.63 ± 0.47 g/100 g DM in the green juice from plants cultivated with 0 mM NaCl and > 10.00 g/100 g DM in other fresh juice samples. Arabinose was not detected in any of the fresh juice samples. The largest amount of lactic acid was detected from the 86 mM NaCl cultivated sample (3.77 ± 0.00 g/100 g DM), but in other juice samples, lactic acid concentrations were low (< 0. 1 g/100gDM).

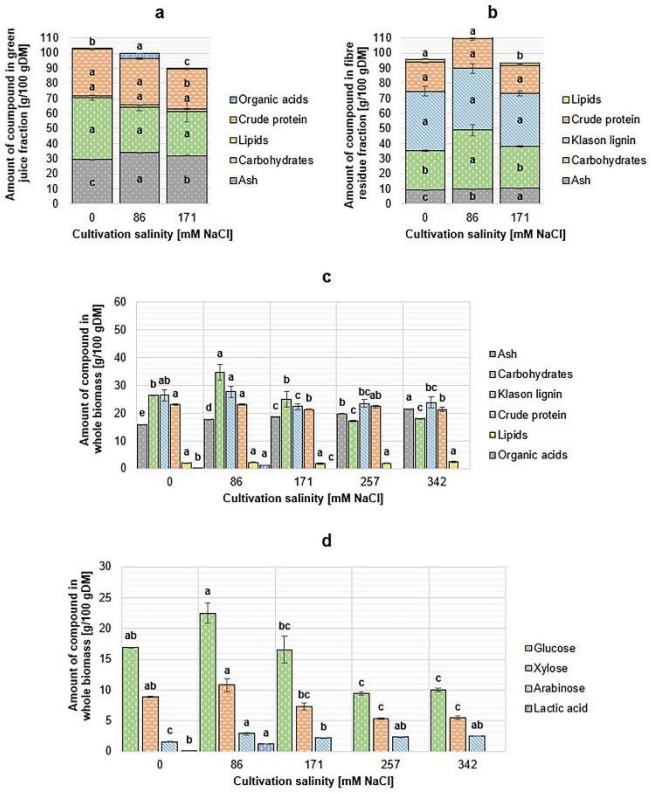

Figure 4. Chemical composition of *Crithmum maritimum* green juice (**a**), fibre residue (**b**) and whole biomass (**c**), and total sugar profile (**d**). DM: dry matter. For 0, 86, and 171 mM NaCl salinity cultivated samples, the content of a compound in whole biomass was calculated from juice and fibre residue fractions. Only structural carbohydrates were analysed for samples cultivated in 257 and 342 mM NaCl. Due to the small concentration of organic acids (< 0.1 g/100 g DM), all bars are not visible in the graphs. Biomass samples which were not fractionated were not analysed for their contents of organic acids. Different letters above the bars denote significantly different ($p < 0.05$) results calculated individually for each biochemical group.

Cultivation salinity is directly related to the ash content of the *C. maritimum* biomass ($p < 0.001$).

Discussion

Halophytes can be divided into two groups: obligate halophytes, which need salt to produce the highest biomass yields, and facultative halophytes, which tolerate salt but have optimal growth in low salinities. In general, dicotyledonous halophyte plants have shown higher salt tolerance compared to monocotyledonous grasses

and glass-like species[43]. As an obligate halophyte, *S. europaea* requires salt for optimal growth, and the highest obtained biomass yield from cultivation at 342 mM NaCl salinity (approximately 34 dS/m) aligns with the previous studies reporting the optimal salinity range for *Salicornia* spp. to be 200–400 mM NaCl (corresponding to approximately 20–40 dS/m)[44–47]. In the cultivation study by Araus et al.[48], brackish water (25 dS/m) irrigation lead to taller plants and higher *S. europaea* biomass production compared to seawater (40 dS/m) irrigation. Adaptability to changing environments was shown, as *S. europaea* exhibited sufficient growth in various salinities. In the study by Cárdenas-Pérez et al.[47] no significant anatomical changes were observed in *S. europaea* cultivated at 200–800 mM salinity, whereas changes in plant cells were observed with extreme salinity treatments (0 mM and 1000 mM NaCl). *S. europaea* exposed to high salinity stress (700 mM) has also exhibited recovery after the stress and as high production of fresh biomass as control plants[49]. *Salicornia* spp. are fully adapted to flooding conditions, which has also been shown to enhance growth[44].

Unlike *Salicornia* spp., *T. pannonicum* and *C. maritimum* are considered facultative halophytes. Uno et al.[50] and Ueda et al.[51] have reported *T. pannonicum* growth inhibition in salinities above 300 mM NaCl. Obtained results are aligned with these observations, as biomass yield was significantly lower when salinity increased to 342 mM NaCl. Wiszniewska et al.[52] also obtained the optimal *T. pannonicum* production at non-saline conditions but reported a non-significant difference in yields in 150 mM and 300 mM NaCl cultivated plants. Turcios et al.[22] reported *T. pannonicum* being able to withstand salinities up to 770 mM (45 g/l NaCl). Even if *T. pannonicum* survived under 684 mM NaCl salinity, the biomass yield was very low, and only small changes in the chemical composition of the biomass were observed. Al-Hawija et al.[53] also reported *T. pannonicum* seeds being able to germinate under salinity up to 600 mM, but the germination percentage decreased from 78 to 24% compared to non-saline conditions. Considering obtained results and previous literature, *C. maritimum* tolerates lower NaCl concentrations than the other two species. This can be explained by the type and the natural habitat of the species. According to eHALOPH database[54], *S. europaea* and *T. pannonicum* are classified as hydrohalophytes found in salt marshes, which can be affected by tidal changes in coastal areas, whereas *C. maritimum* is a chasmophyte found in rocky seashores. In addition, plants have several mechanisms to continue their growth and development under salt stress conditions, including the restriction of Na^+ uptake and exclusion, cellular compartmentalisation of Na^+ in the vacuole, antioxidant regulation, compatible solutes (osmolytes), morphological adaptations, among others. Germination strategies of halophyte seeds also show high variability depending on plant population and their natural habitat[55]. The mechanism used depends on the group of plants, glycophytes or halophytes, and on each species, resulting in a wide range of tolerance to salinity. For example, Ben Amor et al.[56] and Ben Hamed et al.[57] reported a significant reduction in *C. maritimum* yields in salinities higher than 200 mM NaCl and at 300 mM NaCl, respectively, but showed enhanced or unchanged growth under moderate 50 mM NaCl and 100 mM NaCl salinities, respectively. Similarly, Martins-Noguerol et al.[58] defined the optimal cultivation salinity for *C. maritimum* in greenhouse conditions to be 50 mM NaCl (approximately 5 dS/m). Regardless of the lower total biomass production and longer cultivation time, the utilisation of *C. maritimum* could be feasible, as the plant has been reported to be rich in valuable bioactive compounds[35,36].

It must be taken into consideration that the cultivation of plants in different growth media (hydroponic system or cultivation in soil) can also lead to different plant growth responses and biochemical compositions. However, the use of hydroponic cultivation has increased in importance worldwide due to the known advantages, mainly in the efficient use of the resources, being necessary to carry out research in this field. In addition, under hydroponic conditions and for research purposes, the concentrations of salt and nutrients can be easily and precisely controlled, allowing an accurate comparison between the different treatments. The cultivation system to select also depends on other factors. Different cultivation systems provide varying capabilities to control the salinity[7], which among other things, such as targeted products, has to be taken into account when choosing the cultivation practices and following processing methods. As *S. europaea* is an annual plant, it could provide an interesting species for crop rotation, where it would be used in the remediation process and to uptake the excessive salt from the substrate, which may inhibit the growth of other crops.

This study presents a broad overview of the composition of green fractionated halophyte biomasses grown in the hydroponic system, providing information for planning potential biorefinery processes. The crude protein content of studied halophytes was relatively high, and these species could have the potential for protein production. Results for total crude protein content for *S. europaea* were aligned with amounts previously reported for *Salicornia* spp.[19,26]. The content of soluble protein in *S. europaea* has previously shown to be relatively stable and content to decrease only when exposed to very high salinities[47]. The crude protein content was lower in *S. europaea* samples cultivated in more optimal conditions regarding biomass yield; thus, the actual crude protein content was 12.7% higher in plants cultivated in 171 mM NaCl than plants cultivated in 342 mM NaCl. Therefore, the cultivation in 171 mM NaCl could be more desirable for a biorefinery targeting maximum protein production when assuming that all crude protein could be extracted as true protein and changes in the biomass yields would be significant. Even if the total crude protein content is nearly the same in 171 mM NaCl and 684 mM NaCl cultivated samples, due to significantly lower biomass yield obtained with 684 mM, the amount of total protein would decrease. Therefore this cultivation condition cannot be suggested. For *T. pannonicum*, the crude protein content was higher than previously reported for species[22] and six other species in the Asteraceae family[59]. The crude protein content of *T. pannonicum* DM was comparable to widely used legumes, such as chickpea (24.0 g/100 g), lentil (26.1 g/100 g) or green pea (24.9 g/100 g)[60], which makes it the most interesting species for protein production. However, the presence of non-protein free amino acids (e.g. asparagine) may cause an overestimation in protein content[61], as well as high content of nitrate, which depending on cultivation practices, may also become an anti-nutritional factor in *T. pannonicum*[4]. Nitrogen is also present in chlorophylls, and high concentration may affect the estimation of protein content. Therefore, an amino acid analysis would be needed to carry out in further investigations. Also, *C. maritimum* showed relatively high crude protein content, and previous studies have shown optimal cultivation conditions to increase the content of essential amino acids in the plant[58].

Low lipid content was measured from all studied plant species, which is typical for succulent halophytes[62]. For *T. pannonicum*, the total lipid content was lower than values previously reported in the literature[22]. Abiotic stresses, such as high or low salinity, could increase the antioxidant capacity and production of certain protective non-polar compounds, such as carotenoids, in plants[26,47,63]. Therefore, analysis of fatty acid profile and characterisation of other non-polar compounds (e.g. pigments and tocopherols) from lipid-enriched fractions could be desired to evaluate the feasibility of lipid separations as part of the biorefinery process.

With respect to total biomass yield, optimal cultivation conditions seem to increase the lignocellulosic fraction in the *S. europaea* biomass. This can be linked to the larger plant size obtained from the cultivation under optimal salinity[47,48]. Therefore, biomass cultivated in these conditions could be more suitable for biorefinery targeting cellulose and hemicellulose derivatives, cellulose being present mainly in the lignified stems[10]. Regardless of the significantly higher carbohydrate content in *C. maritimum* from 86 mM NaCl salinity cultivation, the actual amount of carbohydrates in obtained fresh biomass was still higher in plants cultivated in 0 mM NaCl due to higher biomass yield. Klason lignin content of *S. europaea* was found to be low, and it is aligned with acid-insoluble lignin contents previously reported for *Salicornia* species[18,19,64]. Low lignin content may indicate biomass to be non-recalcitrant, allowing less severe processing conditions, especially after the removal of extractive material. In *C. maritimum* and *T. pannonicum* biomass, Klason lignin content was higher than *S. europaea*, and the insoluble lignin content was aligned with 18.2 g/100 g DM previously reported for *T. pannonicum*[22]. Studied facultative halophytes had a high concentration of available sugars in the juice, making them interesting for processes utilising micro-organisms. Compared to forage alfalfa juice, which has been suggested as media for lactic acid fermentation[65], the amount of available glucose is similar in *T. pannonicum* and nearly triple in *C. maritimum* cultivated in moderate salinities. The high amount of available xylose could also make *T. pannonicum* a potential feedstock for the production of pentose-derived platform chemicals.

Halophytes are known to accumulate salt and other minerals in their tissues, and high ash contents were also measured from studied biomass. Ushakova et al.[66] showed increased sodium intake and decreased potassium, calcium, and magnesium uptake of *S. europaea*, which aligns with obtained results. Studied halophytes, especially *C. maritimum* and *S. europaea*, were rich in calcium (see Supplementary Table S1 and Supplementary Table S3), which has been shown to have an essential protective role in the salt tolerance of plants growing in saline conditions[67]. Compared to the other studied species cultivated in the same salinities, *C. maritimum* exhibited lower salt accumulation.

Conclusion

S. europaea, *T. pannonicum*, and *C. maritimum* were cultivated in different salinity conditions, and *S. europaea* yielded the most biomass in 342 mM NaCl (approximately 34 dS/m) salinity, whereas facultative halophyte species exhibited the highest biomass production in non-saline conditions. *T. pannonicum*, and especially *S. europaea*, could be potential crops due to their higher biomass yields and shorter cultivation time. Obtained biomass was fractionated to green juice and fibre residue, and the chemical composition of the fractions were analysed. Significant differences were observed between biomass batches cultivated under different salinities, and this study is the first one to report the composition of the species after green fractionation. All species exhibited high crude protein content. Therefore, they can be seen as potential feedstocks for biorefinery targeting green protein production. Obtained results can be used to plan possible processing routes for halophyte-based biorefinery. Still, halophytes and their suitability for different applications should be further explored as a part of the green transition and development of marginal lands.

Data availability

The data generated and analysed during the study is available from the corresponding author on request.

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References

1. Daliakopoulos, I. N. *et al.* The threat of soil salinity: A European scale review. *Sci. Total Environ.* **573**, 727–739 (2016).
2. FAO *Intergovernmental Technical Panel on Soils. Status of the World's Soil Resources*. ISBN 9789251090046 (2015).
3. EIP-AGRI Focus Group Soil Salinisation: Final report. https://ec.europa.eu/eip/agriculture/en/publications/eip-agri-focus-group-soil-salinisation-final.
4. Ventura, Y., Eshel, A., Pasternak, D. & Sagi, M. The development of halophyte-based agriculture: Past and present. *Ann. Bot.* **115**, 529–540. https://doi.org/10.1093/aob/mcu173 (2014).
5. Flowers, T. J. & Colmer, T. D. Salinity tolerance in halophytes. *New Phytol.* **179**, 945–963. https://doi.org/10.1111/j.1469-8137.2008.02531.x (2008).
6. Glenn, E. P., Brown, J. J. & Blumwald, E. Salt tolerance and crop potential of halophytes. *CRC. Crit. Rev. Plant Sci.* **18**, 227–255. https://doi.org/10.1080/07352689991309207 (1999).
7. Ventura, Y. & Sagi, M. Halophyte crop cultivation: The case for *Salicornia* and *Sarcocornia*. *Environ. Exp. Bot.* **92**, 144–153. https://doi.org/10.1016/j.envexpbot.2012.07.010 (2013).
8. Shpigel, M. *et al.* Constructed wetland with *Salicornia* as a biofilter for mariculture effluents. *Aquaculture* **412–413**, 52–63. https://doi.org/10.1016/j.aquaculture.2013.06.038 (2013).
9. Turcios, A. E., Weichgrebe, D. & Papenbrock, J. Uptake and biodegradation of the antimicrobial sulfadimidine by the species *Tripolium pannonicum* acting as biofilter and its further biodegradation by anaerobic digestion and concomitant biogas production. *Bioresour. Technol.* **219**, 687–693. https://doi.org/10.1016/j.biortech.2016.08.047 (2016).
10. Cárdenas-Pérez, S., Piernik, A., Chanona-Pérez, J. J., Grigore, M. N. & Perea-Flores, M. J. An overview of the emerging trends of the *Salicornia* L. genus as a sustainable crop. *Environ. Exp. Bot.* **191**, 104606 (2021).
11. Petropoulos, S. A., Karkanis, A., Martins, N. & Ferreira, I. C. F. R. Edible halophytes of the Mediterranean basin: Potential candidates for novel food products. *Trends Food Sci. Technol.* **74**, 69–84 (2018).

12. Belal, I. E. H. & Al-Dosari, M. Replacement of fish meal with *Salicornia* meal in feeds for Nile Tilapia *Oreochromis niloticus*. *J. World Aquac. Soc.* **30**, 285–289. https://doi.org/10.1111/j.1749-7345.1999.tb00877.x (1999).
13. Attia, F. M., Alsobayel, A. A., Kriadees, M. S., Al-Saiady, M. Y. & Bayoumi, M. S. Nutrient composition and feeding value of *Salicornia bigelovii* torr meal in broiler diets. *Anim. Feed Sci. Technol.* **65**, 257–263. https://doi.org/10.1016/S0377-8401(96)01074-7 (1997).
14. Jiao, Y., Hosseindoust, A., Zhang, W.-L. & Kim, I.-H. Effects of *Salicornia herbacea* on growth performance, meat quality, excreta microbial populations, and noxious gas emissions in broiler chicks. *J. Poult. Sci.* **56**, 44–51. https://doi.org/10.2141/jpsa.0170210 (2019).
15. Abdal, M. S. *Salicornia* production in Kuwait. *World Appl. Sci. J.* **6**, 1033–1038 (2009).
16. Ahmed, M. H. *et al.* Growth performance and carcass characteristics of lambs fed halophytes as a partial or whole replacement of berseem hay. *Small Rumin. Res.* **128**, 1–9. https://doi.org/10.1016/j.smallrumres.2015.05.004 (2015).
17. Masson-Delmotte, V. P. Z. *et al.* IPCC, 2021: summary for policymakers. In *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* (2021).
18. Cybulska, I. *et al.* Chemical characterization and hydrothermal pretreatment of *Salicornia bigelovii* straw for enhanced enzymatic hydrolysis and bioethanol potential. *Bioresour. Technol.* **153**, 165–172. https://doi.org/10.1016/j.biortech.2013.11.071 (2014).
19. Alassali, A., Cybulska, I., Galvan, A. R. & Thomsen, M. H. Wet fractionation of the succulent halophyte *Salicornia sinus-persica*, with the aim of low input (water saving) biorefining into bioethanol. *Appl. Microbiol. Biotechnol.* **101**, 1769–1779. https://doi.org/10.1007/s00253-016-8049-8 (2017).
20. Sharma, R., Wungrampha, S., Singh, V., Pareek, A. & Sharma, M. K. Halophytes as bioenergy crops. *Front. Plant Sci.* **7**, 1372 (2016).
21. Folayan, A. J., Anawe, P. A. L., Ayeni, A. O. & Arellano-Garcia, H. Synthesis and characterization of *Salicornia bigelovii* and *Salicornia brachiata* halophytic plants oil extracted by supercritical CO_2 modified with ethanol for biodiesel production via enzymatic transesterification reaction using immobilized *Candida antarctica* lipase catalyst in tert-butyl alcohol (TBA) solvent. *Cogent Eng.* **6**, 1625847. https://doi.org/10.1080/23311916.2019.1625847 (2019).
22. Turcios, A. E., Weichgrebe, D. & Papenbrock, J. Effect of salt and sodium concentration on the anaerobic methanisation of the halophyte *Tripolium pannonicum*. *Biomass Bioenergy* **87**, 69–77. https://doi.org/10.1016/j.biombioe.2016.01.013 (2016).
23. Severo, I. A. *et al.* Biodiesel facilities: What can we address to make biorefineries commercially competitive?. *Renew. Sustain. Energy Rev.* **112**, 686–705. https://doi.org/10.1016/j.rser.2019.06.020 (2019).
24. Torres, A. I., Ashraf, M. T., Chaturvedi, T., Schmidt, J. E. & Stephanopoulos, G. Hydrothermal pretreatment: Process modeling and economic assessment within the framework of biorefinery processes. In *Hydrothermal Processing in Biorefineries: Production of Bioethanol and High Added-Value Compounds of Second and Third Generation Biomass*, 207–235 ISBN 9783319564579 (Springer International Publishing, 2017).
25. Santamaría-Fernández, M. & Lübeck, M. Production of leaf protein concentrates in green biorefineries as alternative feed for monogastric animals. *Anim. Feed Sci. Technol.* **268**, 114605. https://doi.org/10.1016/j.anifeedsci.2020.114605 (2020).
26. Lima, A. R. *et al.* Influence of cultivation salinity in the nutritional composition, antioxidant capacity and microbial quality of *Salicornia ramosissima* commercially produced in soilless systems. *Food Chem.* **333**, 127525. https://doi.org/10.1016/j.foodchem.2020.127525 (2020).
27. Isca, V. M. S., Seca, A. M. L., Pinto, D. C. G. A., Silva, H. & Silva, A. M. S. Lipophilic profile of the edible halophyte *Salicornia ramosissima*. *Food Chem.* **165**, 330 (2014).
28. Christiansen, A. H. C., Lyra, D. A. & Jørgensen, H. Increasing the value of *Salicornia bigelovii* green biomass grown in a desert environment through biorefining. *Ind. Crops Prod.* **160**, 113105. https://doi.org/10.1016/j.indcrop.2020.113105 (2021).
29. Giordano, R. *et al.* Pharmacological insights into halophyte bioactive extract action on anti-inflammatory, pain relief and antibiotics-type mechanisms. *Molecules* **26**, 3140 (2021).
30. Cybulska, I., Brudecki, G. P., Alassali, A., Thomsen, M. H. & Brown, J. J. Phytochemical composition of some common coastal halophytes of the United Arab Emirates. *Emirates J. Food Agric.* **26**, 1046–1056. https://doi.org/10.9755/ejfa.v26i12.19104 (2014).
31. Chaturvedi, T., Christiansen, A. H. C., Golębiewska, I. & Thomsen, M. H. *Salicornia* species: current status and future potential. In *Future of Sustainable Agriculture in Saline Environments*, 461–482 (CRC Press, 2021).
32. Hulkko, L. S. S., Chaturvedi, T. & Thomsen, M. H. Extraction and quantification of chlorophylls, carotenoids, phenolic compounds, and vitamins from halophyte biomasses. *Appl. Sci.* **12**, 840. https://doi.org/10.3390/app12020840 (2022).
33. Chai, W., Chen, L., Lian, X. Y. & Zhang, Z. Anti-glioma efficacy and mechanism of action of tripolinolate A from *Tripolium pannonicum*. *Planta Med.* **84**, 786–794. https://doi.org/10.1055/s-0044-101038 (2018).
34. Wubshet, S. G., Schmidt, J. S., Wiese, S. & Staerk, D. High-resolution screening combined with HPLC-HRMS-SPE-NMR for identification of potential health-promoting constituents in sea aster and searocket—New nordic food ingredients. *J. Agric. Food Chem.* **61**, 8616–8623. https://doi.org/10.1021/jf402949y (2013).
35. Meot-Duros, L. & Magné, C. Antioxidant activity and phenol content of *Crithmum maritimum* L. leaves. *Plant Physiol. Biochem.* **47**, 37–41. https://doi.org/10.1016/j.plaphy.2008.09.006 (2009).
36. GeneralicMekinić, I. *et al.* Sea fennel (*Crithmum maritimum* L.): Phytochemical profile, antioxidative, cholinesterase inhibitory and vasodilatory activity. *J. Food Sci. Technol.* **53**, 3104–3112. https://doi.org/10.1007/s13197-016-2283-z (2016).
37. Buhmann, A., Hellmueller, L. & Louis, B. Popular culture and communication practice. *Commun. Res. Trends* **34**, 4 (2015).
38. Sluiter, A. *et al. NREL/TP-510-42621 Analytical procedure—Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples* 3–5 (2008).
39. Sluiter, A., *et al. NREL/TP-510-42622 Analytical Procedure—Determination of Ash in Biomass*; (2005).
40. Jones, D. *Factors for Converting Percentages of Nitrogen in Foods and Feeds into Percentages of Proteins*, 16–21 (United States Dep. Agric., 1931).
41. Sluiter, A. *et al. NREL/TP-510-42618 Analytical Procedure—Determination of Structural Carbohydrates and Lignin in Biomass* (2012).
42. Sluiter, A. *et al. Laboratory Analytical Procedure (LAP) Issue Date : 12/08/2006 Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples* (2008).
43. Glenn, E. P. & O'Leary, W. Relationship between salt accumulation and water content of dicotyledonous halophytes. *Plant Cell Environ.* **7**, 253–261. https://doi.org/10.1111/1365-3040.ep11589448 (1984).
44. van Andel, J., Huiskes, A. H. L., Blom, C. W. P. M. & Rozema, J. Vegetation between land and sea: Structure and processes. *Q. Rev. Biol.* **64**, 73–74. https://doi.org/10.1086/416161 (1989).
45. Lv, S. *et al.* Multiple compartmentalization of sodium conferred salt tolerance in *Salicornia europaea*. *Plant Physiol. Biochem.* **51**, 47–52. https://doi.org/10.1016/j.plaphy.2011.10.015 (2012).
46. Katschnig, D., Broekman, R. & Rozema, J. Salt tolerance in the halophyte *Salicornia dolichostachya* Moss: Growth, morphology and physiology. *Environ. Exp. Bot.* **92**, 32–42. https://doi.org/10.1016/j.envexpbot.2012.04.002 (2013).
47. Cárdenas-Pérez, S. *et al. Salicornia europaea* L. functional traits indicate its optimum growth. *Plants* **11**, 1051. https://doi.org/10.3390/plants11081051 (2022).
48. Araus, J. L. *et al.* Effect of irrigation salinity and ecotype on the growth, physiological indicators and seed yield and quality of *Salicornia europaea*. *Plant Sci.* **304**, 110819. https://doi.org/10.1016/j.plantsci.2021.110819 (2021).
49. Calone, R. *et al.* Recovery from salinity and drought stress in the perennial *Sarcocornia fruticosa* vs. the annual *Salicornia europaea* and *S. veneta*. *Plants* **11**, 1058. https://doi.org/10.3390/plants11081058 (2022).

50. Uno, Y., Kanechi, M., Inagaki, N., Sugimoto, M. & Maekawa, S. The evaluation of salt tolerance during germination and vegetative growth of asparagus, table beet and sea aster. *Engei Gakkai zasshi* **65**, 579–585. https://doi.org/10.2503/jjshs.65.579 (1996).
51. Ueda, A., Kanechi, M., Uno, Y. & Inagaki, N. Photosynthetic limitations of a halophyte sea aster (*Aster tripolium* L.) under water stress and NaCl stress. *J. Plant Res.* **116**, 65–70. https://doi.org/10.1007/s10265-002-0070-6 (2003).
52. Wiszniewska, A., Koźmińska, A., Hanus-Fajerska, E., Dziurka, M. & Dziurka, K. Insight into mechanisms of multiple stresses tolerance in a halophyte *Aster tripolium* subjected to salinity and heavy metal stress. *Ecotoxicol. Environ. Saf.* **180**, 12–22. https://doi.org/10.1016/j.ecoenv.2019.04.059 (2019).
53. Al-Hawija, B. N., Partzsch, M. & Hensen, I. Effects of temperature, salinity and cold stratification on seed germination in halophytes. *Nord. J. Bot.* **30**, 627–634. https://doi.org/10.1111/j.1756-1051.2012.01314.x (2012).
54. Santos, J., Al-Azzawi, M., Aronson, J. & Flowers, T. J. eHALOPH a database of salt-tolerant plants: Helping put halophytes to work. *Plant Cell Physiol.* **57**, e10–e10. https://doi.org/10.1093/pcp/pcv155 (2016).
55. Gul, B., Ansari, R., Flowers, T. J. & Khan, M. A. Germination strategies of halophyte seeds under salinity. *Environ. Exp. Bot.* **92**, 4–18. https://doi.org/10.1016/j.envexpbot.2012.11.006 (2013).
56. Ben Amor, N., Ben Hamed, K., Debez, A., Grignon, C. & Abdelly, C. Physiological and antioxidant responses of the perennial halophyte *Crithmum maritimum* to salinity. *Plant Sci.* **168**, 889–899. https://doi.org/10.1016/j.plantsci.2004.11.002 (2005).
57. Ben Hamed, K., Castagna, A., Salem, E., Ranieri, A. & Abdelly, C. Sea fennel (*Crithmum maritimum* L.) under salinity conditions: A comparison of leaf and root antioxidant responses. *Plant Growth Regul.* **53**, 185–194. https://doi.org/10.1007/s10725-007-9217-8 (2007).
58. Martins-Noguerol, R. *et al.* Differences in nutrient composition of sea fennel (*Crithmum maritimum*) grown in different habitats and optimally controlled growing conditions. *J. Food Compos. Anal.* **106**, 104266. https://doi.org/10.1016/j.jfca.2021.104266 (2022).
59. García-Herrera, P. *et al.* Nutrient composition of six wild edible Mediterranean Asteraceae plants of dietary interest. *J. Food Compos. Anal.* **34**, 163–170. https://doi.org/10.1016/j.jfca.2014.02.009 (2014).
60. Iqbal, A., Khalil, I. A., Ateeq, N. & Sayyar Khan, M. Nutritional quality of important food legumes. *Food Chem.* **97**, 331–335. https://doi.org/10.1016/j.foodchem.2005.05.011 (2006).
61. Moore, J. C., DeVries, J. W., Lipp, M., Griffiths, J. C. & Abernethy, D. R. Total protein methods and their potential utility to reduce the risk of food protein adulteration. *Compr. Rev. Food Sci. Food Saf.* **9**, 330–357. https://doi.org/10.1111/j.1541-4337.2010.00114.x (2010).
62. Patel, M. K., Pandey, S., Brahmbhatt, H. R., Mishra, A. & Jha, B. Lipid content and fatty acid profile of selected halophytic plants reveal a promising source of renewable energy. *Biomass Bioenergy* **124**, 25–32. https://doi.org/10.1016/j.biombioe.2019.03.007 (2019).
63. Ghanem, A.-M.F.M., Mohamed, E., Kasem, A. M. M. A. & El-Ghamery, A. A. Differential salt tolerance strategies in three halophytes from the same ecological habitat: Augmentation of antioxidant enzymes and compounds. *Plants* **10**, 1100. https://doi.org/10.3390/plants10061100 (2021).
64. Cybulska, I. *et al.* Comparative study of chemical composition of the halophyte species native to the Persian (Arabian) gulf. *BioResources* **16**, 5524–5537 (2021).
65. Santamaría-Fernández, M., Schneider, R., Lübeck, M. & Venus, J. Combining the production of L-lactic acid with the production of feed protein concentrates from alfalfa. *J. Biotechnol.* **323**, 180–188. https://doi.org/10.1016/j.jbiotec.2020.08.010 (2020).
66. Ushakova, S. A., Kovaleva, N. P., Gribovskaya, I. V., Dolgushev, V. A. & Tikhomirova, N. A. Effect of NaCl concentration on productivity and mineral composition of *Salicornia europaea* as a potential crop for utilization NaCl in LSS. *Adv. Space Res.* **36**, 1349–1353. https://doi.org/10.1016/j.asr.2004.09.017 (2005).
67. Hadi, M. R. & Karimi, N. The role of calcium in plants' salt tolerance. *J. Plant Nutr.* **35**, 2037–2054. https://doi.org/10.1080/01904167.2012.717158 (2012).

Author contributions

L.S.S.H. prepared the original manuscript draft. J.P. obtained the permission to collect seeds and carried out the formal identification of the species. A.T. cultivated and harvested the biomass. L.S.S.H. ran the composition analysis, except the mineral analysis, which was performed by S.K. All authors reviewed the manuscript.

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Competing interests

The authors declare no competing interests.

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Paper II

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Review

Extraction and Quantification of Chlorophylls, Carotenoids, Phenolic Compounds, and Vitamins from Halophyte Biomasses

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Abstract: Halophytes are salt-tolerant plants, and they have been utilised as healthy, nutritious vegetables and medicinal herbs. Various studies have shown halophytes to be rich in health-beneficial compounds with antioxidant activity, anti-inflammatory and antimicrobial effects, and cytotoxic properties. Despite their potential, these plants are still underutilised in agriculture and industrial applications. This review includes the state-of-the-art literature concerning the contents of proanthocyanidins (also known as condensed tannins), total phenolic compounds, photosynthetic pigments (chlorophyll and carotenoids), and vitamins in various halophyte biomasses. Various extraction and analytical methods are also considered. The study shows that various species have exhibited potential for use not only as novel food products but also in the production of nutraceuticals and as ingredients for cosmetics and pharmaceuticals.

Keywords: halophytes; bioactive molecules; saline cultivation; pigments; phenolics

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1. Introduction

According to the European Innovation Partnership for Agricultural Productivity and Sustainability [1], soil salinisation is a significant threat to soils at a global scale, causing agricultural land degradation through natural causes or poor land and water management. As most conventional crops are salt-sensitive glycophytes, the increased salt concentration in cultivation soil causes nutritional imbalances and toxicity in plants, inhibiting their growth and making the soil unsuitable for traditional farming [1]. Loss of arable land affects the economic situation of people who depend on agricultural activities and creates a threat to the world's food security.

Halophytes are salt-tolerant plants adapted to thrive in saline habitats, such as seashores, marshlands, and saline deserts worldwide. Humans have used halophytes for centuries, primarily in medicinal practices, due to their high levels of bioactive compounds [2–4]. Nowadays, fresh shoots of halophytes are sold as gourmet vegetables [5,6]. Cultivation and commercialisation of these naturally salt-loving plants are seen as one of the critical implementations to help the remediation of saline areas and adapt to the changes in soil quality [1].

Vegetables and fruits are typically rich in health-beneficial bioactive compounds, such as phenolics and carotenoids [7,8]. However, these compounds can also be found in high concentrations in botanical extracts obtained from some agricultural and food processing waste, as mentioned in the review by Rauf et al. [7]. Halophytes are likely to produce high levels of these protective compounds due to their adaptation to extreme environmental conditions [9,10]. Besides healthy food, these plants could provide a novel feedstock for bio-functional feed, nutraceuticals, and the pharmaceutical industry [2,11–13]. For example, Lopes et al. [3] studied halophytes from saline habitats of southern Portugal and suggested various species that can potentially be utilised in cosmetic ingredients. These valuable bioactive compounds can also serve as value-added products that can be obtained from

a halophyte-based biorefinery. For some succulent halophytes, such as *Salicornia* species, the food production period is relatively short, as the shrubs become more woody as they mature, making them unpleasant to eat. Due to high salt concentrations accumulated in the plant tissues, halophytes can be directly used as animal feed only when blended with other feed sources [14,15]. Therefore, these partly lignified plants are often considered agricultural waste. However, they could be used to extract phytochemicals as a part of a multi-product biorefinery. This concept is visualised in Figure 1. This would provide a value-added product stream for a biorefinery, whereasresidual extractive-free fibres could be utilised in bioenergy production, or in the production of other lignocellulose-derived products, such as bulk chemicals. Halophyte biomass has previously been tested for bioethanol and biogas production [16–18]. Production of botanical extracts can also be beneficial considering the pretreatment of lignocellulosic fibres, as it makes the fibres less recalcitrant, and lowers the severity of the pretreatment conditions that are needed [16,19]. Extraction with water would also remove salts which could otherwise cause issues during the processing [20]. In terms of other major compounds, since halophytes have exhibited interesting nutritional profiles [21–23], protein production has also been studied [24,25]. Despite their potential, halophytes are still underutilised both in agricultural and industrial applications [3,12,23].

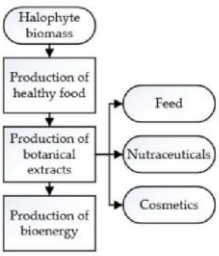

Figure 1. Simplified process chart of halophyte-based biorefinery.

2. Methods

This study reviews the existing literature reporting the phenolic compounds, pigments, and vitamins in halophyte biomass and provides information about the potential of these plants in multi-product biorefinery applications. Several scientific databases were used to retrieve articles, including those of Elsevier, Taylor & Francis, Springer, and MDPI, but other peer-reviewed articles and some book chapters were also considered. Only articles written in English were considered, and the review focused on studies published in and after the year 2010. However, a few papers from 2008 and 2009 were also included. The review was performed to investigate the amount of the following compounds reported for botanical extracts from the biomass of edible halophytes:

- Proanthocyanidins (condensed tannins);
- Phenolic compounds (total phenols);
- Chlorophyll;
- Carotenoids;
- Vitamins.

The main keywords used in the search were: halophytes, phytochemicals, bioactive compounds, proanthocyanidin, condensed tannin, pigments, chlorophyll, carotenoid, and vitamins. Some of the reviewed studies compared the composition of different biomass batches of same species, or extraction methods. From these studies, the data from the

sample with the highest concentration of the compound of interest was presented in the results. This review also briefly summarises the extraction methods and analytical procedures used to obtain, quantify, and identify the bioactive compounds. The strength of the solvent was also noted whenever it was specified in the original study.

3. Proanthocyanidins

Proanthocyanidins (also known as condensed tannins) are pigmented polymerised polyphenolic compounds that contribute to the antioxidant action and cardiovascular disease-preventing properties of botanical extracts, and these medicinal properties make them interesting for the pharmaceutical industry [26–28]. Rauf et al. [7] reviewed the reported health benefits linked to proanthocyanidins, and found that besides antioxidant and cardiovascular protective functions, condensed tannins have cancer-preventing, neuroprotective, and antimicrobial properties. In nature, proanthocyanidins are present in the flowers, leaves, fruits, nuts, seeds, and bark of various plants, protecting them from stress caused by environmental conditions or other living organisms, such as insects, parasitic nematodes, or diseases. More than a thousand derivatives of condensed tannins have been identified, and they have recently attracted increased interest due to their influence on various biological processes [29]. Plants rich in proanthocyanidins have also been used as herbal medicines for mild skin and oral mucosa inflammation and digestion issues [29].

The total amount of condensed tannins is typically determined from a methanol or acetone extract as an amount of catechin equivalent (CE) in the dry weight (DW) of the extract. The most common method for determining total condensed tannins is an applied vanillin assay method developed by Sun et al. [30], which is based on the reaction between catechin or proanthocyanidins and vanillin in methanol solution. However, a colourimetric method using 4-dimethylaminocinnamaldehyde hydrochloric acid (DMACA-HCl), developed by Li et al. [10], is said to have higher sensitivity and specificity compared to a vanillin assay.

Chekroun-Bechlaghem et al. [31] studied the extraction of polyphenolic compounds from halophyte biomasses using different solvents and found the concentration of condensed tannins was highest in aqueous fractions or methanol and acetone soluble fractions. As seen from Table 1, the level of proanthocyanidins in halophyte biomasses varies substantially between different species, even those within the same genus. For example, the concentration of proanthocyanidins in *Mesembryanthemum* species varies between 2.01 ± 0.04 mgCE/gDM [32] and 20.3 ± 0.98 mgCE/gDM [33], and in *Suaeda* species between 1.21 ± 0.05 mgCE/gDM [32] and 15.76 ± 1.43 mgCE/g [34]. The highest concentration of proanthocyanidins (118.43 ± 11.79 mgCE/gDM), measured from water extract, was found from *Tamarix africana* [31]. For comparison, 17.28 ± 1.95 mgCE/gDM of proanthocyanidins were found from *Tamarix gallica* [35] species within the same genus.

Table 1. The total amount of proanthocyanidins (total condensed tannins) measured from halophyte biomasses. Studies that used a vanillin assay applied the method by Sun et al. [30], except Chekroun-Bechlaghemt et al. [31], who used the modified method by Julkunen-Tiitto [36].

Plant Species	Solvent	Method	Concentration [mgCE/gDM]	Ref.
Arthrocnemum macrostachyum	Water	Vanillin assay	7.50 ± 0.80	[31]
Aster tripolium	Acetone (80%)	DMACA-HCl	Not detected	[3]
Cakile maritimum	Methanol	Vanillin assay	14.94 ± 0.04	[37]
Carpobrotus edulis	Ethanol	DMACA-HCl	20.3 ± 0.98	[33]
Cladium mariscus	Acetone (80%)	DMACA-HCl	38.7 ± 2.21	[3]

Table 1. *Cont.*

Plant Species	Solvent	Method	Concentration [mgCE/gDM]	Ref.
Crithmum maritimum	n/a	n/a	1.06 ± 0.77	[38]
	Acetone (80%)	Vanillin assay	0.63	[39]
Inula crithmoides	Acetone (80%)		0.95	[39]
Ipomoea pes-caprae	Methanol (80%)		19.67 ± 2.54	[34]
Limonium delicatulum	Methanol (80%)		48.38 ± 0.75	[40]
Mesembryanthemum edule	Methanol		14.2 ± 0.9	[28]
	Methanol		9.51 ± 1.07	[4]
	Methanol		7.16	[10]
Mesembryanthemum nodiflorum	Methanol	DMACA-HCl	2.01 ± 0.04	[32]
Pluchea lanceolata	Methanol (80%)	Vanillin assay	20.52 ± 4.32	[34]
Reaumuria vermiculata	Dichloromethane		27.98 ± 1.01	[41]
Retama raetam	Acetone (60%)		10.16	[42]
Salicornia ramosissima	Acetone (70%)	DMACA-HCl	32.5 ± 4.6	[5]
	Acetone (80%)		Not detected	[3]
Salsola kali	Methanol	Vanillin assay	2.03 ± 0.62	[43]
Salsola vermiculata	Acetone (80%)		Not detected	[3]
Salvadora persica	Methanol (80%)		22.35 ± 2.87	[34]
Sarcocornia fruticosa	Methanol	DMACA-HCl	0.46 ± 0.04	[32]
Sarcocornia perennis	Methanol	Vanillin assay	1.09	[44]
Suaeda fruticosa	Methanol		7.76 ± 0.28	[31]
	Methanol (80%)		15.76 ± 1.43	[34]
	Acetone		1.50 ± 0.13	[45]
Suaeda maritima	Methanol	DMACA-HCl	1.21 ± 0.05	[32]
Tamarix africana	Water	Vanillin assay	118.43 ± 11.79	[31]
Tamarix gallica	Methanol		17.28 ± 1.95	[35]
Thespesia populnea	Methanol (80%)		20.14 ± 3.54	[34]

4. Phenolic Compounds

Phenols are a diverse group of phytochemicals which include phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids) and flavonoids, amongst other types of compounds [32]. They are potent antioxidants linked to various health benefits, such as anti-inflammatory, anti-obesity, and even anti-cancer properties [11,46]. They have also been shown to prevent cardiovascular diseases and diabetes [46].

Phenolic compounds can be extracted with water, polar organic solvents, and their mixtures [46]. The colourimetric Folin–Ciocalteu method is well established and commonly used to estimate total phenols in plant extracts. In this method, gallic acid is used to determine a reference curve, and total phenols are measured as an amount of gallic acid equivalent (GAE) in the dried extract. The protocols use Folin–Ciocalteu reagent to measure absorbance at 760 nm, and the process is well described by Singleton and Rossi [47] and Dewanto et al. [48]. The total phenols found in halophyte extract are summarised in Table 2. The concentration of phenols may vary between different parts of a plant, and Ksouri et al. [35] and Medini et al. [40] have shown that phenol content is highest in extracts obtained from flowers. The concentration of phenolic compounds depends on biological factors, such as genotype, the part of the plant, and environmental conditions, such as

temperature, salinity, water stress, and light intensity [10]. Therefore, the composition extracts from the same species can vary, as can be seen in methanol extracts of *Salicornia ramosissima* and *Tamarix gallica*.

Table 2. Total phenolics measured from halophyte extracts with the Folin–Ciocalteu method. Chekroun-Bechlaghem et al. [31] Stankovic et al. [49], Qasim et al. [26], Lopes et al. [3], Rodrigues et al. [50], Lima et al. [5], Pereira et al. [51], Zengin et al. [52], and Castañeda-Loaiza et al. [32] used method by Singleton and Rossi [47]. Hamdoon et al. [53] used the method by Quy et al. [54]. Other studies use the protocol by Dewanto et al. [48].

Plant Species	Solvent	Method	Concentration [mgCAE/gDM]	Ref.
Artemisia santonicum	Methanol	Folin–Ciocalteu	212.71 ± 0.68	[49]
Arthrocnemum macrostachyum	Water		10.24 ± 0.01	[31]
	Ethyl acetate		29.54 ± 0.78	[52]
Aster tripolium	Acetone (80%)		223 ± 2.63	[3]
	Methanol		144.75 ± 0.59	[49]
Cladium mariscus	Acetone (80%)		254 ± 2.26	[3]
Carpobrotus edulis	Ethanol		272.82 ± 5.59	[33]
Crithmum maritimum	Acetone (80%)		7.9	[39]
	Water		35.3 ± 2.98	[51]
Halimone portulacoides	Ethyl acetate		14.59 ± 0.21	[52]
Inula crithmoides	Acetone (80%)		14.1	[39]
Ipomoea pes-caprae	Methanol (80%)		54.21 ± 2.31	[34]
Limoniastrum monopetalum	Methanol		15.85	[55]
Limonium algarvense	Methanol		228 ± 2	[50]
Limonium delicatulum	Acetone (80%)		92.9 ± 1.45	[40]
Limonium densiflorum	Methanol		56.18 ± 0.92	[56]
Lycium shawii	Ethanol (95%)		52.72 ± 3.17	[53]
Mesembryanthemum edule	Water		212.2 ± 4.8	[28]
	Methanol		68.75 ± 1.07	[4]
	Methanol		70.07	[10]
Mesembryanthemum nodiflorum	Methanol		6.75 ± 0.08	[32]
Pluchea lanceolata	Methanol (80%)		42.28 ± 3.58	[34]
Reaumuria vermiculata	Methanol		117.12 ± 3.31	[41]
Retama raetam	Water		137.0	[42]
Rumex vesicarius	Ethanol (95%)		28.54 ± 1.13	[53]
Salicornia europaea	Ethyl acetate		24.46 ± 0.16	[52]
	Ethanol/water		~11	[57]
	Methanol		58.20 ± 0.44	[49]
Salicornia ramosissima	Acetone (80%)		74.1 ± 2.49	[3]
	Acetone (80%)		12.9 ± 1.9	[5]
	Water		15.02 ± 2.01	[58]
Salsola kali	Methanol		17.23 ± 1.0	[43]
	Methanol		17.23	[10]

Table 2. *Cont.*

Plant Species	Solvent	Method	Concentration [mgCAE/gDM]	Ref.
Salsola vermiculata	Acetone (80%)	Folin–Ciocalteu	133 ± 2.62	[3]
Salvadora persica	Methanol (80%)		58.23 ± 3.54	[34]
Sarcocornia fruticosa	Methanol		5.69 ± 0.06	[32]
Suaeda fruticosa	Methanol		11.09 ± 0.08	[59]
	Acetone		31.7 ± 0.51	[45]
	Methanol/water		47.73 ± 1.17	[31]
	Methanol (80%)		46.54 ± 4.32	[34]
Suaeda maritima	Methanol		16.6 ± 0.1	[32]
	Methanol		62.88 ± 0.88	[49]
Tamarix africana	Water		61.06 ± 0.40	[31]
Tamarix gallica	Methanol		135.35 ± 7.70	[35]
	Methanol		70.56	[10]
Thespesia populnea	Methanol (80%)		63.91 ± 5.28	[34]
Zaleya pentandra	Methanol		22.60 ± 0.14	[60]

Several different protocols have been established to determine the phenolic profile of plant extracts and measure the concentration of single bioactive compounds using high-performance liquid chromatography (HPLC). The analytical procedure used depends on the compounds of interest; this study presents procedures used in some of the reviewed studies and the main separated compounds detected. Various HPLC methods and phenolic profiles of halophyte extracts has previously been reviewed by Lopes et al. [61]. Rodrigues et al. [50] determined the concentration of phenolic compounds in *Limonium algarvense* extract with a "Mediterranea sea 18" column, a mixture of methanol and aqueous acetic acid as mobile phase, and a diode array detector (DAD),. The targeted compounds with the highest concentrations were gallic acid (3.37 mg/gDM), catechin (2.87 mg/gDM), and salicylic acid (1.89 mg/gDM). However, Medini et al. [32] identified phenolic acids from the closely related *Limonium delicatulum* by means of reverse-phase HPLC with an RPC18 column, aqueous a trifluoroacetic acid and acetonitrile mobile phase, and a DAD, finding the major compounds to be coumaric acid and chlorogenic acid; however, the compounds were not quantified. Castañeda-Loaiza et al. [32] determined phenolic profile using the same method as Rodrigues et al. [50] and found the dry matter from *Mesembryanthemum nodiflorum* extract to be rich in ferulic acid (15.3 µg/mgDW) and caffeic acid (3.85 µg/mgDW). In extracts of *Suaeda maritima* and *Sarcocornia fruticosa*, the phenolic compounds with the highest concentrations were coumaric acid (0.98 µg/mgDW) and chlorogenic acid (2.25 µg/mgDW), respectively [32]. Mariem et al. [42] identified the major phenolic compounds in *Retama raetam* as syringic acid and coumarin, using reverse-phase HPLC with an ODS C18 column and a mixture of acetonitrile and sulphuric acid as the mobile phase. Qasim et al. [34] determined the phenolic profile of *Ipomoea pes-caprae*, finding that the major compounds were chlorogenic acid and gallic acid (7.37 ± 0.11 mg/g and 1.42 ± 0.07 mg/g, respectively). For *Suaeda fruticose*, they found major compounds to be catechin and chlorogenic acid (1.67 ± 0.08 mg/g and 1.27 ± 0.09 mg/g, respectively). Silva et al. [58] used HPLC with a C18 column, a mixture of methanol and water as the mobile phase, and a photodiode array detector (PDA). They found the predominant compound in the extract to be flavonoid myricetin (0.43 ± 0.02 mg/gDM). Jdey et al. [62] analysed phenolic compounds with a C18 column, a methanol and formic acid mobile phase, and DAD; they found the total amount of phenolics in *Frankeani laevis* to be 16 mg/gDM.

5. Chlorophylls

Chlorophylls (chlorophyll *a* and chlorophyll *b*) are photosynthetic pigments responsible for the green colour of plants. In human nutrition, chlorophyll has antioxidant activity, and the rich colour makes products more desirable for consumption [6,63,64]. It has also been shown to have anti-cancer properties, as it can interfere with the absorption of some carcinogenic compounds in the gastrointestinal tract [65]. Chlorophyll-derivative chlorophyllin is also registered as a food additive and natural colourant (E140i), and pigment concentrates can be extracted from edible plants using organic solvents [66].

Most of the studies determine the concentration of pigments as a basis of the dry weight or fresh weight (FW) of the biomass. When considering FW, it is important to note that succulent halophytes, in particular, have a high moisture content which can exceed 80% of the fresh weight [6,67]. The amount of pigment can also be determined based on leaf surface area: Geissler et al. [68] applied this method in their study of photosynthesis of *Aster tripolium*.

Spectrophotometric methods are widely used to determine the amount of chlorophyll in halophyte extracts. Most of the reviewed studies use the technique introduced by Lichtenthaler et al. [69], where wavelengths are used for measuring absorbance, and the conversion coefficients depend on the solvent used. Duarte et al. [67] and Sghaier et al. [70] used a novel quantification technique after ultrasound-assisted acetone extraction which involved applying the Gauss peak spectra method developed by Küpper et al. [71] instead of traditional spectroscopic methods. Some HPLC methods are also available for pigment determination. The method by Mendes et al. [72] was firstly developed for marine algae biomass, and it uses a C8 column with methanol, acetonitrile, and acetone (50:25:25 v%) as the mobile phase.

As seen from Table 3, among halophyte species, the highest chlorophyll content in fresh biomass were found in acetone extracts of *Salicornia europaea* and *Salicornia persica* (approximately 1.25 mg/gFW and 2.21 mg/gFW, respectively) [73]. Values are relatively high compared to other species.

Table 3. Total chlorophyll content measured from halophyte biomasses. Studies that used spectrophotometry applied the method by Lichtenthaler et al. [69], except Lu et al. [22], who used the method proposed by Vernon [74]; Ventura et al. [63], who used the method proposed by Arnon et al. [75]; and Barreira et al. [6]. who used the method proposed by Nagata and Yamashita [76].

Plant Species	Solvent	Method	Concentration	Unit	Ref.
Arthrocnemum macrostachyum	Acetone/hexane	Spectrophotometry	28.3 ± 7.1	mg/100 gDM	[6]
Aster tripolium	Ethanol		~51	mg/cm²	[68]
Halimione portulacoides	Acetone		95.04 ± 23.47	µg/gFW	[67]
Mesembryanthemum nodiflorum	Methanol		~280	mg/100 gDM	[32]
Salicornia bigelovii	Acetone		569.1 ± 9.10	mg/kgFW	[22]
Salicornia brachiata	Acetone		746.5 ± 88.2	µg/gDM	[77]
Salicornia europaea	Acetone		~1.25	mg/gFW	[73]
Salicornia neei	Methanol	HPLC	233.3 ± 42.5	µg/gDM	[78]
Salicornia persica	Acetone	Spectrophotometry	~1.21	mg/gFW	[73]
	Acetone		~325	µg/gFW	[63]
Salicornia prostrata	Acetone		~0.14	mg/gFW	[79]
Salicornia ramosissima	Acetone/hexane		21.56 ± 3.45	mg/100 gDM	[6]
Sarcocornia fruticosa	Methanol		~290	mg/100 gDM	[32]
	Acetone		102.01 ± 18.23	µg/gFW	[67]
	Acetone		~350	µg/gFW	[63]
Sarcocornia perennis	Acetone/hexane		14.78 ± 2.33	mg/100 gDM	[6]
Suaeda maritima	Methanol		~280	mg/100 gDM	[32]
Suaeda prostrata	Acetone		~0.08	mg/gFW	[79]
Tamarix gallica	Acetone		~180	mg	[70]

6. Carotenoids

Carotenoids, which can be divided into carotenes (e.g., β-carotene and lycopene) and xanthophylls (e.g., lutein and zeaxanthin), are potent antioxidants and have various functions in human health, such as pro-vitamin A activity, cancer-preventing properties, and improvements in cognitive function as well as eye and cardiovascular health [8,11]. Immunomodulation activities and prevention of degenerative diseases have also been reported as possible health benefits of carotenoids [80]. In plants, these yellow and orange pigments take part in photosynthesis by protecting photosystems [78].

The level of total carotenoids is often approximated by measuring the absorbance of the acetone extract with a 470 nm wavelength. Conversion calculations depend on the solvent used and whether the calculation focused on the amount of chlorophyll in the extract. Some protocols describing the determination of chlorophyll also include the determination of total carotenoids [69,76]. The total carotenoid content measured in the biomass of various halophytes are summarised in Table 4.

Table 4. Total carotenoid content measured from halophyte biomass. All studies measured the absorbance with 470 nm, except Qasim et al. [34], who used the method by Duxbury and Yentsch [81]; Ventura et al. [63], who used the method by Ben-Amotz et al. [82]; Barreira et al. [26], who used the same method as Uslu et al. [83]; and Kumari et al. [84] and Rangani et al. [85] who used N, N–dimethylformamide (DMF) as a solvent with the method by Chamovitz et al. [86]. The conversion calculations may differ.

Plant Species	Solvent	Method	Concentration	Unit	Ref.
Arthrocnemum macrostachyum	Acetone	Spectrophotometry	210 ± 10	mg/100 gDM	[6]
Aster tripolium	Ethanol		~8	mg/cm^2	[68]
Halimione portulacoides	Acetone		51.47 ± 17.76	µg/gFW	[67]
Ipomoea pes-caprae	Acetone (90%)		0.61 ± 0.01	mg/g	[34]
Mesembryanthemum nodiflorum	Methanol		~21	mg/100 gDM	[32]
Pluchea lanceolata	Acetone (90%)		0.07 ± 0.02	mg/g	[34]
Salicornia bigelovii	Hexane		159.0 ± 5.74	mg/kgFW	[22]
Salicornia brachiata	Acetone		433.8 ± 46.0	µg/gDM	[77]
Salicornia europaea	Acetone		~0.43	mg/gFW	[73]
Salicornia neei	Methanol	HPLC	28.71 ± 7.52	µg/gDM	[78]
Salicornia persica	Acetone	Spectrophotometry	~0.44	mg/gFW	[73]
	Acetone		54.5	µg/gFW	[63]
Salicornia prostrata	Acetone		~0.25	mg/gFW	[79]
Salicornia ramosissima	Ethyl acetate	HPLC	3.49	mg/100 gFW	[5]
	Acetone	Spectrophotometry	290 ± 20	mg/100 gDM	[6]
Salvadora persica	Acetone (90%)		0.84 ± 0.02	mg/g	[34]
	DMF		11.4 ± 2.5	µg/100 gDM	[84]
Sarcocornia fruticosa	Methanol		~16	mg/100 gDM	[32]
	Acetone		56.6	µg/gFW	[63]
	Acetone		34.76 ± 9.03	µg/gFW	[67]
Sarcocornia perennis	Acetone		280 ± 10	mg/100 gDM	[6]
Suaeda fruticosa	Acetone (90%)		0.56 ± 0.01	mg/g	[34]
Suaeda maritima	Methanol		~12	mg/100 gDM	[32]
Suaeda prostrata	Acetone		~0.19	mg/gFW	[79]
Tamarix gallica	Acetone		~70	mg	[70]
Thespesia populnea	Acetone (90%)		0.72 ± 0.02	mg/g	[34]
	DMF		233.5 ± 5.3	mg/100 gDM	[85]

De Souza et al. [78] determined the detailed pigment content of *Salicornia neei* using ultrasound-assisted methanol extraction and the HPLC method proposed by Mendes et al. [72]. They found the β-carotene and the total xanthophyll content of *Salicornia neei* biomass to be 0.99 ± 0.41 µg/gDM and 27.72 ± 7.12 µg/gDM, respectively. Additionally, Lima et al. [5] used HPLC with a reverse-phase RP-18 column and PDA in several wavelengths for the determination of pigments and vitamins from *Salicornia ramosissima* biomass and found 2.37 ± 0.12 mg/100gFW and 1.12 ± 0.05 mg/100gFW of β-carotene and lutein, respectively. Alongside measuring the total carotenoids using a spectrophotometric method, Castañeda-Loaiza et al. [32] determined the amount of carotenes in the methanol extracts using HPLC with a RP-18 column and a PDA in 450 nm, finding *Sarcocornia fruticosa*, *Suaeda maritima*, and *Mesembryanthemum nodiflorum* to be good sources of lutein (8.89–19.7 mg/100 gDM) and latter two also good sources of β-carotene (9.30–20.7 mg/100 gDW).

Duarte et al. [67] determined the carotenoid content of *Halimone portulacoides* and *Sarcocornia fruticosa* and found that concentrations of β-carotene (7.60 ± 2.06 µg/gFW and 5.53 ± 0.94 µg/gFW, respectively), lutein (17.09 ± 6.04 µg/gFW and 14.93 ± 3.91 µg/gFW, respectively) and other xanthophylls (26.78 ± 9.66 µg/gFW and 14.09 ± 4.18 µg/gFW, respectively) decreased when the plants were exposed to salt stress due to lengthened period of relatively high temperatures in harvest region. The opposite effect was observed in salt-stressed *Salicornia ramosissima* in the study by Lima et al. [5], which suggests the higher carotenoid content in biomass may be caused by the increased antioxidant capacity to protect photosystems from photo-oxidation.

7. Vitamins

Vitamins are essential nutrients to ensure average growth and human health. They are a diverse group of organic compounds, classified either as water-soluble (vitamins B and C) or fat-soluble (vitamins A, D, E, and K) [80]. Vitamins have various biochemical roles, and they are already highly commercialised as nutraceutical and functional food additives [80]. Vegetables, fruits, unrefined cereals, seeds, and nuts are key sources of vitamins for humans [87].

Compared to other studied compounds, a large variety of methods are used to analyse vitamin concentrations. Hexane and ethyl acetate are commonly used solvents for the extraction of lipid-soluble vitamins. A wider variety of solvents are used for the extraction of water-soluble compounds. Castañeda-Loaiza et al. [32] and Lima et al. [5] use the extraction and analytical methods proposed by Santos et al. [88] for fat-soluble and water-soluble vitamins. Various HPLC methods involving a photodiode array detector (PDA) or fluorescence detector (FL) are widely used; only Chamkouri et al. [89] use a DAD and Zaier et al. [90] an ultraviolet (UV) detector in their analytical methods.

Ascorbic acid (vitamin C) is an essential nutrient for humans and other animals. Humans cannot synthesise vitamin C, and deficiency due to insufficient intake can cause scurvy disease [80,87]. It has also been reported to help maintain the integrity of cellular membranes and improve skin health [84,85]. It is a co-factor for enzyme reactions and a potent antioxidant which has been shown to have a preventive effect on various chronic conditions [91]. It has also been reported to improve skin health [84,85].

Ascorbic acid degrades relatively fast, and a study by Lu et al. [22] showed a gradual decrease in the ascorbic acid content of *Salicornia bigelovii* during storage: 56% of ascorbic acid was lost after eight days at 0 °C. The succulent *Amaranthaceae* family halophytes *Arthrocnemum indicum*, *Halocnemum strobilaceum*, and *Salicornia bigelovii* have been found to be rich in ascorbic acid [22,90]. Kumari et al. [84] also found considerably high amounts of vitamin C from *Salvadora persica* fruit. Rangani et al. [85] also suggest that the ascorbic acid content in *Thespesia populnea* leaves is enough to fulfil the daily requirements of a healthy individual.

Compounds considered as B complex vitamins are antioxidants and essential co-enzymes, supporting vital biological processes [80,89]. For example, thiamine (B1) is

essential to carbohydrate metabolism, pyridoxine (B6) to protein metabolism, folate (B9) to the synthesis of nucleic acids, and cobalamin (B12) for the nervous system, cell growth, and bone health [5,89].

Chamkouri et al. [89] developed an optimised analytical method to measure the vitamin in B complex in halophyte extracts and suggested that *Suaeda aegyptiaca* and *Suaeda vera* may be promising sources for the production of health-beneficial products for food and pharmaceuticals. *Suaeda aegyptiaca*, in particular, was found to be a significant source of cobalamin [89], with concentrations being higher than in sea buckthorn [92], making it desirable for people following a strictly plant-based diet and who are at risk of vitamin B12 deficiency. The amounts of water-soluble vitamins (vitamin C and B complex vitamins) found in the biomass of various halophytes are summarised in Table 5.

Table 5. Ascorbic acid (vitamin C) and thiamine, pyridoxine, folate, and cobalamin (vitamin B1, B6, B9, and B12, respectively) concentrations measured in halophyte biomasses. The analysed vitamin is marked in parentheses after its concentration.

Plant Species	Solvent	Method	Concentration	Unit	Ref.
Arthrocnemum indicum	m-Phosphoric acid (4.5%)	HPLC-UV	19.17 ± 0.50 (C)	mg/100 gFW	[90]
Halocnemum strobilaceum	m-Phosphoric acid (4.5%)		7.38 ± 0.54 (C)	mg/100 gFW	
Mesembryanthemum nodiflorum	Ammonium acetate/methanol (50:50)	HPLC-PDA	~0.5 (C)	g/100 gDM	[32]
			~24 (B6)	mg/100 gDM	
Salicornia bigelovii	Oxalic acid solution	Indophenol titration	58.4 ± 1.39 (C)	mg/kgFW	[22]
Salicornia ramosissima	Ammonium acetate/methanol (50:50)	HPLC-PDA	30.4 ± 2.0 (B1)	µg/100 gFW	[5]
			2.6 ± 0.1 (B6)	µg/100 gFW	
Salvadora persica	Trichloroacetic acid (6%)	Spectrophotometry	68.0 ± 15.9 (C)	mg/100 gDM	[84]
Sarcocornia fruticosa	Ammonium acetate/methanol (50:50)	HPLC-PDA	~1.0 (C)	g/100 gDM	[32]
			~10 (B6)	mg/100 gDM	
Suaeda aegyptiaca	Methanol	HPLC-DAD	181 ± 2.3 (B6)	mg/kg	[89]
			118 ± 2.2 (B9)	mg/kg	
			466 ± 2.5 (B12)	mg/kg	
Suaeda fruticosa	m-Phosphoric acid (4.5%)	HPLC-UV	2.46 ± 0.07 (C)	mg/100 gFW	[90]
Suaeda maritima	Ammonium acetate/methanol (50:50)	HPLC-PDA	~3 (C)	g/100 gDM	[32]
			~9.5 (B6)	mg/100 gDM	
Suaeda vera	Methanol	HPLC-DAD	102 ± 1.4 (B6)	mg/kg	[89]
			118 ± 2.2 (B9)	mg/kg	
			271 ± 1.7 (B12)	mg/kg	
Thespesia populnea	Trichloroacetic acid (6%)	Spectrophotometry	44.3 ± 5.5 (C)	mg/100 gDM	[85]

Tocopherols (vitamin E) are lipid-soluble antioxidants, and their radical scavenging activity protects cell membranes, lipoproteins, and other molecules against lipid peroxidation caused by oxidative stress [6,11]. Improved cardiovascular health, prevention of degenerative disorders, and anti-cancer properties are also reported as potential health benefits of tocopherols [80]. Tocopherols are present in different isomers, α-tocopherol being reported as the most abundant in halophyte biomasses [11].

Ellouzi et al. [93] studied the effect of salt stress on the α-tocopherol content of glycophyte (*Arabidopsis thaliana*) and halophyte (*Cakile maritima*) species. They found the vitamin E content of glycophytes decreases by 50% soon after being exposed to salt, whereas the vitamin levels of halophyte plants remained significantly higher throughout the cultivation period. Barreira et al. [6] found high concentrations of α-tocopherol in *Arthrocnemum macrostachyum* shoots, making it a good source of vitamin E, similarly to other green vegetables, such as kale and broccoli. The concentration of α-tocopherol measured in halophyte biomasses is summarised in Table 6. Significant variations in vitamin E content are possible

between species within the same genus, which can be seen in the reported results, for example, in *Sarcocornia* and *Suaeda* species.

Table 6. α-Tocopherol (vitamin E) concentrations measured from halophyte biomasses.

Plant Species	Solvent	Method	Concentration	Unit	Ref.
Arthrocnemum indicum	Hexane	HPLC-FL	2.12 ± 0.01	mg/100 gFW	[39]
Arthrocnemum macrostachyum	Hexane		8.74 ± 0.19	mg/100 gDM	[6]
Cakile maritima	Methanol		~200	µg/gDM	[93]
Halocnemum strobilaceum	Hexane		3.35 ± 0.08	mg/100 gFW	[90]
Mesembryanthemum nodiflorum	Ethyl acetate	HPLC-PDA	Not detected		[32]
Salicornia ramosissima	Ethyl acetate		241 ± 10	µg/100 gFW	[5]
	Hexane	HPLC-FL	1.14 ± 0.07	mg/100 gDM	[6]
Sarcocornia fruticosa	Ethyl acetate	HPLC-PDA	~18	mg/100 gDM	[32]
Sarcocornia perennis	Hexane	HPLC-FL	1.11 ± 0.10	mg/100 gDM	[6]
Suaeda fruticosa	Hexane		11.42 ± 0.14	mg/100 gFW	[90]
Suaeda matritima	Ethyl acetate	HPLC-PDA	~12.5	mg/100 gDM	[32]
Teucrium alopecurus	Hexane	HPLC-FL	316.25	mg/kg	[94]
Teucrium polium	Hexane		277.25	mg/kg	[94]
Teucrium nabli	Hexane		296.04	mg/kg	[94]

Concerning another fat-soluble compound, retinyl acetate (vitamin A), Castañeda-Loaiza et al. [32] found concentrations of 4.51 mg/100gDM and 5.39 mg/100gDM in *Mesembryanthemum nodiflorum* and *Suaeda matritima*, respectively. No other studies were found to measure the vitamin A content in halophytes. However, the most important plant-based vitamin A intake for humans comes from the pro-vitamin A activity of carotenoids, mainly β-carotene [95].

Changes in environmental conditions influence plants, and some halophytes exhibit changes in their colour as an adaptation to the stress caused by high UV radiation, temperature, or salinity, factors which also affect the plants' chemical composition, as shown by Zaier et al. [90]. Studies have shown that the phenotypic stage (green or red–purple) has a significant effect on the ascorbic acid content of *Suaeda fruticosa* and the α-tocopherol content of *Suaeda fruticosa* and *Arthocnemum indicum* [90]. As the red–purple phenotype has a lower concentration of these vitamins [90], the possible loss of health-beneficial compounds should be considered when planning the cultivation and harvest of halophytes. Lima et al. [5] studied the effect of cultivation salinity on the nutritional composition of *Salicornia ramosissima* and reported that higher salinity led to a significant increase in thiamine content but a decrease in pyridoxine and α-tocopherol content.

8. Potential Applications

As suggested in Figure 1, potential applications for botanical extracts obtained from biorefinery could include bio-functional feed and nutraceuticals, cosmetics, and even biomedicines. This is due to various health-beneficial properties and bioactivities of the compounds found in such extracts. Halophytes can provide a healthy and nutritious food source, but botanical extracts could also be utilized in bio-functional food and nutraceuticals [6,22,23,32]. Sufficient consumption of phytochemicals, especially phenolic compounds, has been shown to reduce oxidative stress and inflammation, which helps prevent diabetes, cardiovascular diseases, and neurodegenerative diseases [27,29,34,56]. The state-of-the-art literature review concerning the bioactive secondary metabolites in halophytes shows that many species are rich in these protective compounds and vitamins, which are essential nutrients for human health.

Botanical extracts are commonly used as cosmetics ingredients, and the potential of halophyte extracts for the cosmetics industry has been reported [2,3,11,39,96]. Extracts are often used in cosmetics due to their antioxidant activity, but anti-ageing, photoprotective, and skin-whitening properties of halophyte extracts have also been reported [61,96–99].

In some applications, such as utilizing specific pharmacological compounds, it is desirable to isolate specific compounds from botanical extracts. This can be done with filtration or serial solvent extractions when the characteristics of the targeted compounds are known [100], and some of the commercialized plant extracts used for pharmacological purposes have a high market value [101]. However, in plant extracts, various compounds found in the matrix work together and single compounds are rarely responsible for the bioactivity of the botanical extract [100]. Anti-inflammatory effects, radical-scavenging activity, and cytotoxicity have made halophyte extracts interesting for pharmaceutical applications [2,13,41,102,103]. Many studies have also reported the antimicrobial effect of halophyte extracts against pathogenic strains, making them interesting for the biomedicine industry [2,11,35,40,55,61,104].

9. Conclusions

The studies reviewed here show that halophyte biomasses have the potential for nutrient-rich food production in salt-affected areas as well as production of nutraceuticals and ingredients for cosmetics and pharmaceutical industries. Many studies concern the nutritional profile of fresh plants.

The food harvest period for succulent halophyte tips is short, as the shrubs lignify and turn woodier as they mature, making them unpleasant to eat. Leftover shrubs are usually considered agricultural waste. Therefore, to minimise the competition with food resources, it could be desirable to analyse these partly lignified, non-food grade but still-succulent remains for their potential for producing nutraceuticals, bio-functional feed, and other high-value compounds.

Exploring new biomasses and the repurposing of remnants previously seen as waste could help identify new bio-derived sources for valuable compounds and improve the sustainability and economic feasibility of existing production systems. In addition, utilising salt-tolerant plants could bring more value to salt-affected areas which are currently unsuitable for farming and considered marginal lands.

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References

1. EIP-AGRI Focus Group Soil Salinisation: Final report. Publisher EIP Agri. 2020. Available online: https://ec.europa.eu/eip/agriculture/en/publications/eip-agri-focus-group-soil-salinisation-final (accessed on 3 January 2022).
2. Giordano, R.; Saii, Z.; Fredsgaard, M.; Hulkko, L.S.S.; Poulsen, T.B.G.; Thomsen, M.E.; Henneberg, N.; Zucolotto, S.M.; Arendt-Nielsen, L.; Papenbrock, J.; et al. Pharmacological insights into halophyte bioactive extract action on anti-inflammatory, pain relief and antibiotics-type mechanisms. *Molecules* **2021**, *26*, 3140. [CrossRef]
3. Lopes, A.; Rodrigues, M.J.; Pereira, C.; Oliveira, M.; Barreira, L.; Varela, J.; Trampetti, F.; Custódio, L. Natural products from extreme marine environments: Searching for potential industrial uses within extremophile plants. *Ind. Crops Prod.* **2016**, *94*, 299–307. [CrossRef]
4. Falleh, H.; Ksouri, R.; Medini, F.; Guyot, S.; Abdelly, C.; Magné, C. Antioxidant activity and phenolic composition of the medicinal and edible halophyte *Mesembryanthemum edule* L. *Ind. Crops Prod.* **2011**, *34*, 1066–1071. [CrossRef]

5. Lima, A.R.; Castañeda-Loaiza, V.; Salazar, M.; Nunes, C.; Quintas, C.; Gama, F.; Pestana, M.; Correia, P.J.; Santos, T.; Varela, J.; et al. Influence of cultivation salinity in the nutritional composition, antioxidant capacity and microbial quality of Salicornia ramosissima commercially produced in soilless systems. *Food Chem.* **2020**, *333*, 127525. [CrossRef]
6. Barreira, L.; Resek, E.; Rodrigues, M.J.; Rocha, M.I.; Pereira, H.; Bandarra, N.; da Silva, M.M.; Varela, J.; Custódio, L. Halophytes: Gourmet food with nutritional health benefits? *J. Food Compos. Anal.* **2017**, *59*, 35–42. [CrossRef]
7. Rauf, A.; Imran, M.; Abu-Izneid, T.; Iahtisham-Ul-Haq; Patel, S.; Pan, X.; Naz, S.; Sanches Silva, A.; Saeed, F.; Rasul Suleria, H.A. Proanthocyanidins: A comprehensive review. *Biomed. Pharmacother* **2019**, *116*, 108999. [CrossRef] [PubMed]
8. Eggersdorfer, M.; Wyss, A. Carotenoids in human nutrition and health. *Arch. Biochem. Biophys.* **2018**, *652*, 18–26. [CrossRef] [PubMed]
9. Cybulska, I.; Brudecki, G.P.; Brown, J.J.; Hulkko, L.S.S.; Al Hosani, S.; Hedegaard Thomsen, M. Comparative study of chemical composition of the halophyte species native to the Persian (Arabian) gulf. *BioResources* **2021**, *16*, 5524–5537. [CrossRef]
10. Ksouri, R.; Megdiche, W.; Falleh, H.; Trabelsi, N.; Boulaaba, M.; Smaoui, A.; Abdelly, C. Influence of biological, environmental and technical factors on phenolic content and antioxidant activities of Tunisian halophytes. *Comptes Rendus Biol.* **2008**, *331*, 865–873. [CrossRef]
11. Ksouri, R.; Ksouri, W.M.; Jallali, I.; Debez, A.; Magné, C.; Hiroko, I.; Abdelly, C. Medicinal halophytes: Potent source of health promoting biomolecules with medical, nutraceutical and food applications. *Crit. Rev. Biotechnol.* **2012**, *32*, 289–326. [CrossRef] [PubMed]
12. Chaturvedi, T.; Christiansen, A.H.C.; Gołębiewska, I.; Thomsen, M.H. Salicornia Species: Current Status and Future Potential. In *Future of Sustainable Agriculture in Saline Environments*; CRC Press: Boca Raton, FL, USA, 2021; pp. 461–482.
13. Cybulska, I.; Brudecki, G.P.; Alassali, A.; Thomsen, M.H.; Brown, J.J. Phytochemical composition of some common coastal halophytes of the United Arab Emirates. *Emirates J. Food Agric.* **2014**, *26*, 1046–1056. [CrossRef]
14. Abdal, M.S. Salicornia production in Kuwait. *World Appl. Sci. J.* **2009**, *6*, 1033–1038.
15. Belal, I.E.H.; Al-Dosari, M. Replacement of Fish Meal with Salicornia Meal in Feeds for Nile Tilapia Oreochromis niloticus. *J. World Aquac. Soc.* **1999**, *30*, 285–289. [CrossRef]
16. Cybulska, I.; Chaturvedi, T.; Brudecki, G.P.; Kádár, Z.; Meyer, A.S.; Baldwin, R.M.; Thomsen, M.H. Chemical characterization and hydrothermal pretreatment of Salicornia bigelovii straw for enhanced enzymatic hydrolysis and bioethanol potential. *Bioresour. Technol.* **2014**, *153*, 165–172. [CrossRef] [PubMed]
17. Turcios, A.E.; Weichgrebe, D.; Papenbrock, J. Effect of salt and sodium concentration on the anaerobic methanisation of the halophyte Tripolium pannonicum. *Biomass Bioenergy* **2016**, *87*, 69–77. [CrossRef]
18. Bañuelos, J.A.; Velázquez-Hernández, I.; Guerra-Balcázar, M.; Arjona, N. Production, characterization and evaluation of the energetic capability of bioethanol from Salicornia Bigelovii as a renewable energy source. *Renew. Energy* **2018**, *123*, 125–134. [CrossRef]
19. Alassali, A.; Cybulska, I.; Galvan, A.R.; Thomsen, M.H. Wet fractionation of the succulent halophyte Salicornia sinus-persica, with the aim of low input (water saving) biorefining into bioethanol. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 1769–1779. [CrossRef]
20. Cybulska, I.; Chaturvedi, T.; Alassali, A.; Brudecki, G.P.; Brown, J.J.; Sgouridis, S.; Thomsen, M.H. Characterization of the chemical composition of the halophyte salicornia bigelovii under cultivation. *Energy Fuels* **2014**, *28*, 3873–3883. [CrossRef]
21. Martins-Noguerol, R.; Cambrollé, J.; Mancilla-Leytón, J.M.; Puerto-Marchena, A.; Muñoz-Vallés, S.; Millán-Linares, M.C.; Millán, F.; Martínez-Force, E.; Figueroa, M.E.; Pedroche, J.; et al. Influence of soil salinity on the protein and fatty acid composition of the edible halophyte Halimione portulacoides. *Food Chem.* **2021**, *352*, 129370. [CrossRef]
22. Lu, D.; Zhang, M.; Wang, S.; Cai, J.; Zhou, X.; Zhu, C. Nutritional characterization and changes in quality of Salicornia bigelovii Torr. during storage. *LWT Food Sci. Technol.* **2010**, *43*, 519–524. [CrossRef]
23. Agudelo, A.; Carvajal, M.; del Martinez-Ballesta, M.C. Halophytes of the Mediterranean Basin—Underutilized Species with the Potential to Be Nutritious Crops in the Scenario of the Climate Change. *Foods* **2021**, *10*, 119. [CrossRef]
24. Christiansen, A.H.C.; Lyra, D.A.; Jørgensen, H. Increasing the value of Salicornia bigelovii green biomass grown in a desert environment through biorefining. *Ind. Crops Prod.* **2020**, 113105. [CrossRef]
25. Thomsen, M.H.; Alassali, A.; Cybulska, I.; Yousef, A.F.; Brown, J.J.; Andersen, M.; Ratkov, A.; Kiel, P. *Microorganisms for Biorefining of Green Biomass*; Springer: Berlin/Heidelberg, Germany, 2015; pp. 157–181.
26. De Hoyos-Martínez, P.L.; Merle, J.; Labidi, J.; Charrier-El Bouhtoury, F. Tannins extraction: A key point for their valorization and cleaner production. *J. Clean. Prod.* **2019**, *206*, 1138–1155. [CrossRef]
27. Kruger, M.J.; Davies, N.; Myburgh, K.H.; Lecour, S. Proanthocyanidins, anthocyanins and cardiovascular diseases. *Food Res. Int.* **2014**, *59*, 41–52. [CrossRef]
28. Falleh, H.; Ksouri, R.; Boulaaba, M.; Guyot, S.; Abdelly, C.; Magne, C. Phenolic nature, occurrence and polymerization degree as marker of environmental adaptation in the edible halophyte Mesembryanthemum edule. *S. Afr. J. Bot.* **2012**, *79*, 117. [CrossRef]
29. Smeriglio, A.; Barreca, D.; Bellocco, E.; Trombetta, D. Proanthocyanidins and hydrolysable tannins: Occurrence, dietary intake and pharmacological effects. *Br. J. Pharmacol.* **2017**, *174*, 1244–1262. [CrossRef] [PubMed]
30. Sun, B.; Ricardo-da-Silva, J.M.; Spranger, I. Critical Factors of Vanillin Assay for Catechins and Proanthocyanidins. *J. Agric. Food Chem.* **1998**, *46*, 4267–4274. [CrossRef]

31. Chekroun-Bechlaghem, N.; Belyagoubi-Benhammou, N.; Belyagoubi, L.; Gismondi, A.; Nanni, V.; Di Marco, G.; Canuti, L.; Canini, A.; El Haci, I.A.; Atik Bekkara, F. Phytochemical analysis and antioxidant activity of *Tamarix africana*, *Arthrocnemum macrostachyum* and *Suaeda fruticosa*, three halophyte species from Algeria. *Plant Biosyst. Int. J. Deal. Asp. Plant Biol.* **2019**, *153*, 843–852. [CrossRef]
32. Castañeda-Loaiza, V.; Oliveira, M.; Santos, T.; Schüler, L.; Lima, A.R.; Gama, F.; Salazar, M.; Neng, N.R.; Nogueira, J.M.F.; Varela, J.; et al. Wild vs cultivated halophytes: Nutritional and functional differences. *Food Chem.* **2020**, *333*, 127536. [CrossRef]
33. Castañeda-Loaiza, V.; Placines, C.; Rodrigues, M.J.; Pereira, C.; Zengin, G.; Uysal, A.; Jeko, J.; Cziáky, Z.; Reis, C.P.; Gaspar, M.M.; et al. If you cannot beat them, join them: Exploring the fruits of the invasive species Carpobrotus edulis (L.) N.E. Br as a source of bioactive products. *Ind. Crops Prod.* **2020**, *144*, 112005. [CrossRef]
34. Qasim, M.; Abideen, Z.; Adnan, M.Y.; Gulzar, S.; Gul, B.; Rasheed, M.; Khan, M.A. Antioxidant properties, phenolic composition, bioactive compounds and nutritive value of medicinal halophytes commonly used as herbal teas. *S. Afr. J. Bot.* **2017**, *110*, 240–250. [CrossRef]
35. Ksouri, R.; Falleh, H.; Megdiche, W.; Trabelsi, N.; Mhamdi, B.; Chaieb, K.; Bakrouf, A.; Magné, C.; Abdelly, C. Antioxidant and antimicrobial activities of the edible medicinal halophyte Tamarix gallica L. and related polyphenolic constituents. *Food Chem. Toxicol.* **2009**, *47*, 2083–2091. [CrossRef]
36. Julkunen-Tiitto, R. Phenolic Constituents in the Leaves of Northern Willows: Methods for the Analysis of Certain Phenolics. *J. Agric. Food Chem.* **1985**, *33*, 213–217. [CrossRef]
37. Houta, O.; Neffati, M.; Amri, H. Phenolic Contents and Antioxidant Potential of Cakile maritimum. *J. Biol. Act. Prod. Nat.* **2012**, *2*, 387–391. [CrossRef]
38. Houta, O.; Akrout, A.; Amri, H. Phenolic amounts, antioxidant and antimicrobial potential of Crithmum maritimum cultivated in Tunisian arid zones. *Planta Med.* **2011**, *77*, 1416. [CrossRef]
39. Jallali, I.; Zaouali, Y.; Missaoui, I.; Smeoui, A.; Abdelly, C.; Ksouri, R. Variability of antioxidant and antibacterial effects of essential oils and acetonic extracts of two edible halophytes: *Crithmum maritimum* L. and *Inula crithmoides* L. *Food Chem.* **2014**, *145*, 1031–1038. [CrossRef]
40. Medini, F.; Fellah, H.; Ksouri, R.; Abdelly, C. Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant Limonium delicatulum. *J. Taibah Univ. Sci.* **2014**, *8*, 216–224. [CrossRef]
41. Karker, M.; Falleh, H.; Msaada, K.; Smaoui, A.; Abdelly, C.; Legault, J.; Ksouri, R. Antioxidant, Anti-inflammatory and anticancer activities of the medicinal halophyte Reaumuria vermiculata. *EXCLI J.* **2016**, *15*, 297–307. [CrossRef]
42. Mariem, S.; Hanen, F.; Inès, J.; Mejdi, S.; Riadh, K. Phenolic profile, biological activities and fraction analysis of the medicinal halophyte Retama raetam. *S. Afr. J. Bot.* **2014**, *94*, 114–121. [CrossRef]
43. Boulaaba, M.; Medini, F.; Hajlaoui, H.; Mkadmini, K.; Falleh, H.; Ksouri, R.; Isoda, H.; Smaoui, A.; Abdelly, C. Biological activities and phytochemical analysis of phenolic extracts from Salsola kali L. Role of endogenous factors in the selection of the best plant extracts. *S. Afr. J. Bot.* **2019**, *123*, 193–199. [CrossRef]
44. Gargouri, M.; Magné, C.; Dauvergne, X.; Ksouri, R.; El Feki, A.; Metges, M.A.G.; Talarmin, H. Cytoprotective and antioxidant effects of the edible halophyte Sarcocornia perennis L. (swampfire) against lead-induced toxicity in renal cells. *Ecotoxicol. Environ. Saf.* **2013**, *95*, 44–51. [CrossRef] [PubMed]
45. Oueslati, S.; Ksouri, R.; Falleh, H.; Pichette, A.; Abdelly, C.; Legault, J. Phenolic content, antioxidant, anti-inflammatory and anticancer activities of the edible halophyte Suaeda fruticosa Forssk. *Food Chem.* **2012**, *132*, 943–947. [CrossRef]
46. Khoddami, A.; Wilkes, M.; Roberts, T. Techniques for Analysis of Plant Phenolic Compounds. *Molecules* **2013**, *18*, 2328–2375. [CrossRef]
47. Singleton, V.L.; Rossi, J.A. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enol. Vitic.* **1965**, *16*.
48. Dewanto, V.; Xianzhong, W.; Adom, K.K.; Liu, R.H. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.* **2002**, *50*, 3010–3014. [CrossRef] [PubMed]
49. Stanković, M.S.; Petrović, M.; Godjevac, D.; Stevanović, Z.D. Screening inland halophytes from the central balkan for their antioxidant activity in relation to total phenolic compounds and flavonoids: Are there any prospective medicinal plants? *J. Arid Environ.* **2015**, *120*, 26–32. [CrossRef]
50. Rodrigues, M.J.; Soszynski, A.; Martins, A.; Rauter, A.P.; Neng, N.R.; Nogueira, J.M.F.; Varela, J.; Barreira, L.; Custódio, L. Unravelling the antioxidant potential and the phenolic composition of different anatomical organs of the marine halophyte Limonium algarvense. *Ind. Crops Prod.* **2015**, *77*, 315–322. [CrossRef]
51. Pereira, C.G.; Barreira, L.; da Rosa Neng, N.; Nogueira, J.M.F.; Marques, C.; Santos, T.F.; Varela, J.; Custódio, L. Searching for new sources of innovative products for the food industry within halophyte aromatic plants: In vitro antioxidant activity and phenolic and mineral contents of infusions and decoctions of *Crithmum maritimum* L. *Food Chem. Toxicol.* **2017**, *107*, 581–589. [CrossRef]
52. Zengin, G.; Aumeeruddy-Elalfi, Z.; Mollica, A.; Yilmaz, M.A.; Mahomoodally, M.F. In vitro and in silico perspectives on biological and phytochemical profile of three halophyte species—A source of innovative phytopharmaceuticals from nature. *Phytomedicine* **2018**, *38*, 35–44. [CrossRef]
53. Mohammed, H.A.; Ali, H.M.; Qureshi, K.A.; Alsharidah, M.; Kandil, Y.I.; Said, R.; Mohammed, S.A.A.; Al-Omar, M.S.; Al Rugaie, O.; Abdellatif, A.A.H.; et al. Comparative Phytochemical Profile and Biological Activity of Four Major Medicinal Halophytes from Qassim Flora. *Plants* **2021**, *10*, 2208. [CrossRef]

54. Do, Q.D.; Angkawijaya, A.E.; Tran-Nguyen, P.L.; Huynh, L.H.; Soetaredjo, F.E.; Ismadji, S.; Ju, Y.H. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. *J. Food Drug Anal.* **2014**, *22*, 296–302. [CrossRef]
55. Trabelsi, N.; Megdiche, W.; Ksouri, R.; Falleh, H.; Oueslati, S.; Soumaya, B.; Hajlaoui, H.; Abdelly, C. Solvent effects on phenolic contents and biological activities of the halophyte Limoniastrum monopetalum leaves. *LWT Food Sci. Technol.* **2010**, *43*, 632–639. [CrossRef]
56. Medini, F.; Bourgou, S.; Lalancette, K.G.; Snoussi, M.; Mkadmini, K.; Coté, I.; Abdelly, C.; Legault, J.; Ksouri, R. Phytochemical analysis, antioxidant, anti-inflammatory, and anticancer activities of the halophyte Limonium densiflorum extracts on human cell lines and murine macrophages. *S. Afr. J. Bot.* **2015**, *99*, 158–164. [CrossRef]
57. Padalino, L.; Costa, C.; Del Nobile, M.A.; Conte, A. Extract of Salicornia europaea in fresh pasta to enhance phenolic compounds and antioxidant activity. *Int. J. Food Sci. Technol.* **2019**, *54*, 3051–3057. [CrossRef]
58. Silva, A.M.; Lago, J.P.; Pinto, D.; Moreira, M.M.; Grosso, C.; Cruz Fernandes, V.; Delerue-Matos, C.; Rodrigues, F. Salicornia ramosissima Bioactive Composition and Safety: Eco-Friendly Extractions Approach (Microwave-Assisted Extraction vs. Conventional Maceration). *Appl. Sci.* **2021**, *11*, 4744. [CrossRef]
59. Saleem, H.; Khurshid, U.; Sarfraz, M.; Tousif, M.I.; Alamri, A.; Anwar, S.; Alamri, A.; Ahmad, I.; Abdallah, H.H.; Mahomoodally, F.M.; et al. A comprehensive phytochemical, biological, toxicological and molecular docking evaluation of Suaeda fruticosa (L.) Forssk.: An edible halophyte medicinal plant. *Food Chem. Toxicol.* **2021**, *154*, 112348. [CrossRef] [PubMed]
60. Saleem, H.; Zengin, G.; Locatelli, M.; Ahmad, I.; Khaliq, S.; Mahomoodally, M.F.; Hussain, R.; Rengasamy, K.R.R.; Mollica, A.; Zainal Abidin, S.A.; et al. Pharmacological, phytochemical and in-vivo toxicological perspectives of a xero-halophyte medicinal plant: Zaleya pentandra (L.) Jeffrey. *Food Chem. Toxicol.* **2019**, *131*, 110535. [CrossRef]
61. Lopes, M.; Sanches-Silva, A.; Castilho, M.; Cavaleiro, C.; Ramos, F. Halophytes as source of bioactive phenolic compounds and their potential applications. *Crit. Rev. Food Sci. Nutr.* **2021**. [CrossRef] [PubMed]
62. Jdey, A.; Falleh, H.; Ben Jannet, S.; Mkadmini Hammi, K.; Dauvergne, X.; Ksouri, R.; Magné, C. Phytochemical investigation and antioxidant, antibacterial and anti-tyrosinase performances of six medicinal halophytes.*S. Afr. J. Bot.* **2017**, *112*, 508–514. [CrossRef]
63. Ventura, Y.; Wuddineh, W.A.; Myrzabayeva, M.; Alikulov, Z.; Khozin-Goldberg, I.; Shpigel, M.; Samocha, T.M.; Sagi, M. Effect of seawater concentration on the productivity and nutritional value of annual Salicornia and perennial Sarcocornia halophytes as leafy vegetable crops. *Sci. Hortic.* **2011**, *128*, 189–196. [CrossRef]
64. Pangestuti, R.; Kim, S.K. Biological activities and health benefit effects of natural pigments derived from marine algae. *J. Funct. Foods* **2011**, *3*, 255–266. [CrossRef]
65. Egner, P.A.; Muñoz, A.; Kensler, T.W. Chemoprevention with chlorophyllin in individuals exposed to dietary aflatoxin. *Mutat. Res. Fundam. Mol. Mech. Mutagen.* **2003**, *523–524*, 209–216. [CrossRef]
66. Viera, I.; Pérez-Gálvez, A.; Roca, M. Green natural colorants. *Molecules* **2019**, *24*. [CrossRef]
67. Duarte, B.; Santos, D.; Marques, J.C.; Caçador, I. Ecophysiological adaptations of two halophytes to salt stress: Photosynthesis, PS II photochemistry and anti-oxidant feedback - Implications for resilience in climate change. *Plant Physiol. Biochem.* **2013**, *67*, 178–188. [CrossRef] [PubMed]
68. Geissler, N.; Hussin, S.; Koyro, H.-W. Elevated atmospheric CO2 concentration ameliorates effects of NaCl salinity on photosynthesis and leaf structure of *Aster tripolium* L. *J. Exp. Bot.* **2008**, *60*, 137–151. [CrossRef] [PubMed]
69. Lichtenthaler, H.K.; Wellburn, A.R. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* **1983**, *11*, 591–592. [CrossRef]
70. Sghaier, D.B.; Duarte, B.; Bankaji, I.; Caçador, I.; Sleimi, N. Growth, chlorophyll fluorescence and mineral nutrition in the halophyte Tamarix gallica cultivated in combined stress conditions: Arsenic and NaCl. *J. Photochem. Photobiol. B Biol.* **2015**, *149*, 204–214. [CrossRef] [PubMed]
71. Küpper, H.; Seibert, S.; Parameswaran, A. Fast, sensitive, and inexpensive alternative to analytical pigment HPLC: Quantification of chlorophylls and carotenoids in crude extracts by fitting with Gauss peak spectra. *Anal. Chem.* **2007**, *79*, 7611–7627. [CrossRef] [PubMed]
72. Mendes, C.R.; Cartaxana, P.; Brotas, V. HPLC determination of phytoplankton and microphytobenthos pigments: Comparing resolution and sensitivity of a C_{18} and a C_8 method. *Limnol. Oceanogr. Methods* **2007**, *5*, 363–370. [CrossRef]
73. Aghaleh, M.; Niknam, V.; Ebrahimzadeh, H.; Razavi, K. Salt stress effects on growth, pigments, proteins and lipid peroxidation in Salicornia persica and S. europaea. *Biol. Plant.* **2009**, *53*, 243–248. [CrossRef]
74. Vernon, L.P. Spectrophotometry Determination of Chlorophylls and Pheophytins in Plant Extracts. *Anal. Chem.* **1960**, *32*, 1144–1150. [CrossRef]
75. Arnon, D.I. Copper Enymes in Isolated Chloroplasts. Polyphenoloxidase in Beta vulagris. *Plant Physiol.* **1949**, *24*, 1–15. [CrossRef] [PubMed]
76. Nagata, M.; Yamashita, I. Simple Method for Simultaneous Determination of Chlorophyll and Carotenoids in Tomato Fruit. *Nippon Shokuhin Kogyo Gakkaishi* **1992**, *39*, 925–928. [CrossRef]
77. Parida, A.K.; Kumari, A.; Panda, A.; Rangani, J.; Agarwal, P.K. Photosynthetic pigments, betalains, proteins, sugars, and minerals during Salicornia brachiata senescence. *Biol. Plant.* **2018**, *62*, 343–352. [CrossRef]

78. De Souza, M.; Mendes, C.; Doncato, K.; Badiale-Furlong, E.; Costa, C. Growth, Phenolics, Photosynthetic Pigments, and Antioxidant Response of Two New Genotypes of Sea Asparagus (Salicornia neei Lag.) to Salinity under Greenhouse and Field Conditions. *Agriculture* **2018**, *8*, 115. [CrossRef]
79. Akcin, A.; Yalcin, E. Effect of salinity stress on chlorophyll, carotenoid content, and proline in Salicornia prostrata Pall. and Suaeda prostrata Pall. subsp. prostrata (Amaranthaceae). *Rev. Bras. Bot.* **2016**, *39*, 101–106. [CrossRef]
80. Bernal, J.; Mendiola, J.A.; Ibáñez, E.; Cifuentes, A. Advanced analysis of nutraceuticals. *J. Pharm. Biomed. Anal.* **2011**, *55*, 758–774. [CrossRef]
81. Duxbury, A.C.; Yentsch, C.S. Plankton Pigment Nomographs. *J. Mar. Res.* **1956**, *15*, 92–101.
82. Ben-Amotz, A.; Lers, A.; Avron, M. Stereoisomers of β-Carotene and Phytoene in the Alga *Dunaliella bardawil*. *Plant Physiol.* **1988**, *86*, 1286–1291. [CrossRef]
83. Uslu, L.; Durmaz, Y.; Duyar, H.A.; Bandarra, N.M. Fatty acids, α-tocopherol and proximate composition of four red macroalgae in the Sinop Bay (Turkey). *J. Anim. Vet. Adv.* **2013**, *12*, 29–33. [CrossRef]
84. Kumari, A.; Parida, A.K.; Rangani, J.; Panda, A. Antioxidant Activities, Metabolic Profiling, Proximate Analysis, Mineral Nutrient Composition of Salvadora persica Fruit Unravel a Potential Functional Food and a Natural Source of Pharmaceuticals. *Front. Pharmacol.* **2017**, *8*, 61. [CrossRef] [PubMed]
85. Rangani, J.; Kumari, A.; Patel, M.; Brahmbhatt, H.; Parida, A.K. Phytochemical profiling, polyphenol composition, and antioxidant activity of the leaf extract from the medicinal halophyte *Thespesia populnea* reveal a potential source of bioactive compounds and nutraceuticals. *J. Food Biochem.* **2019**, *43*, e12731. [CrossRef] [PubMed]
86. Chamovitz, D.; Sandmann, G.; Hirschberg, J. Molecular and biochemical characterization of herbicide-resistant mutants of cyanobacteria reveals that phytoene desaturation is a rate-limiting step in carotenoid biosynthesis. *J. Biol. Chem.* **1993**, *268*, 17348–17353. [CrossRef]
87. Vitamin and Mineral Requirements in Human Nutrition—World Health Organization, FAO— Google Books. Available online: https://books.google.dk/books?hl=en&lr=&id=NjdkYRkHla0C&oi=fnd&pg=PR1&dq=vitamin+source+human+nutrition&ots=I9UYVSFFzB&sig=BFJ4bRMC2iIqlkmb8wenIOQRQks&redir_esc=y#v=onepage&q=vitaminsourcehumannutrition&f=false (accessed on 18 August 2021).
88. Santos, J.; Mendiola, J.A.; Oliveira, M.B.P.P.; Ibáñez, E.; Herrero, M. Sequential determination of fat- and water-soluble vitamins in green leafy vegetables during storage. *J. Chromatogr. A* **2012**, *1261*, 179–188. [CrossRef]
89. Chamkouri, N.; Khodadoust, S.; Ghalavandi, F. Solid-Phase Extraction Coupled with HPLC-DAD for Determination of B Vitamin Concentrations in Halophytes. *J. Chromatogr. Sci.* **2015**, *53*, bmv080. [CrossRef] [PubMed]
90. Maatallah Zaier, M.; Ciudad-Mulero, M.; Cámara, M.; Pereira, C.; Ferreira, I.C.F.R.; Achour, L.; Kacem, A.; Morales, P. Revalorization of Tunisian wild Amaranthaceae halophytes: Nutritional composition variation at two different phenotypes stages. *J. Food Compos. Anal.* **2020**, *89*, 103463. [CrossRef]
91. Berretta, M.; Quagliariello, V.; Maurea, N.; Di Francia, R.; Sharifi, S.; Facchini, G.; Rinaldi, L.; Piezzo, M.; Ceccarelli, M.; Nunnari, G.; et al. Multiple effects of ascorbic acid against chronic diseases: Updated evidence from preclinical and clinical studies. *Antioxidants* **2020**, *9*, 1–27. [CrossRef] [PubMed]
92. Nakos, M.; Pepelanova, I.; Beutel, S.; Krings, U.; Berger, R.G.; Scheper, T. Isolation and analysis of vitamin B12 from plant samples. *Food Chem.* **2017**, *216*, 301–308. [CrossRef] [PubMed]
93. Ellouzi, H.; Ben Hamed, K.; Cela, J.; Munné-Bosch, S.; Abdelly, C. Early effects of salt stress on the physiological and oxidative status of Cakile maritima (halophyte) and Arabidopsis thaliana (glycophyte). *Physiol. Plant.* **2011**, *142*, 128–143. [CrossRef] [PubMed]
94. Hachicha, S.F.; Barrek, S.; Skanji, T.; Zarrouk, H.; Ghrabi, Z.G. Fatty acid, tocopherol, and sterol content of three Teucrium species from Tunisia. *Chem. Nat. Compd.* **2009**, *45*, 304–308. [CrossRef]
95. Rodriguez-Amaya, D.B. Provitamin A activity. In *Food Carotenoids*; John Wiley & Sons, Ltd.: Chichester, UK, 2015; pp. 255–281.
96. Karan, S.; Turan, C.; Sangun, M.K. Use of Glasswort (Salicona Europaea) Plant as Raw Material in Cosmetics. In Proceedings of the 2nd International Cosmetic Congress "Green Comsetics", Antalya, Turkey, 28–30 October 2018.
97. Sung, J.H.; Park, S.H.; Seo, D.H.; Lee, J.H.; Hong, S.W.; Hong, S.S. Antioxidative and skin-whitening effect of an aqueous extract of salicornia herbacea. *Biosci. Biotechnol. Biochem.* **2009**, *73*, 552–556. [CrossRef] [PubMed]
98. Guillerme, J.B.; Couteau, C.; Coiffard, L. Applications for marine resources in cosmetics. *Cosmetics* **2017**, *4*, 35. [CrossRef]
99. Ferreira, M.S.; Magalhães, M.C.; Oliveira, R.; Sousa-Lobo, J.M.; Almeida, I.F. Trends in the Use of Botanicals in Anti-Aging Cosmetics. *Molecules* **2021**, *26*, 3584. [CrossRef] [PubMed]
100. Brusotti, G.; Cesari, I.; Dentamaro, A.; Caccialanza, G.; Massolini, G. Isolation and characterization of bioactive compounds from plant resources: The role of analysis in the ethnopharmacological approach. *J. Pharm. Biomed. Anal.* **2014**, *87*, 218–228. [CrossRef]
101. Patra, J.K.; Das, G.; Lee, S.; Kang, S.S.; Shin, H.S. Selected commercial plants: A review of extraction and isolation of bioactive compounds and their pharmacological market value. *Trends Food Sci. Technol.* **2018**, *82*, 89–109. [CrossRef]
102. Doughari, J.H. Phytochemicals: Extraction Methods, Basic Structures and Mode of Action as Potential Chemotherapeutic Agents. In *Phytochemicals – A Global Perspective of Their Role in Nutrition and Health*; Rao, D.V., Ed.; IntechOpen: London, UK, 2012; ISBN 978-953-51-0296-0.

103. Essaidi, I.; Brahmi, Z.; Snoussi, A.; Ben Haj Koubaier, H.; Casabianca, H.; Abe, N.; El Omri, A.; Chaabouni, M.M.; Bouzouita, N. Phytochemical investigation of Tunisian Salicornia herbacea L., antioxidant, antimicrobial and cytochrome P450 (CYPs) inhibitory activities of its methanol extract. *Food Control* **2013**, *32*, 125–133. [CrossRef]
104. Meot-Duros, L.; Cérantola, S.; Talarmin, H.; Le Meur, C.; Le Floch, G.; Magné, C. New antibacterial and cytotoxic activities of falcarindiol isolated in Crithmum maritimum L. leaf extract. *Food Chem. Toxicol.* **2010**, *48*, 553–557. [CrossRef]

Paper III

Hulkko, L.S.S.; Rocha, R.M.; Trentin, R.; Fredsgaard, M.; Chaturvedi, T.; Custódio, L.; Thomsen, M.H. Bioactive Extracts from *Salicornia ramosissima* J. Woods Biorefinery as a Source of Ingredients for High-Value Industries. *Plants* **2023**, *12*, 1251.

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Bioactive Extracts from *Salicornia ramosissima* J. Woods Biorefinery as a Source of Ingredients for High-Value Industries

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Abstract: Salt-tolerant plants, also known as halophytes, could provide a novel source of feedstock for biorefineries. After harvesting fresh shoots for food, the lignified fraction of *Salicornia ramosissima* J. Woods could be used to produce bioactive botanical extracts for high-value industries such as nutraceuticals, cosmetics, and biopharmaceuticals. The residual fraction after extraction can be further used for bioenergy or lignocellulose-derived platform chemicals. This work analysed *S. ramosissima* from different sources and growth stages. After pre-processing and extractions, the obtained fractions were analysed for their contents of fatty acids, pigments, and total phenolics. Extracts were also evaluated for their in vitro antioxidant properties and inhibitory effect towards enzymes related to diabetes, hyperpigmentation, obesity, and neurogenerative diseases. The ethanol extract from the fibre residue and the water extract from completely lignified plants showed the highest concentration of phenolic compounds along with the highest antioxidant potential and enzyme-inhibitory properties. Hence, they should be further explored in the context of biorefinery.

Keywords: halophytes; biorefinery; antioxidants; enzyme inhibition; pigments; phytochemicals; biomedicines; nutraceuticals; sustainability

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1. Introduction

Halophytes are salt-tolerant plants that thrive in saline environments and can be found in different locations, including seashores, marshes, and saline deserts. Several species have commercial uses in different areas, including food and cosmetics, and can be commercially cultivated in saline systems. Soil and water salinisation has increased the rate of agricultural land degradation worldwide, and therefore, the cultivation of halophytes is one of the key implementations to solve these issues as they could be used for bioremediation and valorisation of these marginal lands [1]. *Salicornia ramosissima* J. Woods (Amaranthaceae), commonly known as sea asparagus or glasswort, is an edible annual succulent halophyte present in saltmarshes from the Arctic to the Mediterranean region. The importance of *S. ramosissima* as a commercial vegetable is increasing due to its organoleptic properties, including crunchy texture, slightly salty taste, and nutritional and functional properties. It is considered a reliable substitute for salt (NaCl) and, therefore, a promising functional ingredient to prevent cardiovascular diseases with appropriate levels of protein, dietary fibre, and minerals [2–5].

S. ramosissima can be cultivated in salt-affected marginal lands or hydroponic and aquaponic systems using saline water, including seawater [6–8]. However, as the plant matures, it lignifies, making it unpalatable. Due to the high salt content accumulated in the plant matrix, it is appropriate for animal fodder only when blended with other feedstuffs [9,10]. Therefore, this woody residual fraction is often considered agricultural waste; however, it could be utilised as a feedstock for biorefinery to bring additional value to

farmers and provide a way to maximise material valorisation. In this regard, two concepts can be applied, depending on the plant's growth stage: green biorefinery from partly lignified plants or more traditional lignocellulose biorefinery from completely lignified plants after seed production. The green biorefinery approach, where the biomass is first fractionated to green juice and fibre residue, often targeting the production of biochemicals, feed products, and biofuels, has been previously tested for *Salicornia sinus-persica*, *Salicornia bigelovii*, *Salicornia dolichostachya*, and *Salicornia europaea* [11–14].

In multi-product biorefinery, the value-added compounds with high market value can be produced to improve the process's overall feasibility before residual fractions are utilised for bioenergy. Due to adaptation to extreme environmental conditions and a high natural defence against predators and pests, *Salicornia* spp. produce high concentrations of bioactive secondary metabolites, such as phenolic acids, flavanols, flavones, and flavanones [3,15,16]. These metabolites have reported health-beneficial properties, including antioxidant, anti-inflammatory, and anti-diabetic activities [7,15,17–19]. Intake of these compounds can prevent the onset of different diseases, such as cancer, hypertension, and cardiovascular diseases [3,4,20]. Moreover, studies suggest that *S. ramosissima* extracts exhibit photoprotective effects against UV radiation [4,21] and protective effects against testicular toxicity [22].

Botanical extracts or bioactive compounds with different purities could be used in different commercial areas, including nutraceuticals, pharmaceuticals, and cosmetics [16,23,24]. These are high-value industries, and the market size of nutraceuticals is forecasted to reach USD 650.5 billion by 2030. The leading force behind this growth is the increased demand for dietary supplements and functional foods [25]. The natural skin care market is forecasted to reach USD 11.87 billion by 2030, and the demand for natural cosmetics has increased due to changes in consumer behaviour [26]. The interest in biopharmaceuticals is also driven by the trend of shifting from synthetic medicine to plant-derived drugs [27].

However, the existing literature considering the bioactivity and nutritional characteristics of *S. ramosissima* has been mostly focused on fresh food-grade plants [4,18,22,28]. The number of studies considering fully mature or fractionated *Salicornia* biomass for its exploitation for bioactive compounds is limited, and only a few studies have analysed the non-food waste fraction [29] or the effect of the growth stage on the concentration of bioactive compounds. One study showed that the content of flavonoids in *S. herbacea* increases as the plant matures [30]. After extraction and production of value-added compounds, residual fractions could be utilised to produce lignocellulose-derived biochemicals or bioenergy. Considering this, biogas and bioethanol production from *Salicornia* spp. fibres and juice have been previously assessed [11,31].

In this study, the green juice fractions and botanical extracts from partly and completely lignified *S. ramosissima* plants were analysed for their total contents of fatty acids, chlorophylls, carotenoids, and phenolic compounds. In vitro antioxidant activity was also evaluated using assays with different antioxidant mechanisms. The in vitro enzyme inhibition activity was measured against enzymes related to dementia and neurogenerative diseases (such as Alzheimer's and Parkinson's), diabetes, obesity, and skin issues (such as hyperpigmentation and acne). Based on the obtained results, possible applications for the extracts and potential of *S. ramosissima* waste fractions for halophyte-based biorefinery are discussed.

2. Materials and Methods

2.1. Raw Material

Fresh, non-food grade, partly lignified *S. ramosissima* biomass was obtained from two different producers: Les Douceurs du Marais in France (FR) and Riasearch in Portugal (PT). *S. ramosissima* is native to these regions and is already produced (e.g., Les Douceurs du Marais) or collected from the wild for commercial purposes, and the locations have the potential for expansion of biosaline agriculture; thus, the potential for integrated halophyte-based biorefineries. In Les Douceurs du Marais, the plants were produced on an organic

open-field farm in the marsh exposed to tidal changes on the west coast of France near La Turballe. Partly lignified shoots were harvested at the vegetative stage right after the food production period and the start of lignification in May 2020. The data from the closest weather station show that during the last month of cultivation, the average daily maximum and minimum temperatures were 18.6 °C and 9.8 °C, respectively, and the extreme maximum and minimum temperatures were 28.7 °C and 4.9 °C, respectively, and the total rainfall was 35.9 mm divided into 7 days with precipitation [32]. The plants obtain their water uptake from seawater (approximately 3.5 dS/m), but the salinity may vary depending on the occurrence of precipitation or droughts.

In Riasearch, biomass was cultivated in a sandy soil bed in a greenhouse. During the growth phase, plants were irrigated two times a day with a mixture of brackish water aquaculture effluent, which also fertilised the plants, and freshwater to keep the soil salinity at approximately 1.2 dS/m. Additional light fixtures were not used, and during the months with high temperatures and UV radiation, partial removable shade structures were used to protect the plants from drying and premature lignification. On the coast of Central Region of Portugal, during the summer months, the lowest average daily minimum temperature is 15.1 °C (June), and the highest average daily maximum is 24.4 °C (August) [33]. The plants were germinated at the end of February 2020, and for partly lignified biomass, aerial parts were harvested after 26 weeks of cultivation.

Dry, completely lignified, and woody *S. ramosissima* was also obtained from Riasearch. Plants were cultivated under the same conditions as those harvested at the earlier growth stage, but they were allowed to produce seeds before harvesting approximately 8 months after germination. After harvesting, the lignified plants were air-dried for approximately 5 weeks in mesh trays in shade before shipping. The plants were carefully handled after drying to avoid seed loss. The biomass batches and considered fractions and extracts are summarised in Figure 1.

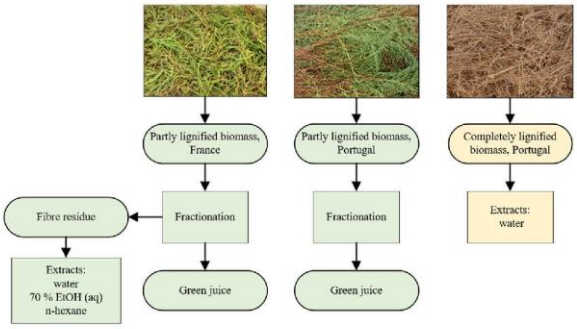

Figure 1. Origin of *Salicornia ramosissima* biomass batches and considered fractions and botanical extracts.

Fresh biomass batches were first fractionated into green juice and fibre residue fractions using a screw press. The green juice fractions were centrifuged at 4000 rpm for 20 min, filtered using a Whatman filter (GE Healthcare), and freeze-dried using a ModulyoD freeze dryer (Thermo Scientific, Waltham, MA, USA). The fibre residue was dried overnight at a 60 °C fan oven, homogenised using a knife-miller to achieve a particle size of less than 2 mm, and stored at room temperature (RT).

The completely lignified plant material was first rinsed with water, dried at RT, and size-reduced into pieces of less than 2 cm using an agricultural straw shredder (AM55, J. N. Jensen og Sønner, Agerskov, Denmark). The dried, shredded biomass was stored at RT.

The dry matter (DM) and ash concentration of the fractions were determined according to the protocols by the National Renewable Energy Laboratory (NREL) [34,35].

2.2. Extract Preparation

From the fibre fraction of partly lignified *S. ramosissima*, after screw press, botanical extracts were prepared using water, 70% aqueous ethanol (EtOH), and *n*-hexane as solvents, and the traditional Soxhlet apparatus with 100 mL extraction chambers. The sample size was approximately 10 g for water and EtOH extractions and 5 g for *n*-hexane extraction. All extractions were run as parallel experiments. The extraction time was 8 h for water and 6 h for organic solvents. Excess solvent was removed using a rotary evaporator, and EtOH and water extracts were freeze-dried. As the fibre residue from partly lignified plants from Portugal was not available for study, only the biomass from France was used in the extractions.

Completely lignified biomass was extracted with a pilot-scale Soxhlet using 25 L of demineralised water. The amount of biomass used was 2 kg with a dry matter content of 88.5%, and the extraction time was 8 h. It must be noted that the pilot-scale equipment does not have the same particle retention as the lab-scale Soxhlet, and in order to avoid the smallest particles in the extract phase, the shredded biomass was sifted through a 2 mm sieve. Only particles with a size more than 2 mm were used for the extraction. The obtained water extract was spray-dried using an inlet temperature of 130 °C and outlet temperature of 80 °C. All dried extracts were re-solubilised in the corresponding solvents at a final concentration of 10 mg/mL and used in the assays.

2.3. Chemical Characterisation of Samples

2.3.1. Determination of Fatty Acids

The fatty acid (FA) profile was determined from the *n*-hexane extract of fresh *S. ramosissima* fibre residue. In transesterification, approximately 0.15 g of lipid extract was dissolved in 1 mL of 0.5 M sodium hydroxide in methanol (MeOH) at 90 °C. Samples were cooled to RT, 1 mL of boron trifluoride and 0.5 mL of hydroquinone solution were added, and samples were kept at 90 °C for another 5 min. For phase separation, 4 mL of saturated NaCl water solution and 3 mL of n-heptane were added. The non-polar fraction was recovered and analysed using gas chromatography (Clarus 500, Perkin Elmer, Waltham, MA, USA) with a capillary column (Elite-WAX, 30 m × 0.25 mm ID × 0.25 µm, Perkin Elmer). Helium was used as a carrier gas, and the temperature program was set to 1 min at 150 °C, heating 10 °C/min until reaching 240 °C, and 10 min at 240 °C. Mass spectrometry was used for the detection and quantification of fatty acids.

2.3.2. Determination of Total Phenolic Compounds

The total phenolic contents (TPC) of the extracts, at the concentration of 10 mg/mL, were determined using the Folin-Ciocalteau assay described by Velioglu et al. [36] and adapted to 96-well plates. The plates were incubated for 90 min at RT, protected from light, and the absorbance was read at 650 nm using a microplate reader (EZ Read 400, Biochrom, Cambridge, United Kingdom). Results were expressed as the amount of gallic acid equivalents (GAE) in the dried extract using a calibration curve ($R^2 = 0.997$).

The total flavonoid content (TFC) was also estimated in the dried extract at a concentration of 10 mg/mL, using the method developed by Pirbalouti [37] adapted to 96-well plates. Aliquots of 50 µL of the samples were mixed with 50 µL of 2% aluminium chloride in MeOH solution. The plates were incubated for 10 min at RT and read at 405 nm. Results were expressed as the amount of quercetin equivalent (QE) per gram of dried extract using a calibration curve ($R^2 = 0.999$).

Total condensed tannins (TCT) of dried extracts at 10 mg/ ml were determined using a method described by Li et al. [38] using p-dimethylaminocinnamaldehyde hydrochloric acid (DMACA-HCl). Briefly, 10 µL of extracts were mixed with 200 µL of 1% DMACA in MeOH and 100 µL of 37% HCl. Plates were incubated for 15 min at RT and read at 640 nm.

Results were expressed as the amount of catechin equivalent (CE) using a calibration curve ($R^2 = 0.991$).

Total anthocyanidins (TAC) were measured as cyanin chloride equivalent (CCE) in the dried extract based on a calibration curve ($R^2 = 0.991$). The method developed by Mazza et al. [39] was followed and adapted to 96-well microplates. In brief, aliquots of 20 µL of the samples at 10 mg/mL were mixed with 20 µL 95% EtOH containing 0.1% HCl and 160 µL 1 M HCl. The absorbance was read at 492 nm.

2.3.3. Determination of Photosynthetic Pigments

The total concentrations of chlorophylls (CHL) and carotenoids (TCA) were determined from green juice fractions and EtOH extracts, as described by Lichtenthaler and Wellburn [40]. The absorbance was measured at 470 nm, 649 nm, and 665 nm using a UV-visible spectrophotometer (Genesys 50, Thermo Scientific, Waltham, MA, USA). The following equations [40] were used to calculate the concentration of pigments:

$$\text{CHL } a = 13.95 \times A_{665} - 6.88 \times A_{649} \quad (1)$$

$$\text{CHL } b = 24.96 \times A_{649} - 7.32 \times A_{665} \quad (2)$$

$$\text{TCA} = (1000 \times A_{470} - 2.05 \times \text{CHL } a - 114.8 \times \text{CHL } b)/245 \quad (3)$$

2.4. In Vitro Antioxidant Activity Assays

Antioxidant properties were tested in vitro using radical-based and metal-based assays. *S. ramosissima* extracts were first tested at a concentration of 10 mg/mL, and the absorbances were read using a microplate reader. Antioxidant activities were calculated as a percentage relative to a control sample. For the samples with activities more than 50%, a minimum of eight different concentrations were evaluated to calculate the half-maximal effective concentration (EC_{50}).

2.4.1. Radical-Based Antioxidant Activity

Radical scavenging activity was tested against 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and nitric oxide (NO). For all assays, 1 mg/mL gallic acid was used as the positive control.

The DPPH assay was carried out using the method developed by Brand-Williams et al. [41] adapted to 96-well microplates by Moreno et al. [42]. A sample of 22 µL was mixed with 200 µL of 120 µM DPPH in EtOH solution. Samples were incubated for 30 min in the dark, and the absorbance was read at 492 nm.

A protocol described by Re et al. [43] was used to determine the ABTS radical scavenging activity. The 7.4 mM ABTS solution was prepared by mixing 100 mL of ABTS with 100 mL of 2.6 mM potassium persulphate in the dark and RT and incubating overnight. The final ABTS solution was diluted with EtOH to obtain an absorbance of approximately 0.7 at 734 nm. In the ABTS assay, 10 µL of the extract was mixed with 190 µL of the final ABTS solution, and plates were incubated for 6 min in the dark and read at 650 nm.

In the NO scavenging assay developed by Baliga et al. [44], aliquots of 50 µL of sample solution and 10 mM sodium nitroprusside were mixed, and plates were incubated for 90 min at RT. Afterwards, 50 µL of Griess reagent (Sigma-Aldrich, Lisbon, Portugal) was added, and the absorbance was read at 562 nm.

2.4.2. Metal-Based Antioxidant Activity

In metal-based assays, iron chelating activity (ICA), copper chelating activity (CCA), and ferric reducing antioxidant power (FRAP) were tested. Positive control samples for metal-based assays were 1 mg/mL gallic acid for FRAP and 1 mg/mL ethylenediaminetetraacetic acid (EDTA) for chelating activity. All metal-based assays were carried out following Megías et al. [45], with minor modifications.

In the ICA assay, 30 µL of sample solution was mixed with 200 µL of distilled H_2O and 30 µL of 0.01% aqueous $FeCl_2$, the plates were incubated for 30 min, 12.5 µL of 40 mM aqueous ferrozine was added, the plates were further incubated for 10 min, and absorbance was read at 562 nm.

For the CCA assay, a 30 µL sample was mixed with 200 µL of 50 mM sodium acetate buffer, 100 µL of 0.005% aqueous $CuSO_4$, and 6 µL of 4 mM aqueous pyrocatechol violet, and plates were immediately read at 620 nm.

The FRAP assay was performed by mixing 50 µL of the sample with 50 µL of distilled H_2O and 50 µL 1% potassium ferrocyanide. The plates were incubated for 20 min at 50 °C oven, 50 µL of 10% aqueous trichloroacetic acid and 10 µL of 0.1% aqueous $FeCl_3$ were added, the plates were incubated for another 10 min at RT, and read at 650 nm.

2.5. *In Vitro Enzyme Inhibition Assays*

Enzyme inhibition activity of 10 mg/mL sample solutions was analysed in vitro using spectrophotometric methods adapted to 96-well plates. Drugs already on the market were used as a reference: acarbose (10 mg/mL, anti-diabetic drug), arbutin (1 mg/mL, tyrosinase inhibitor), galantamine (1 mg/mL, dementia treatment), and orlistat (1 mg/mL, drug used to support weight-loss). Results were expressed as the percentage of inhibition.

The assay used for α-amylase inhibition was developed by Xiao et al. [46] based on the reaction between iodine solution and starch. Aliquots (40 µL) of sample, 0.1% boiled potato starch suspension and 100 U/mL α-amylase in 0.1 M sodium phosphate buffer solution (pH 6.9) were mixed, plates were incubated at 37 °C for 10 min, 20 µL of 1 M HCl and 100 µL of iodine solution (5 mM I_2 and 5 mM KI in distilled H_2O) were added, and absorbance was read at 570 nm. Results were calculated using two negative control samples, one without an enzyme (blank, 100% inhibition) for calculations and one with an enzyme for colour correction:

$$\alpha\text{-Amylase inhibitory activity [\%]} = (A_{570}\ \text{sample} - A_{570}\ \text{colour control})/A_{570}\ \text{blank} \times 100\% \quad (4)$$

Inhibitory activity against α-glucosidase *Saccharomyces cerevisiae* was determined as described by Custódio et al. [47]. An aliquot of 50 µL of sample solution was mixed with 100 µL of 1 U/mL α-glucosidase in phosphate buffer, and plates were incubated at 25 °C for 10 min. Afterwards, 50 µL of 5 mM p-nitrophenyl-α-d-glucopyranoside was added, plates were incubated for another 5 min at 25 °C, and absorbance was read at 405 nm.

Tyrosinase inhibition activity assay was performed as described by Trentin et al. [48] by mixing 70 µL of sample solution with 30 µL of 333 U/mL fungal tyrosinase solution, incubating plates for 5 min in RT, adding 110 µL of substrate solution (2 mM L-tyrosine diluted in 25 mM potassium phosphate buffer, pH 6.5), and incubating plates for 45 min at RT before reading the absorbance at 405 nm.

Inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) were analysed using a method developed by Ellman et al. [49]. A sample of 20 µL was mixed with 140 µL 0.02 M sodium phosphate buffer (pH 8.0) and 20 µL of 0.28 U/mL enzyme solution. Plates were incubated for 15 min at 25 °C, and 10 µL of acetylcholine iodide or butyrylcholine iodide substrate in 4 mg/mL buffer solution, and 20 µL of 5,5′-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent) in 1.2 mg/mL EtOH solution was added. Plates were incubated for another 15 min at 25 °C and read at 405 nm.

The protocol used by McDougall et al. [50] was used to measure the porcine pancreatic lipase inhibition activity. A sample of 20 µL was mixed with 200 µL 100 mM Tris-HCl buffer (pH 8.2), 20 µL of 1 mg/mL enzyme solution, and 20 µL of substrate solution (5.1 mM 4-nitrophenyl dodecanoate in EtOH). Plates were incubated for 10 min at 37 °C, and absorbance was read at 405 nm.

2.6. *Statistical Methods*

Results are given as mean values with standard deviation marked in brackets. For the extraction yields, FA, phenolic acids, HCA, and pigments, the samples were tested in

triplicate ($n = 3$), while for phenolic compounds, antioxidant activity and enzyme inhibition activity, the samples were tested in sexuplicate ($n = 6$). One-way analysis of variance (ANOVA) combined with the Tukey honest significant difference (HSD) test was used to evaluate significant differences between the results, and significantly different results are denoted with different letters. EC50 values were determined for antioxidant activities using an online tool by AAT Bioquest Inc. [51].

3. Results and Discussion

3.1. Extraction Yields

Extraction yields and fractions considered in the study are presented in Table 1. The unfiltered juice fraction corresponded to more than 80% of the fresh weight of partly lignified *S. ramosissima* from France and 66.7% of biomass from Portugal. The biomass from Portugal was fractionated using a lab-scale single-auger juicer, whereas French biomass was juiced using a pilot-scale double-auger juicer with higher fractionation performance. The green juice fraction had a high ash content due to water-soluble salts accumulated in succulent plant tissue. The green juice obtained from the biomass from France exhibited especially high ash content, 81.8%, which could be explained by plants' exposure to seawater flooding and more succulent texture compared to the more woody phenotype from Portugal. The results are aligned to those previously reported for other *Salicornia* spp., as Alassali et al. [11] found 61.1% ash content of *S. sinus-persica* juice, and Christiansen et al. [12] reported more than 80% of the total ash from the fresh *S. bigelovii* was recovered in the juice fraction after screw press. Changes in cultivation salinity do not only affect the ash content of plants but may also change the metabolism of sugars and lipids, which has been previously shown by Hulkko et al. [13] and Duan et al. [52] for *S. europaea* and Magni et al. [53] for *Salicornia perennis*. The DM from lignified plants had a lower ash content than fibre residue from partly lignified plants, but rinsing with water could have removed some of the salts from the biomass surface. The ash content of lignified *S. ramosissima* is also greatly lower than the 43.8% ash content previously reported for *S. bigelovii* [54].

Table 1. Yields of *Salicornia ramosissima* extracts. Results are given as [*w*/*w*%] on the basis of fresh biomass weight for dry matter content and dry matter weight for extractives and ash. n/a: not available. Results are expressed as mean values with standard deviations (in brackets).

Fraction	Dry Matter [*w*/*w*%]	H_2O Extract [*w*/*w*%]	EtOH Extract [*w*/*w*%]	*n*-Hexane Extract [*w*/*w*%]	Ash [*w*/*w*%]
Juice, FR	7.73 (0.01)	n/a	n/a	n/a	81.83 (0.39)
Juice, PT	6.47 (0.21)	n/a	n/a	n/a	64.76 (0.13)
Fibre residue, FR	25.49 (0.27)	33.68 (2.31)	31.45 (0.59)	1.13 (0.06)	20.31 (0.11)
Lignified biomass	89.65 *	18.83 *	n/a	n/a	17.28 (0.98)

* Extraction was run as one batch in a pilot-scale system; not possible to give a standard deviation.

3.2. Fatty Acid Profile

The content of non-polar compounds in *S. ramosissima* fibre residue was found to be low (1.13%). However, the lipid profile can be an important factor when biomass is considered for nutraceutical or feed application. The FA profile of the fibre residue of *S. ramosissima* is presented in Table 2. The total detected FA consisted of polyunsaturated fatty acids (PUFA, 58.2%), followed by saturated fatty acids (SFA, 41.0%), and monounsaturated fatty acids (MUFA, 1.3%). The predominant FA was linoleic acid (34.5%), followed by palmitic acid (30.9%). The obtained ω-6 and ω-3 FA ratio of 1.5 is interesting, as a ratio lower than 5 has been reported to contribute more to the anti-inflammatory state of PUFA, thereby reducing the risk of cardiovascular diseases, cancer, and autoimmune diseases [18].

Table 2. Fatty acid profile of the lipids extracted with *n*-hexane from French *Salicornia ramosissima* fibre residue. Results are given as [% FA/total FA], n.d.: not detected. Results are expressed as mean values with standard deviations (in brackets).

Fatty Acid	Fibres, *n*-Hexane Extract [% FA/total FA]
Myristic acid C14:0	0.4 (0.4)
Palmitic acid C16:0	30.9 (2.3)
Palmitoleic acid C16:1	n.d.
Stearic acid C18:0	2.7 (0.1)
Oleic acid C18:1	1.3 (0.1)
Linoleic acid C18:2	34.5 (0.7)
α-Linolenic acid C18:3	23.7 (1.4)
Arachidic acid C20:0	1.0 (0.9)
Behenic acid C21:0	3.9 (0.1)
Lignoceric acid C24:0	2.1 (1.8)
ΣSFA	41.0 (0.9)
ΣMUFA	1.3 (0.1)
ΣPUFA	58.2 (2.0)
ω-6/ω-3	1.5 (0.1)

Our results are consistent with those reported by Maciel et al. [55], who found the total SFA, MUFA and PUFA in chloroform extract from fresh *S. ramosissima* shoots to be 32.44%, 6.24%, and 61.32%, respectively. However, they reported a lower ratio of ω-6 and ω-3 FA (0.51), and the major difference was the amount of detected α-linolenic acid (39.6%). Isca et al. [18] determined the whole lipophilic profile for *n*-hexane extract from vegetative *S. ramosissima* and found 31.27% SFA and only 3.29% of unsaturated FA in total. Barreira et al. [56] found that 60.8% of the total FA in *S. ramosissima* was SFA, and the contents of arachidic acid (8.6%), behenic acid (10.0%), and lignoceric acid (7.1%) were especially higher compared to results obtained in this study. The differences in the lipids profile may be explained by the different phenotypes and growth stages of *S. ramosissima*. However, no studies have been found to show the variations in the FA profile regarding the growth stage or place of origin of this species. Cultivation conditions also affect the FA profile, and additional irrigation has been shown to increase the content of MUFA and PUFA in *S. ramosissima* [18]. The biomass drying process has been shown to affect the FA composition of *S. ramosissima*, as freeze-dried samples exhibited a higher amount of PUFA compared to oven-dried samples [4].

3.3. Bioactive Compounds

The total amounts of compounds from different phenolic groups and the concentrations of pigments in the fractions are presented in Table 3. The EtOH extract from fibre residue and water extract from lignified plant material were the richest in terms of TPC, with contents of 41.06 mg GAE/g DM and 30.10 mg GAE/g DM, respectively. These results are higher than those previously reported for *S. ramosissima* by Lima et al. [28] for the acetone extract from 200 mM salinity-cultivated fresh shoots (12.9 mg GAE/g DM) and Silva et al. [3] for the water extract from wild-harvested plants (15.02 mg GAE/g DM). However, the study by Lopes et al. [57] reports that 74.1 mg GAE/g DM of TPC were found in the *S. ramosissima* acetone extract from wild-harvested plants. Obtained results are also higher than water and EtOH extracts from *S. europaea* [58]. No significant differences were observed in the content of TFC in extracts where TFC were detected. The concentration of TFC in extracts was lower than previously reported for *Salicornia* spp. extracted with EtOH or MeOH using conventional solid-liquid extraction, such as maceration [59]. TCT was also not detected in the study by Lopes et al. [57]. However, Lima et al. [28] reported relatively high concentrations of TCT (32.5 mg CE/g DM). Polyphenols found in *Salicornia* spp. contain a variety of compounds, such as phenolic and hydroxycinnamic acids and flavonoids, linked to several biological activities of the extracts, such as anti-inflammatory

and antimicrobial effects [3,4,60]. Antioxidant and cardiovascular-protective properties have been linked to compounds previously found in *S. ramosissima*, such as derivates of the flavonoids quercetin, kaempferol, and rutin, and derivates of phenolic acids, such as chlorogenic acid, p-coumaric acid, and protocatechuic acid [60].

Table 3. Bioactive compounds in *S. ramosissima* fractions. TPC: total phenolic compounds [mg GAE/g DM] ($p < 0.001$), TFC: total flavonoids [mg QE/g DM] ($p = 0.240$), TCT: total condensed tannins [mg CE/g DM], TAC: total anthocyanidins [mg CCE/g DM] ($p < 0.001$), CHL: total chlorophyll [µg/g DM] ($p < 0.001$), TCA: total carotenoids [µg/g DM] ($p < 0.001$), n.d.: not detected or concentration lower than the limit of detection, n/a: not available. Results are expressed as mean values with standard deviations (in brackets). Different letters denote significantly different results, calculated individually for each compound group.

Fraction	TPC [mg GAE/g DM]	TFC [mg QE/g DM]	TCT [mg CE/g DM]	TAC [mg CCE/g DM]	CHL (a + b) [µg/g DM]	TCA [µg/g DM]
Juice, FR	1.34 (0.54) ^{d}	n.d.	n.d.	n.d.	15.42 (3.15) ^{c}	73.16 (1.41) ^{b}
Juice, PT	3.66 (0.74) ^{d}	n.d.	n.d.	n.d.	39.26 (0.22) ^{b}	52.31 (2.43) ^{c}
Fibres, H_2O	18.84 (1.04) ^{c}	3.61 (0.67) ^{a}	n.d.	1.46 (0.31) ^{a}	n/a	n/a
Fibres, EtOH	41.06 (4.09) ^{a}	3.22 (0.45) ^{a}	n.d.	n/a	1446.37 (13.99) ^{a}	261.55 (1.56) ^{a}
Lignified	30.10 (1.97) ^{b}	3.86 (0.71) ^{a}	n.d.	0.53 (0.11) ^{b}	n/a	n/a

Many processing parameters can affect the concentration of bioactive secondary metabolites, such as the used extraction method and solvent used [3], as well as drying and storage conditions [4,61]. In addition, intra-specific variability, different plant growth stages [30], biomass fractions, and plants' exposure to abiotic and biotic stresses also affect biomass composition and phytochemical concentration. For example, condensed tannins, also called proanthocyanidins, and other flavonoids have been associated with a protective effect on plants exposed to abiotic stresses such as intensive UV radiation, drought and cold temperatures [62]. High temperatures and waterlogging (flooded conditions) have also been reported to increase the amount of bioactive compounds, as exposure to these conditions includes the production of free radicals [15,53]. Significant differences in chemical composition can be observed between plants harvested even within the same region [53]. Duan et al. [52] showed *S. europaea* cultivated under increased salinity to be enriched in phenolic acids and flavonoids in roots and aerial parts and exhibiting upregulation in bioactive compounds, including protocatechuic acid, quercetin and kaempferol derivatives, p-coumaric acid, and ferulic acid. Cultivated plants have also been shown to have lower phenolics content than wild populations, likely due to more controlled cultivation [63]. These aspects must be taken into consideration when planning potential biorefinery processes to ensure they are robust enough to withstand variations.

Despite the bright red colour of the juice from French plants, anthocyanins were not detected in the sample. The red colour was only observed in the juice from French plants, which could be due to their cultivation methods, could have produced some protective pigments in response to abiotic stresses. However, the concentration of the pigments may have been below the limit of detection, or the stability of the compounds has been compromised due to the neutral pH of the solvent [64]. Among sugar-free anthocyanidins, pelargonidin is known to have colours ranging from orange to red, whereas cyanidin has a strong magenta colour [62]. Thus, there may be a difference in the maximum absorbance of the compounds present in the sample and cyanin chloride used as a standard.

Photosynthetic pigments were detected in the EtOH extract due to the more non-polar nature of these compounds. In the EtOH extract, the concentration of CHL *a* was higher compared to the juice fraction, with a CHL *a*/CHL *b* ratio of 2.3, whereas in the juice fractions from the French and Portuguese biomass, the corresponding ratios were 0.72 and 0.53, respectively. A similar ratio in *Salicornia* EtOH extract has been reported previously for *S. neei* [65]. The amount of total CHL in the EtOH extract was lower than

that previously reported for *S. ramosissima* EtOH extracts by Barreira et al. [56] (21560 µg/g DM) but higher compared to *S. brachiata* (746.5 µg/g DM) and *S. neei* (233.3 µg/g DM) studied by Parida et al. [66] and De Sousa et al. [65], respectively. Salt stress may decrease the amount of CHL in the plants [65], which may explain the lower CHL content in the juice from French plants compared to Portuguese plants, as the plants cultivated in France were exposed to higher salinity.

The concentration of total carotenoids in *Salicornia* spp. varies between different studies [16], and all obtained results lay within the range of reported results. Carotenoids are non-enzymatic antioxidants produced in response to different stressors [67]. The different results reported for the same species are strongly dependent on the conditions where the plants have grown, and light, temperature, and salinity variations are the main factors that may lead to carotenoid production. Carotenoids have been previously reported to have a key role in the salt-tolerance mechanisms of Amaranthaceae, and cultivation salinity has been shown to impact the pigment content of *Salicornia* spp. [65,68,69].

An efficient water-based extraction process with high phenolics yield would be desirable in a biorefinery targeting the production of bioactive compounds, as using solvents such as EtOH increases the capital expenditures and operational costs of the larger-scale facility. Biomass cultivation in a controlled environment, such as a hydroponic system, could provide a possibility to modify the growth conditions and environmental stresses to enhance the production of target compounds, such as phenolic acids and flavonoids.

3.4. Antioxidant Activity

Salinity and other environmental stresses trigger oxidative reactions and the generation of reactive oxygen species, causing cellular damage and metabolic disorders in plants [7,70]. For halophytes, these stresses are more pronounced, and they have developed efficient antioxidant defence mechanisms to cope with extreme environmental stresses. Halophytes produce different classes of antioxidant compounds, such as phenols and carotenoids, which may have a synergic effect as radical scavengers. As seen in Figure 2, all fractions presented antioxidant activity in radical-based assays, and the water extract from the fibre residue exhibited the highest activity in a 10 mg/mL concentration. Compared to the antioxidant activity previously reported for *S. ramosissima* EtOH extract by Barreira et al. [56], the obtained extracts exhibited lower DPPH radical scavenging activity (IC50 5.69 mg/mL). The results for aqueous extracts are also higher than those reported by Faria et al. [71], who found the DPPH and ABTS radical scavenging activity of 10 mg/mL *S. neei* 80% EtOH extract to be 37.1% and 46.1%, respectively. Considering the water extract from the fibre residue, the EC_{50} value of ABTS radical scavenging activity was very similar to that reported by Lima et al. [28] for *S. ramosissima* acetone extract. The activity in the NO assay indicates that the extracts from *S. ramosissima* could have anti-inflammatory properties, as exposure to NO radicals has been directly linked to chronic inflammation [44]. Thus, the extracts could be a potential source of ingredients for dermo-cosmetics and biopharmaceuticals. Extracts from *S. europaea* and *S. brachiata* have been previously reported to have anti-inflammatory properties [72]. Chronic inflammation is involved in various diseases, including but not limited to cardiovascular diseases, diabetes, autoimmune and neurogenerative conditions, and chronic kidney disease [73]. There are no available results for the NO scavenging activity of EtOH extract due to the precipitation of the sample.

The antioxidant activity was generally more pronounced in metal-based assays (Figure 3). Considering the FRAP assay, even though the differences between the results for water and EtOH extracts are non-significant, the EC_{50} values for water extracts are much lower. Therefore, the extracts can be considered more potent, indicating the presence of strong, water-soluble antioxidant compounds in the *S. ramosissima* biomass. Unfortunately, there are no results for CCA of the green juice from Portuguese biomass due to issues with the assay. The EC_{50} values of fibre residue water extract for FRAP, ICA, and CCA were also greatly lower than those reported by Barreira et al. [56] for *S. ramosissima* EtOH extract. For the fibre residue water extract, the obtained EC_{50} (4.91 mg/mL) of CCA

is very similar to the one reported by Lima et al. [28] for *S. ramosissima* acetone extract. Achieving the same antioxidant activity with water extraction and reducing the use of toxic and flammable organic solvents is highly desirable considering large-scale industrial applications. Similarly, obtaining extracts from *S. ramosissima* residues with bioactivity comparable to extracts from food-grade plants highlights the biorefinery and valorisation potential of these residues.

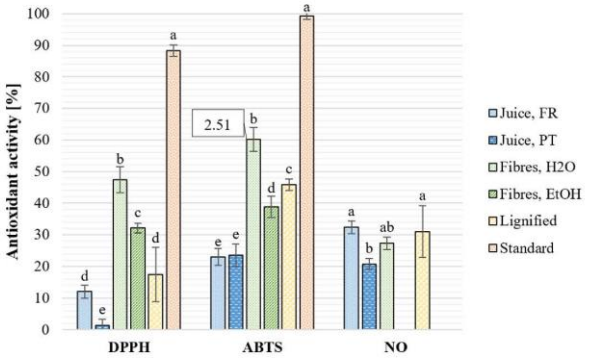

Figure 2. Antioxidant activity [%] of extracts from *S. ramosissima* at the concentration of 10 mg/mL in relation to blank samples with extraction solvent determined by assays with radical-based antioxidant mechanisms. DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), NO: nitric oxide. EC50 values [mg/mL] are presented in callout boxes for *Salicornia* samples with activity more than 50%. The standard compound is gallic acid (1 mg/mL). Different letters denote significantly different results, calculated individually for the results from each assay and for all assays ($p < 0.001$).

Overall, the green juice fractions exhibited the lowest bioactivity, whereas water and EtOH extracts from the fibre residue of French plants had the highest activity. Even though more mature plants have been shown to have higher concentrations of certain compounds with antioxidant activity [30], the extract from completely lignified Portuguese plants exhibited the highest activity only in ICA and FRAP assays. Therefore, the cultivation conditions could explain the higher antioxidant activity of French *S. ramosissima*. Plants grown in an open field have most likely been exposed to more abiotic stresses, such as high UV radiation and temperature difference, as well as higher cultivation salinity, leading to increased antioxidant production. On the contrary, *S. ramosissima* was grown in a more controlled greenhouse environment. The difference can also be observed in the juice fraction, as the juice obtained from French plants had higher antioxidant activity in all assays, except ABTS scavenging activity, compared to Portuguese phenotypes.

The mixture of different bioactive compounds present in the specific biomass fraction or extract could also contribute differently to each antioxidant mechanism, affecting the observed activities. The high concentration of antioxidants produced by halophytes has made them interesting for functional food applications and improving the nutritional quality of everyday products such as bread or pasta [2,7,58].

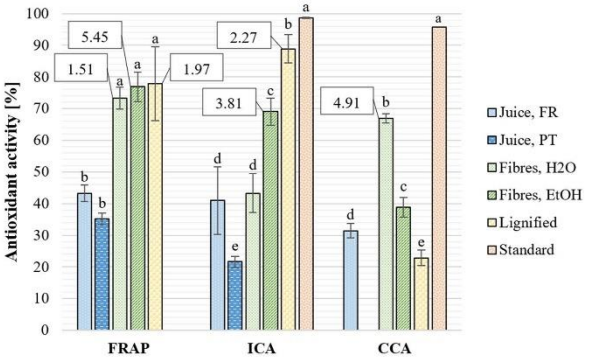

Figure 3. Antioxidant activity [%] of extracts from *S. ramosissima* at the concentration of 10 mg/mL in relation to blank samples with extraction solvent determined by assays with metal-based antioxidant mechanisms. FRAP: ferric reducing antioxidant power, ICA: iron chelating activity, CCA: copper chelating activity. EC50 values [mg/mL] are presented in callout boxes for *Salicornia* samples with activity more than 50%. The standard compound is ethylenediaminetetraacetic acid (1 mg/mL). Different letters denote significantly different results, calculated individually for the results from each assay and for all assays ($p < 0.001$).

3.5. Enzyme Inhibitory Properties

The enzyme inhibitory properties of green juices and extracts are presented in Table 4. Samples displayed different inhibitory activities against the different enzymes, and the EtOH extract with 10 mg/mL concentration exhibited moderate to high inhibition activity against enzymes related to diabetes (α-glucosidase), obesity and acne (lipase), hyperpigmentation (tyrosinase), and neurogenerative diseases (AChE).

Table 4. Enzyme inhibition activity [%] of reference compounds and 10 mg/mL *S. ramosissima* stock solutions in relation to blank samples. n.d.: not detected or activity lower than the limit of detection, n/a: not available. Reference compounds were tested with 1 mg/mL, except acarbose (10 mg/mL). Results are mean values with standard deviations (in brackets). Different letters denote significantly different results, calculated individually for the results from each enzyme assay. For all assays ($p < 0.001$), except for lipase inhibition assay ($p = 0.002$).

Fraction	α-Amylase [%]	α-Glucosidase [%]	Lipase [%]	Tyrosinase [%]	AChE [%]	BuChE [%]
Juice, FR	2.10 (0.41) ^{bc}	5.46 (4.98) ^{c}	19.21 (9.09) ^{b}	10.84 (2.66) ^{b}	16.11 (1.91) ^{c}	24.03 (2.77) ^{b}
Juice, PT	3.29 (1.16) ^{ab}	12.91 (1.73) ^{c}	21.40 (8.77) ^{b}	n.d.	28.44 (4.60) ^{b}	46.25 (1.74) ^{a}
Fibres, H_2O	4.08 (0.72) ^{a}	34.12 (2.10) ^{b}	n.d.	n.d.	n.d.	n.d.
Fibres, EtOH	n/a	68.63 (8.85) ^{a}	41.74 (8.93) ^{a}	71.85 (5.42) ^{a}	68.42 (1.59) ^{a}	17.82 (1.77) ^{c}
Lignified	1.10 (0.47) ^{c}	38.93 (3.90) ^{b}	21.13 (8.64) ^{b}	n.d.	n.d.	n.d.
Acarbose	61.18 (1.30)	85.61 (0.89)	n/a	n/a	n/a	n/a
Orlistat	n/a	n/a	82.33 (1.52)	n/a	n/a	n/a
Arbutin	n/a	n/a	n/a	41.46 (1.51)	n/a	n/a
Galantamine	n/a	n/a	n/a	n/a	88.88 (0.96)	41.41 (4.11)

One treatment for diabetes mellitus is to limit glucose absorption using inhibitors against enzymes responsible for breaking the carbohydrates, such as α-glucosidase and

α-amylase, and the potential of plant-derived extracts as inhibition agents has been investigated [74,75]. Some compounds linked to anti-diabetic properties are phenolics, flavonoids, and anthocyanidins [74]. The α-glucosidase inhibition activity of the EtOH extract (68.63%) is high, especially when compared to acarbose with the same concentration (85.61%) of 10 mg/mL. Raw water extracts, which still include a high amount of salt, had moderate α-glucosidase inhibition activity (34.12% and 8.93% for extracts from fibre residue and completely lignified biomass, respectively), which could be improved by extract purification and increasing the concentration of phenolics. There was no significant difference in the α-glucosidase inhibition activity of water extracts from partly lignified and completely lignified biomass. Considering other *Salicornia* spp., flavonoids isorhamnetin 3-*O*-glucoside and quercetin 3-*O*-glucoside isolated from Korean *S. herbacea* (syn. *S. europaea*) EtOH extract have previously shown potential for blood sugar regulation by α-glucosidase inhibition [76]. Similarly, hydroxycinnamic acid trans-ferulic acid, also found in *S. ramosissima*, has also been reported to have anti-diabetic properties [60]. Hwang et al. [77] also reported α-glucosidase inhibition of 31.9% using desalted 70% aqueous EtOH extracts from fresh shoots of *S. herbacea* with a concentration of 0.5 mg/mL.

For the EtOH extract from *S. ramosissima* fibre residue, the α-amylase inhibition activity was observed visually by the change in sample colour. However, due to precipitation in the sample, it was not possible to reliably measure the absorbance. Green juice fractions and water extracts showed only a very low inhibition of α-amylase.

Obesity, resulting from excessive accumulation of fats, affects metabolic health and its increased prevalence has led to various public health concerns worldwide [78]. To address this issue, phytochemicals with lipase inhibitory properties have been studied as a means of reducing lipid absorption [78]. *S. herbacea* has been shown to have lipase inhibition activity in vitro, and its consumption reduced plasma triglyceride and cholesterol levels in an animal model [77,79]. In addition to their use as anti-obesity agents, lipase inhibitory phytochemicals have been investigated for their potential in treating acne, a common chronic disease, as bacteria related to acne produce lipase to break down triglycerides in sebum to free FA, which then causes skin inflammation [80]. EtOH extract from *S. ramosissima* fibres showed moderate (41.74%) lipase inhibition at a concentration of 10 mg/mL. There was significant differences in the lipase inhibition activity of water extracts from fibre residue and completely lignified biomass. Together with potential anti-diabetic properties, the obtained results indicate that extracts from *S. ramosissima* could provide potential ingredients for nutraceutical and pharmaceutical applications targeting obesity and related lifestyle diseases. However, further investigation and testing are required to confirm the therapeutic properties.

Tyrosinase inhibitors are investigated as skin-whitening agents for cosmetics and for treating hyperpigmentation, but they also have an important role in food and agricultural applications for preventing products oxidation [81]. In order to preserve the appearance and nutritional properties of fresh foodstuff such as cut fruits, safer natural anti-browning agents have been investigated to replace commonly used sulfiting agents [82]. *S. ramosissima* EtOH extract (10 mg/mL) exhibited high tyrosinase inhibition activity of 71.85%. Anti-tyrosinase activity of *Salicornia* spp. has been explored in several studies. According to Ahn et al. [83], the inhibition activity of more than 50% was reached at a concentration of 60 mg/mL of *S. bigelovii* ethyl acetate extract, which is a much higher required concentration. However, the EtOH extract of *S. europaea* has shown 21.04% activity with a concentration of 1 mg/mL [84]. Sung et al. [67] also found that even low concentrations (0.1 µg/mL) of water extract from *S. herbacea* inhibit tyrosinase (>50%) and significantly reduce melanin synthesis in melanoma B16 cells. Copper is a cofactor of tyrosinase, and tyrosinase inhibition activity may be linked to the CCA of extracts [85]. However, no tyrosinase inhibition activity was observed in *S. ramosissima* fibre residue water extract, which exhibited the highest CCA in antioxidant assays. Considering dermo-cosmetic applications, *S. ramosissima* water extract supplemented cream has already been shown to reduce mechanically evoked itching (hyperkinesis), a condition related to atopic der-

matitis, and regulate the skin barrier [85]. Additionally, a cream supplemented with a 3-methoxy-3-methyl-1-butanol extract from Japanese *S. europaea* has shown promising results for UVB-protective properties by improving the skin texture in areas exposed to the sun [21]. Therefore, tyrosinase inhibition activity, together with these effects, could indicate the potential for *Salicornia* extracts for use in dermo-cosmetics. However, further investigation is needed to evaluate the therapeutic properties and effects of the long-term use of extracts.

Cholinesterase enzymes are responsible for the breaking down of acetylcholine and other choline esters, which function as neurotransmitters. Targeting these enzymes with inhibitory agents and increasing the levels of neurotransmitters has been seen as a potential symptomatic treatment for neurogenerative diseases [86,87]. The EtOH extract from fibre residue (10 mg/mL) exhibited high AChE inhibition activity (68.42%), and green juice fractions showed low to moderate AChE and BuChE inhibition activities. Higher inhibition of cholinesterase enzymes was observed using green juice from Portuguese plants compared to French plants. Differences could be explained by phenotypic variations, such as different phytochemicals present in the fractions. However, a detailed metabolomic analysis would be needed to determine the different compounds present in the juice fractions. The studied water extracts did not exhibit cholinesterase inhibition activity. However, Pinto et al. [29] previously determined the AChE inhibition activity of 1 mg/mL water extract from lignified *S. ramosissima* to be 32.34% using a commercial assay kit and showed the extract to be rich in caffeoylquinic acid derivatives. Besides intra-specific variation, the difference could be due to different extraction methods and potential compound degradation, as Pinto et al. [29] used maceration in lower temperatures and shorter resident time than in Soxhlet extraction. Karthivashan et al. [88] reported in vitro AChE inhibition activities of approx. 42% and approx. 78% for 1 mg/mL desalted EtOH extract (rich isorhamnetin and acanthoside B) and enzyme-digested wild-harvested Korean *S. europaea*, respectively, and showed significant suppression in AChE activity in the mice model. The desalted EtOH extract from *S. europaea* has also been tested on subjects complaining of memory dysfunction without dementia, but regardless of some positive results concerning the comprehension of spoken language function and Stroop test results, the study has its limitations due to the small number of subjects and short duration [89]. Phytochemicals, especially phenolics, and the potential synergistic effect of different compounds in plant extract matrices have been suggested to contribute to the neuroprotective properties of botanical extracts [29]. However, further investigation is needed to reveal the potential of botanical extracts as therapeutic agents.

Considering biorefinery, value-added products targeted to the biopharmaceutical industry and cosmetics are desirable, as they often have a relatively high market value and can also be seen as some of the key applications of *Salicornia* species [72]. Full metabolomic profiling and further analysis considering the contribution of specific compounds to different bioactivity are still open for investigation. Several mixtures of phenolic compounds have shown synergistic effects [90], and these mechanisms are still unexplored to a great extent. If the botanical extracts from halophytes could be utilised as a matrix instead of purified isolated compounds, some costly downstream processing steps could be avoided. However, the phenotypical variation due to the biomass harvest stage and cultivation conditions, which could be observed when comparing Portuguese and French biomass, may cause challenges when the extracted matrix is produced for an application requiring high consistency, such as biopharmaceuticals. Generally, biorefinery processes must be designed to withstand some degree of variation in the raw material composition.

4. Conclusions

Bioactive properties of different residue fractions from *S. ramosissima* biomasses were assessed. The FA profile showed a high amount of PUFA. The water extract from completely lignified biomass and EtOH extract from the fibre residue fraction had the highest concentration of phenolic compounds. Aqueous extracts exhibited high antioxidant activ-

ity comparable to extracts with organic solvents, making them interesting for industrial applications. EtOH extract from fibre residue had high and moderate inhibition activity against α-glucosidase and lipase, respectively, indicating the potential for nutraceuticals and biopharmaceutical applications targeting obesity and diabetes. Tyrosinase and lipase inhibition activities also make the extracts interesting for cosmetic applications. Since raw extracts were considered, bioactivity can be improved by purification and increasing the concentration of phytochemicals. Extracts from residual fractions obtained with non-toxic solvents exhibited bioactivities comparable to fresh *S. ramosissima*, which has increased interest as a nutrient-rich commercial vegetable and potential source of bioactive compounds. Overall, residue fractions from *S. ramosissima* could be a potential source of bioactive extracts, making it interesting to investigate possible biorefinery concepts further for maximum feedstock valorisation.

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References

1. EIP-AGRI Focus Group Soil Salinisation: Final Report. Available online: https://ec.europa.eu/eip/agriculture/en/publications/eip-agri-focus-group-soil-salinisation-final (accessed on 20 January 2022).
2. Lopes, M.; Cavaleiro, C.; Ramos, F. Sodium Reduction in Bread: A Role for Glasswort (*Salicornia ramosissima* J. Woods). *Compr. Rev. Food Sci. Food Saf.* **2017**, *16*, 1056–1071. [CrossRef] [PubMed]
3. Silva, A.M.; Lago, J.P.; Pinto, D.; Moreira, M.M.; Grosso, C.; Cruz Fernandes, V.; Delerue-Matos, C.; Rodrigues, F. Salicornia ramosissima Bioactive Composition and Safety: Eco-Friendly Extractions Approach (Microwave-Assisted Extraction vs. Conventional Maceration). *Appl. Sci.* **2021**, *11*, 4744. [CrossRef]
4. Oliveira-Alves, S.C.; Andrade, F.; Prazeres, I.; Silva, A.B.; Capelo, J.; Duarte, B.; Caçador, I.; Coelho, J.; Serra, A.T.; Bronze, M.R. Impact of drying processes on the nutritional composition, volatile profile, phytochemical content and bioactivity of *Salicornia ramosissima* J. Woods. *Antioxidants* **2021**, *10*, 1312. [CrossRef]
5. Ekanayake, S.; Egodawatta, C.; Attanayake, R.N.; Perera, D. From salt pan to saucepan: *Salicornia*, a halophytic vegetable with an array of potential health benefits. *Food Front.* **2023**, *3*, 1–33. [CrossRef]
6. Maciel, E.; Domingues, P.; Domingues, M.R.M.; Calado, R.; Lillebø, A. Halophyte plants from sustainable marine aquaponics are a valuable source of omega-3 polar lipids. *Food Chem.* **2020**, *320*, 126560. [CrossRef] [PubMed]
7. Agudelo, A.; Carvajal, M.; Martinez-Ballesta, M.D.C. Halophytes of the Mediterranean Basin—Underutilized Species with the Potential to Be Nutritious Crops in the Scenario of the Climate Change. *Foods* **2021**, *10*, 119. [CrossRef]
8. Ventura, Y.; Sagi, M. Halophyte crop cultivation: The case for Salicornia and Sarcocornia. *Environ. Exp. Bot.* **2013**, *92*, 144–153. [CrossRef]

9. Belal, I.E.H.; Al-Dosari, M. Replacement of Fish Meal with Salicornia Meal in Feeds for Nile Tilapia Oreochromis niloticus. *J. World Aquac. Soc.* **1999**, *30*, 285–289. [CrossRef]
10. Abdal, M.S. Salicornia production in Kuwait. *World Appl. Sci. J.* **2009**, *6*, 1033–1038.
11. Alassali, A.; Cybulska, I.; Galvan, A.R.; Thomsen, M.H. Wet fractionation of the succulent halophyte Salicornia sinus-persica, with the aim of low input (water saving) biorefining into bioethanol. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 1769–1779. [CrossRef]
12. Christiansen, A.H.C.; Lyra, D.A.; Jørgensen, H. Increasing the value of Salicornia bigelovii green biomass grown in a desert environment through biorefining. *Ind. Crops Prod.* **2020**, *160*, 113105. [CrossRef]
13. Hulkko, L.S.S.; Turcios, A.E.; Kohnen, S.; Chaturvedi, T.; Papenbrock, J.; Thomsen, M.H. Cultivation and characterisation of Salicornia europaea, Tripolium pannonicum and Crithmum maritimum biomass for green biorefinery applications. *Sci. Rep.* **2022**, *12*, 20507. [CrossRef] [PubMed]
14. Monção, M.; Wretborn, T.; Rova, U.; Matsakas, L.; Christakopoulos, P. Salicornia dolichostachya organosolv fractionation: Towards establishing a halophyte biorefinery. *RSC Adv.* **2022**, *12*, 28599–28607. [CrossRef] [PubMed]
15. Cybulska, I.; Brudecki, G.P.; Alassali, A.; Thomsen, M.H.; Brown, J.J. Phytochemical composition of some common coastal halophytes of the United Arab Emirates. *Emir. J. Food Agric.* **2014**, *26*, 1046–1056. [CrossRef]
16. Hulkko, L.S.S.; Chaturvedi, T.; Thomsen, M.H. Extraction and Quantification of Chlorophylls, Carotenoids, Phenolic Compounds, and Vitamins from Halophyte Biomasses. *Appl. Sci.* **2022**, *12*, 840. [CrossRef]
17. Giordano, R.; Saii, Z.; Fredsgaard, M.; Hulkko, L.S.S.; Poulsen, T.B.G.; Thomsen, M.E.; Henneberg, N.; Zucolotto, S.M.; Arendt-Nielsen, L.; Papenbrock, J.; et al. Pharmacological insights into halophyte bioactive extract action on anti-inflammatory, pain relief and antibiotics-type mechanisms. *Molecules* **2021**, *26*, 3140. [CrossRef] [PubMed]
18. Isca, V.M.S.; Seca, A.M.L.; Pinto, D.C.G.A.; Silva, H.; Silva, A.M.S. Lipophilic profile of the edible halophyte Salicornia ramosissima. *Food Chem.* **2014**, *165*, 330. [CrossRef] [PubMed]
19. Ameixa, O.M.C.C.; Rebelo, J.; Silva, H.; Pinto, D.C.G.A. Gall midge *Baldratia salicorniae* Kieffer (Diptera: Cecidomyiidae) infestation on Salicornia europaea L. induces the production of specialized metabolites with biotechnological potential. *Phytochemistry* **2022**, *200*, 113207. [CrossRef]
20. Custodio, L.; Garcia-Caparros, P.; Pereira, C.G.; Castelo-Branco, P. Halophyte Plants as Potential Sources of Anticancer Agents: A Comprehensive Review. *Pharmaceutics* **2022**, *14*, 2406. [CrossRef]
21. Doi, N.; Togari, H.; Minagi, K.; Nakaoji, K.; Hamada, K.; Tatsuka, M. Protective Effects of Salicornia europaea on UVB-Induced Misoriented Cell Divisions in Skin Epithelium. *Cosmetics* **2020**, *7*, 44. [CrossRef]
22. Ferreira, D.; Isca, V.M.S.; Leal, P.; Seca, A.M.L.; Silva, H.; de Lourdes Pereira, M.; Silva, A.M.S.; Pinto, D.C.G.A. Salicornia ramosissima: Secondary metabolites and protective effect against acute testicular toxicity. *Arab. J. Chem.* **2018**, *11*, 70–80. [CrossRef]
23. Buhmann, A.; Papenbrock, J. An economic point of view of secondary compounds in halophytes. *Funct. Plant Biol.* **2013**, *40*, 952. [CrossRef]
24. Chaturvedi, T.; Christiansen, A.H.C.; Gołębiewska, I.; Thomsen, M.H. Salicornia Species: Current Status and Future Potential. In *Future of Sustainable Agriculture in Saline Environments*; CRC Press: Boca Raton, FL, USA, 2021; pp. 461–482.
25. Nutraceuticals Market Size & Share Report, 2021–2030. Available online: https://www.grandviewresearch.com/industry-analysis/nutraceuticals-market (accessed on 8 June 2022).
26. Natural Skin Care Products Market Report, 2022–2030. Available online: https://www.grandviewresearch.com/industry-analysis/natural-skin-care-products-market (accessed on 21 June 2022).
27. Kumar Shakya, A. Medicinal plants: Future source of new drugs. *Int. J. Herb. Med.* **2016**, *4*, 59–64.
28. Lima, A.R.; Castañeda-Loaiza, V.; Salazar, M.; Nunes, C.; Quintas, C.; Gama, F.; Pestana, M.; Correia, P.J.; Santos, T.; Varela, J.; et al. Influence of cultivation salinity in the nutritional composition, antioxidant capacity and microbial quality of Salicornia ramosissima commercially produced in soilless systems. *Food Chem.* **2020**, *333*, 127525. [CrossRef] [PubMed]
29. Pinto, D.; Reis, J.; Silva, A.M.; Salazar, M.; Dall'Acqua, S.; Delerue-Matos, C.; Rodrigues, F. Valorisation of Salicornia ramosissima biowaste by a green approach—An optimizing study using response surface methodology. *Sustain. Chem. Pharm.* **2021**, *24*, 100548. [CrossRef]
30. Kim, J.; Cho, J.-Y.; Moon, J.-H.; Choi, G.-C.; Lee, K.-D.; Ham, K.-S.; Kim, S.-J. Change of phenylpropanoic acid and flavonol contents at different growth stage of glasswort (*Salicornia herbacea* L.). *Food Sci. Biotechnol.* **2014**, *23*, 685–691. [CrossRef]
31. Cayenne, A.; Turcios, A.E.; Thomsen, M.H.; Rocha, R.M.; Papenbrock, J.; Uellendahl, H. Halophytes as Feedstock for Biogas Production: Composition Analysis and Biomethane Potential of Salicornia spp. Plant Material from Hydroponic and Seawater Irrigation Systems. *Fermentation* **2022**, *8*, 189. [CrossRef]
32. Climatologie Globale en Mai à Saint-Nazaire—Montoir—Infoclimat. Available online: https://www.infoclimat.fr/climatologie/globale/mois-de-mai/saint-nazaire-montoir/07217.html (accessed on 6 February 2023).
33. Instituto Português do Mar e da Atmosfera. Available online: https://www.ipma.pt/en/oclima/normais.clima/1981-2010/ (accessed on 7 February 2023).
34. Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. *NREL/TP-510-42622 Analytical Procedure—Determination of Ash in Biomass*; Office of Energy Efficiency & Renewable Energy: Washington, DC, USA, 2005.

35. Sluiter, A.; Hames, B.; Hyman, D.; Payne, C.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Wolfe, J. *NREL/TP-510-42621 Analytical Procedure—Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples*; Office of Energy Efficiency & Renewable Energy: Washington, DC, USA, 2008; pp. 3–5.
36. Velioglu, Y.S.; Mazza, G.; Gao, L.; Oomah, B.D. Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products. *J. Agric. Food Chem.* **1998**, *46*, 4113–4117. [CrossRef]
37. Pirbalouti, A.G.; Siahpoosh, A.; Setayesh, M.; Craker, L. Antioxidant activity, total phenolic and flavonoid contents of some medicinal and aromatic plants used as herbal teas and condiments in Iran. *J. Med. Food* **2014**, *17*, 1151–1157. [CrossRef] [PubMed]
38. Li, Y.G.; Tanner, G.; Larkin, P. The DMACA-HCl protocol and the threshold proanthocyanidin content for bloat safety in forage legumes. *J. Sci. Food Agric.* **1996**, *70*, 89–101. [CrossRef]
39. Mazza, G.; Fukumoto, L.; Delaquis, P.; Girard, B.; Ewert, B. Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia. *J. Agric. Food Chem.* **1999**, *47*, 4009–4017. [CrossRef]
40. Lichtenthaler, H.K.; Wellburn, A.R. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* **1983**, *11*, 591–592. [CrossRef]
41. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT—Food Sci. Technol.* **1995**, *28*, 25–30. [CrossRef]
42. Moreno, S.; Scheyer, T.; Romano, C.S.; Vojnov, A.A. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. *Free Radic. Res.* **2006**, *40*, 223–231. [CrossRef] [PubMed]
43. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef] [PubMed]
44. Baliga, M.S.; Jagetia, G.C.; Rao, S.K.; Babu, S.K. Evaluation of nitric oxide scavenging activity of certain spicesin vitro: A preliminary study. *Nahrung Food* **2003**, *47*, 261–264. [CrossRef]
45. Megías, C.; Pastor-Cavada, E.; Torres-Fuentes, C.; Girón-Calle, J.; Alaiz, M.; Juan, R.; Pastor, J.; Vioque, J. Chelating, antioxidant and antiproliferative activity of Vicia sativa polyphenol extracts. *Eur. Food Res. Technol.* **2009**, *230*, 353–359. [CrossRef]
46. Xiao, Z.; Storms, R.; Tsang, A. A quantitative starch-iodine method for measuring alpha-amylase and glucoamylase activities. *Anal. Biochem.* **2006**, *351*, 146–148. [CrossRef]
47. Custódio, L.; Patarra, J.; Alberício, F.; da Rosa Neng, N.; Nogueira, J.M.F.; Romano, A. Phenolic composition, antioxidant potential and in vitro inhibitory activity of leaves and acorns of Quercus suber on key enzymes relevant for hyperglycemia and Alzheimer's disease. *Ind. Crops Prod.* **2015**, *64*, 45–51. [CrossRef]
48. Trentin, R.; Custódio, L.; Rodrigues, M.J.; Moschin, E.; Sciuto, K.; da Silva, J.P.; Moro, I. Exploring Ulva australis Areschoug for possible biotechnological applications: In vitro antioxidant and enzymatic inhibitory properties, and fatty acids contents. *Algal Res.* **2020**, *50*, 101980. [CrossRef]
49. Ellman, G.L.; Courtney, K.D.; Andres, V.; Featherstone, R.M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **1961**, *7*, 88–95. [CrossRef] [PubMed]
50. McDougall, G.J.; Kulkarni, N.N.; Stewart, D. Berry polyphenols inhibit pancreatic lipase activity in vitro. *Food Chem.* **2009**, *115*, 193–199. [CrossRef]
51. AAT Bioquest Inc. Quest GraphTM IC50 Calculator. Available online: https://www.aatbio.com/tools/ic50-calculator (accessed on 1 April 2022).
52. Duan, H.; Tiika, R.J.; Tian, F.; Lu, Y.; Zhang, Q.; Hu, Y.; Cui, G.; Yang, H. Metabolomics analysis unveils important changes involved in the salt tolerance of Salicornia europaea. *Front. Plant Sci.* **2023**, *13*, 5651. [CrossRef]
53. Magni, N.N.; Veríssimo, A.C.S.; Silva, H.; Pinto, D.C.G.A. Metabolomic Profile of Salicornia perennis Plant's Organs under Diverse in Situ Stress: The Ria de Aveiro Salt Marshes Case. *Metabolites* **2023**, *13*, 280. [CrossRef]
54. Cybulska, I.; Chaturvedi, T.; Brudecki, G.P.; Kádár, Z.; Meyer, A.S.; Baldwin, R.M.; Thomsen, M.H. Chemical characterization and hydrothermal pretreatment of Salicornia bigelovii straw for enhanced enzymatic hydrolysis and bioethanol potential. *Bioresour. Technol.* **2014**, *153*, 165–172. [CrossRef] [PubMed]
55. Maciel, E.; Lillebø, A.; Domingues, P.; Da Costa, E.; Calado, R.; Domingues, M.R.M. Polar lipidome profiling of Salicornia ramosissima and Halimione portulacoides and the relevance of lipidomics for the valorization of halophytes. *Phytochemistry* **2018**, *153*, 94–101. [CrossRef]
56. Barreira, L.; Resek, E.; Rodrigues, M.J.; Rocha, M.I.; Pereira, H.; Bandarra, N.; Da Silva, M.M.; Varela, J.; Custódio, L. Halophytes: Gourmet food with nutritional health benefits? *J. Food Compos. Anal.* **2017**, *59*, 35–42. [CrossRef]
57. Lopes, A.; Rodrigues, M.J.; Pereira, C.; Oliveira, M.; Barreira, L.; Varela, J.; Trampetti, F.; Custódio, L. Natural products from extreme marine environments: Searching for potential industrial uses within extremophile plants. *Ind. Crops Prod.* **2016**, *94*, 299–307. [CrossRef]
58. Padalino, L.; Costa, C.; Del Nobile, M.A.; Conte, A. Extract of *Salicornia europaea* in fresh pasta to enhance phenolic compounds and antioxidant activity. *Int. J. Food Sci. Technol.* **2019**, *54*, 3051–3057. [CrossRef]
59. Lopes, M.; Sanches-Silva, A.; Castilho, M.; Cavaleiro, C.; Ramos, F. Halophytes as source of bioactive phenolic compounds and their potential applications. *Crit. Rev. Food Sci. Nutr.* **2021**, *63*, 1078–1101. [CrossRef]
60. Limongelli, F.; Crupi, P.; Clodoveo, M.L.; Corbo, F.; Muraglia, M. Overview of the Polyphenols in Salicornia: From Recovery to Health-Promoting Effect. *Molecules* **2022**, *27*, 7954. [CrossRef] [PubMed]

61. Lu, D.; Zhang, M.; Wang, S.; Cai, J.; Zhou, X.; Zhu, C. Nutritional characterization and changes in quality of Salicornia bigelovii Torr. during storage. *LWT—Food Sci. Technol.* **2010**, *43*, 519–524. [CrossRef]
62. Falcone Ferreyra, M.L.; Rius, S.P.; Casati, P. Flavonoids: Biosynthesis, biological functions, and biotechnological applications. *Front. Plant Sci.* **2012**, *3*, 222. [CrossRef]
63. Castañeda-Loaiza, V.; Oliveira, M.; Santos, T.; Schüler, L.; Lima, A.R.; Gama, F.; Salazar, M.; Neng, N.R.; Nogueira, J.M.F.; Varela, J.; et al. Wild vs cultivated halophytes: Nutritional and functional differences. *Food Chem.* **2020**, *333*, 127536. [CrossRef] [PubMed]
64. Khoo, H.E.; Azlan, A.; Tang, S.T.; Lim, S.M. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food Nutr. Res.* **2017**, *61*, 1361779. [CrossRef]
65. De Souza, M.; Mendes, C.; Doncato, K.; Badiale-Furlong, E.; Costa, C. Growth, Phenolics, Photosynthetic Pigments, and Antioxidant Response of Two New Genotypes of Sea Asparagus (*Salicornia neei* Lag.) to Salinity under Greenhouse and Field Conditions. *Agriculture* **2018**, *8*, 115. [CrossRef]
66. Parida, A.K.; Kumari, A.; Panda, A.; Rangani, J.; Agarwal, P.K. Photosynthetic pigments, betalains, proteins, sugars, and minerals during Salicornia brachiata senescence. *Biol. Plant.* **2018**, *62*, 343–352. [CrossRef]
67. Sung, J.-H.; Park, S.-H.; Seo, D.-H.; Lee, J.-H.; Hong, S.-W.; Hong, S.-S. Antioxidative and Skin-Whitening Effect of an Aqueous Extract of *Salicornia herbacea*. *Biosci. Biotechnol. Biochem.* **2009**, *73*, 552–556. [CrossRef]
68. Ghanem, A.-M.F.M.; Mohamed, E.; Kasem, A.M.M.A.; El-Ghamery, A.A. Differential Salt Tolerance Strategies in Three Halophytes from the Same Ecological Habitat: Augmentation of Antioxidant Enzymes and Compounds. *Plants* **2021**, *10*, 1100. [CrossRef]
69. Akcin, A.; Yalcin, E. Effect of salinity stress on chlorophyll, carotenoid content, and proline in Salicornia prostrata Pall. and *Suaeda prostrata* Pall. subsp. *prostrata* (Amaranthaceae). *Rev. Bras. Bot.* **2016**, *39*, 101–106. [CrossRef]
70. Ksouri, R.; Ksouri, W.M.; Jallali, I.; Debez, A.; Magné, C.; Hiroko, I.; Abdelly, C. Medicinal halophytes: Potent source of health promoting biomolecules with medical, nutraceutical and food applications. *Crit. Rev. Biotechnol.* **2012**, *32*, 289–326. [CrossRef] [PubMed]
71. Faria, G.Y.Y.; Souza, M.M.; Oliveira, J.R.M.; Costa, C.S.B.; Collares, M.P.; Prentice, C. Effect of ultrasound-assisted cold plasma pretreatment to obtain sea asparagus extract and its application in Italian salami. *Food Res. Int.* **2020**, *137*, 109435. [CrossRef] [PubMed]
72. Cárdenas-Pérez, S.; Piernik, A.; Chanona-Pérez, J.J.; Grigore, M.N.; Perea-Flores, M.J. An overview of the emerging trends of the Salicornia L. genus as a sustainable crop. *Environ. Exp. Bot.* **2021**, *191*, 104606. [CrossRef]
73. Furman, D.; Campisi, J.; Verdin, E.; Carrera-Bastos, P.; Targ, S.; Franceschi, C.; Ferrucci, L.; Gilroy, D.W.; Fasano, A.; Miller, G.W.; et al. Chronic inflammation in the etiology of disease across the life span. *Nat. Med.* **2019**, *25*, 1822–1832. [CrossRef]
74. Kumar, S.; Narwal, S.; Kumar, V.; Prakash, O. α-glucosidase inhibitors from plants: A natural approach to treat diabetes. *Pharmacogn. Rev.* **2011**, *5*, 19–29. [CrossRef] [PubMed]
75. Khadayat, K.; Marasini, B.P.; Gautam, H.; Ghaju, S.; Parajuli, N. Evaluation of the alpha-amylase inhibitory activity of Nepalese medicinal plants used in the treatment of diabetes mellitus. *Clin. Phytosci.* **2020**, *6*, 34. [CrossRef]
76. Lee, D.; Park, J.Y.; Lee, S.; Kang, K.S. In Vitro Studies to Assess the α-Glucosidase Inhibitory Activity and Insulin Secretion Effect of Isorhamnetin 3-O-Glucoside and Quercetin 3-O-Glucoside Isolated from Salicornia herbacea. *Processes* **2021**, *9*, 483. [CrossRef]
77. Hwang, J.-Y.; Lee, S.-K.; Jo, J.-R.; Kim, M.-E.; So, H.-A.; Cho, C.-W.; Seo, Y.-W.; Kim, J.-I. Hypolipidemic effect of Salicornia herbacea in animal model of type 2 diabetes mellitus. *Nutr. Res. Pract.* **2007**, *1*, 371. [CrossRef]
78. Rajan, L.; Palaniswamy, D.; Mohankumar, S.K. Targeting obesity with plant-derived pancreatic lipase inhibitors: A comprehensive review. *Pharmacol. Res.* **2020**, *155*, 104681. [CrossRef]
79. Rahman, M.M.; Kim, M.-J.; Kim, J.-H.; Kim, S.-H.; Go, H.-K.; Kweon, M.-H.; Kim, D.-H. Desalted *Salicornia europaea* powder and its active constituent, *trans* -ferulic acid, exert anti-obesity effects by suppressing adipogenic-related factors. *Pharm. Biol.* **2018**, *56*, 183–191. [CrossRef]
80. Soleymani, S.; Farzaei, M.H.; Zargaran, A.; Niknam, S.; Rahimi, R. Promising plant-derived secondary metabolites for treatment of acne vulgaris: A mechanistic review. *Arch. Dermatol. Res.* **2020**, *312*, 5–23. [CrossRef]
81. Zolghadri, S.; Bahrami, A.; Hassan Khan, M.T.; Munoz-Munoz, J.; Garcia-Molina, F.; Garcia-Canovas, F.; Saboury, A.A. A comprehensive review on tyrosinase inhibitors. *J. Enzyme Inhib. Med. Chem.* **2019**, *34*, 279–309. [CrossRef]
82. Rodrigues, M.J.; Castañeda-Loaiza, V.; Monteiro, I.; Pinela, J.; Barros, L.; Abreu, R.M.V.; Oliveira, M.C.; Reis, C.; Soares, F.; Pousão-Ferreira, P.; et al. Metabolomic Profile and Biological Properties of Sea Lavender (*Limonium algarvense* Erben) Plants Cultivated with Aquaculture Wastewaters: Implications for Its Use in Herbal Formulations and Food Additives. *Foods* **2021**, *10*, 3104. [CrossRef] [PubMed]
83. Ahn, B.K.; Kim, R.; Choi, D.; Kim, Y.S. Effect of *Salicornia bigelovii* extract on the activities of whitening and anti-wrinkle. *Appl. Chem. Eng.* **2011**, *22*, 56–60.
84. Jiratchayamaethasakul, C.; Ding, Y.; Hwang, O.; Im, S.T.; Jang, Y.; Myung, S.W.; Lee, J.M.; Kim, H.S.; Ko, S.C.; Lee, S.H. In vitro screening of elastase, collagenase, hyaluronidase, and tyrosinase inhibitory and antioxidant activities of 22 halophyte plant extracts for novel cosmeceuticals. *Fish. Aquat. Sci.* **2020**, *23*, 6. [CrossRef]
85. Giordano, R.; Aliotta, G.E.; Johannesen, A.S.; Voetmann-Jensen, D.; Laustsen, F.H.; Andersen, L.A.; Rezai, A.; Fredsgaard, M.; Lo Vecchio, S.; Arendt-Nielsen, L.; et al. Effects of Salicornia-Based Skin Cream Application on Healthy Humans' Experimental Model of Pain and Itching. *Pharmaceuticals* **2022**, *15*, 150. [CrossRef] [PubMed]

86. Hassan, M.; Raza, H.; Abbasi, M.A.; Moustafa, A.A.; Seo, S.Y. The exploration of novel Alzheimer's therapeutic agents from the pool of FDA approved medicines using drug repositioning, enzyme inhibition and kinetic mechanism approaches. *Biomed. Pharmacother.* **2019**, *109*, 2513–2526. [CrossRef] [PubMed]
87. Pagano, G.; Rengo, G.; Pasqualetti, G.; Femminella, G.D.; Monzani, F.; Ferrara, N.; Tagliati, M. Cholinesterase inhibitors for Parkinson's disease: A systematic review and meta-analysis. *J. Neurol. Neurosurg. Psychiatry* **2015**, *86*, 767–773. [CrossRef]
88. Karthivashan, G.; Park, S.Y.; Kweon, M.H.; Kim, J.; Haque, M.E.; Cho, D.Y.; Kim, I.S.; Cho, E.A.; Ganesan, P.; Choi, D.K. Ameliorative potential of desalted Salicornia europaea L. extract in multifaceted Alzheimer's-like scopolamine-induced amnesic mice model. *Sci. Rep.* **2018**, *8*, 8826. [CrossRef]
89. Lee, W.J.; Shin, Y.W.; Kim, D.E.; Kweon, M.H.; Kim, M. Effect of desalted *Salicornia europaea* L. ethanol extract (PM-EE) on the subjects complaining memory dysfunction without dementia: A 12 week, randomized, double-blind, placebo-controlled clinical trial. *Sci. Rep.* **2020**, *10*, 19914. [CrossRef]
90. Skroza, D.; Generalić Mekinić, I.; Svilović, S.; Šimat, V.; Katalinić, V. Investigation of the potential synergistic effect of resveratrol with other phenolic compounds: A case of binary phenolic mixtures. *J. Food Compos. Anal.* **2015**, *38*, 13–18. [CrossRef]

Paper IV

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Harnessing the Value of *Tripolium pannonicum* and *Crithmum maritimum* Halophyte Biomass through Integrated Green Biorefinery

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Abstract: Bioactive extracts are often the target fractions in bioprospecting, and halophyte plants could provide a potential source of feedstock for high-value applications as a part of integrated biorefineries. *Tripolium pannonicum* (Jacq.) Dobroez. (sea aster) and *Crithmum maritimum* L. (sea fennel) are edible plants suggested for biosaline halophyte-based agriculture. After food production and harvesting of fresh leaves for food, the inedible plant fractions could be utilized to produce extracts rich in bioactive phytochemicals to maximize feedstock application and increase the economic feasibility of biomass processing to bioenergy. This study analyzed fresh juice and extracts from screw-pressed sea aster and sea fennel for their different phenolic compounds and pigment concentrations. Antioxidant and enzyme inhibition activities were also tested in vitro. Extracts from sea aster and sea fennel had phenolic contents up to 45.2 mgGAE/gDM and 64.7 mgGAE/gDM, respectively, and exhibited > 70% antioxidant activity in several assays. Ethanol extracts also showed > 70% inhibition activity against acetylcholinesterase and > 50% inhibition of tyrosinase and α-glucosidase. Therefore, these species can be seen as potential feedstocks for further investigations.

Keywords: biomass; biorefinery; halophytes; bioprospecting; bioproducts; phytochemicals

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1. Introduction

In order to mitigate global warming and meet the set climate goals, it is necessary to transition from a linear fossil-based economy towards circular bioeconomies and sustainable agricultural intensification, which leads to an expected increase in the world's biomass demand driven by food security, bioproducts and bioenergy [1–3]. Muscat et al. [4] argued that for the effective use of available biomass, producing goods for fulfilling basic human needs, and the chemical industry, due to a lack of sustainable alternatives, should have the highest priority. The need to find bio-based alternatives to various essential products highlights the importance of integrated biorefineries, where multiple products are produced together with bioenergy in a cascade system to maximize the valorization of the available biomass while also increasing the economic feasibility of the processing [2,4–7]. This concept is visualized in Figure 1. Furthermore, these multi-product systems have also been described as an important pillar and tool for circular bioeconomies [2,8]. According to Stegmann et al. [8], products targeting high-value applications, such as biopharmaceuticals and nutraceuticals, are considered to have significant potential within circular bioeconomies. Therefore, bioprospecting the feedstock for these applications can be seen as an inseparable part of future integrated biorefinery design, as maximum value creation can often be vital for process profitability. The agricultural and food processing sectors are significant producers of plant biomass, and currently, residual fractions are often used for biogas or bulk chemical production, compost, or even end up in landfill [7,9–11]. However, Jimenez-Lopez et al. [7] and

Caldeira et al. [12] emphasize the potential of valorizing these fractions through the production of bioactive and functional compounds. Cascading biorefineries targeting products for health, food and feed, and cosmetics industries have also been at the forefront of the discussions related to, for example, algal biomass processing [13]. Due to limited freshwater resources and constrained availability of arable land due to increased soil and water salinity, biosaline agriculture focused on cultivating naturally salt-tolerant plants, i.e., halophytes, have been seen as one of the key solutions to sustainably create added value and rehabilitate marginal degraded areas [14–16]. Marine biomass, including coastal plants, could also contribute to nutrient uptake and recovery as a part of future blue-green bioeconomies [17].

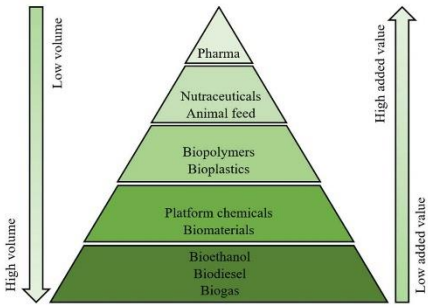


Figure 1. Value pyramid of bioproducts. Adapted from Stegmann et al. [8] and Zabaniotou [2].

According to the definition by Flower and Colmer [18], halophytes are plants that can grow and reproduce under salinity conditions of 200 mM NaCl or more, constituting approximately 1% of the world's plant species. As a part of their adaptation to extreme environmental conditions, such as high salinity, drought, and strong UV radiation, halophytes produce high concentrations of protective bioactive phytochemicals. This way, plants maintain ion homeostasis and protect themselves from cellular damage and the metabolic issues caused by the production and accumulation of reactive oxygen species [19,20]. Due to this pronounced availability of bioactive compounds, halophytes have increased interest in bioprospecting for ingredients for nutraceuticals, cosmetics, and biomedicines [5,19–24]. *Tripolium pannonicum* (Jacq.) Dobrocz. (Asteraceae, syn. *Aster tripolium*, commonly known as sea aster) and *Crithmum maritimum* L. (Apicaceae, commonly known as sea fennel or rock samphire) are edible facultative halophytes used for culinary purposes and suggested species for biosaline agriculture [15,16,25–27]. They can be efficiently grown in hydroponic and aquaponic systems, and they have shown potential for cultivation in degraded soils and marginal land areas [25,28–31]. The extracts from these plants have been reported to have antioxidant, anti-inflammatory, antimicrobial, antiviral, antifungal, neuro-protective, anti-diabetic, and antitumor properties [21,32–41]. In folk medicine, sea fennel was used for its diuretic properties, parasite prevention, and in case of digestive issues; sea aster was used as an expectorant to relieve cough [26,41,42]. Potent essential oils from sea fennel are also suggested for pest control [43–45].

In the green biorefinery concept, the non-food grade fractions of plants are harvested and screw-pressed into liquid (juice) and solid (fiber residue) fractions [6,46]. This approach has been previously tested for sea aster and sea fennel, focusing on the distribution of primary metabolites [31]. Green biorefinery is commonly used for grasses

and forages, where the juice is often used to produce protein and organic acids, and the solid fraction is fed to animals or used for bulk materials and biogas [47–49]. As the high salt content of halophytes may limit their direct use as fodder [25,50], bioactive botanical extracts rich in phytochemicals could provide another value-added stream for halophyte-based biorefinery. This approach could be desirable for edible halophytes, as it would provide not only a nutrition-rich food source but also a way to valorize the residual fractions of the plants for bioproducts after the food production period and harvesting fresh leaves for food, as the plants become unpalatable due to lignification. After the preparation of extracts, the residual lignocellulosic fibers can be used to produce biochemicals and bioenergy in more traditional biorefinery processes, as extraction may also work as a mild pretreatment of often recalcitrant lignocellulose [11,51–55]. In this regard, biogas production from sea aster has been previously studied [53,55,56].

This study analyzed the biological activity of sea aster and sea fennel (Figure 2) juice and extracts from fiber residue fractions to evaluate their suitability for the production of high-value, low-volume products. Non-polar extracts were used to determine fatty acid (FA) profiles, whereas juice fractions and polar extracts were analyzed for the concentration of total phenolic compounds (TPC), total flavonoids compounds (TFC), total condensed tannins (TCT), total anthocyanidins (TAC), as well as photosynthetic pigments. In addition, antioxidant properties were tested in vitro for radical scavenging activity (RSA) against 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and nitric oxide (NO), as well as iron-reducing antioxidant power (FRAP), and iron and copper chelating activities (ICA and CCA, respectively). The inhibition capabilities of juices and polar extracts were also tested in vitro against enzymes involved in neurodegenerative diseases such as Alzheimer's disease (acetylcholinesterase and butyrylcholinesterase), hyperpigmentation (tyrosinase), type 2 diabetes mellitus (α-amylase and α-glucosidase), as well as obesity and acne (lipase). This study provides novel information about the bioactivity of sea fennel, as previous studies have heavily focused on essential oils or the fresh edible fraction of the plant. Also, it describes the bioactivity of sea aster extracts, which have not, to the best of our knowledge, been previously widely reported in the framework of bioprospecting and biorefinery.

Figure 2. *Tripolium pannonicum* (left) and *Crithmum maritimum* biomass.

2. Results and Discussion

2.1. Fractionation and Extraction Yields

After fractionation with a screw press, the unfiltered juice fraction corresponded to > 80% of the total fresh biomass weight in both halophyte species. The composition of lignocellulosic fibers was determined from the residual material after the water extraction in terms of cellulose (glucose and cellobiose), hemicellulose (xylose and arabinose) and Klason lignin. For sea aster fibers, the lignocellulose consisted of 44.9 (5.4)% cellulose, 29.3

(0.5)% hemicellulose, and 31.2 (5.0)% Klason lignin. In sea fennel, the lignocellulose fraction contained 42.9 (0.3)% cellulose, 22.0 (0.2)% hemicellulose, and 35.1 (0.1)% Klason lignin. The dry matter (DM) content, the chemical composition of the juice and fiber fractions, and the yields of the extracts prepared from fiber residue fractions are summarized in Table 1. Extractives yields of 18.7% and 16.5% were obtained from sea aster and sea fennel fibers, respectively, using water as a solvent, whereas with ethanol, 25.9% and 13.6% sea aster and sea fennel extraction yields, respectively, were achieved. Water-soluble salts accumulated in the plant tissues are present in the extracts, and the ash content of dry sea aster and sea fennel water extracts was 22.72 (1.66)% and 27.08 (0.92)%, respectively. Similarly, in the ethanol extracts from sea aster and sea fennel, the ash content was 18.45 (1.80)% and 14.43 (4.71)%, respectively.

Table 1. Dry matter (DM) content and composition of halophyte fractions and extraction yields. Values are expressed as means, with the standard deviation marked in brackets.

Fraction	DM [*w/w* %]	Water extract [g/100gDM]	Ethanol extract [g/100gDM]	n-hexane extract [g/100gDM]	Sugars [g/100gDM]	Lignin [g/100gDM]	Ash [g/100gDM]
Sea aster (*Tripolium pannonicum*)							
Juice	4.01 (0.04)	n/a	n/a	n/a	22.98 (0.71)	n/a	47.71 (0.76)
Fibres	n/a	18.73 (0.50)	25.88 (2.47)	2.87 (0.04)	37.64 (4.56)	16.98 (1.18)	6.30 (0.01)
Sea fennel (*Crithmum maritimum*)							
Juice	7.26 (0.01)	n/a	n/a	n/a	13.24 (3.49)	n/a	36.56 (0.09)
Fibres	35.67 (0.84)	16.54 (0.33)	13.56 [1]	2.20 (0.10)	34.62 (0.67)	18.76 (4.78)	10.19 (0.07)

[1] Due to the small amount of biomass, it was not possible to run the analysis in triplicate and provide the standard deviation.

Even within the same species, the extraction yields may vary greatly due to intraspecific variations and depending on the solvent purity, extraction method, and potential pretreatments, for example, size reduction methods and ultrasound and microwave technologies [51]. Regarding sea fennel, Costa et al. [57] compared supercritical fluid extraction and ultrasound-assisted extraction methods to obtain ethanol extracts from wild-harvested sea fennel aerial parts and reported 11.2% and 33.2% yields with ultrasound-assisted extraction with 100 *v/v*% and 80 *v/v*% ethanol, respectively, whereas supercritical fluid extraction yielded only 4.5% of the extract. Souid et al. [24] reached 25% extract yield with three overnight macerations with 80% ethanol. Considering water-soluble sea fennel extractives, Pereira et al. [34] obtained 47.8% and 50.0% extract yields from "cup-of-tea" infusion and decoction of fresh sea fennel leaves, respectively. Similarly, Pedreiro et al. [58] achieved 36.3% and 46.1% extraction yield with infusion and decoction, respectively. Results from previous studies are significantly higher than the yield of water extract obtained from the fiber fraction, as a high fraction of water-soluble compounds is present in the green juice separated in the initial screw-press fractionation.

Yields of aqueous or ethanol extracts from sea aster have not been previously widely reported, but Wubshet et al. [39] obtained an 8.3% yield in maceration with 70% methanol. Wiszniewska et al. [58] reported that salt stress increases the amount of soluble carbohydrates in sea aster biomass. Therefore, the cultivation conditions may affect the concentration of sugars detected from the juice fractions. The desired composition of the lignocellulose fraction depends on the target application, and cellulose, hemicellulose, and lignin from extractives-free residue could be potentially used to produce various value-added chemicals [50]. The ash content of juice and fiber fractions are similar to those previously reported for sea aster cultivated at 171 mM NaCl and sea fennel cultivated in non-saline hydroponics [30].

2.2. Fatty Acids in Non-Polar Extracts

Considering the FA profile of sea aster, the health-beneficial polyunsaturated fatty acids (PUFA) constituted 78.2% of the total FA, followed by 20.6% saturated fatty acids (SFA) and 1.2% monounsaturated fatty acids (MUFA). The main FA found was α-linolenic acid (53.2%), and the ω-6 and ω-3 ratio was 0.5. Similar results have also been reported by Duarte et al. [59], who also found PUFA to cover > 70% of total FA in sea aster and a < 0.5 ratio of ω-6 and ω-3. Montero et al. [38] reported the content of SFA in sea aster lipids to be 19.3%, which is comparable to obtained results. Both previous studies also report α-linolenic acid as the most pronounced FA [38,59].

The total FA from sea fennel n-hexane extract constituted 49.9% PUFA, 36.7% SFA, and 13.4% MUFA. The main FA was linoleic acid (34.4%), and the ω-6 and ω-3 ratio was 2.2. The results were similar compared to those reported by Ben Hamed et al. [60] for sea fennel cultivated in non-saline conditions in a greenhouse, the main differences being the lower amount of stearic acid (3.2% of total FA compared to obtained 6.3%) and higher amount of α-linolenic acid (24.1% of total FA compared to obtained 15.5%). The obtained ω-6 and ω-3 ratio is also comparable to the ratio of 2.7 determined by Guil-Guerrero and Rodríguez-García [61] for the neutral lipid fraction from young sea fennel leaves. Only 11.5% of oleic acid was found in vegetative plant extract, whereas the seed oil from sea fennel was previously reported to be rich in oleic acid (78% of total FA) [62].

Relative concentrations of fatty acids in n-hexane extracts from halophyte fiber residues are summarized in Table 2. In both species, the ratio of ω-6 and ω-3 FA is low, which has been linked to a reduced risk of cardiovascular diseases and cancer, and the alleviation of diseases with chronic inflammation [63]. However, the total lipids content of halophytes is often low and obtained yields for n-hexane extracts were only 2.9% and 2.2% from sea aster and sea fennel fibers, respectively. Therefore, even if the lipids are rich in health-beneficial PUFA, the role of lipids from these species in industrial biorefinery applications may not be as significant as other extracts.

Table 2. Relative concentration of fatty acids (FA) in n-hexane extracts obtained from fiber residues of sea aster (*Tripolium pannonicum*) and sea fennel (*Crithmum maritimum*). Values are expressed as means, with the standard deviation marked in brackets.

Fatty acids	Sea aster [% FA/total FA]	Sea fennel [% FA/total FA]
Myristic acid	n.d.	1.5 (2.2)
Palmitic acid	19.0 (0.2)	28.9 (1.8)
Palmitoleic acid	n.d.	1.9 (2.7)
Stearic acid	1.6 (0.0)	6.3 (0.3)
Oleic acid	1.2 (0.5)	11.5 (0.2)
Linoleic acid	24.9 (0.2)	34.4 (1.8)
α-Linolenic acid	53.2 (0.3)	15.5 (0.7)
Arachidic acid	n.d.	n.d.
Behenic acid	n.d.	n.d.
Lignoceric acid	n.d.	n.d.
Σ SFA	20.6 (0.2)	36.7 (4.3)
Σ MUFA	1.2 (0.5)	13.4 (2.9)
Σ PUFA	78.2 (0.5)	49.9 (1.2)
ω-6 / ω-3	0.5 (0.0)	2.2 (0.2)

n.d.: not detected, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

2.3. Bioactive Compounds

The total contents of phenolic compounds and pigments determined in juice and extracts of sea aster and sea fennel are presented in Table 3. The highest concentration of

TPC was found in the ethanol extract from sea fennel (64.7 mgGAE/gDM). The two species had no significant difference in the TPC content of aqueous extracts, with results being 32.3 mgGAE/gDM and 33.5 mgGAE/gDM for sea aster and sea fennel, respectively. However, extracts from sea aster fibers had a higher concentration of TFC, 5.4 mgQE/gDM and 6.6 mgQE/gDM in aqueous and ethanol extracts, respectively. Condensed tannins were only found in low concentrations from sea aster ethanol extract, whereas they were not detected in other samples. Depending on the target application, the lack of condensed tannins, also called proanthocyanidins, can be seen as an advantage, as tannins can be considered potential antinutrients due to their protein-binding properties, depending on the intake [64]. However, tannins have been previously studied for their anti-parasitic effects [65]. It was not possible to reliably determine the content of total anthocyanidins for ethanol extract, as precipitation was observed in samples. Photosynthetic pigments were found mainly in the ethanol extracts, and especially sea aster was rich in chlorophyll (3631.95 µg/gDM) and carotenoids (299.05 µg/gDM).

Table 3. Dry matter (DM) content and composition of halophyte fractions and extraction yields. Values are expressed as means, with the standard deviation marked in brackets. Different letters denote significantly different results ($p \leq 0.05$), calculated individually for each compound group.

Fraction	TPC [mgGAE/gDM]	TFC [mgQE/gDM]	TCT [mgCE/gDM]	TAC [mgCCE/gDM]	CHL a [µg/gDM]	CHL b [µg/gDM]	TCA [µg/gDM]
Sea aster (*Tripolium pannonicum*)							
Juice	n.d.	n.d.	n.d.	n.d.	89.42 (14.56) ^{c}	148.30 (24.23) ^{c}	125.75 (8.85) ^{c}
Water extract	32.34 (6.80) ^{c}	5.41 (0.68) ^{ab}	n.d.	4.37 (0.74) ^{a}	n/a	n/a	n/a
Ethanol extract	45.20 (5.27) ^{b}	6.58 (1.49) ^{a}	0.43 (0.60)	n/a	2614.08 (12.19) ^{a}	1017.87 (17.94) ^{a}	299.05 (3.79) ^{a}
Sea fennel (*Crithmum maritimum*)							
Juice	14.97 (4.56) ^{d}	n.d.	n.d.	n.d.	13.62 (0.69) ^{d}	44.28 (0.73) ^{d}	85.34 (3.20) ^{d}
Water extract	33.53 (2.53) ^{c}	4.90 (0.81) ^{b}	n.d.	1.90 (0.21) ^{b}	n/a	n/a	n/a
Ethanol extract	64.70 (9.01) ^{a}	1.84 (1.15) ^{c}	n.d.	n/a	1008.44 (14.74) ^{b}	430.92 (9.31) ^{b}	261.79 (2.03) ^{b}

n.d.: not detected or concentration lower than the limit of detection, n/a: not available, TPC: total phenolic compounds [mgGAE/gDM], TFC: total flavonoids [mgQE/gDM], TCT: total condensed tannins [mgCE/gDM], TAC: total anthocyanins [mgCCE/gDM], CHL: chlorophyll [µg/gDM], TCA: total carotenoids [µg/gDM], GAE: gallic acid equivalent, QE: quercitin equivalent, CE: catechin equivalent, CCE: cyanidin chloride equivalent, DM: dry matter.

Meot-Duros et al. [35] found 33 mgGAE/gDM of TPC, which is similar to the result from the aqueous extract, in a water-methanol extract from sea fennel harvested from the wild in late summer. However, Pereira et al. [34] reported approximately 70.5 mgGAE/gDM TPC in sea fennel leaf extracts but also found 114.1 mg rutin equivalent/gDM TFC and 1.6 mgCE/gDM TCT; however, the extracts from sea fennel stems exhibited much lower concentrations of TPC, and phytochemicals are distributed differently to plant organs. Similarly, Mekinić et al. [37] measured greatly different amounts of TPC from sea fennel leaves (35.1 mgGAE/gDM) and stems (7.6 mgGAE/gDM). Politeo et al. [66] also reported a similar trend in aqueous sea fennel extracts.

Considering sea aster, Stankovic et al. [67] reported that a methanol extract obtained by maceration contained 144.75 mgGAE/gDM phenolic compounds and 55.43 mg rutin equivalent/gDM flavonoids. This study utilized the extract from screw-pressed fibers from the whole aerial part of the biomass instead of the whole fresh plant or selected part of plants, such as leaves, which may explain the differences in the content of phenolics. Some phytochemicals are also shown to be sensitive to different pH conditions or elevated temperatures, which, used in different extraction methods, may lead to compound instability and degradation [68]. Differences in sea fennel flavonoid content could also be explained by abiotic stresses and intraspecific variation but also by different assays used. The effect of abiotic stresses on the content of phenolic compounds is highlighted in various studies [35,64,69–71]. For example, salinity and exposure to heavy metals have been

shown to increase the content of total phenols and antioxidant activity in sea aster [69,71,72].

Cultivation conditions and abiotic stresses also affect the content of photosynthetic pigments in plants, and Wiszniewska et al. [72] reported that the salinity stress increased the amount of chlorophyll in sea aster, whereas chlorophyll and carotenoid contents decreased when plants were exposed to heavy metals. On the contrary, Ventura et al. [71] reported an inverse relationship between cultivation salinity, and chlorophyll and β-carotene content of sea aster leaves. Duarte et al. [70] also showed that the content of chlorophyll and some carotenoids decreased when sea aster plants were exposed to heat or cold waves; however, the exposure to heat waves did not affect the content of β-carotene, lutein and certain xanthophylls. Geissler et al. [73] determined the content of chlorophyll and carotenoids in the sea aster leaf surface to be approximately 51 mg/cm^2 and 8 mg/cm^2, respectively. Chlorophyll is especially sensitive to degradation, and different drying methods have been shown to affect the chlorophyll concentration of sea fennel; microwave-assisted drying and freeze-drying are significantly more gentle compared to air drying in an oven [42]. Considering carotenoids in sea fennel, Sarrou et al. [74] identified lutein and neoxanthin as major carotenoid compounds.

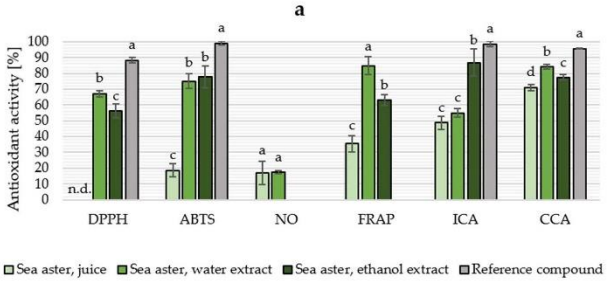
2.4. Antioxidant Properties

The antioxidant activity of sea aster and sea fennel juice fractions and extracts is presented in Figure 3. The juice faction from sea fennel exhibited high ABTS scavenging activity (60.75%) and FRAP (77.67%), and CCA (71.19%) in 10 mg/mL concentration; however, the half-maximum effective concentration (EC_{50}) values were higher compared to extracts (Table 4). The DPPH and ABTS scavenging activity of both sea fennel and sea aster aqueous extracts were similar, but sea aster had more pronounced antioxidant activity in metal-based assays. On the contrary, sea fennel fractions had higher NO scavenging activity, estimating the extract's anti-inflammatory properties, than sea aster fractions. Especially in ethanol extracts, there was a large variation in the antioxidant activity between different assays, highlighting the importance of running multiple assays to evaluate the extracts' bioactivity as a single assay provides only a limited view of the antioxidant potential. The results from the total contents of different phenolic groups also cannot directly be reflected in the antioxidant activities, bringing interest to specific phytochemicals or potential synergic interactions between them. The ethanol-soluble samples turned cloudy during the NO radical scavenging assay when the Griess reagent was added; therefore, it was not possible to measure the activity.

Table 4. The half-maximum effective concentrations (EC_{50}) [mg/mL] of antioxidant activity of juice and fiber residue extracts from sea aster (*Tripolium pannonicum*) and sea fennel (*Crithmum maritimum*).

Fraction	DPPH	ABTS	NO	FRAP	ICA	CCA
Sea aster (*Tripolium pannonicum*)						
Juice	n.d.	> 10	> 10	> 10	> 10	3.18
Water extract	< 10	2.24	> 10	1.91	< 10	3.40
Ethanol extract	7.67	4.86	n/a	1.66	1.15	2.37
Sea fennel (*Crithmum maritimum*)						
Juice	> 10	4.59	> 10	3.46	> 10	6.79
Water extract	3.53	4.36	> 10	1.13	> 10	7.03
Ethanol extract	2.84	3.95	n/a	< 10	3.10	3.32

DPPH: not detected, 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), NO: nitric oxide, FRAP: ferric reducing antioxidant power, ICA: iron chelating activity, CCA: copper chelating activity, n.d.: no activity detected, n/a: not available.



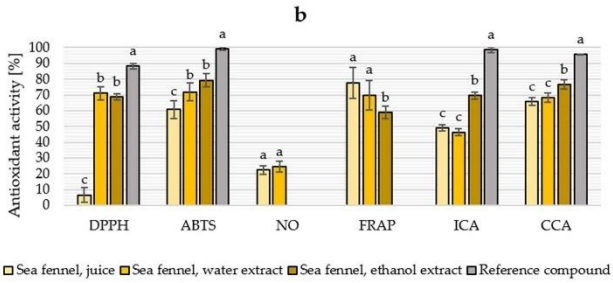


Figure 3. Antioxidant activity [%] of the 10 mg/mL sea aster (*Tripolium pannonicum*, **a**) and sea fennel (*Crithmum maritimum*, **b**) juice and extracts in relation to negative control. The reference compounds are gallic acid 1 mg/mL (DPPH and ABTS) and ethylenediaminetetraacetic acid 1 mg/mL (ICA and CCA). Different letters denote significantly different results, calculated individually for each assay. DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), NO: nitric oxide, FRAP: ferric reducing antioxidant power, ICA: iron chelating activity, CCA: copper chelating activity, n.d.: no activity detected. Different letters denote significantly different results ($p \leq 0.05$), calculated individually for each assay and plant species.

Pereira et al. [34] reported high DPPH and ABTS scavenging activity (> 86%), moderate NO scavenging activity (> 37%), and high FRAP (> 98%) of aqueous sea fennel extracts in low concentrations ("cup-of-tea" infusion and decoction), whereas the ICA and CCA were lower than the results obtained in this study. Moreover, Pedreiro et al. [58] reported EC_{50} values of 36.5 µg/mL and 37.3 µg/mL for DPPH and ABTS scavenging activity for aqueous sea fennel infusion. Those results are nearly a hundred-fold lower than the results obtained in this study. Siracusa et al. [75] also reported a 88% DPPH scavenging activity of 0.4 mg/mL sea fennel infusion with potential relation to high chlorogenic acid content. The differences in results could be explained by the plant growth stage and fractions used but also growth conditions, as cultivated plants have previously been shown to have lower phytochemical concentration and antioxidant activity compared to wild plants due to controlled conditions with less pronounced abiotic stresses [64,76].

Whereas the antioxidant activity of different sea fennel fractions is covered in literature, the use of sea aster for health-promoting purposes is rather unexplored. Stankovic et

al. [67] reported an EC_{50} value of 0.13 mg/mL for the DPPH scavenging activity of sea aster methanol extract, which is significantly lower compared to the obtained results in this study. On the other hand, Wubshet et al. [40] used nuclear magnetic resonance technology and found caffeoyl esters, flavonoids, and flavonoid glycosides in sea aster extract, which they described as potential compounds to explain the ABTS scavenging activity.

Exposure to excessive reactive oxygen species (ROS) and following oxidative stress has been linked to the development and progress of various health issues, such as neurodegenerative, metabolic and autoimmune diseases and cancer, often due to their multifactorial nature [77,78]. Furthermore, as the individual's nutrition, level of exercise, and exposure to toxins significantly contribute to the production of ROS, many of the related issues are called "lifestyle diseases" [77]. Therefore, antioxidant products capable of preventing or alleviating these emerging conditions are often targets of bioprospecting. Due to their active properties, botanical extracts are common ingredients in various high-value products, for example, in the nutraceuticals and cosmetics sectors. However, identifying and concentrating or separating compounds responsible for specific activities could be desired for some applications, such as biomedicines. Bioguided fractionation is a potential analysis method to find the target compounds. Resin absorption, chromatography, and different filtration methods are examples of technologies studied to purify and isolate compounds from extracts and separate compounds from the green juice fraction in green biorefineries [51,79,80].

2.5. Enzyme Inhibition

Inhibition activity against enzymes linked to different diseases is summarized in Figure 4. It was not possible to determine the inhibition activity against butyrylcholinesterase (BuChE) for sea fennel water extract, tyrosinase for sea fennel juice, or lipase for juice fractions from both species due to instability issues with the assays. The inhibition of α-amylase was visually observed in ethanol-based samples. However, due to sample precipitation, it was not possible to reliably measure the absorbance and determine the activity. In general, the enzyme inhibition activity was higher in ethanol extracts compared to juice fractions or aqueous extracts.

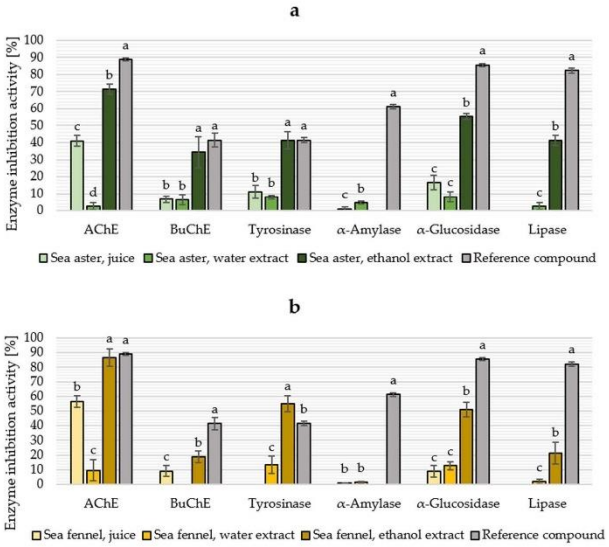


Figure 4. Enzyme inhibition activity of 10 mg/mL sample solutions from sea aster (*Tripolium pannonicum*, **a**) and sea fennel (*Crithmum maritimum*, **b**) juice and extracts. Reference compounds: galantamine 1 mg/mL (AChE and BuChE), arbutin 1 mg/mL (tyrosinase), acarbose 10 mg/mL (α-amylase and α-glucosidase), and orlistat 1 mg/mL (lipase). Different letters denote significantly different results, calculated individually for each assay. AChE: acetylcholinesterase, BuChE: butyrylcholinesterase. Different letters denote significantly different results ($p \leq 0.05$), calculated individually for each assay and plant species.

For sea aster ethanol extract, the acetylcholinesterase (AChE) inhibition activity (71.5%) is higher than previously reported for ethanol extracts (concentration not disclosed) from other Asteraceae species [81], and BuChE inhibition activity (34.2%) was the highest from the tested samples. To the best of our knowledge, this is the first study to report the AChE and BuChE inhibition activity of sea aster fractions. Aghraz et al. [82] determined the neuroprotective properties of 0.4 mg/mL infusions from two other halophytes from the Asteraceae family and shower moderate and low inhibition activities against AChE and BuChE, respectively, comparable to obtained results for the sea aster juice fraction.

In sea fennel samples, the AChE inhibition activity of ethanol extract (86.7%) was significantly higher, and the inhibition of BuChE is nearly the same compared to the study by Mekinić et al. [37], who reported 27.2% and 19.6% inhibition of AChE and BuChE, respectively, using sea fennel ethanol extract (concentration not disclosed). Nguir et al. [83] reported 31.2% AChE inhibition activity of 1 mg/mL sea fennel essential oils; indeed, many studies of sea fennel bioactivity focus on the essential oil fraction. The juice fraction from both species exhibited higher inhibition of AChE compared to aqueous extracts, indicating that the compounds potentially responsible for the activity are mostly ethanol-

soluble or free water-soluble compounds, which end up in the juice fraction in the initial fractionation process.

Tyrosinase inhibitors have a role in the food, cosmetics, and pharmaceutical industries. As a type of polyphenol oxidase, tyrosinase causes an undesired browning in plant-derived food products [84]. In animals, tyrosinase produced in melanocytes participates in melanin production, and tyrosinase inhibitors are used in cosmetics as skin-whitening agents [85,86] and have increased interest in the treatment of skin disorders with hyperpigmentation and melanoma skin cancer [23]. Tested ethanol extracts exhibited moderate tyrosinase inhibition at 10 mg/mL, 41.2% and 55.1% for sea aster and sea fennel, respectively. Calvo et al. [87] studied the potential of 50% aqueous ethanol halophyte extracts to prevent melanosis in shrimp, and they reported both sea fennel and sea aster to have diphenol oxidase inhibition activity of 30–40% at 10 mg/mL. Sea fennel cell culture has already been commercialized as an active cosmetic ingredient due to its tyrosinase-inhibitory properties [88]. The 50% ethanol extracts from halophyte species *Inula crithmoides* and *Daucus carota*, belonging to Asteraceae and Apicaceae families, respectively, have also previously shown tyrosinase inhibition activity; major phenolic compounds in the extracts are chlorogenic acid and rutin for *Inula crithmoides*, and chlorogenic acid and quercetin for *Daucus carota* [23].

There is a limited amount of existing literature considering the anti-diabetic and lipase-inhibitory properties of raw extracts from sea fennel and sea aster. Wubshet et al. [40] found quercitin and kaempferol derivatives from the sea aster to be present in the extract fraction with α-glucosidase activity. Souid et al. [24] found chlorogenic acid, neochlorogenic acid, and quercitin derivates to be the most abundant phenolics in 80% aqueous ethanol sea fennel extract, and these phytochemicals have been described as potential anti-diabetic compounds [89–91]. These compounds inhibit α-amylase and α-glucosidase, the key enzymes responsible for the digestion of carbohydrates, delaying and limiting glucose absorption and potentially preventing hyperglycemia in people with type II diabetes mellitus [90,91]. Obesity has been shown to increase an individual's risk of a multitude of health concerns, and one of the suggested treatments to control the excessive accumulation of body fat has been to inhibit the activity of pancreatic lipase, an enzyme with a key role in fat metabolism [92]. Phytochemicals, such as flavonoids, alkaloids, saponins and terpenoids, have been shown to have lipase inhibition activity [93]. Ethanol extract of sea aster exhibited moderate lipase inhibition at 10 mg/mL, and this sample also exhibited the highest content of TFC, whereas other tested extracts showed only low lipase inhibition activity. Extracts from other medicinal halophytes have previously been reported to have lipase inhibition with half maximal inhibitory concentrations of 0.16 mg/mL and 1.33 mg/mL for *Limonium sinuatum* and *Lobularia maritima* methanolic extracts, respectively, and 41.7% lipase inhibition at 10 mg/mL with ethanol extract from *Salicornia ramosissima* [94–96]. The anti-obesity properties of sea fennel tincture have also been studied considering the increased energy expenditure (thermogenic drug), but no positive effects were observed [97].

Extracting phytochemicals for medicinal purposes and targeting chronic diseases with plant-derived products has attracted attention in recent years [21,68,77,98]. The bioprospecting of novel feedstocks is beneficial for finding new potential treatments for health concerns. Targeting products for biomedical and nutraceutical applications is also important for the economic feasibility of integrated biorefineries and sustainable bioeconomies. The results show that extracts from residual halophyte fraction can have similar or higher biological activity as extracts from fresh food-grade plants, highlighting the potential of the residual fractions for full feedstock valorization. In tested samples, the highest bioactivities in enzyme inhibition assays were observed in ethanol extracts. In large-scale biorefinery applications, minimizing the use of organic solvent is often desired, and the optimization of the extraction method is key in the process development; however, each method has advantages and disadvantages related to extraction efficiency, chemical use and toxicity, and energy consumption [51,68,94].

3. Materials and Methods

3.1. Raw Material

Plant material was obtained from the Institute of Botany of Leibniz University Hannover, where it was grown in a greenhouse. Halophytes were harvested when the plants were fresh but partially lignified (non-food grade). Sea aster was cultivated in a hydroponic system under 120 mM NaCl salinity in similar conditions as previously described by Hulkko et al. [31] and harvested at the vegetative growth stage in June 2021. Sea fennel was cultivated in non-saline conditions in pots with sandy soil and harvested at the flowering stage in September 2021. At the harvest stage, the biomass was partly lignified and had developed a fibrous texture. Biomass was frozen after harvesting and kept in the freezer at −24 °C before further processing.

3.2. Biomass Fractionation and Extraction

Both biomasses were fractionated to green juice and fiber residue using a pilot-scale double-auger juicer. Green juice fractions were centrifuged and filtered (Whatman, GE Healthcare, Chicago, IL,USA), concentrated using a rotary evaporator, freeze-dried (ModulyoD, Thermo Scientific, Waltham, MA, USA), and re-solubilized to distilled water with a concentration of 10 mg/mL. Fiber residue fractions were dried overnight in a 60 °C fan oven(Memmert, Schwabach, Germany), size-reduced by knife-milling to particle size < 2 mm, and stored at room temperature (RT) protected from the light before analysis. The dry matter (DM), ash, and carbohydrate content of the fractions were determined using analytical protocols by National Renewable Energy Laboratory [99–101]. For structural carbohydrates and Klason lignin, the analysis was run in duplicates for samples and recovery standards. Separated sugars were glucose, xylose and arabinose, and samples were analyzed using high-performance liquid chromatography (126 Infinity II, Agilent Technologies, Santa Clara, CA, USA) with 5 mM H_2SO_4 as mobile phase, organic acid column (Aminex HPX-87H, Bio-Rad Laboratories, Hercules, CA, USA) at 63 °C, and refractive index detector at 30 °C.

Plant extracts were obtained from fiber residue fraction using conventional Soxhlet apparatus with a 100 mL extraction chamber and 250 mL of different solvents, namely: demineralized water, 70% aqueous ethanol, and n-hexane. All extractions were run as parallel experiments. For water and ethanol extractions, samples (10 g) were extracted for 8 h, and for n-hexane extraction, samples (5 g) were extracted for 6 h. The excess solvent was recovered at 40 °C using a rotary evaporator combined with a vacuum pump (KNF, Stockholm, Sweden). Obtained water and ethanol extracts were freeze-dried and re-solubilized to the corresponding solvents at a concentration of 10 mg/mL.

3.3. Transesterification and Determination of Fatty Acids

FA present in the n-hexane extracts were converted into fatty acid methyl esters (FAME) by transesterification. A small amount of obtained lipids from the n-hexane extraction (0.15 g) was dissolved in 0.5 M methanolic sodium hydroxide (1 mL) at 90 °C. Samples were cooled to RT, boron trifluoride (1 mL) and hydroquinone solution (0.5 mL) was added, and samples were heated at 90 °C for 5 min. Phase separation was achieved using saturated NaCl aqueous solution (4 mL) and n-heptane (3 mL). After separation, the n-heptane soluble fraction was recovered. FA profile was determined using gas chromatography (Clarus 500, Perkin Elmer, Waltham, MA, USA), capillary column (Elite-WAX, 30 m × 0.25 mm ID × 0.25 µm, Perkin Elmer, Waltham, MA, USA) and helium as carrier gas. The temperature program was set to 1 min at 150 °C, heating 10 °C/min until 240 °C and keeping it at 240 °C for 10 min. Compounds were detected with mass spectrometry, and the results were compared to the National Institute of Standards and Technology (NIST) library and given as relative concentrations.

3.4. Total Photosynthetic Pigments

The total concentrations of chlorophylls (CHL) and carotenoids (TCA) were determined in green juice fractions and ethanol extracts by spectrophotometry. The absorbance was measured at 470 nm, 649 nm, and 665 nm using a UV-visible spectrophotometer (Genesys 50, Thermo Scientific, Waltham, CA, USA) with a quartz cuvette. Turbidity at 750 nm was considered. Calculations were carried out using the formulas described by Lichtenthaler and Wellburn [102]:

$$\text{CHL a} = 13.95 \times A_{665} - 6.88 \times A_{649}, \quad (1)$$

$$\text{CHL b} = 24.96 \times A_{649} - 7.32 \times A_{665}, \quad (2)$$

$$\text{TCA} = (1000 \times A_{470} - 2.05 \times \text{CHL a} - 114.8 \times \text{CHL b}) / 245 \quad (3)$$

3.5. Phenolic Compounds in Plant Fractions

The total contents of different phenolic compound groups were determined in juice fractions, and water and ethanol extracts. TPC were determined as the amount of gallic acid equivalent (GAE) in the juice or extract samples by the Folin–Ciocalteau (F-C) assay described by Velioglu et al. [103] adapted to 96-well plates. Samples (5 µL) were mixed with F-C reagent in ethanol (100 µL) and incubated in RT for 10 min, after which 75 g/L aqueous sodium carbonate (100 µL) was added, and plates were further incubated for 90 min. The calibration curve ($R^2 = 0.997$) was made using gallic acid, and absorbance was read at 650 nm using a microplate reader (EZ Read 400, Biochrom, Cambridge, UK).

TFC were determined using a method by Pirbalouti et al. [104] by mixing samples (50 µL) with 2% aluminum chloride in methanol solution (50 µL), incubating plates for 10 min at RT, and reading the plates at 405 nm. Quercetin was used as a standard for calibration ($R^2 = 0.999$), and results are expressed as the amount of quercetin equivalent (QE) in the mass unit of the dried sample.

TCT, also known as proanthocyanidins, were determined using the assay by Li et al. [105] with p-dimethylaminocinnamaldehyde hydrochloric acid (DMACA-HCl). Juice and extract samples (10 µL) were mixed with 1% DMACA in methanol (200 µL) and 37% HCl (100 µL). Plates were incubated for 15 min at RT, and absorbance was read at 640 nm. Results are expressed as catechin equivalent (CE) in the dry extract and calculated based on the calibration curve ($R^2 = 0.991$).

TAC were determined as described by Mazza et al. [106] by mixing an aliquot of the samples (20 µL) with 95% ethanol containing 0.1% HCl (20 µL) and 1 M HCl (160 µL). The absorbance was read at 492 nm, and the results are expressed as cyanidin chloride equivalent (CCE) based on the calibration curve ($R^2 = 0.991$).

3.6. In Vitro Radical Scavenging Activity

RSA of juice and water and ethanol extracts was tested using colorimetric methods towards the following radicals: DPPH, ABTS, and NO. Samples were tested at 10 mg/mL concentrations, absorbances were read using a microplate reader, and RSA was calculated as a percentage relative to a negative control sample (blank). Gallic acid at a 1 mg/mL concentration was used as a positive control in all RSA assays. For the DPPH assay by Brand-Williams et al. [107], samples (22 µL) were mixed with 120 µM DPPH in ethanol (200 µL), incubated for 30 min in the dark, and reading the absorbance at 492 nm.

The ABTS scavenging assay was carried out as described by Re et al. [108]. First, the $ABTS^{\bullet+}$ solution with a molarity of 7.4 mM was obtained by mixing ABTS (100 mg) with 2.6 mM potassium persulphate (100 mL) in RT in the dark and incubating the mixture overnight. Afterward, the $ABTS^{\bullet+}$ solution was diluted with ethanol until an absorbance of approximately 0.7 at 734 nm was achieved. This diluted solution was used in the assay,

where samples (10 µL) were mixed with $ABTS^{\bullet+}$ solution (190 µL) and incubated for 6 min in the dark. The absorbance was read at 650 nm.

The NO scavenging assay was carried out by the method described by Baliga et al. [109]. First, samples (50 µL) were mixed with 10 mM sodium nitroprusside (50 µL) in 96-well plates and incubated for 90 min in RT. Afterward, Griess reagent (50 µL) was added, and plates were read at 562 nm.

3.7. In Vitro Antioxidant Activity by Metal-Based Assays

Metal-based assays were also carried out using juice and water and ethanol extract samples at a concentration of 10 mg/mL. Results are expressed as a percentage relative to a negative control sample. The capability to bind and hold iron and copper was analyzed with chelating assays described by Megías et al. [110], and ethylenediaminetetraacetic acid (EDTA) with a concentration of 1 mg/mL was used as a positive control. In the ICA assay, samples (30 µL) were mixed with water (200 µL) and 0.01 % aqueous $FeCl_2$ (30 µL) in 96-well plates. Plates were incubated for 30 min, and after incubation, 40 mM aqueous ferrozine (12.5 µL) was added. Plates were further incubated for 10 min, and absorbance was read at 562 nm. In the CCA assay, samples (30 µL) were mixed with 50 mM sodium acetate buffer (200 µL), 0.005 % aqueous $CuSO_4$ (100 µL) and 4 mM aqueous pyrocatechol violet (6 µL), and plates were immediately read at 620 nm.

The FRAP assay was performed according to Megías et al. [110] by mixing equal parts (50 µL) of samples, distilled water, and 1 % potassium ferrocyanide in 96-well plates and incubating for 20 min at 50 °C. Afterward, 10 % aqueous trichloroacetic acid (50 µL) and 0.1 % aqueous $FeCl_3$ (10 µL) were added, plates were incubated for 10 min at RT, and absorbance was read at 650 nm. Gallic acid at 1 mg/mL concentration was used as a positive control.

3.8. In Vitro Enzyme Inhibition Assays

Inhibition activity against enzymes related to different diseases was tested in vitro using green juice fractions and water and ethanol extracts at a concentration of 10 mg/mL. Results were expressed as the percentage of inhibition related to a negative control sample. Potential anti-diabetic properties of halophyte juices and extracts were determined by measuring the inhibition of α-amylase and α-glucosidase. Acarbose, a known anti-diabetic drug on the market, was used as a reference at a concentration of 10 mg/mL. For α-amylase inhibition activity, an assay developed by Xiao et al. [111] was used, which is based on the reaction between iodine solution and starch. Equals parts (40 µL) of samples, 0.1 % boiled potato starch solution, and 100 U/mL α-amylase in 0.1 M sodium phosphate buffer solution (pH 6.9) were mixed in 96-well plates and incubated at 37 °C for 10 min. Afterward, 1 M HCl (20 µL) and iodine solution (100 µL), consisting of 5 mM I_2 and 5 mM KI in distilled water, were added, and absorbance was read at 570 nm. A negative control sample with no enzyme (100% inhibition, blank) was used in the basis of calculation, whereas a control sample with an enzyme was used for color correction. Results were calculated as follows:

$$\alpha\text{-amylase inhibition activity [\%]} = (A_{570}\,\text{sample} - A_{570}\,\text{color control}) / A_{570}\,\text{blank} \times 100\% \quad (4)$$

In the α-glucosidase inhibition activity assay described by Custódio et al. [112], samples (50 µL) were mixed with 1 U/mL *Saccharomyces cerevisiae* α-glucosidase in phosphate buffer (100 µL, pH 7.0), and plates were incubated at 25 °C for 10 min. After incubation, 5 mM p-nitrophenyl-α-d-glucopyranoside (50 µL) was added, and plates were incubated for a further 5 min at 25 °C before reading the absorbance at 405 nm.

The ability to inhibit tyrosinase was tested as described by Trentin et al. [113] by mixing samples (70 µL) with 333 U/mL tyrosinase solution (30 µL) in 25 mM potassium phosphate buffer (pH 6.5) and incubating for 5 min at RT. After incubation, a substrate solution (110 µL) of 2 mM L-tyrosine diluted in buffer was added, and plates were incubated for 30–45 min in RT before reading the absorbance at 405 nm. Arbutin, a compound known

to inhibit tyrosinase and prevent melanin formation, was used as a reference at a concentration of 1 mg/mL.

Inhibition of AChE and BuChE was measured using the method by Ellman et al. [114] by mixing samples (20 µL) with 0.02 M sodium phosphate buffer (140 µL, pH 8.0) and 0.28 U/mL AChE or BuChE enzyme solution in pH 7.0 buffer (20 µL), respectively. Plates were incubated for 15 min at 25 °C, and afterward, acetylcholine iodide or butyrylcholine iodide, respectively, in 4 mg/mL pH 8.0 buffer solution (10 µL) was added. Then, Ellman's reagent (5,5'-dithiobis(2-nitrobenzoic acid)) in 1.2 mg/mL ethanol (20 µL) was added. Samples were incubated for another 15 min at 25 °C, and absorbance was read at 405 nm. Galantamine, a drug used to treat dementia, was used as a reference at 1 mg/mL concentration.

Lipase inhibition activity was measured using a protocol based on that described by McDougall et al. [115]. Samples (20 µL) were mixed with 100 mM Tris-HCl buffer (200 µL, pH 8.2), 1 mg/mL porcine pancreatic lipase in buffer solution (20 µL), and 5.1 mM 4-nitrophenyl dodecanoate in ethanol (20 µL). Plates were incubated at 37 °C for 10 min before reading the absorbance at 405 nm. Orlistat, a medicinal compound used to support weight loss, was used as a reference at a 1 mg/mL concentration.

3.9. Statistical Methods

Results are given as the mean values with standard deviation marked in brackets unless stated otherwise. The number of replicates was ($n = 6$) for analysis run in microplates and ($n = 3$) for other analyses unless stated otherwise. One-way analysis of variance (ANOVA) and Tukey honest significant difference (HSD) test were run to evaluate differences between the results, and significantly different results are marked with different letters. For the antioxidant activities, the half-maximum effective concentration (EC_{50}) was calculated using an online tool by AAT Bioquest Inc (Pleasanton, CA, USA). [116] when the obtained activity at 10 mg/mL concentration was higher than 50%.

4. Conclusions

The chemical composition and biological activities of the juice and fiber residue extracts from sea aster and sea fennel after fractionation with screw-press were analyzed to evaluate the potential of non-food grade residuals to produce high-value, low-volume bioproducts as a part of halophyte-based integrated biorefinery. Analyzed plant fractions exhibited interesting properties for potential added value creation of residual biomass, such as antioxidant activity and the inhibition of enzymes related to chronic diseases. Halophytes from biosaline agriculture could provide not only a healthy food source but also a feedstock to produce extracts for biomedicines, nutraceuticals, and cosmetics. Process development and optimization, as well as phytochemical analysis and bioavailability assays, are needed to maximize the potential and valorization of halophyte biomass. Overall, sea aster and sea fennel can be seen as interesting species for further biorefinery investigations, and in general, expanding the utilization of halophytes could provide an important contribution to sustainable bioeconomies.

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References

1. Intergovernmental Panel on Climate Change (IPCC). *Synthesis Report of the IPCC Sixth Assessment Report—Summary for Policymakers*; IPCC: Geneva, Switzerland, 2023.
2. Zabaniotou, A. Redesigning a bioenergy sector in EU in the transition to circular waste-based Bioeconomy—A multidisciplinary review. *J. Clean. Prod.* **2018**, *177*, 197–206. https://doi.org/10.1016/j.jclepro.2017.12.172.
3. Mauser, W.; Klepper, G.; Zabel, F.; Delzeit, R.; Hank, T.; Putzenlechner, B.; Calzadilla, A. Global biomass production potentials exceed expected future demand without the need for cropland expansion. *Nat. Commun.* **2015**, *6*, 1–11. https://doi.org/10.1038/ncomms9946.
4. Muscat, A.; de Olde, E.M.; Ripoll-Bosch, R.; Van Zanten, H.H.E.; Metze, T.A.P.; Termeer, C.J.A.M.; van Ittersum, M.K.; de Boer, I.J.M. Principles, drivers and opportunities of a circular bioeconomy. *Nat. Food* **2021**, *2*, 561–566.
5. Hulkko, L.S.S.; Chaturvedi, T.; Thomsen, M.H. Extraction and Quantification of Chlorophylls, Carotenoids, Phenolic Compounds, and Vitamins from Halophyte Biomasses. *Appl. Sci.* **2022**, *12*, 840. https://doi.org/10.3390/app12020840.
6. Chaturvedi, T.; Torres, A.I.; Stephanopoulos, G.; Thomsen, M.H.; Schmidt, J.E. Developing process designs for biorefineries-definitions, categories, and unit operations. *Energies* **2020**, *13*, 1493. https://doi.org/10.3390/en13061493.
7. Jimenez-Lopez, C.; Fraga-Corral, M.; Carpena, M.; García-Oliveira, P.; Echave, J.; Pereira, A.G.; Lourenço-Lopes, C.; Prieto, M.A.; Simal-Gandara, J. Agriculture waste valorisation as a source of antioxidant phenolic compounds within a circular and sustainable bioeconomy. *Food Funct.* **2020**, *11*, 4853–4877.
8. Stegmann, P.; Londo, M.; Junginger, M. The circular bioeconomy: Its elements and role in European bioeconomy clusters. *Resour. Conserv. Recycl. X* **2020**, *6*, 100029.
9. Yaashikaa, P.R.; Senthil Kumar, P.; Varjani, S. Valorization of agro-industrial wastes for biorefinery process and circular bioeconomy: A critical review. *Bioresour. Technol.* **2022**, *343*, 126126. https://doi.org/10.1016/j.biortech.2021.126126.
10. Fermoso, F.G.; Serrano, A.; Alonso-Fariñas, B.; Fernández-Bolaños, J.; Borja, R.; Rodríguez-Gutiérrez, G. Valuable Compound Extraction, Anaerobic Digestion, and Composting: A Leading Biorefinery Approach for Agricultural Wastes. *J. Agric. Food Chem.* **2018**, *66*, 8451–8468.
11. Manhongo, T.T.; Chimphango, A.F.A.; Thornley, P.; Röder, M. Current status and opportunities for fruit processing waste biorefineries. *Renew. Sustain. Energy Rev.* **2022**, *155*, 111823.
12. Caldeira, C.; Vlysidis, A.; Fiore, G.; De Laurentiis, V.; Vignali, G.; Sala, S. Sustainability of food waste biorefinery: A review on valorisation pathways, techno-economic constraints, and environmental assessment. *Bioresour. Technol.* **2020**, *312*, 123575. https://doi.org/10.1016/j.biortech.2020.123575.
13. Uzir, B.; Saha, A. Biorefinery Approach for Production of Biofuel from Algal Biomass. In *Biorefinery Production Technologies for Chemicals and Energy*; Wiley: Hoboken, NJ, USA, 2020; pp. 335–345.
14. EIP-AGRI Focus Group Soil Salinisation: Final Report. Available online: https://ec.europa.eu/eip/agriculture/en/publications/eip-agri-focus-group-soil-salinisation-final (accessed on 20 January 2022).
15. Nikalje, G.C.; Srivastava, A.K.; Pandey, G.K.; Suprasanna, P. Halophytes in biosaline agriculture: Mechanism, utilisation, and value addition. *Land Degrad. Dev.* **2018**, *29*, 1081–1095. https://doi.org/10.1002/ldr.2819.
16. Duarte, B.; Caçador, I. Iberian Halophytes as Agroecological Solutions for Degraded Lands and Biosaline Agriculture. *Sustainability* **2021**, *13*, 1005. https://doi.org/10.3390/su13021005.
17. Thomas, J.E.; Sinha, R.; Strand, Å.; Söderqvist, T.; Stadmark, J.; Franzén, F.; Ingmansson, I.; Gröndahl, F.; Hasselström, L. Marine biomass for a circular blue-green bioeconomy? A life cycle perspective on closing nitrogen and phosphorus land-marine loops. *J. Ind. Ecol.* **2022**, *26*, 2136–2153. https://doi.org/10.1111/jiec.13177.
18. Flowers, T.J.; Colmer, T.D. Salinity tolerance in halophytes. *New Phytol.* **2008**, *179*, 945–963. https://doi.org/10.1111/j.1469-8137.2008.02531.x.
19. Ksouri, R.; Ksouri, W.M.; Jallali, I.; Debez, A.; Magné, C.; Hiroko, I.; Abdelly, C. Medicinal halophytes: Potent source of health promoting biomolecules with medical, nutraceutical and food applications. *Crit. Rev. Biotechnol.* **2012**, *32*, 289–326. https://doi.org/10.3109/07388551.2011.630647.
20. Faustino, M.V.; Faustino, M.A.F.; Pinto, D.C.G.A. Halophytic grasses, a new source of nutraceuticals? A review on their secondary metabolites and biological activities. *Int. J. Mol. Sci.* **2019**, *20*, 1067.
21. Giordano, R.; Saii, Z.; Fredsgaard, M.; Hulkko, L.S.S.; Poulsen, T.B.G.; Thomsen, M.E.; Henneberg, N.; Zucolotto, S.M.; Arendt-Nielsen, L.; Papenbrock, J.; et al. Pharmacological insights into halophyte bioactive extract action on anti-inflammatory, pain relief and antibiotics-type mechanisms. *Molecules* **2021**, *26*, 3140.

22. Giordano, R.; Aliotta, G.E.; Johannesen, A.S.; Voetmann-Jensen, D.; Laustsen, F.H.; Andersen, L.A.; Rezai, A.; Fredsgaard, M.; Lo Vecchio, S.; Arendt-Nielsen, L.; et al. Effects of Salicornia-Based Skin Cream Application on Healthy Humans' Experimental Model of Pain and Itching. *Pharmaceuticals* **2022**, *15*, 150. https://doi.org/10.3390/ph15020150.
23. Jdey, A.; Falleh, H.; Ben Jannet, S.; Mkadmini Hammi, K.; Dauvergne, X.; Ksouri, R.; Magné, C. Phytochemical investigation and antioxidant, antibacterial and anti-tyrosinase performances of six medicinal halophytes. *South Afr. J. Bot.* **2017**, *112*, 508–514. https://doi.org/10.1016/j.sajb.2017.05.016.
24. Souid, A.; Della Croce, C.M.; Frassinetti, S.; Gabriele, M.; Pozzo, L.; Ciardi, M.; Abdelly, C.; Hamed, K.B.; Magné, C.; Longo, V. Nutraceutical Potential of Leaf Hydro-Ethanolic Extract of the Edible Halophyte *Crithmum maritimum* L. *Molecules* **2021**, *26*, 5380. https://doi.org/10.3390/molecules26175380.
25. Ventura, Y.; Eshel, A.; Pasternak, D.; Sagi, M. The development of halophyte-based agriculture: Past and present. *Ann. Bot.* **2014**, *115*, 529–540. https://doi.org/10.1093/aob/mcu173.
26. Accogli, R.; Tomaselli, V.; Direnzo, P.; Perrino, E.V.; Albanese, G.; Urbano, M.; Laghetti, G. Edible Halophytes and Halo-Tolerant Species in Apulia Region (Southeastern Italy): Biogeography, Traditional Food Use and Potential Sustainable Crops. *Plants* **2023**, *12*, 549. https://doi.org/10.3390/plants12030549.
27. Biscotti, N.; Pieroni, A.; Luczaj, L. The hidden Mediterranean diet: Wild vegetables traditionally gathered and consumed in the Gargano area, Apulia, SE Italy. *Acta Soc. Bot. Pol.* **2015**, *84*, 327–338. https://doi.org/10.5586/asbp.2015.031.
28. Vlahos, N.; Levizou, E.; Stathopoulou, P.; Berillis, P.; Antonopoulou, E.; Bekiari, V.; Krigas, N.; Kormas, K.; Mente, E. An Experimental Brackish Aquaponic System Using Juvenile Gilthead Sea Bream (Sparus aurata) and Rock Samphire (*Crithmum maritimum*). *Sustainability* **2019**, *11*, 4820. https://doi.org/10.3390/su11184820.
29. Custódio, M.; Villasante, S.; Cremades, J.; Calado, R.; Lillebø, A. Unravelling the potential of halophytes for marine integrated multi-trophic aquaculture (IMTA)—A perspective on performance, opportunities and challenges. *Aquac. Environ. Interact.* **2017**, *9*, 445–460. https://doi.org/10.3354/aei00244.
30. Zenobi, S.; Fiorentini, M.; Ledda, L.; Deligios, P.; Aquilanti, L.; Orsini, R. *Crithmum maritimum* L. Biomass Production in Mediterranean Environment. *Agronomy* **2022**, *12*, 926. https://doi.org/10.3390/agronomy12040926.
31. Hulkko, L.S.S.; Turcios, A.E.; Kohnen, S.; Chaturvedi, T.; Papenbrock, J.; Thomsen, M.H. Cultivation and characterisation of Salicornia europaea, Tripolium pannonicum and *Crithmum maritimum* biomass for green biorefinery applications. *Sci. Rep.* **2022**, *12*, 20507. https://doi.org/10.1038/s41598-022-24865-4.
32. Jallali, I.; Zaouali, Y.; Missaoui, I.; Smeoui, A.; Abdelly, C.; Ksouri, R. Variability of antioxidant and antibacterial effects of essential oils and acetonic extracts of two edible halophytes: *Crithmum maritimum* L. and Inula crithmoïdes L. *Food Chem.* **2014**, *145*, 1031–1038. https://doi.org/10.1016/j.foodchem.2013.09.034.
33. Park, J.C.; Hur, J.M.; Park, J.G.; Hatano, T.; Yoshida, T.; Miyashiro, H.; Min, B.S.; Hattori, M. Inhibitory effects of Korean medicinal plants and camelliatannin H from Camellia japonica on human immunodeficiency virus type 1 protease. *Phyther. Res.* **2002**, *16*, 422–426. https://doi.org/10.1002/ptr.919.
34. Pereira, C.G.; Barreira, L.; da Rosa Neng, N.; Nogueira, J.M.F.; Marques, C.; Santos, T.F.; Varela, J.; Custódio, L. Searching for new sources of innovative products for the food industry within halophyte aromatic plants: In vitro antioxidant activity and phenolic and mineral contents of infusions and decoctions of *Crithmum maritimum* L. *Food Chem. Toxicol.* **2017**, *107*, 581–589. https://doi.org/10.1016/j.fct.2017.04.018.
35. Meot-Duros, L.; Magné, C. Antioxidant activity and phenol content of *Crithmum maritimum* L. leaves. *Plant Physiol. Biochem.* **2009**, *47*, 37–41. https://doi.org/10.1016/j.plaphy.2008.09.006.
36. Houta, O.; Akrout, A.; Amri, H. Phenolic amounts, antioxidant and antimicrobial potential of *Crithmum maritimum* cultivated in Tunisian arid zones. *Planta Med.* **2011**, *77*, 1416–1416. https://doi.org/10.1055/s-0031-1282826.
37. Generalić Mekinić, I.; Blažević, I.; Mudnić, I.; Burčul, F.; Grga, M.; Skroza, D.; Jerčić, I.; Ljubenkov, I.; Boban, M.; Miloš, M.; et al. Sea fennel (*Crithmum maritimum* L.): Phytochemical profile, antioxidative, cholinesterase inhibitory and vasodilatory activity. *J. Food Sci. Technol.* **2016**, *53*, 3104–3112. https://doi.org/10.1007/s13197-016-2283-z.
38. Montero, M.P.; Sánchez-Faure, A.; Rico, D.; Martín-Diana, A.B.; de Palencia, P.F.; Sánchez-Guisado, M.J.; Gómez-Guillén, C.; López-Caballero, M.E.; Martínez-Alvarez, O. Sea Aster (Aster Tripolium) As a Source of Bioactive Molecules. In Proceedings of the 49th WEFTA Conference, Tórshavn, Faroe Islands, 15–17 October 2019.
39. Chai, W.; Chen, L.; Lian, X.Y.; Zhang, Z. Anti-glioma Efficacy and Mechanism of Action of Tripolinolate A from Tripolium pannonicum. *Planta Med.* **2018**, *84*, 786–794. https://doi.org/10.1055/s-0044-101038.
40. Wubshet, S.G.; Schmidt, J.S.; Wiese, S.; Staerk, D. High-resolution screening combined with HPLC-HRMS-SPE-NMR for identification of potential health-promoting constituents in sea aster and searocket—New nordic food ingredients. *J. Agric. Food Chem.* **2013**, *61*, 8616–8623. https://doi.org/10.1021/jf402949y.
41. Lopes, A.; Rodrigues, M.J.; Pereira, C.; Oliveira, M.; Barreira, L.; Varela, J.; Trampetti, F.; Custódio, L. Natural products from extreme marine environments: Searching for potential industrial uses within extremophile plants. *Ind. Crops Prod.* **2016**, *94*, 299–307. https://doi.org/10.1016/j.indcrop.2016.08.040.
42. Renna, M.; Gonnella, M.; Caretto, S.; Mita, G.; Serio, F. Sea fennel (*Crithmum maritimum* L.): From underutilised crop to new dried product for food use. *Genet. Resour. Crop Evol.* **2017**, *64*, 205–216. https://doi.org/10.1007/s10722-016-0472-2.
43. Pavela, R.; Maggi, F.; Lupidi, G.; Cianfaglione, K.; Dauvergne, X.; Bruno, M.; Benelli, G. Efficacy of sea fennel (*Crithmum maritimum* L., Apiaceae) essential oils against Culex quinquefasciatus Say and Spodoptera littoralis (Boisd.). *Ind. Crops Prod.* **2017**, *109*, 603–610. https://doi.org/10.1016/j.indcrop.2017.09.013.

44. Tsoukatou, M.; Tsitsimpikou, C.; Vagias, C.; Roussis, V. Chemical intra-mediterranean variation and insecticidal activity of *Crithmum maritimum*. *Z. Fur Naturforsch.—Sect. C J. Biosci.* **2001**, *56*, 211–215. https://doi.org/10.1515/znc-2001-3-407.
45. Polatoğlu, K.; Karakoç, Ö.C.; Yücel Yücel, Y.; Gücel, S.; Demirci, B.; Başer, K.H.C.; Demirci, F. Insecticidal activity of edible *Crithmum maritimum* L. essential oil against Coleopteran and Lepidopteran insects. *Ind. Crops Prod.* **2016**, *89*, 383–389. https://doi.org/10.1016/j.indcrop.2016.05.032.
46. Höltinger, S.; Schmidt, J.; Schönhart, M.; Schmid, E. A spatially explicit techno-economic assessment of green biorefinery concepts. *Biofuels Bioprod. Biorefining* **2014**, *8*, 325–341. https://doi.org/10.1002/bbb.1461.
47. Mandl, M.G. Status of green biorefining in Europe. *Biofuels Bioprod. Biorefining* **2010**, *4*, 268–274. https://doi.org/10.1002/bbb.219.
48. Prieler, M.; Lindorfer, J.; Steinmüller, H. Life-cycle assessment of green biorefinery process options. *Biofuels Bioprod. Biorefining* **2019**, *13*, 1391–1401. https://doi.org/10.1002/bbb.2022.
49. Novalin, S.; Zweckmair, T. Renewable resources—Green biorefinery: Separation of valuable substances from fluid-fractions by means of membrane technology. *Biofuels Bioprod. Biorefining* **2009**, *3*, 20–27. https://doi.org/10.1002/bbb.118.
50. Masters, D.G.; Benes, S.E.; Norman, H.C. Biosaline agriculture for forage and livestock production. *Agric. Ecosyst. Environ.* **2007**, *119*, 234–248.
51. Chaturvedi, T.; Hulkko, L.S.S.; Fredsgaard, M.; Thomsen, M.H. Extraction, Isolation, and Purification of Value-Added Chemicals from Lignocellulosic Biomass. *Processes* **2022**, *10*, 1752. https://doi.org/10.3390/pr10091752.
52. De Bhowmick, G.; Sarmah, A.K.; Sen, R. Lignocellulosic biorefinery as a model for sustainable development of biofuels and value added products. *Bioresour. Technol.* **2018**, *247*, 1144–1154.
53. Turcios, A.E.; Weichgrebe, D.; Papenbrock, J. Effect of salt and sodium concentration on the anaerobic methanisation of the halophyte Tripolium pannonicum. *Biomass Bioenergy* **2016**, *87*, 69–77. https://doi.org/10.1016/j.biombioe.2016.01.013.
54. Abideen, Z.; Ansari, R.; Khan, M.A. Halophytes: Potential source of ligno-cellulosic biomass for ethanol production. *Biomass Bioenergy* **2011**, *35*, 1818–1822. https://doi.org/10.1016/j.biombioe.2011.01.023.
55. Turcios, A.E.; Cayenne, A.; Uellendahl, H.; Papenbrock, J. Halophyte Plants and Their Residues as Feedstock for Biogas Production—Chances and Challenges. *Appl. Sci.* **2021**, *11*, 2746. https://doi.org/10.3390/app11062746.
56. Turcios, A.E.; Weichgrebe, D.; Papenbrock, J. Uptake and biodegradation of the antimicrobial sulfadimidine by the species Tripolium pannonicum acting as biofilter and its further biodegradation by anaerobic digestion and concomitant biogas production. *Bioresour. Technol.* **2016**, *219*, 687–693. https://doi.org/10.1016/j.biortech.2016.08.047.
57. Cristina, C.; Lucia, P.; Sara, S.; Francesco, S.; Nobile, D.; Alessandro, M.; Conte, A.; Amalia, C. Study of the Efficacy of Two Extraction Techniques from *Crithmum maritimum* and Salicornia europaea. *J. Food Nutr. Res.* **2018**, *6*, 456–463. https://doi.org/10.12691/jfnr-6-7-6.
58. Pedreiro, S.; Figueirinha, A.; Cavaleiro, C.; Cardoso, O.; Donato, M.M.; Salgueiro, L.; Ramos, F. Exploiting the *Crithmum maritimum* L. Aqueous Extracts and Essential Oil as Potential Preservatives in Food, Feed, Pharmaceutical and Cosmetic Industries. *Antioxidants* **2023**, *12*, 252. https://doi.org/10.3390/antiox12020252.
59. Duarte, B.; Cabrita, M.T.; Gameiro, C.; Matos, A.R.; Godinho, R.; Marques, J.C.; Caçador, I. Disentangling the photochemical salinity tolerance in *Aster tripolium* L.: Connecting biophysical traits with changes in fatty acid composition. *Plant Biol.* **2017**, *19*, 239–248. https://doi.org/10.1111/plb.12517.
60. Ben Hamed, K.; Ben Youssef, N.; Ranieri, A.; Zarrouk, M.; Abdelly, C. Changes in content and fatty acid profiles of total lipids and sulfolipids in the halophyte *Crithmum maritimum* under salt stress. *J. Plant Physiol.* **2005**, *162*, 599–602. https://doi.org/10.1016/j.jplph.2004.11.010.
61. Guil-Guerrero, J.L.; Rodríguez-García, I. Lipids classes, fatty acids and carotenes of the leaves of six edible wild plants. *Eur. Food Res. Technol.* **1999**, *209*, 313–316. https://doi.org/10.1007/s002170050501.
62. Atia, A.; Debez, A.; Barhoumi, Z.; Abdelly, C.; Smaoui, A. Localization and composition of seed oils of *Crithmum maritimum* L. (Apiaceae). *Afr. J. Biotechnol.* **2010**, *9*, 6482–6485. https://doi.org/10.5897/AJB09.724.
63. Simopoulos, A.P. Omega-6/Omega-3 Essential Fatty Acid Ratio and Chronic Diseases. *Food Rev. Int.* **2004**, *20*, 77–90. https://doi.org/10.1081/FRI-120028831.
64. Castañeda-Loaiza, V.; Oliveira, M.; Santos, T.; Schüler, L.; Lima, A.R.; Gama, F.; Salazar, M.; Neng, N.R.; Nogueira, J.M.F.; Varela, J.; et al. Wild vs cultivated halophytes: Nutritional and functional differences. *Food Chem.* **2020**, *333*, 127536. https://doi.org/10.1016/j.foodchem.2020.127536.
65. Hoste, H.; Jackson, F.; Athanasiadou, S.; Thamsborg, S.M.; Hoskin, S.O. The effects of tannin-rich plants on parasitic nematodes in ruminants. *Trends Parasitol.* **2006**, *22*, 253–261.
66. Politeo, O.; Popović, M.; Veršić Bratinčević, M.; Kovačević, K.; Urlić, B.; Generalić Mekinić, I. Chemical Profiling of Sea Fennel (*Crithmum maritimum* L., Apiaceae) Essential Oils and Their Isolation Residual Waste-Waters. *Plants* **2023**, *12*, 214. https://doi.org/10.3390/plants12010214.
67. Stanković, M.S.; Petrović, M.; Godjevac, D.; Stevanović, Z.D. Screening inland halophytes from the central balkan for their antioxidant activity in relation to total phenolic compounds and flavonoids: Are there any prospective medicinal plants? *J. Arid Environ.* **2015**, *120*, 26–32. https://doi.org/10.1016/j.jaridenv.2015.04.008.
68. Kapadia, P.; Newell, A.S.; Cunningham, J.; Roberts, M.R.; Hardy, J.G. Extraction of High-Value Chemicals from Plants for Technical and Medical Applications. *Int. J. Mol. Sci.* **2022**, *23*, 10334.

69. Boestfleisch, C.; Wagenseil, N.B.; Buhmann, A.K.; Seal, C.E.; Wade, E.M.; Muscolo, A.; Papenbrock, J. Manipulating the antioxidant capacity of halophytes to increase their cultural and economic value through saline cultivation. *AoB Plants* **2014**, *6*, plu046. https://doi.org/10.1093/aobpla/plu046.
70. Duarte, B.; Goessling, J.W.; Marques, J.C.; Caçador, I. Ecophysiological constraints of Aster tripolium under extreme thermal events impacts: Merging biophysical, biochemical and genetic insights. *Plant Physiol. Biochem.* **2015**, *97*, 217–228. https://doi.org/10.1016/j.plaphy.2015.10.015.
71. Ventura, Y.; Myrzabayeva, M.; Alikulov, Z.; Cohen, S.; Shemer, Z.; Sagi, M. The importance of iron supply during repetitive harvesting of Aster tripolium. *Funct. Plant Biol.* **2013**, *40*, 968–976.
72. Wiszniewska, A.; Koźmińska, A.; Hanus-Fajerska, E.; Dziurka, M.; Dziurka, K. Insight into mechanisms of multiple stresses tolerance in a halophyte Aster tripolium subjected to salinity and heavy metal stress. *Ecotoxicol. Environ. Saf.* **2019**, *180*, 12–22. https://doi.org/10.1016/j.ecoenv.2019.04.059.
73. Geissler, N.; Hussin, S.; Koyro, H.-W. Elevated atmospheric CO_2 concentration ameliorates effects of NaCl salinity on photosynthesis and leaf structure of Aster tripolium L. *J. Exp. Bot.* **2008**, *60*, 137–151. https://doi.org/10.1093/jxb/ern271.
74. Sarrou, E.; Siomos, A.S.; Riccadona, S.; Aktsoglou, D.C.; Tsouvaltzis, P.; Angeli, A.; Franceschi, P.; Chatzopoulou, P.; Vrhovsek, U.; Martens, S. Improvement of sea fennel (*Crithmum maritimum* L.) nutritional value through iodine biofortification in a hydroponic floating system. *Food Chem.* **2019**, *296*, 150–159. https://doi.org/10.1016/j.foodchem.2019.05.190.
75. Siracusa, L.; Kulisic-Bilusic, T.; Politeo, O.; Krause, I.; Dejanovic, B.; Ruberto, G. Phenolic composition and antioxidant activity of aqueous infusions from Capparis spinosa L. and *Crithmum maritimum* L. before and after submission to a two-step in vitro digestion model. *J. Agric. Food Chem.* **2011**, *59*, 12453–12459. https://doi.org/10.1021/jf203096q.
76. Zafeiropoulou, V.; Tomou, E.-M.; Karioti, A.; Skaltsa, H.; Ioannidou, O. Sea fennel: Phytochemical analysis of Greek wild and cultivated *Crithmum maritimum* L. populations, based on HPLC-PDA-MS and NMR methods. *J. Pharmacogn. Phytochem.* **2020**, *9*, 998–1004.
77. Sharifi-Rad, M.; Anil Kumar, N.V.; Zucca, P.; Varoni, E.M.; Dini, L.; Panzarini, E.; Rajkovic, J.; Tsouh Fokou, P.V.; Azzini, E.; Peluso, I.; et al. Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. *Front. Physiol.* **2020**, *11*, 694.
78. García-Sánchez, A.; Miranda-Díaz, A.G.; Cardona-Muñoz, E.G. The Role of Oxidative Stress in Physiopathology and Pharmacological Treatment with Pro- and Antioxidant Properties in Chronic Diseases. *Oxid. Med. Cell. Longev.* **2020**, *2020*, 2082145.
79. Kamm, B.; Schönicke, P.; Kamm, M. Biorefining of green biomass—Technical and energetic considerations. *Clean—Soil Air Water* **2009**, *37*, 27–30. https://doi.org/10.1002/clen.200800122.
80. Thang, V.H.; Novalin, S. Green Biorefinery: Separation of lactic acid from grass silage juice by chromatography using neutral polymeric resin. *Bioresour. Technol.* **2008**, *99*, 4368–4379. https://doi.org/10.1016/j.biortech.2007.08.045.
81. Mekinic, I.G.; Burčul, F.; Blaževic, I.; Skroza, D.; Kerum, D.; Katalinic, V. Antioxidative / acetylcholinesterase inhibitory activity of some Asteraceae plants. *Nat. Prod. Commun.* **2013**, *8*, 471–474. https://doi.org/10.1177/1934578x1300800416.
82. Aghraz, A.; Gonçalves, S.; Rodríguez-Solana, R.; Dra, L.A.; Di Stefano, V.; Dugo, G.; Cicero, N.; Larhsini, M.; Markouk, M.; Romano, A. Antioxidant activity and enzymes inhibitory properties of several extracts from two Moroccan Asteraceae species. *South Afr. J. Bot.* **2018**, *118*, 58–64. https://doi.org/10.1016/j.sajb.2018.06.017.
83. Nguir, A.; Besbes, M.; Ben Jannet, H.; Flamini, G.; Harzallah-Skhiri, F.; Ali Hamza, M. Chemical Composition, Antioxidant and Anti-acetylcholinesterase activities of Tunisian *Crithmum maritimum* L. Essential oils. *Mediterr. J. Chem.* **2011**, *1*, 173–179. https://doi.org/10.13171/mjc.1.4.2011.03.12.23.
84. Kubo, I.; Chen, Q.X.; Nihei, K.I. Molecular design of antibrowning agents: Antioxidative tyrosinase inhibitors. *Food Chem.* **2003**, *81*, 241–247. https://doi.org/10.1016/S0308-8146(02)00418-1.
85. Guillerme, J.B.; Couteau, C.; Coiffard, L. Applications for marine resources in cosmetics. *Cosmetics* **2017**, *4*, 35.
86. Pillaiyar, T.; Manickam, M.; Namasivayam, V. Skin whitening agents: Medicinal chemistry perspective of tyrosinase inhibitors. *J. Enzyme Inhib. Med. Chem.* **2017**, *32*, 403–425. https://doi.org/10.1080/14756366.2016.1256882.
87. Calvo, M.M.; Tzamourani, A.; Martínez-Alvarez, O. Halophytes as a potential source of melanosis-inhibiting compounds. Mechanism of inhibition of a characterised polyphenol extract of purslane (Portulaca oleracea). *Food Chem.* **2021**, *355*, 129649. https://doi.org/10.1016/j.foodchem.2021.129649.
88. Georgiev, V.; Slavov, A.; Vasileva, I.; Pavlov, A. Plant cell culture as emerging technology for production of active cosmetic ingredients. *Eng. Life Sci.* **2018**, *18*, 779–798. https://doi.org/10.1002/elsc.201800066.
89. Ma, C.M.; Hattori, M.; Daneshtalab, M.; Wang, L. Chlorogenic acid derivatives with alkyl chains of different lengths and orientations: Potent α-glucosidase inhibitors. *J. Med. Chem.* **2008**, *51*, 6188–6194. https://doi.org/10.1021/jm800621x.
90. Wang, S.; Li, Y.; Huang, D.; Chen, S.; Xia, Y.; Zhu, S. The inhibitory mechanism of chlorogenic acid and its acylated derivatives on α-amylase and α-glucosidase. *Food Chem.* **2022**, *372*, 131334. https://doi.org/10.1016/j.foodchem.2021.131334.
91. Li, Y.Q.; Zhou, F.C.; Gao, F.; Bian, J.S.; Shan, F. Comparative evaluation of quercetin, isoquercetin and rutin as inhibitors of α-glucosidase. *J. Agric. Food Chem.* **2009**, *57*, 11463–11468. https://doi.org/10.1021/jf903083h.
92. Liu, T.T.; Liu, X.T.; Chen, Q.X.; Shi, Y. Lipase Inhibitors for Obesity: A Review. *Biomed. Pharmacother.* **2020**, *128*, 110314.
93. Rajan, L.; Palaniswamy, D.; Mohankumar, S.K. Targeting obesity with plant-derived pancreatic lipase inhibitors: A comprehensive review. *Pharmacol. Res.* **2020**, *155*, 104681.

94. Hulkko, L.S.S.; Rocha, R.M.; Trentin, R.; Fredsgaard, M.; Chaturvedi, T.; Custódio, L.; Thomsen, M.H. Bioactive Extracts from Salicornia ramosissima J. Woods Biorefinery as a Source of Ingredients for High-Value Industries. *Plants* **2023**, *12*, 1251. https://doi.org/10.3390/plants12061251.
95. Baysal, I.; Ekizoglu, M.; Ertas, A.; Temiz, B.; Agalar, H.G.; Yabanoglu-Ciftci, S.; Temel, H.; Ucar, G.; Turkmenoglu, F.P. Identification of Phenolic Compounds by LC-MS/MS and Evaluation of Bioactive Properties of Two Edible Halophytes: Limonium effusum and L. sinuatum. *Molecules* **2021**, *26*, 4040. https://doi.org/10.3390/molecules26134040.
96. Marrelli, M.; Argentieri, M.P.; Avato, P.; Conforti, F. Lobularia maritima (L.) Desv. Aerial Parts Methanolic Extract: In Vitro Screening of Biological Activity. *Plants* **2020**, *9*, 89. https://doi.org/10.3390/plants9010089.
97. Martinet, A.; Hostettmann, K.; Schutz, Y. Thermogenic effects of commercially available plant preparations aimed at treating human obesity. *Phytomedicine* **1999**, *6*, 231–238. https://doi.org/10.1016/S0944-7113(99)80014-2.
98. Zhang, Y.J.; Gan, R.Y.; Li, S.; Zhou, Y.; Li, A.N.; Xu, D.P.; Li, H.B.; Kitts, D.D. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. *Molecules* **2015**, *20*, 21138–21156.
99. Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. *NREL/TP-510-42622 Analytical Procedure—Determination of Ash in Biomass*; National Renewable Energy Laboratory: Golden, CO, USA, 2005.
100. Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. *NREL/TP-510-42618 Analytical Procedure—Determination of Structural Carbohydrates and Lignin in Biomass*; National Renewable Energy Laboratory: Golden, CO, USA, 2012.
101. Sluiter, A.; Hames, B.; Hyman, D.; Payne, C.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Wolfe, J. *NREL/TP-510-42621 Analytical Procedure—Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples*; National Renewable Energy Laboratory: Golden, CO, USA, 2008; pp. 3–5.
102. Lichtenthaler, H.K.; Wellburn, A.R. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* **1983**, *11*, 591–592. https://doi.org/10.1042/bst0110591.
103. Velioglu, Y.S.; Mazza, G.; Gao, L.; Oomah, B.D. Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products. *J. Agric. Food Chem.* **1998**, *46*, 4113–4117. https://doi.org/10.1021/jf9801973.
104. Pirbalouti, A.G.; Siahpoosh, A.; Setayesh, M.; Craker, L. Antioxidant activity, total phenolic and flavonoid contents of some medicinal and aromatic plants used as herbal teas and condiments in Iran. *J. Med. Food* **2014**, *17*, 1151–1157. https://doi.org/10.1089/jmf.2013.0057.
105. Li, Y.G.; Tanner, G.; Larkin, P. The DMACA-HCl protocol and the threshold proanthocyanidin content for bloat safety in forage legumes. *J. Sci. Food Agric.* **1996**, *70*, 89–101. https://doi.org/10.1002/(SICI)1097-0010(199601)70:1<89::AID-JSFA470>3.0.CO;2-N.
106. Mazza, G.; Fukumoto, L.; Delaquis, P.; Girard, B.; Ewert, B. Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia. *J. Agric. Food Chem.* **1999**, *47*, 4009–4017. https://doi.org/10.1021/jf990449f.
107. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT—Food Sci. Technol.* **1995**, *28*, 25–30.
108. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorisation assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3.
109. Baliga, M.S.; Jagetia, G.C.; Rao, S.K.; Babu K.. Evaluation of nitric oxide scavenging activity of certain spices in vitro: A preliminary study. *Nahrung/Food* **2003**, *47*, 261–264. https://doi.org/10.1002/food.200390061.
110. Megías, C.; Pastor-Cavada, E.; Torres-Fuentes, C.; Girón-Calle, J.; Alaiz, M.; Juan, R.; Pastor, J.; Vioque, J. Chelating, antioxidant and antiproliferative activity of Vicia sativa polyphenol extracts. *Eur. Food Res. Technol.* **2009**, *230*, 353–359. https://doi.org/10.1007/s00217-009-1178-x.
111. Xiao, Z.; Storms, R.; Tsang, A. A quantitative starch-iodine method for measuring alpha-amylase and glucoamylase activities. *Anal. Biochem.* **2006**, *351*, 146–148. https://doi.org/10.1016/j.ab.2006.01.036.
112. Custódio, L.; Patarra, J.; Albericio, F.; da Rosa Neng, N.; Nogueira, J.M.F.; Romano, A. Phenolic composition, antioxidant potential and in vitro inhibitory activity of leaves and acorns of Quercus suber on key enzymes relevant for hyperglycemia and Alzheimer's disease. *Ind. Crops Prod.* **2015**, *64*, 45–51. https://doi.org/10.1016/j.indcrop.2014.11.001.
113. Trentin, R.; Custódio, L.; Rodrigues, M.J.; Moschin, E.; Sciuto, K.; da Silva, J.P.; Moro, I. Exploring Ulva australis Areschoug for possible biotechnological applications: In vitro antioxidant and enzymatic inhibitory properties, and fatty acids contents. *Algal Res.* **2020**, *50*, 101980. https://doi.org/10.1016/j.algal.2020.101980.
114. Ellman, G.L.; Courtney, K.D.; Andres, V.; Featherstone, R.M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **1961**, *7*, 88–95. https://doi.org/10.1016/0006-2952(61)90145-9.
115. McDougall, G.J.; Kulkarni, N.N.; Stewart, D. Berry polyphenols inhibit pancreatic lipase activity in vitro. *Food Chem.* **2009**, *115*, 193–199. https://doi.org/10.1016/j.foodchem.2008.11.093.
116. AAT Bioquest Inc. Quest Graph™ IC50 Calculator. Available online: https://www.aatbio.com/tools/ic50-calculator (accessed on 1 April 2022).

Paper V

Hulkko, L.S.S.; Chaturvedi, T.; Thomsen, M.H. Valorisation of Residual Biomass Fractions from Biosaline Agriculture Through Green Biorefinery. *Submitted manuscript.*

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Paper VI

Giordano, R.; Saii, Z.; Fredsgaard, M.; Hulkko, L.S.S.; Poulsen, T.B.G.; Thomsen, M.E.; Henneberg, N.; Zucolotto, S.M.; Arendt-Nielsen, L.; Papenbrock, J.; Thomsen, M.H.; Stensballe, A. Pharmacological Insights into Halophyte Bioactive Extract Action on Anti-Inflammatory, Pain Relief and Antibiotics-Type Mechanisms. *Molecules* **2021**, 26, 3140.

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Review

Pharmacological Insights into Halophyte Bioactive Extract Action on Anti-Inflammatory, Pain Relief and Antibiotics-Type Mechanisms

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Abstract: The pharmacological activities in bioactive plant extracts play an increasing role in sustainable resources for valorization and biomedical applications. Bioactive phytochemicals, including natural compounds, secondary metabolites and their derivatives, have attracted significant attention for use in both medicinal products and cosmetic products. Our review highlights the pharmacological mode-of-action and current biomedical applications of key bioactive compounds applied as anti-inflammatory, bactericidal with antibiotics effects, and pain relief purposes in controlled clinical studies or preclinical studies. In this systematic review, the availability of bioactive compounds from several salt-tolerant plant species, mainly focusing on the three promising species *Aster tripolium*, *Crithmum maritimum* and *Salicornia europaea*, are summarized and discussed. All three of them have been widely used in natural folk medicines and are now in the focus for future nutraceutical and pharmacological applications.

Keywords: secondary metabolites; halophytes; hydroxycinnamic acid; inflammation; nutraceuticals

1. Halophyte Species for Current and Future Biomedical Applications

Salt-tolerant plant species, also called halophytes, belong to many botanical families. Halophytes are defined as plants that can grow and complete their life-cycle in a salt concentration of at least 200 mM NaCl [1]. They do not form a systematical group and, phylogenetically, they are not related to each other. Through evolution, they have adapted to saline conditions in several ways: morphologically, physiologically, and biochemically. Hence, halophyte species are found to produce high levels of bioactive compounds and free radical-scavenging secondary metabolites, potentially due to their adaptation to harsh environmental conditions. High salinity during growth and development also increases the level of free radicals in plants [2,3]. Such potential beneficial dietary factors in small doses and complex combinations (e.g., polyphenols, fibers, polyunsaturated fatty acids, etc.) for lifestyle changes can lead to reduced inflammation and improved health; however, metabolic disturbances are key contributors to disease progression [4]. Screening and testing of extracts from medicinal plants species, including halophytes, against a variety of pharmacological targets and disease conditions in order to benefit from the

immense natural chemical diversity is a research focus in many laboratories and companies worldwide [5,6]. For halophytes, the relevant compound classes are a combination of components typical of lignocellulosic biomass and components unique for a family or species (Figure 1). The majority of the free radical-scavenging phenolic compounds are biosynthesized through the shikimic acid, acetic acid, and phenylpropanoid pathways, resulting in phenylpropanoids, simple phenols, and phenolics, respectively [7,8].

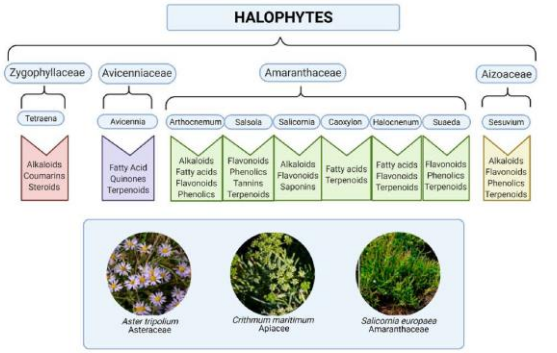

Figure 1. Phytochemical composition of some common coastal halophytes. Created with BioRender.com. (Pictures reproduced with permission [9]).

Over time, nature has been a rich source for various natural bioactive substances that have many applications in folk medicine and ethnopharmaceuticals [10–12]. For thousands of years, plants were the only source of medicine due to the absence of other compounds available, and they were used for a broad spectrum of medical purposes as therapies based on their contents of secondary metabolites and their bioactive properties [13].

The present review will focus on reviewing the current state of the art for biomedical applications of bioactive molecules present in three halophytic plant species, *Aster tripolium* (Jacq.) Dobrocz., *Crithmum maritimum* L., and *Salicornia europaea* agg. (illustrated in Figure 1), due to their potential use in nutraceutical foods, cosmetics, and also as bioactive components for medicinal applications because they contain health-promoting compounds such as minerals, fibers, oils, phenolics and vitamins [14]. The biological qualities of these three halophytic plants species can overall be divided into anti-inflammatory, antioxidant-rich and antibacterial activities, and future use in medicinal practice. The bioactive compounds are listed in Table 1 and summarized in Section 2. The chemical structure and biological activities of secondary metabolites isolated from *Salicornia europaea* L. have recently been reviewed [15].

Table 1. Anti-inflammatory and antibacterial activities of several halophytes based on their bioactive compounds.

	Anti-Inflammatory	Antibacterial	Antioxidative	References
Aster tripolium	- Caffeoyl esters (isomer of chlorogenic acid) - Quercetin (flavonoid)	- Caffeic acid (from chlorogenic acid)	- Quercetin (flavonoid)	[11,12,14,16–21]
Crithmum maritimum	- Chlorogenic acid (hydroxycinnamic acids) - p-Cymene - β-Phellandrene, gamma-terpinene, thymol methyl ether and dillapiole (essential oils)	- Essential oils - Falcarindiol	- Chlorogenic acid (hydroxycinnamic acids)	[11,12,14,21–24]
Salicornia europaea	- Acacetin (flavone) - Chlorogenic acid, rosmarinic acid (esters) - Cinnamic acid, p-coumaric acid, caffeic acid, ferulic acid, sinapic acid, (hydroxycinnamic acids) - Gallic acid, salicylic acid, protocatechuic acid, quinic acid (phenolic acids) - Irilin B (isoflavonoid) - Hesperetin (flavanone) - Galangin isorhamnetin, kaempferol, myricetin, quercetin, rhamnetin, (anthoxanthins flavonol)	- Phenols - Fatty acids	- Tungtungmadic acid - Quercetin - Chlorogenic acid - Caffeoylquinic acid	[3,15,25–28]

2. Bioactive Components Including Primary Metabolites, Phenolics and Their Antioxidant Properties

The most common bioactive components include primary metabolites such as amino acids, proteins, bioactive polysaccharides, lipids, and lignin [29]. Dietary fibers and minerals (Mg, Ca, Fe, K) are also present in large amounts [3]. Immunomodulatory proteins, peptides, and polysaccharides have been isolated and characterized from *Salicornia* spp., that may explain some of the therapeutic efficacies which have been used in folk medicine to treat various diseases, including cancer [30]. The class of secondary metabolites or phytochemicals include compounds of pharmacological and biological importance, including alkaloids, fatty acids, and lipids, flavonoids, phenolics, quinines, tannins, terpenoids, steroids and saponins, and coumarins. The content of the secondary metabolites may vary depending on the particular habitat where the plant grows. The secondary metabolites from plants are challenging to categorize and replicate in the industry because their metabolic pathways of synthesis, features, and mechanisms of action often have similarities and overlap as well as there being an incomplete understanding of synthesis. However, a possible classification is based on their biosynthetic pathways which include large molecule categories: (a) phenolics including phenolic acids, their derivatives, and flavonoids; (b) terpenes and steroids; and (c) alkaloids [10].

Antioxidants can be found in high quantities in different foods, such as vegetables, berries and fruits, because most plants contain phenolic compounds, which are secondary metabolites with health beneficial properties, proven in in vivo and in vitro studies [31–36]. The common structural characteristics all phenolics share are aromatic rings, hydroxyl groups and, commonly, electron double bonds. Phenolics have been extracted using ethanol, methanol, water, ethyl acetate, and dichloromethane by the use of simple solvent extraction under reflux, Soxhlet extraction, assisted ultrasound extraction, and assisted microwave extraction [37–41]. Different extraction methods and solvents have been shown to target the extraction of different phenolics [41,42]. Especially, phenolic monomers have been shown to be thermolabile, and thereby prone to thermal degradation. Therefore, prolonged extractions using high temperatures should be avoided, and the extraction method should be chosen accordingly [38,43]. Rice-Evans et al. [43] investigated the impact of a number of hydroxyl groups on antioxidant activity of each molecule compared to vitamin E and proposed a correlation between the number of hydroxyl groups in HCAs, phenolic acids, flavonoids, their respective derivatives, and their antioxidant capacity. Here, the antioxidative capacity of the catechins epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) is due to the molecular presence of seven and eight hydroxyl groups, respectively [44]. The latter compounds are well known antioxidants, relevant to cancer and neurodegenerative diseases [45,46].

Not only do a high amount of hydroxyl groups have high antioxidant activity, but Cano et al. [47] found the dimer of the hydroxycinnamic acid ferulic acid, 5–5′diferulic acid, to have a high superoxide anion free radical scavenging capacity compared to its monomer, ferulic acid. With a 35.7% inhibition of superoxide anion formation by 5–5′diferulic acid, compared to ferulic acid showing no direct inhibition of superoxide anion formation, Cano et al. [46] concluded that the type of linkage between ferulic acid monomers alters the superoxide anion free radical scavenging capacity, independent of the number of hydroxyl groups. The same trend of inhibition of superoxide anion formation after dimerization was also shown to be true for dimers of the flavonols kaempferol and quercetin containing multiple hydroxyl groups [47].

The beneficial properties of antioxidants are due to their ability to scavenge free radicals. Free radicals are atoms, molecules, or ions with unpaired electrons, and in biological systems are often derived from molecules containing oxygen, nitrogen, or sulfur, called reactive oxygen/nitrogen/sulfur species, or ROS, RNS, and RSS, respectively [48]. Common examples of ROS are the superoxide anion (O_2^-), hydroxyl (OH), and hydroperoxyl (HO_2). Varieties of ROS are produced in living cells as products of oxygen utilization, with a possibility for these to lead to cellular damage, metabolic disorders, or cause DNA damage.

Usually, enzymatic pathways can neutralize these free radicals, e.g., superoxide dismutase, catalase, or glutathione peroxidase [49]. However, these pathways might not be sufficient in all cases, and a non-enzymatic nutritional supplement of antioxidants is necessary.

Diet management using plant-based formulations may improve the metabolic status of patients, including those with diabetes mellitus, where an increased oxidative stress and chronic low-grade inflammation is observed as a consequence of the complex syndrome including long-term alterations of protein and lipid metabolisms [50]. Here, researchers found that a plant-based antidiabetic formulation including antioxidants was able to enhance total serum antioxidant defense and improve overall serum redox status and HDL redox function. More than 5000 flavonoids have been identified and are distributed in a wide range of plants; flavonoids possess documented anticancer activity, both in animal and cellular model systems [51]. Here, luteolin is an important natural antioxidant which has potent anticancer effects under both in vitro and in vivo conditions.

Flavonoids are a large family of polyphenolic compounds that consist of 12 major subclasses according to their chemical composition. Some of these are flavan-3-ols, flavonols, flavanones, flavones, isoflavones, and glycosylated flavonols [52]. These compounds provide an essential source of antioxidants in the human diet. They essentially exist in all foods, which have an origin from plants [20]. Although flavonoids and phenolics, in general, are thought to be non-nutritive agents, they are believed to have a possible health-promoting impact on multiple diseases such as cancer and atherosclerosis [20,53]. The nutraceutical value of *S. herbacea*-derived glucopyranosides as potent anti-obesity agents have been attributed to the alleviation of lipid accumulation [54]. Despite the broad range of key bioactive components, more clinical research is needed to substantiate the composition and quantity thereof in specific halophytes, as well as to determine the biomedical effects in health and disease.

3. Nutraceutical and Pharmacological Mode-of-Action of Key Secondary Metabolites in Halophytes

Wild edible plants (WEPs) are considered as promising sources of essential compounds, needed not only in the human diet including carbohydrates, proteins, and lipids, but also of other minor compounds such as phenols, vitamins, or carotenoids [55]. The presence of phenolic compounds in these vegetal matrices is supposed to provide a prophylactic effect against further pathogenesis and disorders related to aging or oxidative stresses. The utilization and valorization of phytochemicals have focused on nutraceutical use. Hence, the modes-of-action, pharmacological attributes, and medicinal properties target multiple common therapeutic areas, such as neuroprotective, antioxidant, analgesic, immunomodulatory, antimicrobial, antidiabetic and cardioprotective activities (Figure 2) [56]. Neuroprotection is attributed to high levels of antioxidants, including tungmadic acid, quercetin, and chlorogenic acid, enabling the scavenging of reactive oxygen species (ROS), e.g., H_2O_2, efficiently. These electrophilic compounds exert antioxidant activity as well as induce antioxidant enzymes through the Nrf2 signaling pathway, thereby exerting protective effects against ROS-induced neuronal cell damage [57]. Nrf2 regulation contributes to anti-inflammatory processes by orchestrating the recruitment of inflammatory cells, thus regulating gene expression through the antioxidant response element (ARE) [58]. NRF2 activation provides cytoprotection against numerous pathologies including chronic diseases of the lung and liver, autoimmune, neurodegenerative and metabolic disorders, and cancer initiation [59]. However, unidentified compounds may be co-responsible for the neuroprotective effect [60]. Linked to neuroprotection and ROS scavenging then HCAs and their derivatives also display antioxidant, anti-collagenase, anti-inflammatory, antimicrobial and anti-tyrosinase activities, as well as ultraviolet (UV) protective effects. This suggests that HCAs can be exploited as anti-aging and anti-inflammatory agents, preservatives and hyperpigmentation-correcting ingredients [61]. Recent findings suggest that the reversal of UVB-induced damages to skin may be prevented by the protecting effects of aqueous extracts of *S. europaea* affecting basal keratinocytes [62].

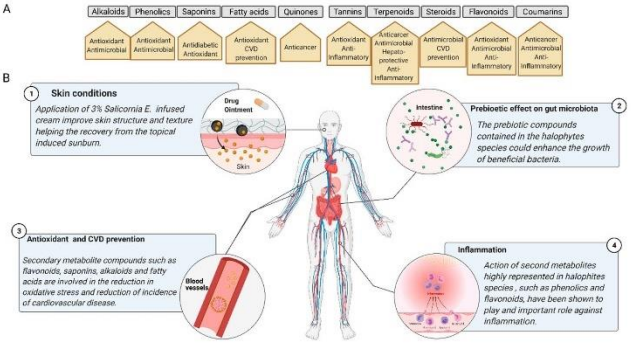

Figure 2. (**A**) Principal physiological processes that involve the action of secondary metabolites present in halophytic species. CVD, cardiovascular disease. (**B**) Main biomedical mechanisms of secondary metabolites' activities of halophytes. Created with BioRender.com.

The involvement of *A. tripolium*, *C. maritimum*, and *S. europaea* bioactive extracts in pain and itch mechanisms remains scarce, although several studies have been conducted, in vitro and in vivo, in order to demonstrate the effects of secondary metabolites present in the three species in relation to nociception and analgesia [63–68]. In a previous study, the authors looked at the analgesic action of chlorogenic acid (5-caffeoylquinic acid, CGA) in animal neuropathic pain models. CGA is a polyphenol formed by the esterification of caffeic and quinic acid, which can be found in plants, vegetables, and fruits [69], showing antioxidant, anti-inflammatory, antigenotoxic, anticancer, and cytostatic activities [63,70]. Dos Santos and colleagues [57] investigated the effect of pure CGA in the formalin-induced pain test, where CGA was reported to possess antinociceptive activity in neuropathic pain models, drastically reducing the pain behavior of mice after the injection of formalin [63]. Neuropathic pain could arise from tissue damage, inflammation, or injury to the nervous system, and is characterized by three sensory abnormalities which include increased sensitivity to painful stimuli (hyperalgesia), perception of innocuous stimuli as painful (allodynia), and spontaneous pain [64]. In this direction, another study showed the antinociceptive action of CGA in the neuropathic pain rat model [65]. The authors showed that the administration of CGA produces significant dose- and time-dependent anti-hyperalgesic effects in chronic constrictive nerve injury (CCI) rat models, and that a chronic treatment for 14 days reduces mechanical hyperalgesia in rats, suggesting action of the CGA on the inhibition of reactive oxygen species (ROS) [65]. Moreover, another study demonstrated that the administration of CGA systemically or intrathecally improves mechanical and cold hyperalgesia in rat neuropathic pain models [68]. In 2014, Qu et al. [61] suggested that the usage of CGA may exert analgesic action by modulating acid-sensing ion channels (ASICS) in rat dorsal root ganglion neurons [67]. The effect of plant extracts and secondary metabolites on itch or skin diseases is a topic that needs to be further evaluated, although a recent study looking into the effect of the prolonged application of *S. europaea*-based cream on sunburned skin of women showed that eight weeks of treatment can improve skin structure and texture, helping the recovery from topical induced sunburn [62].

4. Anti-Inflammatory and Antimicrobial Activities of the Three Halophytes

4.1. Characteristics and Medicinal Properties of Aster Tripolium

4.1.1. Anti-Inflammatory Compounds of *Aster Tripolium*

Aster tripolium (syn. *Tripolium pannonicum*) is a halophyte that belongs to the Asteraceae family, which is one of the most consumed wild-gathered food in several European countries [71]. It is often found in coastal areas and sometimes in salty bogs [72]. It consists of multiple bioactive compounds, which gives *A. tripolium* considerable potential as a functional food ingredient. Apart from *A. tripolium* being highly useful for fodder and food, such as a salad or vegetable, it has high levels of nutrients in its leaves [73]. Additionally, these functional food ingredients may have a positive outcome on numerous diseases such as diabetes [74]. Some of the bioactive compounds are three types of caffeoyl esters which are isomers of CGA. Besides acting as anti-inflammatory and antihypertensive agents, as stated previously, evidence suggests that CGA from coffee possesses insulin sensitivity effects with the same mechanism of action as metformin [75]. Metformin is an antibiotic but has been approved as a first-line treatment for type 2 diabetes [76,77]. Glucose tolerance has been investigated in obese men, which revealed that treatment with CGA improved insulin responses. Both in vitro and in humans, researchers have demonstrated that CGA increases cell insulin secretion and thus glucose uptake [78,79]. These qualities make CGA valuable in the treatment of diabetes and obesity. Another bioactive compound found in *A. tripolium* at unreported levels is quercetin, which belongs to the flavonol subclass of flavonoids [53]. Quercetin is a very strong antioxidant, also found in foods such as apples, onions, and tea [16,20]. Accordingly, quercetin can chelate metals, scavenge oxygen-free radicals, and prevent the oxidation of low-density lipoprotein (LDL) in vitro [17]. Therefore, quercetin could thus contribute significantly to the antioxidant defenses present in blood plasma; it is reportedly able to inhibit the oxidation of LDL in atherosclerotic lesions and thereby be a natural anti-atherosclerotic diet component or be used in T2DM to achieve adequate glycemic control [80–82].

4.1.2. Antimicrobial Compounds of *Aster Tripolium*

To the best of our knowledge, there is currently no evidence that suggests or clarifies whether *A. tripolium* possesses antimicrobial properties. Antibiotic resistance mechanisms are an increasing global health concern; therefore, research in this field with *A. tripolium* in mind is crucial. In general, antibiotics possess the ability to exert selective toxic or growth-limiting effects on bacteria. This selective toxic effect of the antibiotics is nontoxic for human cells but can simultaneously inhibit the functions and target the structures in the bacteria cell [83]. Antibiotics are commonly classified into bactericidal and bacteriostatic agents based on their antimicrobial action. The above-mentioned classification discriminates antibiotics that kill bacteria, referred to as bactericidal, and antibiotics that inhibit bacterial growth or reproduction, called bacteriostatic [84]. One way that bactericidal antibiotics kill bacteria is by inhibiting cell wall synthesis. Another mechanism of action includes the inhibition of key bacterial enzymes or protein translation. On the other hand, bacteriostatic antibiotics limit the growth of bacteria by interfering with bacterial protein production, DNA replication, or other aspects of bacterial cellular metabolism [85]. *A. tripolium* also contains CGAs, similarly to other halophytic species. CGA is a family of esters constructed between certain trans-cinnamic acids and trans-quinic acid [22]. Interestingly, however, if CGA isomers are hydrolyzed to quinic and caffeic acids, the latter have shown antimicrobial effectiveness against certain Gram-bacteria. Elegir et al. [18] demonstrated that caffeic acid revealed antibacterial effects against *Staphylococcus aureus*, and when increasing the concentration, the acid was also capable of exerting bactericidal activity against *Escherichia coli* [18,19]. According to multiple studies, caffeic acid has the strongest antibacterial effects observed when compared to other phenolic acids such as *p*-coumaric acid. It has been postulated that this might be due to one or more hydroxyl groups substituted at the caffeic acid phenol ring. Additionally, because caffeic acid is less polar, it is capable of exerting lipophilicity and thus impacts the permeability of the

cell membrane of the bacteria and interferes with the aerobic metabolism. The cell membrane is crucial for the integrity of the bacterium, which explains why caffeic acid acts bactericidal [18,19].

4.2. Characteristics and Medicinal Properties of Crithmum maritimum

4.2.1. Anti-Inflammatory Compounds of *Crithmum maritimum*

Crithmum maritimum is known as sea fennel or rochepatok samphire and is a member of the Apiaceae family. It grows on maritime cliffs and, more rarely, in the sand. Normally, the leaves are used as a condiment or are eaten as salad [14]. Already used in folk medicine, sea fennel seems to be a very promising candidate for both the pharmaceutical and food industry in order to produce new functional products due to its content of vitamin C, iodine, carotenoids, and great amounts of phenolics compared to other species [23,86]. Additionally, *C. maritimum* has gained increasing attention because it is strongly believed to possess antioxidant and antimicrobial activities [11,12,14,87]. The essential oil (EO) of sea fennel contains several volatile compounds such as limonene, α-pinene, sabinene, p-cimene, β-terpinene, β-myrcene, thymol, γ-terpinene, carvacrol, p-cymol, β-ionone, dillapiole, anisaldehyde, β-caryophyllene, carvone, and myristicin [86,88]. Bioactive compounds identified in *C. maritimum* are HCAs and CGA. It is postulated that CGA is produced as a self-defense mechanism during environmental stresses, such as boron and nitrogen deficit or against ROS. CGA is known for several qualities, including antimicrobial, anti-inflammatory, and immune properties [11,12,14,87]. In terms of medical use, the effect of CGA on hypertension has been proven by multiple studies [89–92]. Several mechanisms have been postulated on how CGA decreases blood pressure. Some of the postulations are the stimulation of nitric oxide (NO) production through the endothelial-dependent pathway, reduction in free radicals by blocking NAD(P)H oxidase expression and activity and, importantly, by the inhibition of angiotensin-converting enzyme [70,93]. Chauhan et al. (2012) demonstrated the anti-inflammatory and immune properties of CGA. Their study revealed the suppression of Th1 cell cytokines such as IL-2 and IL-12 which play a major role and an essential role, respectively, in tolerance in the thymus and the regulation of IFNy and TNFa [94]. Simultaneously, their study revealed an elevation of Th2 cell cytokines such as IL-10 and IL-4, of which principal functions include the negative regulation of Th1 cells, cytokines, and anti-inflammatory response. Thus, CGA seems to exhibit effects that may prove useful in treating/battling/improving different autoimmune or inflammatory diseases such as rheumatoid arthritis and diabetes mellitus that exerts hypoglycemic and hypolipidemic effects [21]. In the latter, CGA seems to mitigate the damaging effects induced by hyperglycemic conditions in both pre-and post-treatment of human hepatocytes cells [95].

Crithmum maritimum is also rich in EOs which are proposed to be produced as a self-defense mechanism due to stressful events, and their amount reaches about 0.8% in fruits and from 0.15 to 0.3% in leaves [96]. The EOs mainly comprise monoterpene hydrocarbons and oxygenated monoterpenes. The major oil components are *p*-cymene, *β*-phellandrene, *β*-terpinene, thymol methyl ether, and dillapiole [23]. The monoterpenes, especially the thymol, are believed to play an important role in the odors and taste of the plant [16]. Additionally, a study by Jallali et al. [13] showed that the EOs have antioxidant effects. However, it was low compared to the acetone extract [23]. The oils revealed beneficial protective abilities, indicating that they can protect a lipid matrix from an oxidative event by the formation of hydroperoxydienes (primary oxidation) and by reducing the degradation of these (secondary oxidation) [23,24,36]. Altogether, *C. maritimum* contains several bioactive compounds exhibiting anti-inflammatory and antioxidant properties. Thus, the non-volatile EOs extract rich in hydroxycinnamic acids and flavonoid glycosides, obtained after the hydrodistillation process, have important biological activities, thus endorsing the industrial exploitation of this plant [97].

4.2.2. Antimicrobial Compounds of *Crithmum maritimum*

As mentioned, EOs have antioxidant effects. However, the important role of EO is antimicrobial activity, despite it being less potent than that of synthetic antibiotics. Nevertheless, due to numerous divergent mechanisms of action, they may have the potential to withstand resistant strains of microorganisms [23,24]. Meot-Duros et al. [8,9] have demonstrated that the *C. maritimum* has excellent antimicrobial activity against bacteria such as *Pseudomonas aeruginosa*, *Candida albicans*, and *Escherichia coli* [11,12]. From the *C. maritimum* leaf, they purified and identified falcarindiol, a polyacetylene with several biological activities such as antibacterial, anti-inflammatory, and scavenging activity [11,12,14]. Additionally, they revealed that falcarindiol has antimycobacterial properties against *Mycobacterium tuberculosis* [11,12]. Several studies have also demonstrated cytotoxic properties of falcarindiol against cell lines such as lymphocytic leukemia and human myeloma [12,98–100]. These findings have potential for future research in the field of overcoming antibiotic resistance, where *C. maritimum* is an obvious and possible candidate.

4.3. Characteristics and Medicinal Properties of Salicornia Species

4.3.1. Anti-Inflammatory Compounds of *Salicornia europaea* and other *Salicornia species*

Salicornia europaea is one of the most salt-tolerant species worldwide and belongs to the Amaranthaceae family. Halophytes belonging to the Amaranthaceae family occur in salt marshes and are exposed to excessive environmental salt concentrations as well as physiological drought. Although not restricted to wetlands, these species dominate saline wetlands, such as inland and coastal salt marshes. They grow in coastal regions across mainly the Mediterranean and East Asia, as well as northern European countries including Denmark and Germany. The species is also known as glasswort or marsh samphire in English. The leaves of the plant are used as a salt substitute, to season vegetable, and as a nutritious fermented food, mostly in Korea but also in some European countries. The nutritional profile, the antioxidant capacity, and microbial quality of the produced plants have been evaluated, including minerals and vitamins [101]. This is due to its nutritional and therapeutic importance for treating constipation, obesity, and diabetes [25,54]. It grows in extreme saltwater areas, such as seashores, marsh lands, and salted deserts; therefore, it produces a rich variety of secondary metabolite compounds such as flavonoids, saponins, and alkaloids. These compounds are believed to play a major role in the biological properties of the plant, such as antioxidative, antitumor, antidiabetic, and neuroprotective potential [25]. Botanical extracts from *S. europaea* have also been reported to include saponin compounds, oleanolic acid glucoside, and chikusetsusaponin methyl ester, which have been shown to work in diabetes prevention and as anti-obesity agents [2]. The number of bioactive compounds are relatively higher in matured plants, in comparison to young plants, and the amounts of phenylpropanoic acids and flavonols in *S. herbacea* ethanol extract have shown to increase by 32.6% and 42.4%, respectively, as the shrubs mature [102]. *Salicornia europaea* have been reported to include β-cyanines and isoflavones, which are known for their strong anti-inflammatory and free radical scavenging properties [2]. Flavonoids can be grouped into bioflavonoids, isoflavonoids, and neoflavonoids, and are derived from the same structures, such as flavone, flavonol, and 4-phenylcoumarin, and have been previously investigated for their anti-neuroinflammatory effects [25,103]. In their study, Kim and colleagues [22] show that the isoflavonoid irilin B extracted from *S. europaea* revealed anti-ROS and anti-inflammatory activities in BV-2 microglial cells (in vitro). Thus, their study suggests that irilin B successfully improves the damaging effect of microglia-mediated neuroinflammation and stimulates antioxidative effects. Neuronal death and oxidative stress are some of the hallmarks of neurodegenerative diseases such Parkinson's. Therefore, the irilin B extract from *S. europaea* may possess anti-Parkinson's disease-like (anti-PD) potential [25]. Besides *S. europaea*, *S. herbacea* and other *Salicornia* species have also shown to be rich in numerous bioactive compounds, and ethanol extract from the aerial parts of *S. ramosissima* have been reported to include antioxidant alkyl ferulates and coumarin, LDL cholesterol-lowering stigmastanol (syn. sitostanol), and ethyl(E)-2-hydroxycinnamate,

which is mostly known for its anti-cancer potential [104]. Sterols and HCAs, such as ferulic acid, have also been detected from the n-hexane extract of *S. ramosissima* [105]. Another succulent halophyte species in the Salicornioideae subfamily is *Arthrocnemum macrostachyum*. Despite their similarities, their total phenolic content seems different, suggesting variability in their chemical composition. AM appears to have a sixfold more active extract than the other species, and thus can exert the highest scavenging activity of reactive species [106]. Flavonoid glycosides including quercetin-3-glucoside and isorhamnetin-3-glucoside can be structurally transformed into minor aglycone molecules, which play a significant role in exerting physiological responses in vivo. Ahn et al. demonstrated that such microbials catalyzed the transformation into quercetin, and isorhamnetin promoted improved anti-inflammatory activity vs. the original source molecules against lipopolysaccharide-induced macrophages [107]. This verifies the anti-inflammatory and antioxidant effects of the *Salicornia* species. Due to these properties, the plants exhibit therapeutic and preventive/protective effects on skin conditions by reducing inflammation of the skin, with the possibility to treat wounds effectively. In some cases, this could be applied in a patch and/or in/on a bandage or simply applied by the hand [106]. In vitro cell assays demonstrated that *Arthrocnemum macrostachyum* possesses a significant concentration-dependent inhibitory performance on matrix metalloproteinase-1 (MMP-1) release by aged fibroblasts. MMP-1 is a collagenase involved in the breakdown of the extracellular matrix [108]. The inhibition of this process reduces, delays, and prevents the breakdown of collagen in the skin and maintains natural collagen levels. Thus, the inhibition of MMP-1 by the halophytic *Arthrocnemum macrostachyum* suggests that the plant can protect the extracellular matrix against damaging outcomes and supply anti-aging effects when applied to the skin. This means that the plant is valuable as a cosmetic for the use of anti-wrinkle cream/extract/agent by reducing or delaying the aging of the skin. The effect could be achieved by applying the invention by several administration routes such as by injection, spray, sponge, and/or directly by the hand [106]. Apart from that, it has been demonstrated that *S. europaea* consists of several other compounds, such as the flavone acacetin [3]. Acacetin, which is an O-methylated flavone, has anti-inflammatory and antioxidant effects that may have a positive effect on sepsis, for example. The explanation for this could be that acacetin strongly inhibits the expression of several proinflammatory cytokines, such as inducible nitric oxide synthase, cyclooxygenase-2, superoxide dismutases, and heme oxygenase-1 [26,60]. Nevertheless, *S. herbacea* also consists of hesperetin, a flavanone found in citrus fruits which showed to have inhibitory effects on microglia-mediated neuroinflammation [3,27]. It was proposed that hesperetin was capable of suppressing MAPK pathways and inflammatory cytokines such as interleukin-1b and IL-6 which are released by activated microglial cells in neurodegenerative diseases, for example [27].

4.3.2. Antimicrobial Compounds of *S. europaea*

As specified earlier, *S. europaea* has anti-inflammatory effects. Nonetheless, its antimicrobial effects have been demonstrated by Essaidi et al. [2], who showed the antimicrobial effect of *S. europaea* against several pathogenic mechanisms, such as *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumonia*, among others [3]. The extract from *S. europaea* mostly affected Gram-positive bacteria, because they showed the most sensitivity by having a greater inhibition zone than the Gram-negative bacteria [3]. The antimicrobial activity of *S. europaea* is suggested to be attributed to multiple compounds in the plant. These compounds may have bacteriostatic effects because some bacteria are especially sensitive to the extracts from halophytes. Compounds such as phenols are thought to have an impact on this antimicrobial activity. However, this activity is also thought to be associated with components other than phenols; for instance, fatty acids. In addition, Essaidi et al. [2] stated that these extracted compounds from *S. europaea* are potential inhibitors of cytochrome P450 enzymes, such as CYP2D6, CYP1A2, and CYP3A4. This is because *S. europaea* contains flavonols such as quercetin, which have been shown to have inhibitory activity on several cytochrome P450 enzymes. Therefore, it is thought that the inhibition of multiple cy-

tochrome P450 enzymes is related to the presence of phenolic compounds such as phenolic acids and flavonoids in *S. europaea* [3]. The application of mass spectrometry techniques in preclinical investigations and to evaluate the potential biologically active compounds in halophytic plants is encouraged for future studies [109].

5. Conclusions

In summary, we have reviewed the current literature and current state of the art for biomedical applications of halophytes such as *A. tripolium*, *C. maritimum*, and *S. europaea*. Conclusively, a number of halophyte species can be used in many applications such as functional food, functional feed, cosmetic products, and finally, as bioactive pharmaceutical compounds. Their properties emphasize their potential for use as medicinal agents such as antibiotics or prebiotics. The massive use of antibiotic treatment has resulted in increased antibiotic resistance, which is one of the most critical treatment problems worldwide. Therefore, there is a growing request for new antimicrobial drugs. Due to the situation, natural derivates and biologically active compounds isolated from plants can be beneficial resources for such new drugs. Halophytic plants are an obvious resource because several studies have proved their antimicrobial effectiveness. Due to the multiple positive effects on health aspects such as antibiotic resistance, regulation of the inflammatory response, and pain analgesia, it increases the need to further investigate the mechanisms and pathways in which these plant species and their secondary metabolites are involved.

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References

1. Flowers, T.J.; Colmer, T.D. Salinity tolerance in halophytes*. *New Phytol.* **2008**, *179*, 945–963. [CrossRef] [PubMed]
2. Cybulska, I.; Brudecki, G.; Alassali, A.; Thomsen, M.; Brown, J. Phytochemical composition of some common coastal halophytes of the United Arab Emirates. *Emirates J. Food Agric.* **2014**, *26*, 1046–1056. [CrossRef]
3. Essaidi, I.; Brahmi, Z.; Snoussi, A.; Ben Haj Koubaier, H.; Casabianca, H.; Abe, N.; El Omri, A.; Chaabouni, M.M.; Bouzouita, N. Phytochemical investigation of Tunisian *Salicornia herbacea* L., antioxidant, antimicrobial and cytochrome P450 (CYPs) inhibitory activities of its methanol extract. *Food Control* **2013**, *32*, 125–133. [CrossRef]
4. Margină, D.; Ungurianu, A.; Purdel, C.; Tsoukalas, D.; Sarandi, E.; Thanasoula, M.; Tekos, F.; Mesnage, R.; Kouretas, D.; Tsatsakis, A. Chronic inflammation in the context of everyday life: Dietary changes as mitigating factors. *Int. J. Environ. Res. Public Health* **2020**, *17*, 4135. [CrossRef] [PubMed]
5. Lautié, E.; Russo, O.; Ducrot, P.; Boutin, J.A. Unraveling Plant Natural Chemical Diversity for Drug Discovery Purposes. *Front. Pharmacol.* **2020**, *11*, 397. [CrossRef]
6. İbrahimova, U.; Kumari, P.; Yadav, S.; Rastogi, A.; Antala, M.; Suleymanova, Z.; Zivcak, M.; Tahjib-Ul-Arif, M.; Hussain, S.; Abdelhamid, M.; et al. Progress in understanding salt stress response in plants using biotechnological tools. *J. Biotechnol.* **2021**, *329*, 180–191. [CrossRef] [PubMed]
7. Hollman, P.C.H. Evidence for health benefits of plant phenols: Local or systemic effects? *J. Sci. Food Agric.* **2001**, *81*, 842–852. [CrossRef]
8. Giada, M.D.L.R. *Oxidative Stress and Chronic Degenerative Diseases—A Role for Antioxidants*; Chapter: Food Phenolic Compounds: Main Classes, Sources and Their Antioxidant Power; Morales-Gonzalez, J.A., Ed.; IntechOpen Limited: London, UK, 2013; ISBN 953-51-1123-X.
9. Hilbers, D.; van Kruijsbergen, W.; Boll, H. Photograph on Creative Commons Licence; Saxifraga. Available online: http://www.saxifraga.nl/ (accessed on 10 March 2021).
10. Bodas, R.; Prieto, N.; García-González, R.; Andrés, S.; Giráldez, F.J. Manipulation of rumen fermentation and methane production with plant secondary metabolites. *Anim. Feed Sci. Technol.* **2012**, *176*, 78–93. [CrossRef]

11. Meot-Duros, L.; Le Floch, G.; Magné, C. Radical scavenging, antioxidant and antimicrobial activities of halophytic species. *J. Ethnopharmacol.* **2008**, *116*, 258–262. [CrossRef]
12. Meot-Duros, L.; Cérantola, S.; Talarmin, H.; Le Meur, C.; Le Floch, G.; Magné, C. New antibacterial and cytotoxic activities of falcarindiol isolated in *Crithmum maritimum* L. leaf extract. *Food Chem. Toxicol.* **2010**, *48*, 553–557. [CrossRef]
13. Alassali, A.; Cybulska, I.; Brudecki, G.P.; Farzanah, R.; Thomsen, M.H. Methods for upstream extraction and chemical characterization of secondary metabolites from algae biomass. *Adv. Tech. Biol. Med.* **2015**, *4*, 1–16. [CrossRef]
14. Meot-Duros, L.; Magné, C. Antioxidant activity and phenol content of *Crithmum maritimum* L. leaves. *Plant Physiol. Biochem.* **2009**, *47*, 37–41. [CrossRef] [PubMed]
15. Kim, S.; Lee, E.-Y.; Hillman, P.F.; Ko, J.; Yang, I.; Nam, S.-J. Chemical structure and biological activities of secondary metabolites from *Salicornia europaea* L. *Molecules* **2021**, *26*, 2252. [CrossRef] [PubMed]
16. Heinrich, M.; Barnes, J.; Gibbons, S.; Williamson, E.M.; Kinghorn, A.D. *Fundamentals of Pharmacognosy And Phytotherapy*; Elsevier Ltd: London, UK, 2012; ISBN 9780702033889.
17. de Whalley, C.V.; Rankin, S.M.; Hoult, J.R.; Jessup, W.; Leake, D.S. Flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages. *Biochem. Pharmacol.* **1990**, *39*, 1743–1750. [CrossRef]
18. Elegir, G.; Kindl, A.; Sadocco, P.; Orlandi, M. Development of antimicrobial cellulose packaging through laccase-mediated grafting of phenolic compounds. *Enzyme Microb. Technol.* **2008**, *43*, 84–92. [CrossRef]
19. Kępa, M.; Miklasińska-Majdanik, M.; Wojtyczka, R.D.; Idzik, D.; Korzeniowski, K.; Smoleń-Dzirba, J.; Wasik, T.J. Antimicrobial potential of caffeic acid against staphylococcus aureus clinical strains. *Biomed Res. Int.* **2018**. [CrossRef]
20. Hertog, M.G.L.; Hollman, P.C.H.; Katan, M.B.; Daan, D.; Kromhout, K. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. *Nutr. Cancer* **1993**, *20*, 21–29. [CrossRef]
21. Chauhan, P.S.; Satti, N.K.; Sharma, P.; Sharma, V.K.; Suri, K.A.; Bani, S. Differential effects of chlorogenic acid on various immunological parameters relevant to rheumatoid arthritis. *Phyther. Res.* **2012**, *26*, 1156–1165. [CrossRef]
22. Clifford, M.N.; Johnston, K.L.; Knight, S.; Kuhnert, N. Hierarchical scheme for LC-MSn identification of chlorogenic acids. *J. Agric. Food Chem.* **2003**, *51*, 2900–2911. [CrossRef]
23. Jallali, I.; Zaouali, Y.; Missaoui, I.; Smeoui, A.; Abdelly, C.; Ksouri, R. Variability of antioxidant and antibacterial effects of essential oils and acetonic extracts of two edible halophytes: *Crithmum maritimum* L. and *Inula crithmoïdes* L. *Food Chem.* **2014**, *145*, 1031–1038. [CrossRef] [PubMed]
24. Ruberto, G.; Baratta, M.T.; Deans, S.G.; Dorman, H.J.D. Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* essential oils. *Planta Med.* **2000**, *66*, 687–693. [CrossRef]
25. Kim, J.; Karthivashan, G.; Kweon, M.H.; Kim, D.H.; Choi, D.K. The ameliorative effects of the ethyl acetate extract of *Salicornia europaea* L. and its bioactive candidate, Irilin B, on LPS-induced microglial inflammation and MPTP-intoxicated PD-like mouse model. *Oxid. Med. Cell. Longev.* **2019**. [CrossRef] [PubMed]
26. Sun, L.C.; Zhang, H.B.; Gu, C.D.; Guo, S.D.; Li, G.; Lian, R.; Yao, Y.; Zhang, G.Q. Protective effect of acacetin on sepsis-induced acute lung injury via its anti-inflammatory and antioxidative activity. *Arch. Pharm. Res.* **2018**, *41*, 1199–1210. [CrossRef] [PubMed]
27. Jo, S.H.; Kim, M.E.; Cho, J.H.; Lee, Y.; Lee, J.; Park, Y.D.; Lee, J.S. Hesperetin inhibits neuroinflammation on microglia by suppressing inflammatory cytokines and MAPK pathways. *Arch. Pharm. Res.* **2019**, *42*, 695–703. [CrossRef] [PubMed]
28. Zengin, G.; Aumeeruddy-Elalfi, Z.; Mollica, A.; Yilmaz, M.A.; Mahomoodally, M.F. In vitro and in silico perspectives on biological and phytochemical profile of three halophyte species—A source of innovative phytopharmaceuticals from nature. *Phytomedicine* **2018**, *38*, 35–44. [CrossRef]
29. Bandaranayake, W.M. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. *Wetl. Ecol. Manag.* **2002**, *10*, 421–452. [CrossRef]
30. Im, S.A.; Kim, K.; Lee, C.K. Immunomodulatory activity of polysaccharides isolated from *Salicornia herbacea*. *Int. Immunopharmacol.* **2006**, *6*, 1451–1458. [CrossRef]
31. Apea-Bah, F.B.; Minnaar, A.; Bester, M.J.; Duodu, K.G. Sorghum–cowpea composite porridge as a functional food, Part II: Antioxidant properties as affected by simulated in vitro gastrointestinal digestion. *Food Chem.* **2016**, *197*, 307–315. [CrossRef]
32. Du, N.; Cao, S.; Yu, Y. Research on the adsorption property of supported ionic liquids for ferulic acid, caffeic acid and salicylic acid. *J. Chromatogr. B.* **2011**, *879*, 1697–1703. [CrossRef]
33. Xiang, J.; Zhang, M.; Apea-Bah, F.B.; Beta, T. Hydroxycinnamic acid amide (HCAA) derivatives, flavonoid C-glycosides, phenolic acids and antioxidant properties of foxtail millet. *Food Chem.* **2019**, *295*, 214–223. [CrossRef]
34. Tavares-Da-Silva, E.J.; Varela, C.L.; Pires, A.S.; Encarnação, J.C.; Abrantes, A.M.; Botelho, M.F.; Carvalho, R.A.; Proença, C.; Freitas, M.; Fernandes, E.; et al. Combined dual effect of modulation of human neutrophils' oxidative burst and inhibition of colon cancer cells proliferation by hydroxycinnamic acid derivatives. *Bioorg. Med. Chem.* **2016**, *24*, 3556–3564. [CrossRef] [PubMed]
35. Karthivashan, G.; Kweon, M.-H.; Park, S.-Y.; Kim, J.-S.; Kim, D.-H.; Ganesan, P.; Choi, D.-K. Cognitive-enhancing and ameliorative effects of acanthoside B in a scopolamine-induced amnesic mouse model through regulation of oxidative/inflammatory/cholinergic systems and activation of the TrkB/CREB/BDNF pathway. *Food Chem. Toxicol.* **2019**, *129*, 444–457. [CrossRef] [PubMed]
36. Choudhury, P.K.; Salem, A.Z.M.; Jena, R.; Kumar, S.; Singh, R.; Puniya, A.K. Rumen microbiology: An overview. In *Rumen Microbiology: From Evolution to Revolution*; Springer India: Punjab, India, 2015; pp. 1–379. ISBN 9788132224013.

37. Manzano Durán, R.; Sánchez, J.E.F.; Velardo-Micharet, B.; Gómez, M.J.R. Multivariate optimization of ultrasound-assisted extraction for the determination of phenolic compounds in plums (*Prunus salicina* Lindl.) by high-performance liquid chromatography (HPLC). *Instrum. Sci. Technol.* **2020**, *48*, 113–127. [CrossRef]
38. He, B.; Zhang, L.L.; Yue, X.Y.; Liang, J.; Jiang, J.; Gao, X.L.; Yue, P.X. Optimization of ultrasound-assisted extraction of phenolic compounds and anthocyanins from blueberry (*Vaccinium ashei*) wine pomace. *Food Chem.* **2016**, *204*, 70–76. [CrossRef] [PubMed]
39. Padalino, L.; Costa, C.; Del Nobile, M.A.; Conte, A. Extract of *Salicornia europaea* in fresh pasta to enhance phenolic compounds and antioxidant activity. *Int. J. Food Sci. Technol.* **2019**, *54*, 3051–3057. [CrossRef]
40. Roselló-Soto, E.; Martí-Quijal, F.J.; Cilla, A.; Munekata, P.E.S.; Lorenzo, J.M.; Remize, F.; Barba, F.J. Influence of temperature, solvent and pH on the selective extraction of phenolic compounds from tiger nuts by-products: Triple-TOF-LC-MS-MS characterization. *Molecules* **2019**, *24*, 797. [CrossRef]
41. Galanakis, C.; Goulas, V.; Tsakona, S.; Manganaris, G.; Gekas, V. A knowledge base for the recovery of natural phenols with different solvents. *Int. J. food Prop.* **2013**, *16*, 382–396. [CrossRef]
42. Corbin, C.; Fidel, T.; Leclerc, E.A.; Barakzoy, E.; Sagot, N.; Falguiéres, A.; Renouard, S.; Blondeau, J.P.; Ferroud, C.; Doussot, J.; et al. Development and validation of an efficient ultrasound assisted extraction of phenolic compounds from flax (*Linum usitatissimum* L.) seeds. *Ultrason. Sonochem.* **2015**, *26*, 176–185. [CrossRef]
43. Camel, V. Microwave-assisted solvent extraction of environmental samples. *TrAC-Trends Anal. Chem.* **2000**, *19*, 229–248. [CrossRef]
44. Rice-Evans, C.A.; Miller, N.J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic. Biol. Med.* **1996**, *20*, 933–956. [CrossRef]
45. Pervin, M.; Unno, K.; Takagaki, A.; Isemura, M.; Nakamura, Y. Function of green tea catechins in the brain: Epigallocatechin gallate and its metabolites. *Int. J. Mol. Sci.* **2019**, *20*, 3630. [CrossRef] [PubMed]
46. Du, G.J.; Zhang, Z.; Wen, X.D.; Yu, C.; Calway, T.; Yuan, C.S.; Wang, C.Z. Epigallocatechin gallate (EGCG) is the most effective cancer chemopreventive polyphenol in green tea. *Nutrients* **2012**, *4*, 1679–1691. [CrossRef]
47. Cano, A.; Arnao, M.; Williamson, G.; Garcia-Conesa, M.-T. Superoxide scavenging by polyphenols: Effect of conjugation and dimerization. *Redox Rep.* **2002**, *7*, 379–383. [CrossRef] [PubMed]
48. Lü, J.-M.; Lin, P.H.; Yao, Q.; Chen, C. Chemical and molecular mechanisms of antioxidants: Experimental approaches and model systems. *J. Cell. Mol. Med.* **2010**, *14*, 840–860. [CrossRef] [PubMed]
49. Ighodaro, O.; Akinloye, O. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alex. J. Med.* **2018**, *54*, 287–293. [CrossRef]
50. Ungurianu, A.; Şeremet, O.; Gagniuc, E.; Olaru, O.T.; Guţu, C.; Grădinaru, D.; Ionescu-Tîrgovişte, C.; Margină, D.; Dănciulescu-Miulescu, R. Preclinical and clinical results regarding the effects of a plant-based antidiabetic formulation versus well established antidiabetic molecules. *Pharmacol. Res.* **2019**, *150*, 104522. [CrossRef] [PubMed]
51. Imran, M.; Rauf, A.; Abu-Izneid, T.; Nadeem, M.; Shariati, M.A.; Khan, I.A.; Imran, A.; Orhan, I.E.; Rizwan, M.; Atif, M.; et al. Luteolin, a flavonoid, as an anticancer agent: A review. *Biomed. Pharmacother.* **2019**, *112*, 108612. [CrossRef]
52. Panche, A.N.; Diwan, A.D.; Chandra, S.R. Flavonoids: An overview. *J. Nutr. Sci.* **2016**, *5*, e47. [CrossRef]
53. Hollman, P.C.H.; Van Trijp, J.M.P.; Buysman, M.N.C.P.; Martijn, M.S.; Mengelers, M.J.B.; De Vries, J.H.M.; Katan, M.B. Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. *FEBS Lett.* **1997**, *418*, 152–156. [CrossRef]
54. Kim, Y.A.; Kong, C.S.; Um, Y.R.; Lim, S.Y.; Yea, S.S.; Seo, Y. Evaluation of *Salicornia herbacea* as a potential antioxidant and anti-inflammatory agent. *J. Med. Food* **2009**, *12*, 661–668. [CrossRef]
55. Pereira, A.G.; Fraga-Corral, M.; García-Oliveira, P.; Jimenez-Lopez, C.; Lourenço-Lopes, C.; Carpena, M.; Otero, P.; Gullón, P.; Prieto, M.A.; Simal-Gandara, J. Culinary and nutritional value of edible wild plants from northern Spain rich in phenolic compounds with potential health benefits. *Food Funct.* **2020**, *11*, 8493–8515. [CrossRef] [PubMed]
56. Balkrishna, A.; Thakur, P.; Varshney, A. Phytochemical profile, pharmacological attributes and medicinal properties of convolvulus prostratus–A cognitive enhancer herb for the management of neurodegenerative etiologies. *Front. Pharmacol.* **2020**, *11*, 171. [CrossRef]
57. Chung, Y.C.; Chun, H.K.; Yang, J.Y.; Kim, J.Y.; Han, E.H.; Kho, Y.H.; Jeong, H.G. Tungtungmadic acid, a novel antioxidant, from *Salicornia herbacea*. *Arch. Pharm. Res.* **2005**, *28*, 1122–1126. [CrossRef]
58. Ahmed, S.M.U.; Luo, L.; Namani, A.; Wang, X.J.; Tang, X. Nrf2 signaling pathway: Pivotal roles in inflammation. *Biochim. Biophys. Acta Mol. Basis Dis.* **2017**, *1863*, 585–597. [CrossRef] [PubMed]
59. Cuadrado, A.; Rojo, A.I.; Wells, G.; Hayes, J.D.; Cousin, S.P.; Rumsey, W.L.; Attucks, O.C.; Franklin, S.; Levonen, A.-L.; Kensler, T.W.; et al. Therapeutic targeting of the NRF2 and KEAP1 partnership in chronic diseases. *Nat. Rev. Drug Discov.* **2019**, *18*, 295–317. [CrossRef] [PubMed]
60. Kim, M.S.; Seo, J.Y.; Oh, J.; Jang, Y.K.; Lee, C.H.; Kim, J.-S. Neuroprotective effect of halophyte *Salicornia herbacea* L. is mediated by activation of heme oxygenase-1 in mouse hippocampal HT22 cells. *J. Med. Food* **2017**, *20*, 140–151. [CrossRef]
61. Taofiq, O.; González-Paramás, A.M.; Barreiro, M.F.; Ferreira, I.C.F.R.; McPhee, D.J. Hydroxycinnamic acids and their derivatives: Cosmeceutical significance, challenges and future perspectives, a review. *Molecules* **2017**, *22*, 281. [CrossRef]
62. Doi, N.; Togari, H.; Minagi, K.; Nakaoji, K.; Hamada, K.; Tatsuka, M. Protective effects of *Salicornia europaea* on UVB-induced misoriented cell divisions in skin epithelium. *Cosmetics* **2020**, *7*, 44. [CrossRef]
63. Dos Santos, M.D.; Almeida, M.C.; Lopes, N.P.; De Souza, G.E.P. Evaluation of the anti-inflammatory, analgesic and antipyretic activities of the natural polyphenol chlorogenic acid. *Biol. Pharm. Bull.* **2006**, *29*, 2236–2240. [CrossRef]

64. Moalem, G.; Tracey, D.J. Immune and inflammatory mechanisms in neuropathic pain. *Brain Res. Rev.* **2006**, *51*, 240–264. [CrossRef]
65. Bagdas, D.; Cinkilic, N.; Ozboluk, H.Y.; Ozyigit, M.O.; Gurun, M.S. Antihyperalgesic activity of chlorogenic acid in experimental neuropathic pain. *J. Nat. Med.* **2013**, *67*, 698–704. [CrossRef]
66. Bagdas, D.; Ozboluk, H.Y.; Cinkilic, N.; Gurun, M.S. Antinociceptive effect of chlorogenic acid in rats with painful diabetic neuropathy. *J. Med. Food* **2014**, *17*, 730–732. [CrossRef] [PubMed]
67. Qu, Z.W.; Liu, T.T.; Qiu, C.Y.; Li, J.D.; Hu, W.P. Inhibition of acid-sensing ion channels by chlorogenic acid in rat dorsal root ganglion neurons. *Neurosci. Lett.* **2014**, *567*, 35–39. [CrossRef] [PubMed]
68. Hara, K.; Haranishi, Y.; Kataoka, K.; Takahashi, Y.; Terada, T.; Nakamura, M.; Sata, T. Chlorogenic acid administered intrathecally alleviates mechanical and cold hyperalgesia in a rat neuropathic pain model. *Eur. J. Pharmacol.* **2014**, *723*, 459–464. [CrossRef] [PubMed]
69. Clifford, M.N. Chlorogenic acids and other cinnamates - Nature, occurrence and dietary burden. *J. Sci. Food Agric.* **1999**, *79*, 362–372. [CrossRef]
70. Sato, Y.; Itagaki, S.; Kurokawa, T.; Ogura, J.; Kobayashi, M.; Hirano, T.; Sugawara, M.; Iseki, K. In vitro and in vivo antioxidant properties of chlorogenic acid and caffeic acid. *Int. J. Pharm.* **2011**, *403*, 136–138. [CrossRef] [PubMed]
71. Ramani, B.; Reeck, T.; Debez, A.; Stelzer, R.; Huchzermeyer, B.; Schmidt, A.; Papenbrock, J. *Aster tripolium* L. and *Sesuvium portulacastrum* L.: Two halophytes, two strategies to survive in saline habitats. *Plant Physiol. Biochem.* **2006**, *44*, 395–408. [CrossRef]
72. Duarte, B.; Goessling, J.W.; Marques, J.C.; Caçador, I. Ecophysiological constraints of *Aster tripolium* under extreme thermal events impacts: Merging biophysical, biochemical and genetic insights. *Plant Physiol. Biochem.* **2015**, *97*, 217–228. [CrossRef]
73. Geissler, N.; Hussin, S.; Koyro, H.W. Interactive effects of NaCl salinity and elevated atmospheric CO_2 concentration on growth, photosynthesis, water relations and chemical composition of the potential cash crop halophyte *Aster tripolium* L. *Environ. Exp. Bot.* **2009**, *65*, 220–231. [CrossRef]
74. Wubshet, S.G.; Schmidt, J.S.; Wiese, S.; Staerk, D. High-resolution screening combined with HPLC-HRMS-SPE-NMR for identification of potential health-promoting constituents in sea aster and searocket—New nordic food ingredients. *J. Agric. Food Chem.* **2013**, *61*, 8616–8623. [CrossRef]
75. Ong, K.W.; Hsu, A.; Tan, B.K.H. Chlorogenic acid stimulates glucose transport in skeletal muscle via AMPK activation: A contributor to the beneficial effects of coffee on diabetes. *PLoS ONE* **2012**, *7*, e32718. [CrossRef]
76. McCarty, M.F. A chlorogenic acid-induced increase in GLP-1 production may mediate the impact of heavy coffee consumption on diabetes risk. *Med. Hypotheses* **2005**, *64*, 848–853. [CrossRef]
77. McCreight, L.J.; Bailey, C.J.; Pearson, E.R. Metformin and the gastrointestinal tract. *Diabetologia* **2016**, *59*, 426–435. [CrossRef] [PubMed]
78. Tousch, D.; Lajoix, A.D.; Hosy, E.; Azay-Milhau, J.; Ferrare, K.; Jahannault, C.; Cros, G.; Petit, P. Chicoric acid, a new compound able to enhance insulin release and glucose uptake. *Biochem. Biophys. Res. Commun.* **2008**, *377*, 131–135. [CrossRef] [PubMed]
79. Johnston, K.L.; Clifford, M.N.; Morgan, L.M. Coffee acutely modifies gastrointestinal hormone secretion and glucose tolerance in humans: Glycemic effects of chlorogenic acid and caffeine. *Am. J. Clin. Nutr.* **2003**, *78*, 728–733. [CrossRef] [PubMed]
80. Gaballah, H.H.; Zakaria, S.S.; Mwafy, S.E.; Tahoon, N.M.; Ebeid, A.M. Mechanistic insights into the effects of quercetin and/or GLP-1 analogue liraglutide on high-fat diet/streptozotocin-induced type 2 diabetes in rats. *Biomed. Pharmacother.* **2017**, *92*, 331–339. [CrossRef] [PubMed]
81. Patel, R.V.; Mistry, B.M.; Shinde, S.K.; Syed, R.; Singh, V.; Shin, H.-S. Therapeutic potential of quercetin as a cardiovascular agent. *Eur. J. Med. Chem.* **2018**, *155*, 889–904. [CrossRef] [PubMed]
82. Margina, D.; Ilie, M.; Gradinaru, D. Quercetin and epigallocatechin gallate induce in vitro a dose-dependent stiffening and hyperpolarizing effect on the cell membrane of human mononuclear blood cells. *Int. J. Mol. Sci.* **2012**, *13*, 4839–4859. [CrossRef]
83. Blair, J.M.A.; Webber, M.A.; Baylay, A.J.; Ogbolu, D.O.; Piddock, L.J.V. Molecular mechanisms of antibiotic resistance. *Nat. Rev. Microbiol.* **2015**, *13*, 42–51. [CrossRef]
84. Nemeth, J.; Oesch, G.; Kuster, S.P. Bacteriostatic versus bactericidal antibiotics for patients with serious bacterial infections: Systematic review and meta-analysis. *J. Antimicrob. Chemother.* **2015**, *70*, 382–395. [CrossRef]
85. Kohanski, M.A.; Dwyer, D.J.; Collins, J.J. How antibiotics kill bacteria: From targets to networks. *Nat. Rev. Microbiol.* **2010**, *8*, 423–435. [CrossRef] [PubMed]
86. Renna, M. Reviewing the prospects of sea fennel (*Crithmum maritimum* L.) as emerging vegetable crop. *Plants* **2018**, *7*, 92. [CrossRef] [PubMed]
87. Flamini, G.; Mastrorilli, E.; Cioni, P.L.; Panizzi, L. Essential oil from *Crithmum maritimum* grown in liguria (Italy): Seasonal variation and antimicrobial activity. *J. Essent. Oil Res.* **1999**, *11*, 788–792. [CrossRef]
88. Zafeiropoulou, V.; Tomou, E.-M.; Douros, A.; Skaltsa, H. The effect of successive harvesting on the volatile constituents of two essential oils of cultivated populations of sea fennel (*Crithmum maritimum* L.) in Greece. *J. Essent. Oil Bear. Plants* **2021**, *24*, 1–11. [CrossRef]
89. Watanabe, T.; Arai, Y.; Mitsui, Y.; Kusaura, T.; Okawa, W.; Kajihara, Y.; Saito, I. The blood pressure-lowering effect and safety of chlorogenic acid from green coffee bean extract in essential hypertension. *Clin. Exp. Hypertens.* **2006**, *28*, 439–449. [CrossRef]
90. Yamaguchi, T.; Chikama, A.; Mori, K.; Watanabe, T.; Shioya, Y.; Katsuragi, Y.; Tokimitsu, I. Hydroxyhydroquinone-free coffee: A double-blind, randomized controlled dose-response study of blood pressure. *Nutr. Metab. Cardiovasc. Dis.* **2008**, *18*, 408–414. [CrossRef]

91. Kozuma, K.; Tsuchiya, S.; Kohori, J.; Hase, T.; Tokimitsu, I. Antihypertensive effect of green coffee bean extract on mildly hypertensive subjects. *Hypertens. Res.* **2005**, *28*, 711–718. [CrossRef]
92. Ochiai, R.; Jokura, H.; Suzuki, A.; Tokimitsu, I.; Ohishi, M.; Komai, N.; Rakugi, H.; Ogihara, T. Green coffee bean extract improves human vasoreactivity. *Hypertens. Res.* **2004**, *27*, 731–737. [CrossRef]
93. Kanegae, M.P.P.; da Fonseca, L.M.; Brunetti, I.L.; de Oliveira Silva, S.; Ximenes, V.F. The reactivity of ortho-methoxy-substituted catechol radicals with sulfhydryl groups: Contribution for the comprehension of the mechanism of inhibition of NADPH oxidase by apocynin. *Biochem. Pharmacol.* **2007**, *74*, 457–464. [CrossRef]
94. Pastori, C.; Wahlestedt, C. Involvement of long noncoding RNAs in diseases affecting the central nervous system. *RNA Biol.* **2012**, *9*, 860–870. [CrossRef]
95. Bazool Farhood, H.; Balas, M.; Gradinaru, D.; Margină, D.; Dinischiotu, A. Hepatoprotective effects of chlorogenic acid under hyperglycemic conditions. *Rom. Biotechnol. Lett.* **2019**, *24*, 301–307. [CrossRef]
96. Atia, A.; Barhoumi, Z.; Mokded, R.; Abdelly, C.; Smaoui, A. Environmental eco-physiology and economical potential of the halophyte *Crithmum maritimum* L. (Apiaceae). *J. Med. Plants Res.* **2011**, *5*, 3564–3571.
97. Alves-Silva, J.M.; Guerra, I.; Gonçalves, M.J.; Cavaleiro, C.; Cruz, M.T.; Figueirinha, A.; Salgueiro, L. Chemical composition of *Crithmum maritimum* L. essential oil and hydrodistillation residual water by GC-MS and HPLC-DAD-MS/MS, and their biological activities. *Ind. Crops Prod.* **2020**, *149*, 112329. [CrossRef]
98. Cunsolo, F.; Ruberto, G.; Amico, V.; Plattelli, M. Bioactive metabolites from sicilian marine fennel, *Crithmum maritimum*. *J. Nat. Prod.* **1993**, *56*, 1598–1600. [CrossRef]
99. Zidorn, C.; Jöhrer, K.; Ganzera, M.; Schubert, B.; Sigmund, E.M.; Mader, J.; Greil, R.; Ellmerer, E.P.; Stuppner, H. Polyacetylenes from the apiaceae vegetables carrot, celery, fennel, parsley, and parsnip and their cytotoxic activities. *J. Agric. Food Chem.* **2005**, *53*, 2518–2523. [CrossRef] [PubMed]
100. Quan, C.C.; Lee, J.P.; Jin, W.Y.; Youn, U.J.; Kim, H.J.; Ik, S.L.; Zhang, X.F.; Song, K.S.; Seong, Y.H.; Bae, K.H. Cytotoxic constituents from *Angelicae sinensis radix*. *Arch. Pharm. Res.* **2007**, *30*, 565–569. [CrossRef]
101. Lima, A.R.; Castañeda-Loaiza, V.; Salazar, M.; Nunes, C.; Quintas, C.; Gama, F.; Pestana, M.; Correia, P.J.; Santos, T.; Varela, J.; et al. Influence of cultivation salinity in the nutritional composition, antioxidant capacity and microbial quality of *Salicornia ramosissima* commercially produced in soilless systems. *Food Chem.* **2020**, *333*, 127525. [CrossRef] [PubMed]
102. Kim, J.; Cho, J.-Y.; Moon, J.-H.; Choi, G.-C.; Lee, K.-D.; Ham, K.-S.; Kim, S.-J. Change of phenylpropanoic acid and flavonol contents at different growth stage of glasswort (*Salicornia herbacea* L.). *Food Sci. Biotechnol.* **2014**, *23*, 685–691. [CrossRef]
103. McNaught, A.D.; Wilkinson, A. Flavonoids (isoflavonoids and neoflavonoids). In *IUPAC Compendium of Chemical Terminology*; IUPAC: Research Triangle, NC, USA, 2008.
104. Ferreira, D.; Isca, V.; Leal, P.; Seca, A.; Silva, H.; Pereira, M.; Silva, A.; Pinto, D. *Salicornia ramosissima*: Secondary metabolites and protective effect against acute testicular toxicity. *Arab. J. Chem.* **2018**, *11*, 70–80. [CrossRef]
105. Isca, V.M.S.; Seca, A.M.L.; Pinto, D.C.G.A.; Silva, H.; Silva, A.M.S. Lipophilic profile of the edible halophyte *Salicornia ramosissima*. *Food Chem.* **2014**, *165*, 330. [CrossRef]
106. Yousef, L.F.; Alkhoori, S.A.; Thomsen, M.H. Compositions from Halophyte Plants and Methods of Use Thereof. U.S. Patent WO 2018125694 A1, 5 July 2018.
107. Ahn, H.J.; You, H.J.; Park, M.S.; Li, Z.; Choe, D.; Johnston, T.V.; Ku, S.; Ji, G.E. Microbial biocatalysis of quercetin-3-glucoside and isorhamnetin-3-glucoside in *Salicornia herbacea* and their contribution to improved anti-inflammatory activity. *RSC Adv.* **2020**, *10*, 5339–5350. [CrossRef]
108. Pendás, A.M.; Santamaría, I.; Alvarez, M.V.; Pritchard, M.; López-Otín, C. Fine physical mapping of the human matrix metalloproteinase genes clustered on chromosome 11q22.3. *Genomics* **1996**, *37*, 266–269. [CrossRef] [PubMed]
109. Dantas-Medeiros, R.; Furtado, A.A.; Zanatta, A.C.; Torres-Rêgo, M.; Guimarães Lourenço, E.M.; Ferreira Alves, J.S.; Galinari, É.; Alexandre de Oliveira Rocha, H.; Bernardo Guerra, G.C.; Vilegas, W.; et al. Mass spectrometry characterization of *Commiphora leptophloeos* leaf extract and preclinical evaluation of toxicity and anti-inflammatory potential effect. *J. Ethnopharmacol.* **2021**, *264*, 113229. [CrossRef] [PubMed]

Paper VII

Cybulska, I.; Brudecki, G. P.; Brown, J. J.; Hulkko, L. S. S.; Al Hosani, S.; Thomsen, M. H. Comparative study of chemical composition of the halophyte species native to the Persian (Arabian) gulf. *BioResources* **2021** *16(3)*, 5524-5537.

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Paper VIII

Chaturvedi, T.; Hulkko, L.S.S.; Fredsgaard, M.; Thomsen, M.H. Extraction, Isolation, and Purification of Value-Added Chemicals from Lignocellulosic Biomass. *Processes* **2022**, *10*, 1752.

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Review

Extraction, Isolation, and Purification of Value-Added Chemicals from Lignocellulosic Biomass

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Abstract: This review covers the operating conditions for extracting top value-added chemicals, such as levulinic acid, lactic acid, succinic acid, vanillic acid, 3-hydroxypropionic acid, xylitol, 2,5-furandicarboxylic acid, 5-hydroxymethyl furfural, chitosan, 2,3-butanediol, and xylo-oligosaccharides, from common lignocellulosic biomass. Operating principles of novel extraction methods, beyond pretreatments, such as Soxhlet extraction, ultrasound-assisted extraction, and enzymatic extraction, are also presented and reviewed. Post extraction, high-value biochemicals need to be isolated, which is achieved through a combination of one or more isolation and purification steps. The operating principles, as well as a review of isolation methods, such as membrane filtration and liquid–liquid extraction and purification using preparative chromatography, are also discussed.

Keywords: value-added chemicals; lignocellulose biomass; pretreatment; extraction; isolation

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1. Introduction

Lignocellulosic biomass is the most widely available feedstock for biofuel production. As a second-generation feedstock, lignocellulosic biomass does not compete with crops used for food products, such as corn, sugarcane, beetroot, and others. Traditionally, large-scale biomass processing facilities have focused on two main bio-based products, i.e., biofuels and bioenergy. Biofuels include ethanol, butanol, biodiesel, etc. [1,2]. Methane from biogas plants and syngas constitute the primary bioenergy products obtained from biomass processing [3,4]. Whereas these products have spurred the quest for cleaner fuels, they have fallen short of presenting a sustainable business model for production of high-value biomass-derived products. Moving away from single feedstock to a single-product approach leads toward the contemporary biorefinery approach, whereby multi feedstock processing leads to multiple bio-based products, including but not limited to biofuel, bioenergy, biochemicals, proteins, and other high-value bioproducts.

In 2004, the U.S. Department of Energy (USDOE) identified the top 12 platform chemicals (based on their market potential) that can be derived from biomass [5]. Four carbon (C4) 1,4-dicarboxylic acids (succinic, fumaric, and malic acid), 2,5-furandicarboxylic acid, 3-hydroxypropionic acid, aspartic acid, glucaric acid, glutamic acid, itaconic acid, 3-hydroxybutyrolactone, glycerol, sorbitol, and xylitol were identified as the top 12 candidates that can subsequently be converted into numerous high-value biomass-derived products. 'Top Value Added Chemicals from Biomass' was the first volume of a two-volume report, with the second volume published in 2007, in which the list was updated to include lignin-based derivatives, such as vanillin, vanillic acid, syringaldehyde, aromatic diacids, and quinones, amongst others [6]. Several promising technologies to obtain these building block/intermediate chemicals are identified in this report based on technological maturity and reported product yields.

2. Methodology

In this paper, we build on the foundation laid by these two reports and aim to consolidate the literature with respect to the effectiveness of extracting some of these value-added

chemicals from the most common lignocellulosic biomasses. The most common lignocellulosic biomasses include corn stover, sugarcane bagasse, pine, wheat straw, rice straw, softwood, aspen wood, etc. This review identifies which value-added chemicals can be derived from the most common lignocellulosic biomass and presents their respective yields. Furthermore, we evaluate the technologies and the operating conditions used to extract value-added chemicals from the most common lignocellulosic biomasses. Subsequently, methods for the isolation of chemicals are also discussed.

Three factors govern the processing of lignocellulosic biomass: availability of the type of biomass, accessibility and maturity of processing technology, and the intended bioproduct and its market demand. We begin this review begins by identifying the critical value-added chemicals based on their availability and potential to be extracted from lignocellulosic biomass. Next, the literature-reported concentrations, yield, and productivity of extracting value-added chemicals from some of the most common lignocellulosic biomasses are presented. Lastly, the most promising technologies for the isolation of bioproducts are reported.

Lignocellulosic biomass can be broken down into three major building blocks: cellulose, hemicellulose, and lignin. Value-added chemicals can be categorized according to their functional groups of origin, and based on processing routes as shown in Figure 1.

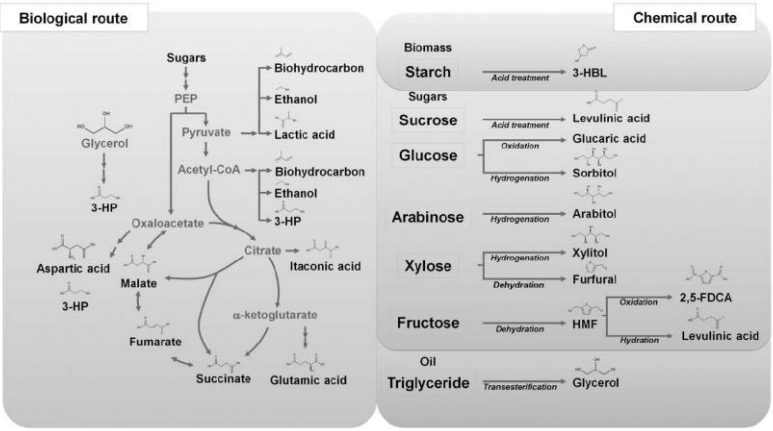

Figure 1. Biological and chemical routes for the production of top value-added chemicals derived from lignocellulosic biomass. Reprinted/adapted with permission from Ref. [7].

In this paper, we will discuss the following derivatives of cellulose:

1. Levulinic acid;
2. Lactic acid;
3. 3-hydroxypropionic acid (3-HP);
4. Succinic acid;
5. Vanillic acid and vanillin;
6. Itaconic acid;
7. Adipic acid;
8. 2,5-furandicarboxylic acid (FDCA); and
9. 5-hydroxymethylfurfural (HMF).

The value-added chemicals extracted from hemicellulose and lignin discussed in this paper are:

1. Xylitol;
2. Furfural;
3. Chitosan;
4. 2,3-butanediol (2,3-BD); and
5. Xylo-oligosaccharides (XOs)

3. Top Value-Added Chemicals

3.1. Levulinic Acid

Levulinic acid can be extracted by the dehydration of sugars, hydration of hydroxymethylfurfural (HMF) or hydrolysis of furfuryl alcohol, both of which are derived from xylose, which is a hemicellulose sugar [5]. A simple structure of levulinic acid is seen in Figure 2. Levulinic acid has applications in additives, pharmaceutical, and plastic industries [7]. Biofine Technology (Boston, MA, USA), GFBiochemicals (Paris, France), and Avantium (Amsterdam, The Netherlands) are companies that are invested in the commercial production of levulinic acid. Table 1 summarizes the levulinic acid yield extracted from lignocellulosic biomass under optimized pretreatment conditions (acid concentration, time, and operating temperature). Levulinic acid can serve as a precursor for succinic acid, diphenolic acid, valeric acid, γ-valerolactone, acetyl acrylic acid, 1,4-butanediol, and other value-added chemicals [8,9]. The ease of deriving levulinic acid from a variety of lignocellulosic crops and its important position in the supply chain as an intermediate for the production of resins, herbicides, plasticizers, solvents, fuels, food, flavoring, and fragrance components makes it one of the top value-added chemicals that can be derived in a lignocellulosic biorefinery.

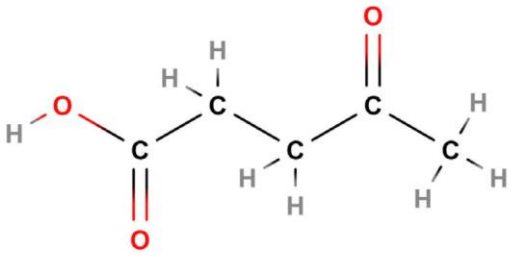

Figure 2. Structure of levulinic acid ($C_5H_8O_3$).

Table 1. Review of extraction methods for levulinic acid from lignocellulosic biomass.

Biomass	Cellulose Content %	Acid Concentration	Operating Temperature (°C)	Time (h)	Theoretical Yield (mol%)	Reference
Kraft Paper Pulp Residue	80	1–5% H_2SO_4	1st Stage 210–230 2nd Stage 195–215	N/A	70–80	[10]
Wheat Straw	40	3.5% H_2SO_4	210	N/A	68.8	[11]
	40.4	4.5% H_2SO_4	220	N/A	79.6	[12]
		3.5% H_2SO_4	210	0.63	19.8	[13]
Bagasse		4.5% HCl	220	N/A	82.7	[12]
	42	1.5% H_2SO_4	25–195	2	17.5	[14]
Glucose	32	20% HCl	100	24	15	[15]
	29	6.5% HCl	162	1	24	[16]
	27	Amberlite IR-120	Room Temperature	124	5.8	[17]
	5–20	0.1–4% H_2SO_4	160–240	N/A	35.4	[18]
	10	6% HCl	160	0.25	41.4	[19]
	12	3% Clay Catalyst (Fe-pillared montmorillonite)	150	24	12	[20]
	12	3% HY Zeolite	150	24	6	[21]
Rice Hull	N/A	1% HCl	160	3	10.3	
Rice Straw	N/A	1% HCl	160	3	5.5	[22]
Corn Stalks	N/A	1% HCl	160	3	7.5	
Wood Sawdust	N/A	1.5% HCl	190	0.5	9	[23]
Oakwood	N/A	3% H_2SO_4	180	3	17.5	[24]
Aspen, Pine, and Spruce	N/A	5% H_2SO_4	200–240	2–4	13–18	[13]
Cellulose	N/A	1–5% H_2SO_4	150–250	2–7	<25	[25]
		1–5% HCl			<28	
		1–5% HBr			<27	
Aspen Wood	N/A	1–5% H_2SO_4	150–250	2–7	<15.5	
		1–5% HCl			<12.4	
		1–5% HBr			<13	

3.2. *Lactic Acid*

Lactic acid (structure in Figure 3) has become increasingly popular as a biomass-derived chemical due to its utility for production of polylactic acid (PLA). Currently, Corbin (Amsterdam, The Netherlands), Futerro (Escanaffles, Belgium), NatureWorks (Minnetonka, MN, USA), and Myriant (Quincy, MA, USA) are commercial-scale users of lactic acid [7]. Some of the most common lactic acid bacteria used for lactic acid production from lignocellulosic-derived sugars include *L. planterum*, *L. pentosus*, *L. delbrueckii*, *L. casei*, *L. brevis*, *E. mundtii*, *E. faecalis*, *L. coryniformis*, *L. rhamnosus*, *L. salivarius*, *L. amylovorans*, and *L. amylophilus*, amongst others [26]. Lactic acid also serves as a critical platform chemical for production of lactide (the intermediate for polylactic acid production), propanoic acid, 1,2-propanediol, polyurethanes, pyruvic acid, acrylic acid, 2,3-pentanedione, and others. Lactic acid concentration, yield, and productivity from some of the most common lignocellulosic biomasses are shown in Table 2.

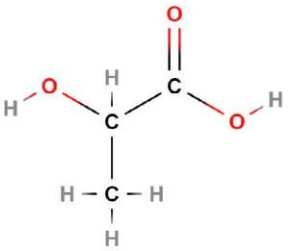

Figure 3. Structure of Lactic Acid ($C_3H_6O_3$).

Table 2. Lactic acid's concentration, yield, and productivity from some of the most common lignocellulosic biomasses.

Biomass	Strain	Concentration (g/L)	Yield [a] (g/g)	Productivity [b] (g/L/h)	Reference
Wood Hydrolysate	*E. mundtii* QU 25	93	0.93	1.7	[27]
Corn Cob/Stover	*Lb. brevis*	39.1	0.7	0.81	[28]
	L. delbrueckii ZU-S2	48.7/44.2	0.95/0.92	1.01/5.7	[29]
	L. pentosus	26	0.53	0.34	[30]
	L. pentosus ATCC 8041	74.8	0.65	N/A	[31]
	L. rhamnosus and *L. brevis*	20.95	0.7	0.58	[32]
Wheat Straw	*L. brevis* and *L. pentosus*	7.1	0.95	N/A	[33]
Softwood	*L. casei* subsp. *rhamnosus*	21.1–23.75	0.74–0.83	0.15–0.23	[34]
Sugarcane Bagasse	*L. delbrueckii* subsp. *delbrueckii* Mutant Uc-3	67	0.83	0.93	[35]
	L. lactis IO-1	10.9	0.36	0.17	[36]
Rice and Wheat Barn	*L. rhamnosus* ATCC 9595 (CET288)	129	0.95	2.9	[37]
Brewer's Spent Grain	*L. delbrueckii* UFV H2B20	35.5	0.99	0.59	[38]

[a] Ratio of the yield of lactic acid produced (g) to substrate consumed (g). [b] Lactic acid productivity.

3.3. 3-Hydroxypropionic Acid

3-hydroxypropionic acid (3-HP) is an important C3 platform chemical, primarily due to its contribution as precursor for the production of 1,3-propanediol. 3-HP is also a platform chemical used for the production of malonic acid, acrylic acid, acrylonitrile, polyamides, and 3-hydroxypropionate esters and its structure can be seen in Figure 4. BASF-Cargill-Novozymes (Ludwigshafen, Germany/Wayzata, MN, USA/Bagsværd, Denmark), and Dow (Midland, MI, USA) are commercial-scale producers of 3-HP [7].

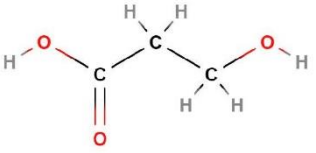

Figure 4. Structure of 3-hydroxypropionic acid ($C_3H_6O_3$).

3-HP can be produced via microbial fermentation pathways from two major substrates: glucose and glycerol [39]. Matsakas et al. [40] presented a comprehensive review on 3-HP production and its production pathway mechanisms. Table 3 provides an overview of how various microorganism compare in terms of their concentrations and productivity of 3-HP, with glycerol and glucose as the two main substrate sources.

Table 3. 3-hydroxypropionic acid concentration and productivity based on various microbes and two main substrate sources, i.e., glucose and glycerol.

Substrate	Host Microorganism	Concentration (g/L)	Productivity (g/L/h)	Reference
Glucose	*S. cerevisiae*	9.8	0.1	[41]
		13.7	0.17	[42]
		7.4	0.06	[43]
	E. coli	10.1	0.28	[44]
		40.6	0.56	[45]
		31.1	0.63	[7]
		29.7	0.54	[46]
	S. pombe	7.6	0.25	[47]
	C. glutamicum	62.6	0.87	[48]
Glycerol	*K. pneumoniae*	18	0.77	[49]
		48.9	1.75	[50]
		43	0.9	[51]
		83.8	1.16	[52]
		0.9	0.04	[53]
		24.4	1.02	[50]
		16	0.3	[54]
		11.3	0.94	[55]
		22.7	0.38	[56]
		28.1	0.58	[57]
		22	0.46	[58]
		60.5	1.12	[59]
	E. coli	42.1	1.32	[60]
		71.9	1.8	[61]
		40.5	1.35	[62]
		56.4	1.18	[63]
		41.5	0.86	[64]
		31	0.43	[65]
		38.7	0.54	[66]
		6.06	0.13	[67]
		5.05	0.105	[68]
	L. reuteri	10.6	1.08	[69]
		3.3	0.09	[70]
	L. collinoides	0.55	0.07	[71]

3.4. Succinic Acid

Succinic acid is a four-carbon dicarboxylic acid (structure in Figure 5) that has been produced via chemical routes in the past but is gaining popularity for production via the biological route. Myriant Technologies now PTT Global Chemical (Bangkok, Thailand) and Reverdia (Utrecht, the Netherlands) have commercial-scale facilities for the production of bio-based succinic acid [7].

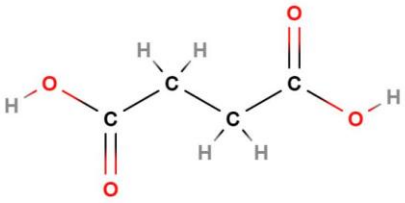

Figure 5. Structure of succinic acid ($C_4H_6O_4$).

Succinic acid is a key intermediate chemical used for the production of several derivatives of industrial importance, such as 1,4-butanediol, tetrahydrofuran, N-methylpyrrolidone, and γ-butyrolactone. These derivatives are, in turn, utilized in the production of polyurethanes, polyesters, and polyvinylpyrrolidone (PVP). Succinic acid can be extracted from various lignocellulosic biomasses. Table 4 provides an overview of succinic acid concentrations, alongside the concentrations of other value-added chemicals that can be derived simultaneously under varying pretreatment conditions, from some of the most common lignocellulosic biomasses.

3.5. Vanillic Acid and Vanillin

Vanillin is a widely used food flavoring agent that is most typically extracted from *Vanilla* spp.; however, it is currently produced inexpensively via petrochemical routes (structure of vanillic acid and vanillin in Figure 6). Besides the food industry, vanillin finds applications in the pharmaceutical and fragrance industries. The increasing demand for this molecule has propelled the search for biomass-derived pathways for vanillin production.

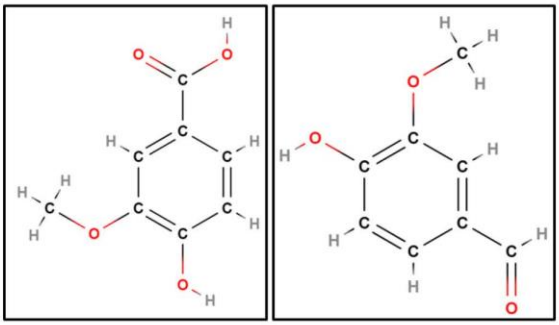

Figure 6. Structure of vanillic acid ($C_8H_8O_4$) (**left**) and vanillin ($C_8H_8O_3$) (**right**).

Vanillin can be extracted from lignin, ferulic acid, glucose, vanillic acid, aromatic amino acids, isoeugenol, waste residue, and other substrates [72]. Currently, Borregaard (Sarpsborg, Norway), a Norwegian company, claims to be the only producer of biovanillin, which it produces from wood. Table 4 summarizes some lignocellulosic biomasses that can be used to derive vanillin and vanillic acid.

3.6. Itaconic Acid

Itaconic acid is a C5 dicarboxylic acid (see Figure 7) that does not have as large a market share as the likes of succinic acid, levulinic acid, and lactic acid. However, it remains an intermediate building block of interest due to its significance in producing other value-added chemicals with larger market shares. Itaconic acid is a precursor for polymethyl methacrylate (PMMA), 3-methyltetrahydrofuran, polyitaconic acid, and styrene-butadiene rubber latex. Additionally, itaconic acid can be converted to methyl pyrrolidones, 2-methylbutanediol, 3-methyltetrahydrofuran, 4-methyl-γ-butyrolactone, and 4-methyl-γ-butyrolactone. Itaconic acid is recommended as a replacement for maleic acid/anhydride and sodium tripolyphosphate, which are, in turn, used for the production of polyester resin and detergent [5].

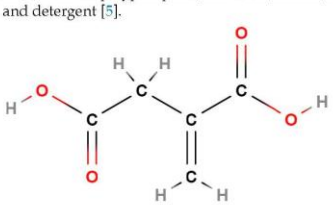

Figure 7. Structure of itaconic acid ($C_5H_6O_4$).

Qingdao Kehai Biochemistry (Jiaonan City, China), Zhejiang Guoguang Biochemistry (Quzhou City, China), Jinan Huaming Biochemistry (Mingshui Zhangqiu City, China), and Itaconix (Stratham, NH, USA) are producers of itaconic acid on a commercial scale [7]. Table 4 provides a summary of lignocellulosic biomasses and their respective pretreatment conditions for extraction of itaconic acid.

3.7. Adipic Acid

Adipic acid is a C6 dicarboxylic acid (see Figure 8) with applications in the production of nylon -6,6 fibers, resins, plasticizers, polyester polyols, food ingredients, and lubricants [5].

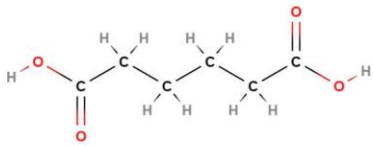

Figure 8. Structure of adipic acid ($C_6H_{10}O_4$).

Adipic acid is also a precursor for the production of muconic acid and glucaric acid. Biomass-derived adipic acid can be obtained from glucose as a starting substrate and *E. coli*, *S. cerevisiae*, and *P. putida* as the chassis [73]. Lignin, lipids, xylose, and amino acids can also serve as substrates to obtain bio-derived adipic acid. Genomatica (San Diego, CA, USA) and DSM (Heerlen, The Netherlands) are developing strategies to produce commercial-scale quantities of biomass-derived adipic acid [74].

3.8. Furfural

Furfural is a C5 molecule (as shown in Figure 9) most often derived from the hemicellulose fraction in lignocellulosic biomass. Furfural is a top value-added chemical with several commercial-scale facilities operating across China, South Africa, and the Dominican Republic [75]. The largest fractions of furfural are used in the production of furfuryl alcohol, which, in turn, is used in resin production.

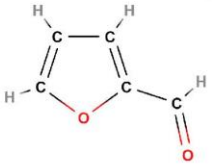

Figure 9. Structure of furfural ($C_5H_4O_2$).

Furfural is the precursor for the production of furoic acid, tetrahydrofuran, fumaric acid, 2-methyltetrahydrofuran, tetrahydrofurfuryl alcohol, and furfurylamine [7]. The highest reported furfural yields from lignocellulosic biomass vary from 34 to 87 weight% (wt%) [76–78]. Table 4 highlights the concentrations of furfural obtained after pretreatment from some of the most common lignocellulosic biomasses.

3.9. 5-Hydroxymethylfurfural (HMF)

5-hydroxymethylfurfural (HMF) is a C6 derivative obtained through dehydration of glucose and fructose, a structure of which can be seen in Figure 10 [79]. For lignocellulosic biomasses, cellulose is the main contributor to HMF production. HMF production, in principle, is possible from all biomasses containing hexoses and its oligomers, providing a wide range of possible feedstock for HMF production [75].

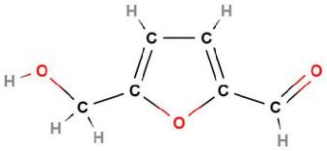

Figure 10. Structure of 5-HMF ($C_6H_6O_3$).

HMF is a precursor for the production of adipic acid, levulinic acid, 2,5-dimethylfuran, caprolactone, polyamide 6, 2,5-furandicarboxylic acid, and others. Despite the presence of hydroxyl and aldehyde functional groups in this molecule, industrial-scale production of HMF remains unfeasible, predominantly due to the high costs of fructose and low reactivity of cellulose. AVA Biochem (Muttenz, Switzerland), is one of the few companies close to commercial-scale production of HMF [7]. Table 4 shows HMF concentrations obtained for different lignocellulosic biomasses after pretreatment.

Table 4 provides an overview of succinic acid, glutaric acid, vanillin, vanillic acid, itaconic acid, adipic acid, furfural, and 5-HMF concentrations, alongside the concentrations of other value-added chemicals that can be derived simultaneously under varying pretreatment conditions from some of the most common lignocellulosic biomasses. Table 4 does not present the highest possible concentrations of each biochemical but, rather presents the biorefinery view, wherein multiple value-added products can be obtained from lignocellulosic biomass.

Table 4. Pretreatment conditions and concentrations of selected furans, organic acids, and phenolics.

Biomass	Feedstock Content	Pretreatment/ Catalyst	Operating Temp. (°C)	Time (min)	Concentrations											Unit	Ref.
					Furans			Organic Acids					Phenolics				
					Furoic Acid	Furfural	5 HMF	Lactic Acid	Succinic Acid	Glutaric Acid	Itaconic Acid	Adipic Acid	Vanillin	Vanillic Acid	Ferulic Acid		
Corn Stover	34.4% glucan, 22.8% xylan, and 11% lignin	0.7% H_2SO_4 (*w*/*w*)	180	8	2.4	220	44	20	2.9	0.57	7.2	0.11	4	3.3	6.6	g/L	[80]
		0.07% H_2SO_4 (*w*/*w*)			1.1	26	11	17.8	1.7	0.24	2	0.14	2.8	1.5	2.6	g/L	
		Liquid hot water			0.88	8	2.3	5.5	2.2	0.23	1.2	0.15	2.6	2.6	2.2	g/L	
		Deionized water saturated with oxygen at 174 psi			1.2	6.5	2.8	24	5.2	0.65	2.1	0.2	6.7	4.3	1	g/L	
		Aqueous ammonia 0.1% (*w*/*w*)			1.1	0.4	0.89	38	6.5	1.2	3.2	0.18	2.6	3.2	4.2	g/L	
	34.4% glucan, 22.4% xylan, 4.2% arabinan, 0.6% mannan, 1.4% galactan, 11% lignin, 2.3% protein, 6.1% ash, and 3.8% uronic acids	Ammonia fiber expansion (AFEX)	130	15	0.006	0.003	0.642	0.318	0.596	0.008	0.022	0.003	0.195	0.046	0.103	g/g DM	[81]
		30% H_2SO_4 (*w*/*w*)	190		0.155	7.94	15.7	1.5	0.26	0.012	0.58	0.005	0.281	0.124	1.314	g/g DM	
	N/A	1% H_2SO_4 (*w*/*w*)	160	8	N/A	18.7	0.701	41	N/A	N/A	N/A	N/A	0.06	0.034	N/A	mM	[82]
Poplar	43.8% glucan, 14.85% xylan, 3.94% mannan, and 29.12% lignin	0.7% H_2SO_4 (*w*/*w*)	180	8	3.1	220	64	29	2.5	0.61	0.11	0.057	5.5	5.9	0.19	g/L	[80]
		0.07% H_2SO_4 (*w*/*w*)			1.7	31	4	19	0.93	0.26	0.13	0.1	5.6	5.7	0.46	g/L	
		Liquid hot water			0.94	2.6	0.45	1.8	2.3	0.23	0.093	0.048	3.1	4.1	0.23	g/L	
		Deionized water saturated with oxygen at 12 bar			0.76	2.1	0.39	22	2.4	0.25	0.17	0.14	9.1	5.3	0.07	g/L	
		Aqueous ammonia 0.1% (*w*/*w*)			0.49	0.5	0.079	26	1.7	0.35	0.088	0.13	2.8	2.5	0.13	g/L	
	48.9% glucan, 15.7% xylan, 27.7% lignin, and 1.2% ash	Steam explosion	214	6	N/A	5.9	2.6	N/A	N/A	N/A	N/A	N/A	0.035	N/A	N/A	mg/g DM	[83]

Table 4. *Cont.*

Biomass	Feedstock Content	Pretreatment/ Catalyst	Operating Temp. (°C)	Time (min)	Concentrations											Unit	Ref.
					Furans			Organic Acids					Phenolics				
					Furoic Acid	Furfural	5 HMF	Lactic Acid	Succinic Acid	Glutaric Acid	Itaconic Acid	Adipic Acid	Vanillin	Vanillic Acid	Ferulic Acid		
Pine	40% glucan, 8.9% xylan, 16% mannan, and 27.7% lignin	0.7% H_2SO_4 (*w*/*w*)	180	8	1.1	190	170	3.7	0.73	0.37	0.07	0.076	4.6	5.2	0.12	g/L	[80]
		0.07% H_2SO_4 (*w*/*w*)			0.8	13	9.5	4.5	0.34	0.18	0.032	0.09	5.8	3.6	0.22	g/L	
		Liquid hot water			0.83	2.5	1.3	8.7	0.75	0.16	0.09	0.054	2.4	2.3	0.31	g/L	
		Deionized water saturated with oxygen at 12 bar			0.91	1.9	0.61	18	1.8	0.31	0.24	0.18	7.1	4.8	0.14	g/L	
		Aqueous ammonia 0.1% (*w*/*w*)			0.55	0.65	0.16	36	2.39	0.66	0.099	0.13	3.2	4.8	0.16	g/L	
Spruce	41.6% glucan, 11.5% mannan, 4.7% xylan, 2% galactan, 1.1% arabinan, 25.7% lignin, and 5.4% extractives	0.5% H_2SO_4 (*w*/*w*)	222	7	N/A	1	5.9	N/A	N/A	N/A	N/A	N/A	0.12	0.034	N/A	g/L	[84]
Wheat Straw	36.3% cellulose, 20.9% hemicellulose, and 7.1% lignin	6.5 g/L Na_2CO_3	185	10	N/A	N/A	N/A	0.461	0.899	N/A	N/A	N/A	0.008	0.004	0.009	g/100 g DM	[85]
		2 g/L Na_2CO_3	195	15	0.017	0.116	0.016	N/A	0.447	N/A	N/A	N/A	0.096	0.084	0.015		
Barley Straw	33% glucan, 20% xylan, 3.8% arabinan, 1% galactan, 16.1% lignin, 7.6% ash, and 13.8% extractives	N/A	210	5	N/A	0.28	0.08	N/A	N/A	N/A	N/A	N/A	25	4.4	10	mg/100 g DM	[86]

DM: dry matter. Adapted from [87].

3.10. 2,5-Furandicarboxylic Acid

2,5-Furandicarboxylic acid (FDCA) is considered a promising alternative for petroleum-derived terephthalic acid for the production of bioplastics, such as polyamides, polyesters, and polyethylene furandicarboxylate. FDCA can be synthesized from 5-HMF or 2-furoic acid derived from lignocellulose-based C6 and C5 sugars, respectively. The structure of FDCA can be seen in Figure 11. Besides the required pretreatment for lignocellulose saccharification, the low efficiency of the dehydration process from hexoses to 5-HMF has been an obstacle for commercial production [88,89]. For example, reported yields from the conversion of fructose to HMF vary between 26 and 92% [90].

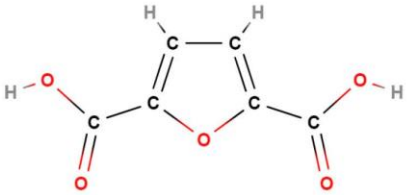

Figure 11. Structure of FDCA ($C_6H_4O_5$).

No commercial process has been established for FDCA production from lignocellulosic biomass, and the production of sugar-derived FDCA has only been tested at the pilot scale [91]. Some studies have presented direct conversion (so-called one-pot, two-step method) for 5-HMF synthetized from sugars and oxidation to FDCA [92–95]. Another challenge has been the need for a cost-efficient catalyst for the oxidation of furans, as utilization of noble-metal-assisted catalysts without improved recycling can be costly [89,91]. Zhou et al. [89] developed a partly lignin-derived catalyst, which showed high selectivity, as well as promising substrate conversion and FDCA yields. Some research has been conducted with consideration of enzymatic oxidation, which is suggested as a renewable alternative for catalytic conversion, although the area should be further explored [91,96]. Table 5 provides a summary of the most common oxidation processes used to derive 5-HMF and 2-furoic acid.

Table 5. Review of oxidation processes from sugar-derived 5-hydroxymethylfurfural (5-HMF) and 2-furoic acid to 2,5-furandicarboxylic acid (FDCA).

Substrate	Catalyst	Reagents	Temp. (°C)	Time (h)	Pressure (Bar)	Substr. Conv. (%)	FDCA Yield (%)	Ref.
5-HMF	Lignin-derived Co SAs/N@C	Na_2CO_3, O_2	85	3	1	99.4	74.4	[89]
			85	8	1	100	99.5	
	MnO_2	$NaHCO_3$	100	24	10	>99	91	[97]
	Au-TiO_2	N/A	65	8	10	N/A	>99	[98]
	5% Pt/C	O_2	100	20	40	N/A	94	[92]
	Magnetic $ZnFe_{1.65}Ru_{0.35}O$	Dimethyl sulfoxide	130	16	N/A	N/A	91.2	[94]
	Ru/HAP	N/A	160	4	20	N/A	34.2	[94]
	Pd/CC	K_2CO_3, O_2	140	30	N/A	N/A	85	[95]
	Ru (4%)/$MnCo_2O_4$	N/A	120	10	24	100	99.1	[99]
	Fungal enzymes: aryl alcohol oxidase, peroxygenase, galactose oxidase	H_2O_2, phosphate buffer	N/A	>24	N/A	N/A	80	[96]
2-furoic acid	N/A	Cs_2CO_3, CO_2	200	5	8	N/A	77	[88]
	Lignin-derived Co SAs/N@C	Cs_2CO_3, CO_2	260	36	Flowing	85.8	71.1	[89]

3.11. Xylitol

Xylitol is featured as a top value-added product from biorefineries in both reports published by the USDOE and is one of the most studied molecules due to its applications in the pharmaceutical, cosmetic, and food industries [100]. The Asia Pacific region (China

in particular) represents a disproportionally large share of xylitol as compared to the rest of the world, with chewing gum being the major market, representing 80–90% of the total demand in Asia [100,101]. Xylitol demand grew from 6000 tons in 1978 to 190 thousand metric tons in 2016, which was valued at USD 725.9 million [102].

Xylitol (structure in Figure 12) can be derived through the hydrogenation of xylose-by-xylose reductase; however, microbial production of xylitol using yeast fungi and bacteria has proven to be the more promising route for production. *Candia* spp. is the most studied fungus with respect to xylitol production [103]. Xylitol serves as a precursor for the production xylaric acid, ethylene glycol, and propylene glycol. Table 6 summarizes reported xylitol production from lignocellulosic biomass. Whereas the pretreatment conditions are quite diverse, the hydrolysate concentration is a good indicator of the relative effectiveness of pretreatment.

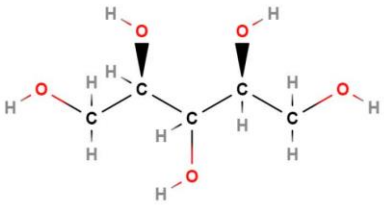

Figure 12. Structure of xylitol ($C_5H_{12}O_5$).

3.12. Chitosan

Chitosan (deacetylated chitin) is biopolymer used in food, pharmaceutical, and cosmetics industries due to its non-toxicity, biocompatibility, biodegradability, and antimicrobial properties [104,105]. It mainly consists of amino sugar D-glucosamine bounded to small amounts of N-acetyl-D-glucosamine [105]. Satari et al. [106] approximated the amount of chitosan in a biomass sample by measuring the amount of D-glucosamine. A representative structure of chitosan can be seen in Figure 13.

Traditionally, chitosan is produced from waste exoskeletons of shellfish, but as a high concentration (45–60%) NaOH is needed to extract the chitosan from chitin, fungus-derived chitosan is a suitable alternative, as the more diluted alkaline required for the extraction decreases the environmental pollution caused by the process [105]. Sigma-Aldrich (St. Louis, MO, USA) and ChitoLytic (Toronto, ON, Canada) are companies producing non-animal-derived chitosan at the commercial level. Chitosan can be found in the cell wall of *Zygomycetes* fungi, and strains from *Rhizopus* and *Mucor* genera have been tested for fermentation of lignocellulose prehydrolysates [104–107]. Xylose, which is digested by microorganisms, is released from lignocellulose by treating the biomass using hydrothermal or acid-assisted pretreatment at moderate temperatures, which can be followed by enzymatic hydrolysis [108]. Despite being inhibitors, some sugar degradation compounds, such as formic acid and acetic acid, in moderate concentrations have been shown to stimulate the fungal growth and increase the accumulation of protective chitosan in the fungal cell wall [104]. However, severe pretreatment conditions must be avoided to prevent the excessive production of inhibitory compounds for fungal growth, such as furfural. Chitosan can be extracted from fungal biomass by first separating the alkaline, insoluble material, which is subsequently extracted using diluted acetic acid or acetate [104–107]. Tai et al. [104] demonstrated fermentation in hemicellulose-based hydrolysate to enhance the fungal growth and chitosan production compared to fermentation in synthetic glucose and xylose-containing medium. Table 7 provides a summary of fungal chitosan production from lignocellulosic biomass.

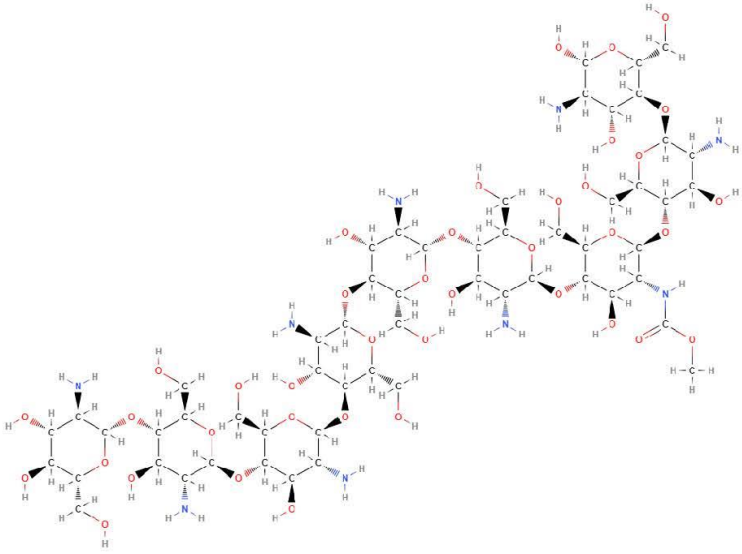

Figure 13. Representative structure of chitosan.

3.13. 2,3 Butanediol (2,3-BD)

Long-chain alcohol 2,3-butanediol (2,3-BD), structure seen in Figure 14, is an important platform bulk chemical that is used as a fuel additive and in various other industries, including chemicals, plastic manufacturing, pharmaceuticals, cosmetics, and even food [109–111]. Currently, it is mainly sourced from the petrochemical industry, but the increasing interest in biorefining and sustainable bio-based chemicals has made microbial production of 2,3-BD a desirable alternative [112–114].

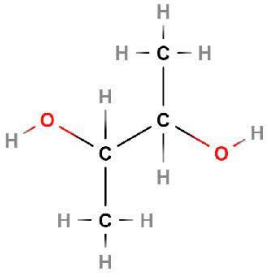

Figure 14. Structure of 2,3-BD ($C_4H_{10}O_2$).

2,3-BD can be produced by fermentation of lignocellulose-derived sugars using bacterial strains that can simultaneously utilize glucose and xylose. Many industrial strains can biologically produce 2,3-BD and have been shown to be suitable for fermentation. However, to enhance the production and ensure sufficient yields, metabolic engineering of the strains has been studied [109,110,112,114,115]. The pathogenic nature of most robust strains in *Klebsiella* and *Enterobacter* genera remains an obstacle to commercialization of microbial 2,3-BD production. On the other hand, generally recognized as safe (GRAS) organisms, such strains in the *Bacillus* genus, have shown lower fermentation efficiency [109,115]. Lignocellulose is pretreated and hydrolyzed before fermentation, usually by sodium hydroxide or sulfuric acid treatment followed by enzymatic hydrolysis. Joo et al. [116] studied the effect of inhibitory sugar degradation compounds and reported that formic acid, furans, and phenolic compounds have negative effects on cell growth and 2,3-BD production, which enhances the importance of appropriate pretreatment conditions. Table 8 provides a summary of fermentation techniques used to derive 2,3-BD from lignocellulosic biomass.

3.14. Xylo-Oligosaccharides (XOs)

Xylo-oligosaccharides (XOs) are non-digestible carbohydrates with prebiotic and other beneficial health properties that have gained commercial interest due to their potential for use as nutraceuticals; they can be produced by hydrolyzing xylan, the main component of hemicellulose. [117,118]. This provides a route to produce high-value compounds from abundant and inexpensive lignocellulosic feedstock, such as agricultural residues [119,120]. A representative structure of XOs can be seen in Figure 15.

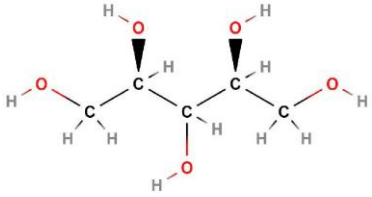

Figure 15. A representative structure of XOs $(C_5H_{12}O_5)_n$.

Several methods have been tested for XO production from lignocellulose, the most common of which is hydrothermal or alkaline pretreatment followed by enzymatic or acid hydrolysis. Hydrothermal pretreatment [120], acid hydrolysis [121], and enzymatic hydrolysis [122] without pretreatment have also been tested. However, the high temperatures required for hydrothermal pretreatment and autohydrolysis increase the energy consumption of the process. Therefore, it is suggested that alkaline pretreatment at moderate temperatures, together with enzymatic hydrolysis, could be the most sustainable processing route [118]. Downstream processing is required to purify XO-containing liquors (75–90% purity required for food applications) from unwanted toxic and unhealthy compounds, such as furfural and 5-HMF, and this process directly impacts the production costs [118]. Production of XOs from agricultural residues has been extensively studied in recent years, and in the reviewed studies, the reported yields, depending on the xylan extraction and hydrolysis method, vary between 10.2% from wheat straw [119] up to >99% from sugarcane bagasse [123]. One of the most promising feedstocks is corn cob, which has shown high yields in various studies [124–127]. Table 9 summarizes XO production from lignocellulose.

Table 6. Review of extraction methods for xylitol from lignocellulosic biomass.

Biomass	Feedstock Content	Acid Conc.	Operating Temp. (°C)	Time (min)	Hydrolysate	Strain	Xylitol Reported	Reference
Corncob	20.91 g/L of Xylose	1% H_2SO_4 (*v*/*v*)	121	30	40.16 g/L and 52.71 g/L of xylose (hydrolysate concentrated through rotavapor and microwave, respectively)	*Candida tropicalis*	1.2-fold increase in yield and 1.8-fold increase in productivity	[128]
	31.2 g/L of xylose and 3.3 g/L of glucose	1% H_2SO_4 (*v*/*v*)	121	40	160 g/L of xylose	*Candida tropicalis* As 2.1776	Yield of 0.83 g/g and productivity of 1.01 g/L/h. Maximum xylitol production of 96.5 g/L	[129]
	41.2% cellulose, 33.4% hemicellulose, and 18.7% lignin	1% H_2SO_4 (*v*/*v*)	125	60	21.67 g/L of xylose	*Candida tropicalis* CCTCC M2012462	Maximal xylitol concentration of 38.8 g/L. Yield of 0.7 g/g of xylose and a productivity of 0.46 g/L/h	[130]
	42.7% cellulose, 34.3% hemicellulose, and 17.5% lignin	1% H_2SO_4 (*v*/*v*)	120	60	28.7 g/L of xylose	*Candida tropicalis* W103	Maximal xylitol concentration of 68.4 g/L. Yield of 0.7 g/g xylose and a productivity of 0.95 g/L/h	[131]
	32% cellulose, 35% hemicellulose, 20% lignin, and 4% ash and others	1% H_2SO_4 (*v*/*v*)	121	60	24.9 g/L of Xylose	*Candida magnoliae*	Production rate of 0.51 g/L/h and 18.7 g xylitol/L	[132]
Sugarcane bagasse	-	-	-	-	65% xylose, 15% arabinose, and 8% glucose	*Debaryomyces hansenii*	Maximum yield of 0.76 and 0.82 g/g using free and immobilized cells, respectively, with corresponding volumetric productivities of 0.44 and 0.46 g/L/h at 100 g/L initial xylose concentration	[133]
	17.5% DM loading	1% H_2SO_4	150	30	15.73 g/L of D-xylose	*Candida guilliermondii* FTI 20037	Maximal xylitol production of 50.5 g/L. Yield of 0.81 g/g of xylose and productivity of 0.6 g/L/h	[134]

Table 6. *Cont.*

Biomass	Feedstock Content	Acid Conc.	Operating Temp. (°C)	Time (min)	Hydrolysate	Strain	Xylitol Reported	Reference
Sugarcane bagasse	9.3 xylose, 15.2 glucose, and 8.5 lignin (% of DM)	100 mg of sulfuric acid per g of bagasse (dry wt)	121	10	Hydrolysate 64.7 g/L xylose, 3.08 g/L glucose, 4.23 g/L arabinose, and 1.84 g/ L acetic acid	*Candida guilliermondii*	Maximum xylitol concentration of 28.7 g/L, xylitol yield on consumed xylose of 0.49 g/g, and a xylitol volumetric productivity of 0.24 g/L/h	[135]
	10% DM loading	1% H_2SO_4 (v/v)	121	60	Sugar composition in hydrolysate xylose 56%, glucose 15%, and arabinose 24%	*Candida tropicalis*	Xylitol yield was 0.65 g/g of xylose	[136]
Corn Fiber	20% DM loading	1% H_2SO_4 (v/v)	121	60	Sugar composition in hydrolysate xylose 30%, glucose 38%, arabinose 22%, and galactose 4%.	*Candida tropicalis*	Xylitol yield was 0.65 g/g of xylose	

DM: dry matter.

Table 7. Review of production methods for fungal chitosan from lignocellulose.

Biomass	Treatment Conditions	Xylose (g/L)	Fungal Strain	Chitosan Extraction	Biomass Production (g/L/day)	Chitosan Content (g/g Biomass)	Comment	Ref.
Corn Stover	2% H_2SO_4 100 °C 2 h	22.4	*Rhizopus oryzae* ME-F12	1 M NaOH at 121 °C for 15 min + 2% acetic acid at 95 °C for 24 h	5.2	0.09	Total production	[104]
	Autoclave at 121 °C for 20 min	N/A	*Aspergillus niger*	1 M NaOH at 121 °C for 20 min + 2% acetic acid at 95 °C for 6–8 h	15.8	6.8	(g/kg) Solid-state fermentation	[107]
	Autoclave at 21 °C 20 for min	N/A	*Rhizopus oryzae*	1 M NaOH at 121 °C for 20 min + 2% acetic acid at 95 °C for 6-8 h	14.6	8.6		
	Acid-assisted steam explosion, 0.8 MPa	30	*Rhizopus oryzae* AS 3.819	1 M NaOH at 121 °C for 15 min + 2% acetate at 95 °C for 24 h	3.7	0.09	N/A	[105]
Elm Wood	85% H_3PO_4 at 60 ° for C 45 min + enzymatic	ND	*Mucor indicus* CCUG 22424	0.5 M NaOH at 121 °C for 20 min (alkali-insoluble material)	3.3	0.06	Determined as the amount of glucosamine	[106]
Pine Wood	85% H_3PO_4 at 60 °C for 45 min + enzymatic	6.9	*Mucor indicus* CCUG 22424	0.5 M NaOH at 121 °C for 20 min (alkali-insoluble material)	2.8	0.06		
Rice Straw	85% H_3PO_4 at 60 °C for 45 min + enzymatic	ND	*Mucor indicus* CCUG 22424	0.5 M NaOH at 121 °C for 20 min (alkali-insoluble material)	3.1	0.06		
Wheat Straw	NMMO * 120 °C 3 h + enzymatic	19.8	*Mucor indicus* CCUG 22424	Autolysis + NaOH treatment + extraction	N/A	0.13	N-methylmorpholine -N-oxide	[137]

* N-methylmorpholine-N-oxide.

Table 8. Review of fermentation methods for 2,3-butanediol (2,3-BD) from lignocellulose.

Biomass	Pretreatment	Hydrolysis	Strain	Productivity (g/L/h)	2,3-BD Conc. (g/L)	Yield (%)	Ref.
Corn cob	2% NaOH at 80 °C for 2 h	Enzymatic	*Enterobacter cloacae* CICC 10011	0.9	N/A	42	[138]
Corn stover	0.1 M NaOH at 80 °C	Enzymatic	*Zymomonas mobilis*	N/A	10	N/A	[114]
	N/A	Enzymatic	*Paenibacillus polymyxa*	1.1	18.8	31	[139]
	N/A	N/A	*Bacillus licheniformis*	2.3	119.4	95	[112]
Jerusalem Artichoke Stalk	1% H_2SO_4 at 130 °C for 90 min	Enzymatic	*Klebsiella pneumoniae*	N/A	80.5	16.8	[111]
Oil Plan Frond	3% NaOH at 121 °C for 20 min	Enzymatic	*Enterobacter cloacae* SG1	0.3	30.7	N/A	[113]
Pine Tree	N/A	N/A	*Klebsiella oxytoca* CHA006	0.7	5.8	30	[110]
Rice Straw	0.375 M NaOH at 120 °C for 20 min	Enzymatic	*Klebsiella* sp. Zmd30	2.4	N/A	62	[140]
Rice Waste	Na_2CO_3 + $NaHCO_3$ + Na_2SO_4 at 100 °C for 3 h	Enzymatic	*Klebsiella pneumoniae* KMK-05	0.48	11.5	38.4	[141]
Sorghum Stalk	1.25% NaOH at 121 °C for 30 min	Enzymatic	*Bacillus licheniformis* DSM 8785	1	N/A	45	[109]
Sugarcane Bagasse	0.375 M NaOH at 120 °C for 20 min	Enzymatic	*Klebsiella* sp. Zmd30	0.7	N/A	15	[140]
	10% NaOH at 90 °C for 90 min	Enzymatic	*Klebsiella pneumoniae* CGMCC 1.9131	N/A	9	N/A	[142]
	1% H_2SO_4 at 121 °C for 30 min	N/A	*Enterobacter aerogenes* EMY-22	0.8	66.4	42	[110]
	5% Na_2CO_3 + 5% Na_2SO_3 at 100 °C for 4 h	N/A	*Enterobacter aerogenes* EMY-22	N/A	N/A	39.5	[143]
Sunflower Stalk	N/A	N/A	*Klebsiella oxytoca* CHA006	0.8	4.3	34	[110]
Wood	24 N H_2SO_4 at 30 °C for 60 min + diluted acid at 105 °C for 60 min	N/A	*Enterobacter aerogenes*	N/A	9.9	N/A	[116]
	N/A	N/A	*Bacillus licheniformis* DSM 8785	1.6	N/A	40	[109]

Table 9. Review of xylo-oligosaccharide (XO) production from lignocellulose.

Biomass	Hemicellulose (%)	Xylan (%)	Extraction/Pretreatment	Hydrolysis	XO Conc. (g/L)	Yield	Unit	Ref.
Barley Husk	N/A	26.8	N/A	Autohydrolysis at 220 °C for 0.75 h	N/A	27.1	%	[120]
Corn Cob	N/A	30.6	N/A	Non-isotherm autohydrolysis at 202 °C	N/A	78.7	gXOs/100 g xylan	[126]
	38.8	N/A	12% NaOH – steam at 121 °C for 45 min	0.25 M H_2SO_4 at 90 °C for 60 h	0.9	N/A	N/A	[144]
	38.9	N/A	4–16% NaOH + steam at 121 °C for 45 min	Enzymatic at 40.9–41.4 °C for 16.6–17.3 h	<2.0	N/A	N/A	[145]
	N/A	N/A	1.25 M NaOH at 37 °C for 180 min	Enzymatic at 45 °C for 8 h	6.7	60	%	[124]
	N/A	31.3	N/A	Autohydrolysis at 220 °C for 0.75 h	N/A	24.8	%	[120]
	N/A	31.9	2% NaOH at 20 °C for 6 h	Enzymatic at 50 °C for 24–36 h	8.2	86.7	%	[125]
	N/A	34.8	1.0 g/L H_2SO_4 + steam at 135 °C for 30 min	Enzymatic at 50 °C for 24 h	N/A	67.7	gXOs/100 g xylan	[127]
Eucalyptus Wood	N/A	16.6	N/A	Autohydrolysis at 220 °C for 0.75 h	N/A	15.4	%	[120]
Maize Silage	35.1	N/A	1 M NaOH – steam at 121 °C for 15 min	Enzymatic at 50 °C for 24 h	3.5	N/A	N/A	[117]
Oil Palm Frond	30.4	N/A	Steam at 121 °C for 60 min	Enzymatic at 40 °C for 24 h	N/A	17.5	w/w%	[146]
Reed	N/A	21.2	Steam at 170 °C for 30 min	Enzymatic at 50 °C for 48 h	N/A	68.1	gXOs/100 g xylan	[147]
Rice Husk	N/A	15.6	N/A	Autohydrolysis at 220 °C for 0.75 h	N/A	18	%	[120]
	25	N/A	18% NaOH – steam at 120 °C for 45 min	Enzymatic at 50 °C for 9 h	N/A	34.7	gXOs/100 g xylan	[148]
	11.2	N/A	N/A	Enzymatic at 50 °C for 24 h	N/A	69	gXOs/100 g xylan	[122]
Ryegrass Silage	36.6	N/A	1 M NaOH – steam at 121 °C for 15 min	Enzymatic at 50 °C for 24 h	2.4	N/A	N/A	[117]

Table 9. *Cont.*

Biomass	Hemicellulose (%)	Xylan (%)	Extraction/Pretreatment	Hydrolysis	XO Conc. (g/L)	Yield	Unit	Ref.
Sugar Cane Bagasse	N/A	20.6	Aqueous ammonia + steam at 121 °C for 30 min	Enzymatic at 50 °C for 30 h	N/A	>99	%	[123]
	N/A	N/A	N/A	0.1% H_2SO_4 at 140 °C for 1 h	N/A	92.28	gXOs/100 g xylan	[121]
	N/A	N/A	6% Alkaline peroxide at 20 °C for 180 min	Enzymatic at 50 °C for 96 h	N/A	31.5	%	[149]
	N/A	N/A	10% Acetic acid + steam at 150 °C for 45 min	Enzymatic at 30 °C for 1.25 h	N/A	39.1	gXOs/100 g xylan	[150]
	23.2	N/A	12% NaOH – steam at 121 °C for 15 min	Enzymatic at 40 °C for 8 h	1.72	N/A	N/A	[151]
Sunflower Stalk	N/A	18.9	24% KOH at 35 °C for 120 min	Enzymatic at 40 °C for 24 h	3.2	N/A	N/A	[152]
	N/A	19.1	24% KOH at 35 °C for 120 min	0.25 M H_2SO_4 at 100 °C for 30 h	N/A	12.6	gXOs/100 g xylan	[118]
Wheat Straw	N/A	20.6	24% KOH at 35 °C for 120 min	Enzymatic 4 at 0 °C for 24 h	2.3	N/A	N/A	[152]
	N/A	20.9	24% KOH at 35 °C for 120 min	0.25 M H_2SO_4 at 100 °C for 30 h	N/A	10.2	gXOs/100 g xylan	[119]
	N/A	N/A	2% NaOH at 80 °C for 90 min	Enzymatic at 60 °C for 15 h	N/A	39.8	gXOs/100 g xylan	[153]

4. Extraction Methods

Physical pretreatment is one of the first steps to open the molecular structure of lignocellulosic biomass. Several physical, chemical, and physiochemical methods have been studied and optimized to provide access to cellulose and hemicellulose by opening up the binding lignin structure [154,155]. Whereas the primary goal of pretreatment is to provide accessibility to cellulose and hemicellulose sugars, with time and increased interest in biomass-derived production of value-added chemicals, molecules that were once considered pretreatment inhibitors, such as HMF and furfural, are now the primary compounds of interest. This newfound interest prioritizes the secondary goal, which is opening the lignin structure. Whereas accessibility to polysaccharides still holds relevance, if high-value compounds can be extracted via low-cost extraction methods, then this step precedes the step of extraction of polysaccharides into monomeric sugars. As a result of this shift, new extractions methods are continually being investigated with respect to their efficacy in obtaining high-value biochemicals. Conventional pretreatment operating conditions, as described in Tables 1–9 are often energy-intensive, requiring reactor temperatures above 150 °C and reactor pressure between 1 and 20 atm and are frequently performed in the presence of a catalyst. Novel extraction methods include less energy-intensive alternatives that allow for recovery of high-value chemicals. The attractive prices and wide-ranging applications of specialty chemicals derived from biomass justify the development of new extraction methods. In the following sections, extraction methods for targeted high-value compounds from lignocellulosic biomass are reviewed. The isolation and encapsulation techniques used to stabilize high-value compounds are also discussed and reviewed.

For efficient extraction of high-value compounds from lignocellulosic biomass, physiochemical pretreatment is followed by extraction methods. The extraction method is governed by the characteristics of the targeted compounds identified for extraction. Some compounds are thermolabile and therefore prone to thermal degradation. Therefore, prolonged extractions using high temperatures should be chosen with caution [156,157]. For a solvent to dissolve the solute, the diffusion, solubilization, and/or transfer are governed by the thermodynamic properties of the solute and the solvent. Solvents are widely used for extraction of targeted molecules, partly due to the simplicity of the method, its scalability, and low cost [158]. Solvents used to extract high-value compounds discussed in this review include water, ethanol (EtOH), methanol (MeOH), ethyl acetate (EtOAc), dichloromethane (DCM), acetone, dimethyl sulfoxide (DMSO), deep eutectic and ionic solvents (DESs), acids and bases, and supercritical CO_2 [159–166].

Phenolics are secondary metabolites with a chemical structure comprising one or more aromatic rings attached to one or more hydroxyl groups. Phenolics can be derived from food plants and lignocellulosic plants. With more than known 8000 phenolics, there is certainly a growing interest in the ability to extract these compounds from a variety of biomass sources [158]. In an in silico study, Galanakis et al. [167] investigated the ability of solvents to solubilize phenolics, e.g., hydroxycinnamic acids (HCA), flavonoids, phenolic aldehydes, or hydroxybenzoic acids, all of which are molecules with at least one hydroxyl group (polar group) connected to an aromatic ring (non-polar group). The activity coefficient and polarity, which can be predicted by computer models, were the main parameters used to determine the solubility of a solute in a solvent. The authors stated that different solvents target different phenolic groups [167]. Of the 15 investigated phenolic groups, the solvents with the best properties for extracting phenolics were EtOH, MeOH, EtOAc, and DCM. However, these solvents do not consider the liberation of phenolics from the lignocellulosic matrix.

4.1. Hansen Solubility Parameters

Hansen solubility parameters (HSPs) are associated with the method of theoretically calculating a solute's solubility in a solvent based on the thermodynamical properties of dispersion, polarity, and hydrogen bonding. Compounds with similar HSP values have high miscibility, solubility, diffusivity, and affinity for each other. Such compounds have

similar HSP values because they have comparable atomic dispersion forces, molecular dipole forces, and electron exchanges, denoted by δ_D, δ_P, and δ_H, respectively.

A compound is defined as soluble in a solvent if the solvent lies inside in three-dimensional solubility parameter spheroid in the Hansen space [168,169]. Hansen emphasizes the impracticality of using water to predict solubility behaviors with HPS. Water is a small molecule with strong polar interactions, as well as strong hydrogen-bonding and hydrogen-donor capabilities. Therefore, the HPS values depend on the local environment, and Hansen does not recommend water predictions of solubility with the HPS method [168,169]. Ionic compounds are not well-described by the HSP method. Tables 10 ad 11 consolidate HSP for common solvents and solutes.

Table 10. HSP values of solvents as described by Hansen [169].

Compound	δ_D	δ_P	δ_H	Compound	δ_D	δ_P	δ_H
1,4-Dioxane	19	1.8	7.4	Iso-butanol	15.1	5.7	15.9
1-Butanol	16	5.7	15.8	Methanol	14.7	12.3	22.3
1-Propanol	16	6.8	17.4	Methyl cyclohexane	16	0	1
2-Butanol	15.8	5.7	14.5	Methyl ethyl ketone	16	9	5.1
2-Propanol	15.8	6.1	16.4	Methyl isobutyl ketone	15.3	6.1	4.1
Acetone	15.5	10.4	7	Methylene dichloride	18.2	6.3	6.1
Acetonitrile	15.3	18	6.1	N,N-dimethyl acetamide	16.8	11.5	10.2
Benzene	18.4	0	2	N,N-dimethyl formamide	17.4	13.7	11.3
Benzyl Alcohol	18.4	6.3	13.7	n-Butyl acetate	15.8	3.7	6.3
Carbon tetrachloride	17.8	0	0.6	n-Heptane	15.3	0	0
Chlorobenzene	19	4.3	2	n-Hexane	14.9	0	0
Chloroform	17.8	3.1	5.7	n-Nonane	15.7	0	0
Cyclohexane	16.8	0	0.2	n-Octane	15.5	0	0
Cyclohexanone	17.8	6.3	5.1	n-Pentane	15.6	0	0
Decalin (cis)	18	0	0	sec-Butyl acetate	15	3.7	7.6
Dichloromethane	8.9	3.1	3	Styrene	18.6	1	4.1
Diethyl Ether	14.5	2.9	4.6	Tetralin	19.6	2	2.9
Dimethyl Phthalate	18.6	10.8	4.9	Tetramethylene sulfoxide	18.2	11	9.1
Dimethyl Sulfoxide	18.4	16.4	10.2	Toluene	18	1.4	2
Ethanol	15.8	8.8	19.4	Water	18.1	17.1	16.9
Ethyl Acetate	15.8	5.3	7.2	Xylene	17.6	1	3.1
Ethyl Benzene	17.8	0.6	1.4	γ-Butyrolactone	19	16.6	7.4
Ethylene Carbonate	19.4	21.7	5.1				

Units are in $MPa^{1/2}$.

Table 11. HSP values of solutes [169–171].

Solute	δ_D	δ_P	δ_H
Lactic acid	17.0	8.3	28.4
Adipic acid	17.1	9.0	14.6
Vanillin *	19.4	9.8	11.2
Furfural	18.6	14.9	5.1
Ferulic acid *	19.0	6.6	15.1
4-Hydroxy cinnamic acid *	19.1	6.7	15.9
Chitosan	23.0	17.3	25.7
Xylo-oligosaccharides	25.4	7.4	15.5

Units are in $MPa^{1/2}$. * Phenolic compound.

By applying the solvent HSP values to the solute HSP values using Equation (1), the distance in Hansen space (R_{AB}) can be calculated to determine the theoretically best solvents for individual solutes of interest, as shown in Table 12, where the solvents with the lowest distance to the solute in the Hansen space as defined by R_1, the second-lowest is defined by R_2, etc.

$$R_{AB} = \sqrt{4 \cdot \Delta\delta_D^2 + \Delta\delta_P^2 + \Delta\delta_H^2} \quad (1)$$

Table 12. Chosen solutes of interest and the solvents closest to them in Hansen space.

Solute	R_1	R_2	R_3	R_4
Lactic acid	MeOH	EtOH	1-Propanol	2-Propanol
Succinic acid Adipic acid	EtOAc	n-Butyl acetate	Sec-Butyl acetate	Chloroform
Vanillin *	Tetramethylene sulfoxide	Benzyl alcohol	N,N-dimethyl acetamide	N,N-dimethyl formamide
Furfural	γ-Butyrolactone	Dimethyl phthalate	Dimethyl sulfoxide	Tetramethylene sulfoxide
Ferulic acid *	Benzyl alcohol	1-Butanol	1-Propanol	2-Butanol
4-Hydroxy cinnamic acid *	Benzyl alcohol	1-Butanol	1-Propanol	2-Propanol
Chitosan	Water	MeOH	EtOH	Dimethyl sulfoxide
Xylo-oligosaccharides	Benzyl alcohol	1,4-Dioxane	Tetramethylene sulfoxide	Dichloromethane

R_1 denotes the shortest distance between solute and solvent in Hansen space, R_2 denotes the second shortest distance, etc. * Phenolic compound.

As shown in Table 12, the best solvent for chitosan is water, according to calculations based on HSP values. However, this does not mean that chitosan is soluble in water, as chitosan is a polymer, and a second chemical is therefore needed to change the ionic charge of the solute and solvent, e.g., by the addition of a weak acid [172].

If a solvent with good HSP values compared to the compound of interest is unfit for handling due to safety concerns, high cost, or due to processing inability or environmental restrictions, other miscible solvents with desirable characteristics can be chosen and mixed in ratios that will result in similar HSP values. If mixed on the basis of a volume-weighted average, a new R_{AB} can be calculated such that the mixture of solvents might be low-cost, safer, or more environmentally accepted, with a possibly lower R_{AB} distance in Hansen-space. By applying Equations (2)–(4), new HSP values can be calculated for a mix of solvents. This technique can also shift the solubility of a solute in the mix of solvents to induce crystallization of the solute without extensive and expensive downstream processing.

$$\delta_D = \frac{\delta_{D1} \cdot V_1 + \delta_{D2} \cdot V_2 + \delta_{Dn} \cdot V_n}{V_{tot}} \quad (2)$$

$$\delta_P = \frac{\delta_{P1} \cdot V_1 + \delta_{P2} \cdot V_2 + \delta_{Pn} \cdot V_n}{V_{tot}} \quad (3)$$

$$\delta_H = \frac{\delta_{H1} \cdot V_1 + \delta_{H2} \cdot V_2 + \delta_{Hn} \cdot V_n}{V_{tot}} \quad (4)$$

4.2. Solvent Extraction

Maceration extraction (ME) is the most common and easy solvent extraction technique. This method is often called simple solvent extraction or conventional extraction [173,174]. Compounds can be extracted by simply submerging the biomass in a solvent and heating below the boiling point. As ME relies on the diffusion transfer of compounds, physical pretreatment is often required for efficient extraction to increase the surface area of the biomass and possibly to open the plant cell walls of lignocellulosic material. Such physical pretreatment can be achieved by milling or crushing. ME can also happen before, during, or after fermentation. This is the case for winemaking, where the solvent slowly changes from sugar-rich water to a mix of water–sugar–ethanol, shifting the solvent properties, e.g., polarity, dielectric constant, and surface tension [175]. As many organic solvents are perfectly miscible with water, the effect of shifting solvent properties can be well-controlled.

Decoction extraction (DE) involves boiling the plant material in a solvent to extract compounds of interest. This method is also commonly referred to as hot maceration or boiling maceration. Silva et al. [176] investigated DE and microwave-assisted extraction of bioactive compounds from lignocellulosic halophyte *Salicornia ramosissima*. The biomass was milled to open the lignocellulosic structure, and particles of 1 mm were obtained. DE was conducted by boiling 300 mg biomass in 10 mL distilled water for 5 min and leaving it to cool for 25 min. The extract was filtered and freeze-dried into a powder. The extracts

were analyzed by a Folin–Ciocalteu total phenolic assay, with DE showing 80% higher extraction of total phenolics compared to microwave-assisted extraction.

Most analysis methods require liquid samples, as analytical equipment cannot handle solid samples. For analysis, a solid–liquid extraction method is needed to solubilize the solute of interest. Soxhlet extraction is a commonly used method for solid–liquid extraction, as the method is well-described, reliable, safe, and easy to operate. Soxhlet extraction works on the principle of continuous evaporation and simultaneous condensation of a solvent. The biomass is gradually submerged in the solvent. Once the extraction chamber containing the biomass is full of condensed solvent at near-boiling temperature, the solvent is siphoned off, and a new condensed solvent can fill up the extraction chamber. The transfer equilibrium is thereby shifted and does not determine the mass transfer of the desired compound into the solvent. This allows for an unmonitored operation of the Soxhlet apparatus, and the operator can stop the operation when desired. The continuous and cyclic nature of this extraction method ensures a concentrated extract. The solvent is usually determined by the polarity of the solute, but other factors, such as flammability, toxicity, and price, can be used as parameters for the selection of a solvent [177].

Soxhlet extraction can also be applied for the extraction of oils, fats, waxes, sterols, and other non-polar compounds from lignocellulosic biomass using a non-polar solvent. More polar compounds, e.g., phenolic compounds, can be extracted using ethanol, methanol, or ethyl acetate. A schematic of this setup is shown in Figure 16. Cascade extraction using a Soxhlet apparatus can achieve relatively pure extract phases and yield a lignocellulosic fraction with a low concentration of residual material, such as lipids or ash [178–180].

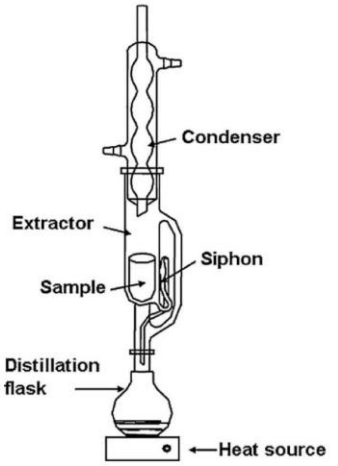

Figure 16. Experimental setup for experimental Soxhlet extraction. Adapted from [177].

Medini et al. [178] studied the phytochemical composition, antioxidant capacity, anti-inflammatory effect, and anticancer activities of the halophyte *Limonium densiflorum*. The tested extracts were extracted by Soxhlet extraction using a successive cascade with hexane, dichloromethane, ethanol, and methanol. These solvents were carefully chosen to extract as much non-lignocellulosic material as possible and thereby afford a pure lignocellulosic fraction after extraction [178].

4.3. Subcritical Water Extraction

Subcritical water extraction (SWE) utilizes increasing temperatures and pressure of water of 100–374 °C and 0.1–22.1 MPa, respectively, while remaining below the vapor pressure. SWE utilizes the shift in chemical properties, such as viscosity, dielectric constant, surface tension, diffusivity characteristics, polarity, and pH value. Changing these properties can cause the water assimilate acids and organic solvents, allowing the operator to select the compounds of interest and yielding an aqueous extract free of harmful acids and solvents [181]. Zhang et al. [181] reviewed the extraction of phenolic compounds by SWE and found the optimal extraction conditions for total phenolic content (TPC) and total flavonoid content (TFC) of lignocellulosic biomass to be approximately 160–220 °C for 16–45 min at 2–6 MPa. Table 13 summarizes the parameters and raw materials reviewed by Zhang et al. [181]. The optimized extraction parameters are changed by increasing the pressure, and the extraction temperature can be lowered significantly to 100–140 °C for 5–130 min at 10–15 MPa.

Table 13. Optimal extraction parameters by subcritical water extraction measured by Folin–Ciocalteu total phenolics assay. Literature reported in a review by Zhang et al. [181].

Detection Method	Raw Material	Temperature (°C)	Pressure (MPa)	Time (min)	Ref.
Folin–Ciocalteu	*Oleaceae europaea* pulp residue	160	-	30	[182]
	Vitis vinifera pomace	140	15	130	[183]
	Vitis vinifera pomace	120	10	120	[184]
	Plantago major	100	10	2	[185]
	Plantago lanceolata	100	10	2	[185]
	Japonica-type rice (*Oryza sativa*)	100–360	18	10–30	[186]

4.4. Extraction at Varying pH Values

Changing pH during an extraction process can purify the product from other compounds that will dissolve in acidic or alkali solutions and hence extract unwanted compounds. Examples include polymers and resin chitosan, 2,5-furandicarboxylic acid polymers, and furfural resins.

Treating biomass using alkalis is a common method for pretreatment or extraction of certain compounds [85,187]. To avoid irreversible degradation of many compounds with low pK_a values, such as organic acids, the stabilities of compounds of interest should be reviewed. Friedman et al. [187] demonstrated the irreversible degradation of caffeic, chlorogenic, and gallic acids at high pH, and degradation was shown to occur even at acidic pH levels, which emphasizes the necessity of low pH values during storage and extraction of phenolic compounds. Chlorogenic acids were shown to degrade into their subcomponent monophenolic compounds, caffeic acid and quinic acid. Flavonoids were shown to be more stable than monophenolic compounds or heterodimers, such as chlorogenic acid. Friedman et al. explained that this was due to the exposed carboxyl groups on the less complex phenolic compounds [187]. However, Peanparkdee et al. contested that protocatechuic acid, similar in molecular structure to gallic acid and thus considered to be more stable, exhibited degradation at lower pH levels [188]. Table 14 summarizes the stability and degradation of phenolic compounds at varying pH.

Table 14. Review of stability and degradation of phenolic compounds at varying pH levels.

Compound	Stable at pH	High Degradation at pH	Refs.
Gallic acid	<7	>10	[187]
Protocatechuic acid	<5	>7	[188]
Vanillic acid	<5	>7	[188]
Caffiec acid	<7	>10	[187]
Ferulic acid	<9	>11	[187,188]
Quercetin	N/A	>6	[53]
(-)-catechin	<7	>10	[187]
Rutin	<11	N/A	[187]
Neochlorogenic acid	<6	>9	[189]
Cryptochlorogenic acid	<5	>9	[190]
Chlorogenic acid	<5	>7	[191,192]

4.5. Ultrasound Extraction

Ultrasound extraction (USE) has been studied extensively with the purpose of providing enhanced accessibility to cellulose and hemicellulose. Bussemaker and Zhang [193] reviewed the application of ultrasound on various lignocellulosic biomasses, such as sorghum, corn stover, sugarcane bagasse, rice hull, and rice straw. However, the focus, like that of other reviews, was the extraction of lignin from biomass or the extraction of cellulose or hemicellulose [194–196]. Fang et al.'s [197] book, '*Production of biofuels and chemicals with ultrasound*' covers the extraction of biofuels and enhanced biogas production using ultrasound, as well as the extraction of chemicals from algae. The use of ultrasound to extract value-added chemicals from lignocellulosic biomass remains scarcely researched. This expands the review slightly to accommodate other biomasses that are not necessarily lignocellulosic but have been utilized for the extraction of high-value chemicals using ultrasound.

Corbin et al. [198] investigated the USE of flax seeds for efficient extraction of phenolic compounds. The phenolics extracted by Corbin et al. [198] were bound to a glucoside group, as the phenolics in flaxseed are bound in the seed coat, with high content of glucosidic bonds. The molecules are still considered HCA, despite the glucosidic bonds. Corbin et al. [198] used slightly alkaline operation conditions (0.2 N NaOH) for the compounds bound in the seed coat matrix to release the polyphenolic lignans, polyphenolic flavonoids, and monophenolic hydroxycinnamic acids. The study by Corbin et al. [198] also compared phenolic extractions in flax seeds by optimized microwave extraction (MWE), USE, enzymatic-assisted extraction (EASE), and heated reflux. This shows MWE to be superior for the extraction of ferulic acid glucoside (76 w% higher than USE), USE to be superior for the extraction of *p*-coumaric acid glucoside (20 w% higher than MWE), and MWE and USE to show similar properties in the extraction of caffeic acid glucoside. The extraction of other polyphenolic compounds was also dominated by MWE and USE extractions in flaxseed, with USE showing superior extraction properties [198].

USE has also been investigated with respect to the extraction of phenolics from saline lignocellulosic biomass and halophytes. Padalino et al. [199] extracted phenolics from vacuum-dried fresh *Salicornia europaea* to increase the phenolic content and antioxidant capacity of freshly made pasta. USE was executed at an extraction temperature of 50 °C with an ethanol/water ratio of 40/60 *v*/*v*% and a DM loading of 1:30 *w*/*v*. The researchers achieved an increase in antioxidant capacity of 148% [199].

4.6. Enzymatic Extraction

Arabinoxylan–lignin and glucan–lignin linkage with the HCAs ferulic acid, *p*-coumaric acid, and flavonoid tricin, amongst others, were shown to be present in wheat straw by Zikeli et al. [200]. Of the carbohydrate–lignin linkages in wheat straw, the ferulic acid linkage is the most predominant, with high amounts of tricin linkages also shown between glucan and lignin. These intermolecular bonds are too strong to be broken solvents; hence,

enzymatic hydrolysis could be investigated as part of an extraction cascade, as different extraction methods and solvents have been shown to target the extraction of different phenolics [161,167,198].

Zhu et al. [161] demonstrated that *p*-coumaric acid, ferulic acid, and caffeic acid in dehulled barley cannot be (or are poorly) extracted by regular solvent extraction using acetone but very easily extracted after digestion in 2 M NaOH in the presence of nitrogen. It should be noted that Zikeli et al. [200] found both ferulic acid and *p*-coumaric acid in the carbohydrate–lignin linkage, which is supported by Bartolomé and Gómez-Cordovés' [201] characterization of the purified enzymes from microbial cultures, ferulic acid, and *p*-coumaric acid esterases. Extraction of barley using enzymatic digestion with pepsin, pancreatin, Pronase E, and Viscozyme L, compared to solvent extraction of free and bound phenolics, increased the extraction of (+)-catechin by 232–239 wt% and that of *p*-coumaric acid 29–82 w%, with no significant increase in the extraction of ferulic acid or caffeic acid [162].

Torres-Mancera et al. [202] described enzymatic extraction methods, whereby the majority of the phenolics in ground coffee pulp were recorded to be bound in the plant cell wall. Pectinase was used as a coenzyme to break the structure of the cell wall, and *Rhizomucor pusillus* strain 23aIV was used in solid-state fermentation to extract the HCAs. The phenolics were extracted downstream with solvents [202].

In the case of high protein content in the lignified biomass, protein removal should be considered, as high protein content can inhibit enzymatic hydrolysis, as described by Faulds et al. [203]. For low-protein biomasses, a hydrothermal pretreatment with a low severity factor, followed by enzymatic hydrolysis using commercial enzymatic blend DEPOL 740 L containing ferulic acid and *p*-coumaric acid esterases and subsequent Soxhlet extraction, can be considered. Table 15 describes extraction methods for phenols and hydroxycinnamic acids from lignocellulosic biomass.

Table 15. Review of extraction methods for phenolics.

Biomass	Method	Optimal or Experimental Conditions	References
Flax	USE	0.2 M NaOH in water at 25 °C for 60 min at 30 kHz	[198]
Crithmum maritimum and *Salicornia europaea*	USE	Water:ethanol, 40:60 *v*/*v*% at 50 °C for 20 min	[199,204]
Wheat straw	Solvent	Water:ethanol, 60:40 *v*/*v*%, 8 w% NaOH at 70 °C for 18 h	[200]
Barley straw	Alkaline + solvent	Pretreatment: 2 M NaOH for 1 h, nitrogen atmosphere. Solvent: EtOAc.	[161,162]
	Enzymatic	Pepsin, Pancreatin, Pronase E, Viscozyme L	
Used coffee bean pulp	Enzymatic + fungi + solvent	Pectinase, *Rhizomucor pusillus*, and EtOAc	[202]
Brewer's spent grain	Enzymatic	DEPOL 740 L, pH 8 at 50 °C	[203]

5. Isolation and Purification Methods

Once the value-added compounds have been extracted from the biomass, the next step is to isolate and purify said compounds. Here, we discuss membrane filtration, liquid–liquid extraction, and purification using preparative high-performance liquid chromatography (Prep-HPLC).

5.1. Membrane Filtration

Membrane filtration can be seen as an easily scalable and inexpensive method for the filtration of extracts. Amongst the various membrane filtration technologies, micro-(MF), ultra-(UF), and nano-(NF) filtration are pressure-driven technologies. These technologies have the benefits of a low energy input, high separation efficiency, simple operation, no use of expensive solvents or effluents, and scalability [205]. A disadvantage of membrane separation is the inability to separate specific compounds of similar polarities and molecular

weights (MWs), as the membranes only retain compounds above a certain molecular size or approximate MW.

Galanakis and Castro-Muñoz et al. [206–208] reviewed the separation of functional macro- and micromolecules using ultra- and nanofiltration (NF). NF with a pore size of 120 Da was used to separate phenolic compounds, achieving a separation efficiency of 99%. The smallest possible phenolic acid, benzoic acid, has an MW of 122 Da, which means all phenolics should be retained by the 120 Da membrane filter. Most phenolics with three or fewer aromatic rings have an MW of <650, which means that an initial filtration to remove larger molecules and particles, such as bacteria, hemicellulose, cellulose, lignin, proteins, and starch, is necessary, as such particles would clog the NF membrane. Even if the range of MW is established as 141–650 Da for membrane separation of phenolic compounds, this does not imply that the molecules will not be retained in larger pores. Galanakis et al. [206] investigated the separation of the phenolic compounds of HCA derivatives and flavanol from olive mill wastewater using UF and NF. Four UF pore sizes and one size of NF (100, 25, 10, 2 kDa, and 120 Da, respectively) were found to retain <1, 32, 44, 53, and 99% HCA, respectively, and 10, 37, 56, 62, and 99% flavonols, respectively [206]. This indicates the ability of these molecules to attach to other molecules or in lignocellulosic structures. Furthermore, 79, 98, 98, 99, 99% of pectin was retained with a pore size of 100, 25, 10, 2 kDa, and 120 Da, respectively, indicating that the optimal UF pore size for retention of pectin and purification of a phenolic-rich fraction is between 25 and 100 kDa, with a secondary NF membrane filtration with a pore size of 120 Da.

Whereas membrane filtration is one of the most promising technologies for recovery of macro- and micromolecules derived from lignocellulosic biomass, it is associated with some drawbacks. Some of the major drawbacks of membrane filtration are membrane fouling, leading to a decrease in permeate flux, as well as reduced efficiency of the process, and the high cost associated with cleaning and maintenance of membranes.

5.2. *Liquid–Liquid Extraction*

Many organic solvents used for liquid–liquid purposes have lower relative polarity than water, and these might coextract lipids, waxes, and other non-polar compounds, along with the targeted compounds. Therefore, a prior liquid–liquid extraction using a non-polar non-water-soluble solvent, such as hexane, dichloromethane, or chloroform, can positively affect the isolation and purification of the targeted compounds, yielding a more concentrated product with fewer contaminates.

Stiger-Pouvreau et al. [209] originally developed a cascade liquid–liquid extraction for the isolation of phlorotannin, a specific phenolic compound group in macroalgae *Sargassacaea* spp. crude extract (Figure 17). A hydroethanolic maceration obtained the extract (*v*/*v*, 1:1). The method implies the use of various consequent washings with organic solvents by liquid–liquid extraction in a separatory funnel, evaporation of solvents, and resuspension of the extracted material in an aqueous phase. Liquid–liquid extraction using dichloromethane extracted the lipids from the crude aqueous extract. Sugars and proteins were separated by low-temperature acetone and ethanol washings. Liquid–liquid extraction using ethyl acetate purified the phenolic compounds and isolated them in the organic solvent [209]. Kim et al. [210] resuspended a powdered methanolic extract of *Salicornia herbacea* in water and successively partitioned it with n-hexane, chloroform, EtOAc, and n-butanol. These compounds are immiscible with water or have a low solubility in water, which can be further decreased by introducing salt [210,211]. The solvent layers were easily separated from the aqueous layers and concentrated in a vacuum at 38 °C. Both the ethyl acetate and the n-butanol phases showed high free-radical scavenging activity, and the compound's two layers were further isolated by column chromatography.

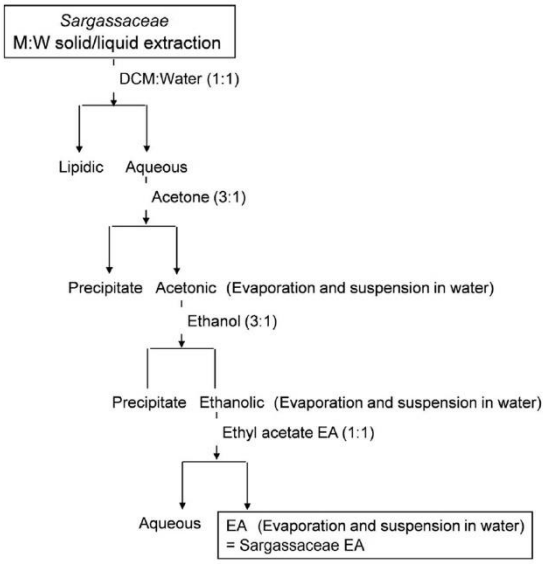

Figure 17. Procedure of liquid–liquid extraction of phenolic compounds from *Sargassaceae* spp. [209]. M:W: methanol:water; DCM: dichloromethane.

Similar to membrane filtration, liquid–liquid extraction also has its drawbacks. Bokhary et al. [212] highlighted these challenges, some of which include poor biocompatibility of solvents with microbial species, energy-intensive processes for solvent recovery, solvent toxicity, high cost of solvents, extensive safety protocols, and environmental risks associated with using large quantities of solvents at high temperatures.

5.3. Purification Using Preparative-HPLC

Prep-HPLC, like analytical HPLC, is a method of isolating chemical compounds by attracting force retention of compounds in the column stationary phase. While the mixture is passing through the column, the compounds separate in retention time, as detected by ultraviolet (UV) reflection at specific wavelengths, but in prep-HPLC, unlike analytical HPLC, the compounds are quantitatively separated and collected after detection. After collecting the separated compounds, i.e., extract purification, the separated compounds can be further processed and stabilized using encapsulation methods if necessary. Compared to analytical HPLC, Prep-HPLC usually has a larger column diameters and stationary-phase particle diameters, and the operator aims for maximum allowed sample weight, which requires more attention with respect to injection flow rate. To compare the retention times of the separated compounds, the retention factor of compound n (k'_n) can be calculated. This is a dimensionless number representing how long a compound is retained in the stationary phase [213–215].

$$k'_n = \frac{t_n - t_0}{t_0} \quad (5)$$

where t_n is the retention time for compound n, and t_0 is the column dead time, i.e., the shortest retention time.

Making the peak width as narrow as possible is important to determine the fraction concentration. Unlike a narrow peak with the same area, a broad peak decreases the concentration of the compound of interest, as a more mobile phase dilutes the concentration of the separated compound. The peak width is determined at half the peak height and denoted as $w_{\frac{1}{2}}$. The peak number of compound n (N_n) is derived from fractional distillation theory and is a height equivalent to a theoretical plate. The higher the peak number, the narrower the peak in the chromatogram and the higher the concentration of the separated compound [213–215]. The peak number is calculated as:

$$N_n = 5.54 \cdot \left(\frac{t_n}{w_{\frac{1}{2}}} \right) \quad (6)$$

Prep-HPLC can be scaled-up to allow for the separation of larger volumes with a column diameter of up to 30 cm, whereas industrial prep-HPLC can separate up to 3 kg h^{-1} of the analyte, although overloading effects are common. Overloading volume or mass in the column can result in non-optimal peak characteristics, leading to a diluted collected sample, so the maximum injection volume must determined [213,215].

Some of the challenges of using prep-HPLC for large-scale extraction of high-value compounds include the high cost of solvents, challenges in scaling up stationary phase chemistry, and the relationship between the quantity of material recovered and the size of the column [216,217]. However, many such challenges have been successfully addressed in the pharmaceutical sector, which suggests that prep-HPLC is viable for extraction of high-value products [218].

6. Conclusions

Research and development activity with respect to the extraction of high-value biomass-derived chemicals have increased considerably since the first report published by USDOE in 2004, highlighting the future of biochemicals. Although the list of top contenders has been altered and appended in the last two decades, several of the top 12 featured compounds have already reached commercial-scale production, such as succinic acid, xylitol, 2,5-FDCA, itaconic acid, levulinic acid, and furfural. Advances in genetically modified microbial strains have also boosted derivation of these compounds using biological routes as an alternative to petrochemical pathways. The considerable advances with respect to processing of lignocellulosic biomasses has enabled researchers to explore cost-effective extraction, isolation, and purification methods that directly target high-value molecules. Whereas pretreatment remains one of the primary steps in opening up the lignocellulosic structure, the severity of pretreatment can be reduced in lieu of the possibility of protecting the unstable phenolic groups, which have a high market value. Although there are still many challenges with respect to the extraction and isolation of high-value compounds, shifting the biorefinery approach to maximize the utility of lignocellulosic biomasses will aid in the cost-effective discovery of novel methods to produce biochemicals from lignocellulosic biomasses.

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References

1. Dutta, K.; Daverey, A.; Lin, J.G. Evolution Retrospective for Alternative Fuels: First to Fourth Generation. *Renew. Energy* **2014**, *69*, 114–122. [CrossRef]
2. Cherubini, F.; Strømman, A.H. Principles of Biorefining. In *Biofuels: Alternative Feedstocks and Conversion Processes*; Pandey, A., Larroche, C., Ricke, S.C., Dussap, C.-G., Gnansounou, E., Dussap, C.-G., Eds.; Academic Press: Cambridge, MA, USA, 2011; pp. 3–24. ISBN 9780123850997.
3. Cherubini, F.; Jungmeier, G.; Wellisch, M.; Willke, T.; Skiadas, I.; Van Ree, R.; de Jong, E. Toward a Common Classification Approach for Biorefinery Systems. *Biofuels Bioprod. Biorefining* **2009**, *3*, 534–546. [CrossRef]
4. Henrich, E.; Dahmen, N.; Dinjus, E. Cost Estimate for Biosynfuel Production via Biosyncrude Gasification. *Biofuels, Bioprod. Biorefining* **2009**, *3*, 28–41. [CrossRef]
5. Werpy, T.; Petersen, G. *Top Value Added Chemicals from Biomass Volume I—Results of Screening for Potential Candidates from Sugars and Synthesis Gas*; U.S. Department of Energy: Washington, DC, USA, 2004; Volume 1.
6. Holladay, J.E.; White, J.F.; Bozell, J.J.; Johnson, D. *Top Value Added Chemicals from Biomass-Volume II, Results of Screening for Potential Candidates from Biorefinery Lignin*; Pacific Northwest National Laboratory (PNNL): Richland, WA, USA; National Renewable Energy Laboratory (NREL): Golden, CO, USA, 2007; Volume 2.
7. Choi, S.; Song, C.W.; Shin, J.H.; Lee, S.Y. Biorefineries for the Production of Top Building Block Chemicals and Their Derivatives. *Metab. Eng.* **2015**, *28*, 223–239. [CrossRef]
8. Girisuta, B. Levulinic Acid from Lignocellulosic Biomass. Ph.D. Thesis, University of Gronigen, Groningen, The Netherlands, 2007.
9. Rackemann, D.W.; Doherty, W.O.S. The Conversion of Lignocellulosics to Levulinic Acid. *Biofuels Bioprod. Biorefining* **2011**, *5*, 198–214. [CrossRef]
10. Fitzpatrick, S.W. Production of Levulinic Acid from Carbohydrate-Containing Materials. U.S. Patent 5,608,105, 4 March 1997.
11. Chang, C.; Cen, P.; Ma, X. Levulinic Acid Production from Wheat Straw. *Bioresour. Technol.* **2007**, *98*, 1448–1453. [CrossRef]
12. Yan, L.; Yang, N.; Pang, H.; Liao, B. Production of Levulinic Acid from Bagasse and Paddy Straw by Liquefaction in the Presence of Hydrochloride Acid. *CLEAN–Soil Air Water* **2008**, *36*, 158–163. [CrossRef]
13. Efremov, A.A.; Pervyshina, G.G.; Kuznetsov, B.N. Production of Levulinic Acid from Wood Raw Material in the Presence of Sulfuric Acid and Its Salts. *Chem. Nat. Compd.* **1998**, *34*, 182–185. [CrossRef]
14. Binder, J.B.; Raines, R.T. Fermentable Sugars by Chemical Hydrolysis of Biomass. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 4516–4521. [CrossRef]
15. Sah, P.P.T.; Ma, S.-Y. Levulinic Acid and Its Esters. *J. Am. Chem. Soc.* **1930**, *52*, 4880–4883. [CrossRef]
16. Thomas, R.W.; Schuette, H.A. Studies on Levulinic Acid. I. Its Preparation from Carbohydrates by Digestion with Hydrochloric Acid under Pressure. *J. Am. Chem. Soc.* **1931**, *53*, 2324–2328. [CrossRef]
17. Redmon, B.C. Process for the Production of Levulinic Acid. U.S. Patent 2,738,367, 13 March 1956.
18. McKibbins, S.W. *Kinetics of the Acid Catalyzed Conversion of Glucose to 5-Hydroxymethyl-2-Furaldehyde and Levulinic Acid*; The University of Wisconsin-Madison: Madison, WI, USA, 1958; ISBN 1084403552.
19. Carlson, L.J. Process for the Manufacture of Levulinic Acid. U.S. Patent 3,065,263, 20 November 1962.
20. Lourvanij, K.; Rorrer, G.L. Dehydration of Glucose to Organic Acids in Microporous Pillared Clay Catalysts. *Appl. Catal. A Gen.* **1994**, *109*, 147–165. [CrossRef]
21. Lourvanij, K.; Rorrer, G.L. Reaction Rates for the Partial Dehydration of Glucose to Organic Acids in Solid-acid, Molecular-sieving Catalyst Powders. *J. Chem. Technol. Biotechnol. Int. Res. Process. Environ. Clean Technol.* **1997**, *69*, 35–44. [CrossRef]
22. Sumiki, Y. Levulinic Acid. Japanese Patent 176,438, 30 June 1948.
23. Sassenrath, C.P.; Shilling, W.L. Preparation of Levulinic Acid from Hexose-Containing Material. U.S. Patent 3,258,481, 28 June 1966.
24. Kin, Z.; Stawecka, J. Use of Waste Lignocellulose for the Production of Levulinic Acid. *Prz. Pap.* **1968**, 303–306.
25. Efremov, A.A.; Pervyshina, G.G.; Kuznetsov, B.N. Thermocatalytic Transformations of Wood and Cellulose in the Presence of HCl, HBr, and H_2SO_4. *Chem. Nat. Compd.* **1997**, *33*, 84–88. [CrossRef]
26. Abdel-Rahman, M.A.; Tashiro, Y.; Sonomoto, K. Lactic Acid Production from Lignocellulose-Derived Sugars Using Lactic Acid Bacteria: Overview and Limits. *J. Biotechnol.* **2011**, *156*, 286–301. [CrossRef]
27. Wee, Y.-J.; Yun, J.-S.; Park, D.-H.; Ryu, H.-W. Biotechnological Production of L (+)-Lactic Acid from Wood Hydrolyzate by Batch Fermentation of Enterococcus Faecalis. *Biotechnol. Lett.* **2004**, *26*, 71–74. [CrossRef]
28. Guo, W.; Jia, W.; Li, Y.; Chen, S. Performances of Lactobacillus Brevis for Producing Lactic Acid from Hydrolysate of Lignocellulosics. *Appl. Biochem. Biotechnol.* **2010**, *161*, 124–136. [CrossRef]
29. Shen, X.; Xia, L. Lactic Acid Production from Cellulosic Material by Synergetic Hydrolysis and Fermentation. *Appl. Biochem. Biotechnol.* **2006**, *133*, 251–262. [CrossRef]
30. Moldes, A.B.; Torrado, A.; Converti, A.; Dominguez, J.M. Complete Bioconversion of Hemicellulosic Sugars from Agricultural Residues into Lactic Acid by Lactobacillus Pentosus. *Appl. Biochem. Biotechnol.* **2006**, *135*, 219–227. [CrossRef]
31. Zhu, Y.; Lee, Y.Y.; Elander, R.T. Conversion of Aqueous Ammonia-Treated Corn Stover to Lactic Acid by Simultaneous Saccharification and Cofermentation. *Appl. Biochem. Biotechnol.* **2007**, *137*, 721–738. [PubMed]

32. Cui, F.; Li, Y.; Wan, C. Lactic Acid Production from Corn Stover Using Mixed Cultures of Lactobacillus Rhamnosus and Lactobacillus Brevis. *Bioresour. Technol.* **2011**, *102*, 1831–1836. [CrossRef] [PubMed]
33. Garde, A.; Jonsson, G.; Schmidt, A.S.; Ahring, B.K. Lactic Acid Production from Wheat Straw Hemicellulose Hydrolysate by Lactobacillus Pentosus and Lactobacillus Brevis. *Bioresour. Technol.* **2002**, *81*, 217–223. [CrossRef]
34. Iyer, P.V.; Thomas, S.; Lee, Y.Y. High-Yield Fermentation of Pentoses into Lactic Acid. In Proceedings of the Twenty-First Symposium on Biotechnology for Fuels and Chemicals, Fort Collins, CO, USA, 2–6 May 1999; Walt, D.R., Ed.; Springer: Berlin/Heidelberg, Germany, 2000; pp. 665–677.
35. Adsul, M.; Khire, J.; Bastawde, K.; Gokhale, D. Production of Lactic Acid from Cellobiose and Cellotriose by Lactobacillus Delbrueckii Mutant Uc-3. *Appl. Environ. Microbiol.* **2007**, *73*, 5055–5057. [CrossRef] [PubMed]
36. Laopaiboon, P.; Thani, A.; Leelavatcharamas, V.; Laopaiboon, L. Acid Hydrolysis of Sugarcane Bagasse for Lactic Acid Production. *Bioresour. Technol.* **2010**, *101*, 1036–1043. [CrossRef]
37. Yun, J.-S.; Wee, Y.-J.; Kim, J.-N.; Ryu, H.-W. Fermentative Production of Dl-Lactic Acid from Amylase-Treated Rice and Wheat Brans Hydrolyzate by a Novel Lactic Acid Bacterium, *Lactobacillus sp. Biotechnol. Lett.* **2004**, *26*, 1613–1616. [CrossRef]
38. Mussatto, S.I.; Fernandes, M.; Mancilha, I.M.; Roberto, I.C. Effects of Medium Supplementation and PH Control on Lactic Acid Production from Brewer's Spent Grain. *Biochem. Eng. J.* **2008**, *40*, 437–444. [CrossRef]
39. Kumar, V.; Ashok, S.; Park, S. Recent Advances in Biological Production of 3-Hydroxypropionic Acid. *Biotechnol. Adv.* **2013**, *31*, 945–961. [CrossRef]
40. Matsakas, L.; Hrůzová, K.; Rova, U.; Christakopoulos, P. Biological Production of 3-Hydroxypropionic Acid: An Update on the Current Status. *Fermentation* **2018**, *4*, 13. [CrossRef]
41. Kildegaard, K.R.; Jensen, N.B.; Schneider, K.; Czarnotta, E.; Özdemir, E.; Klein, T.; Maury, J.; Ebert, B.E.; Christensen, H.B.; Chen, Y.; et al. Engineering and Systems-Level Analysis of Saccharomyces Cerevisiae for Production of 3-Hydroxypropionic Acid via Malonyl-CoA Reductase-Dependent Pathway. *Microb. Cell Factories* **2016**, *15*, 1–13. [CrossRef]
42. Borodina, I.; Kildegaard, K.R.; Jensen, N.B.; Blicher, T.H.; Maury, J.; Sherstyk, S.; Schneider, K.; Lamosa, P.; Herrgård, M.J.; Rosenstand, I.; et al. Establishing a Synthetic Pathway for High-Level Production of 3-Hydroxypropionic Acid in Saccharomyces Cerevisiae via β-Alanine. *Metab. Eng.* **2015**, *27*, 57–64. [CrossRef]
43. Kildegaard, K.R.; Wang, Z.; Chen, Y.; Nielsen, J.; Borodina, I. Production of 3-Hydroxypropionic Acid from Glucose and Xylose by Metabolically Engineered Saccharomyces Cerevisiae. *Metab. Eng. Commun.* **2015**, *2*, 132–136. [CrossRef] [PubMed]
44. Cheng, Z.; Jiang, J.; Wu, H.; Li, Z.; Ye, Q. Enhanced Production of 3-Hydroxypropionic Acid from Glucose via Malonyl-CoA Pathway by Engineered *Escherichia coli*. *Bioresour. Technol.* **2016**, *200*, 897–904. [CrossRef]
45. Liu, C.; Ding, Y.; Zhang, R.; Liu, H.; Xian, M.; Zhao, G. Functional Balance between Enzymes in Malonyl-CoA Pathway for 3-Hydroxypropionate Biosynthesis. *Metab. Eng.* **2016**, *34*, 104–111. [CrossRef]
46. Jung, I.Y.; Lee, J.W.; Min, W.K.; Park, Y.C.; Seo, J.H. Simultaneous Conversion of Glucose and Xylose to 3-Hydroxypropionic Acid in Engineered *Escherichia coli* by Modulation of Sugar Transport and Glycerol Synthesis. *Bioresour. Technol.* **2015**, *198*, 709–716. [CrossRef]
47. Suyama, A.; Higuchi, Y.; Urushihara, M.; Maeda, Y.; Takegawa, K. Production of 3-Hydroxypropionic Acid via the Malonyl-CoA Pathway Using Recombinant Fission Yeast Strains. *J. Biosci. Bioeng.* **2017**, *124*, 392–399. [CrossRef]
48. Chen, Z.; Huang, J.; Wu, Y.; Wu, W.; Zhang, Y.; Liu, D. Metabolic Engineering of Corynebacterium Glutamicum for the Production of 3-Hydroxypropionic Acid from Glucose and Xylose. *Metab. Eng.* **2017**, *39*, 151–158. [CrossRef]
49. Su, M.; Li, Y.; Ge, X.; Tian, P. 3-Hydroxypropionaldehyde-Specific Aldehyde Dehydrogenase from Bacillus Subtilis Catalyzes 3-Hydroxypropionic Acid Production in Klebsiella Pneumoniae. *Biotechnol. Lett.* **2014**, *37*, 717–724. [CrossRef]
50. Huang, Y.; Li, Z.; Shimizu, K.; Ye, Q. Co-Production of 3-Hydroxypropionic Acid and 1,3-Propanediol by Klebseilla Pneumoniae Expressing AldH under Microaerobic Conditions. *Bioresour. Technol.* **2013**, *128*, 505–512. [CrossRef] [PubMed]
51. Ko, Y.; Seol, E.; Sundara Sekar, B.; Kwon, S.; Lee, J.; Park, S. Metabolic Engineering of Klebsiella Pneumoniae J2B for Co-Production of 3-Hydroxypropionic Acid and 1,3-Propanediol from Glycerol: Reduction of Acetate and Other by-Products. *Bioresour. Technol.* **2017**, *244*, 1096–1103. [CrossRef]
52. Li, Y.; Wang, X.; Ge, X.; Tian, P. High Production of 3-Hydroxypropionic Acid in Klebsiella Pneumoniae by Systematic Optimization of Glycerol Metabolism. *Sci. Reports* **2016**, *6*, 1–10. [CrossRef]
53. Wang, K.; Tian, P. Engineering Plasmid-Free Klebsiella Pneumoniae for Production of 3-Hydroxypropionic Acid. *Curr. Microbiol.* **2016**, *74*, 55–58. [CrossRef]
54. Ko, Y.; Ashok, S.; Zhou, S.; Kumar, V.; Park, S. Aldehyde Dehydrogenase Activity Is Important to the Production of 3-Hydroxypropionic Acid from Glycerol by Recombinant Klebsiella Pneumoniae. *Process Biochem.* **2012**, *47*, 1135–1143. [CrossRef]
55. Kumar, V.; Sankaranarayanan, M.; Jae, K.; Durgapal, M.; Ashok, S.; Ko, Y.; Sarkar, R.; Park, S. Co-Production of 3-Hydroxypropionic Acid and 1,3-Propanediol from Glycerol Using Resting Cells of Recombinant Klebsiella Pneumoniae J2B Strain Overexpressing Aldehyde Dehydrogenase. *Appl. Microbiol. Biotechnol.* **2012**, *96*, 373–383. [CrossRef]

56. Kumar, V.; Sankaranarayanan, M.; Durgapal, M.; Shengfang, Z.; Yeounjoo, K.; Ashok, S.; Sarkar, R.; Park, S. Simultaneous Production of 3-Hydroxypropionic Acid and 1,3-Propanediol from Glycerol Using Resting Cells of the Lactate Dehydrogenase-Deficient Recombinant Klebsiella Pneumoniae Overexpressing an Aldehyde Dehydrogenase. *Bioresour. Technol.* **2013**, *135*, 555–563. [CrossRef]
57. Ashok, S.; Raj, S.M.; Rathnasingh, C.; Park, S. Development of Recombinant Klebsiella Pneumoniae ΔdhaT Strain for the Co-Production of 3-Hydroxypropionic Acid and 1,3-Propanediol from Glycerol. *Appl. Microbiol. Biotechnol.* **2011**, *90*, 1253–1265. [CrossRef]
58. Ashok, S.; Sankaranarayanan, M.; Ko, Y.; Jae, K.-E.; Ainala, S.K.; Kumar, V.; Park, S. Production of 3-Hydroxypropionic Acid from Glycerol by Recombinant Klebsiella Pneumoniae ΔdhaTΔyqhD Which Can Produce Vitamin B12 Naturally. *Biotechnol. Bioeng.* **2013**, *110*, 511–524. [CrossRef]
59. Zhao, L.; Lin, J.; Wang, H.; Xie, J.; Wei, D. Development of a Two-Step Process for Production of 3-Hydroxypropionic Acid from Glycerol Using Klebsiella Pneumoniae and Gluconobacter Oxydans. *Bioprocess Biosyst. Eng.* **2015**, *38*, 2487–2495. [CrossRef]
60. Jung, W.S.; Kang, J.H.; Chu, H.S.; Choi, I.S.; Cho, K.M. Elevated Production of 3-Hydroxypropionic Acid by Metabolic Engineering of the Glycerol Metabolism in *Escherichia coli*. *Metab. Eng.* **2014**, *23*, 116–122. [CrossRef]
61. Chu, H.S.; Kim, Y.S.; Lee, C.M.; Lee, J.H.; Jung, W.S.; Ahn, J.-H.; Song, S.H.; Choi, I.S.; Cho, K.M. Metabolic Engineering of 3-Hydroxypropionic Acid Biosynthesis in *Escherichia coli*. *Biotechnol. Bioeng.* **2015**, *112*, 356–364. [CrossRef]
62. Lim, H.G.; Noh, M.H.; Jeong, J.H.; Park, S.; Jung, G.Y. Optimum Rebalancing of the 3-Hydroxypropionic Acid Production Pathway from Glycerol in *Escherichia coli*. *ACS Synth. Biol.* **2016**, *5*, 1247–1255. [CrossRef] [PubMed]
63. Sankaranarayanan, M.; Somasundar, A.; Seol, E.; Chauhan, A.S.; Kwon, S.; Jung, G.Y.; Park, S. Production of 3-Hydroxypropionic Acid by Balancing the Pathway Enzymes Using Synthetic Cassette Architecture. *J. Biotechnol.* **2017**, *259*, 140–147. [CrossRef] [PubMed]
64. Sankaranarayanan, M.; Ashok, S.; Park, S. Production of 3-Hydroxypropionic Acid from Glycerol by Acid Tolerant Escherichia Coli. *J. Ind. Microbiol. Biotechnol.* **2014**, *41*, 1039–1050. [CrossRef] [PubMed]
65. Raj, S.M.; Rathnasingh, C.; Jung, W.-C.; Park, S. Effect of Process Parameters on 3-Hydroxypropionic Acid Production from Glycerol Using a Recombinant *Escherichia coli*. *Appl. Microbiol. Biotechnol.* **2009**, *84*, 649–657. [CrossRef]
66. Rathnasingh, C.; Raj, S.M.; Jo, J.-E.; Park, S. Development and Evaluation of Efficient Recombinant *Escherichia Coli* Strains for the Production of 3-Hydroxypropionic Acid from Glycerol. *Biotechnol. Bioeng.* **2009**, *104*, 729–739. [CrossRef]
67. Tsuruno, K.; Honjo, H.; Hanai, T. Enhancement of 3-Hydroxypropionic Acid Production from Glycerol by Using a Metabolic Toggle Switch. *Microb. Cell Factories* **2015**, *14*, 1–14. [CrossRef]
68. Honjo, H.; Tsuruno, K.; Tatsuke, T.; Sato, M.; Hanai, T. Dual Synthetic Pathway for 3-Hydroxypropionic Acid Production in Engineered *Escherichia coli*. *J. Biosci. Bioeng.* **2015**, *120*, 199–204. [CrossRef]
69. Dishisha, T.; Pereyra, L.P.; Pyo, S.-H.; Britton, R.A.; Hatti-Kaul, R. Flux Analysis of the Lactobacillus Reuteri Propanediol-Utilization Pathway for Production of 3-Hydroxypropionaldehyde, 3-Hydroxypropionic Acid and 1,3-Propanediol from Glycerol. *Microb. Cell Fact.* **2014**, *13*, 1–11. [CrossRef]
70. Zaushitsyna, O.; Dishisha, T.; Hatti-Kaul, R.; Mattiasson, B. Crosslinked, Cryostructured Lactobacillus Reuteri Monoliths for Production of 3-Hydroxypropionaldehyde, 3-Hydroxypropionic Acid and 1,3-Propanediol from Glycerol. *J. Biotechnol.* **2017**, *241*, 22–32. [CrossRef]
71. Garai-Ibabe, G.; Ibarburu, I.; Berregi, I.; Claisse, O.; Lonvaud-Funel, A.; Irastorza, A.; Dueñas, M.T. Glycerol Metabolism and Bitterness Producing Lactic Acid Bacteria in Cidermaking. *Int. J. Food Microbiol.* **2008**, *121*, 253–261. [CrossRef]
72. Kaur, B.; Chakraborty, D. Biotechnological and Molecular Approaches for Vanillin Production: A Review. *Appl. Biochem. Biotechnol.* **2013**, *169*, 1353–1372. [CrossRef] [PubMed]
73. Kruyer, N.S.; Peralta-Yahya, P. Metabolic Engineering Strategies to Bio-Adipic Acid Production. *Curr. Opin. Biotechnol.* **2017**, *45*, 136–143. [CrossRef] [PubMed]
74. Polen, T.; Spelberg, M.; Bott, M. Toward Biotechnological Production of Adipic Acid and Precursors from Biorenewables. *J. Biotechnol.* **2013**, *167*, 75–84. [CrossRef]
75. Steinbach, D.; Kruse, A.; Sauer, J. Pretreatment Technologies of Lignocellulosic Biomass in Water in View of Furfural and 5-Hydroxymethylfurfural Production—A Review. *Biomass Convers. Biorefinery* **2017**, *7*, 247–274. [CrossRef]
76. Zeitsch, K.J. *The Chemistry and Technology of Furfural and Its Many by-Products*; Elsevier: Amsterdam, The Netherlands, 2000; ISBN 0080528996.
77. Ji, L.; Tang, Z.; Yang, D.; Ma, C.; He, Y.-C. Improved One-Pot Synthesis of Furfural from Corn Stalk with Heterogeneous Catalysis Using Corn Stalk as Biobased Carrier in Deep Eutectic Solvent–Water System. *Bioresour. Technol.* **2021**, *340*, 125691. [CrossRef]
78. Kim, H.; Lee, S.; Lee, J.; Won, W. Simultaneous Production of 1,6-Hexanediol, Furfural, and High-Purity Lignin from White Birch: Process Integration and Techno-Economic Evaluation. *Bioresour. Technol.* **2021**, *331*, 125009. [CrossRef]
79. Bozell, J.J.; Petersen, G.R. Technology Development for the Production of Biobased Products from Biorefinery Carbohydrates—the US Department of Energy's "Top 10" Revisited. *Green Chem.* **2010**, *12*, 539. [CrossRef]
80. Du, B.; Sharma, L.N.; Becker, C.; Chen, S.-F.; Mowery, R.A.; van Walsum, G.P.; Chambliss, C.K. Effect of Varying Feedstock–Pretreatment Chemistry Combinations on the Formation and Accumulation of Potentially Inhibitory Degradation Products in Biomass Hydrolysates. *Biotechnol. Bioeng.* **2010**, *107*, 430–440. [CrossRef]

81. Chundawat, S.P.S.; Vismeh, R.; Sharma, L.N.; Humpula, J.F.; da Costa Sousa, L.; Chambliss, C.K.; Jones, A.D.; Balan, V.; Dale, B.E. Multifaceted Characterization of Cell Wall Decomposition Products Formed during Ammonia Fiber Expansion (AFEX) and Dilute Acid Based Pretreatments. *Bioresour. Technol.* **2010**, *101*, 8429–8438. [CrossRef]
82. Chen, S.F.; Mowery, R.A.; Castleberry, V.A.; Van Walsum, G.P.; Chambliss, C.K. High-Performance Liquid Chromatography Method for Simultaneous Determination of Aliphatic Acid, Aromatic Acid and Neutral Degradation Products in Biomass Pretreatment Hydrolysates. *J. Chromatogr. A* **2006**, *1104*, 54–61. [CrossRef]
83. Cantarella, M.; Cantarella, L.; Gallifuoco, A.; Spera, A.; Alfani, F. Effect of Inhibitors Released during Steam-Explosion Treatment of Poplar Wood on Subsequent Enzymatic Hydrolysis and SSF. *Biotechnol. Prog.* **2004**, *20*, 200–206. [CrossRef]
84. Larsson, S.; Reimann, A.; Nilvebrant, N.-O.; Jönsson, L.J. Comparison of Different Methods for the Detoxification of Lignocellulose Hydrolyzates of Spruce. *Larssonal. Appl. Biochem. Biotechnol.* **1999**, *77*, 77–79.
85. Klinke, H.B.; Ahring, B.K.; Schmidt, A.S.; Thomsen, A.B. Characterization of Degradation Products from Alkaline Wet Oxidation of Wheat Straw. *Bioresour. Technol.* **2002**, *82*, 15–26. [CrossRef]
86. García-Aparicio, M.P.; Ballesteros, I.; González, A.; Oliva, J.M.; Ballesteros, M.; Negro, M.J. Effect of Inhibitors Released During Steam-Explosion Pretreatment of Barley Straw on Enzymatic Hydrolysis. *Twenty-Seventh Symp. Biotechnol. Fuels Chem.* **2006**, *129*, 278–288. [CrossRef]
87. van der Pol, E.C.; Bakker, R.R.; Baets, P.; Eggink, G. By-Products Resulting from Lignocellulose Pretreatment and Their Inhibitory Effect on Fermentations for (Bio) Chemicals and Fuels. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 9579–9593. [CrossRef]
88. Banerjee, A.; Dick, G.R.; Yoshino, T.; Kanan, M.W. Carbon Dioxide Utilization via Carbonate-Promoted C-H Carboxylation. *Nature* **2016**, *531*, 215–219. [CrossRef]
89. Zhou, H.; Xu, H.; Wang, X.; Liu, Y. Convergent Production of 2,5-Furandicarboxylic Acid from Biomass and CO_2. *Green Chem.* **2019**, *21*, 2923–2927. [CrossRef]
90. Boisen, A.; Christensen, T.B.; Fu, W.; Gorbanev, Y.Y.; Hansen, T.S.; Jensen, J.S.; Klitgaard, S.K.; Pedersen, S.; Riisager, A.; Ståhlberg, T.; et al. Process Integration for the Conversion of Glucose to 2,5-Furandicarboxylic Acid. *Chem. Eng. Res. Des.* **2009**, *87*, 1318–1327. [CrossRef]
91. Deshan, A.D.K.; Atanda, L.; Moghaddam, L.; Rackemann, D.W.; Beltramini, J.; Doherty, W.O.S. Heterogeneous Catalytic Conversion of Sugars Into 2,5-Furandicarboxylic Acid. *Front. Chem.* **2020**, *8*, 659. [CrossRef] [PubMed]
92. Motagamwala, A.H.; Won, W.; Sener, C.; Alonso, D.M.; Maravelias, C.T.; Dumesic, J.A. Toward Biomass-Derived Renewable Plastics: Production of 2,5-Furandicarboxylic Acid from Fructose. *Sci. Adv.* **2018**, *4*, eaap9722. [CrossRef]
93. Yang, Z.; Qi, W.; Su, R.; He, Z. Selective Synthesis of 2,5-Diformylfuran and 2,5-Furandicarboxylic Acid from 5-Hydroxymethylfurfural and Fructose Catalyzed by Magnetically Separable Catalysts. *Energy Fuels* **2017**, *31*, 533–541. [CrossRef]
94. Yangyang, J.Z.; Zhou, F.; Ma, H.X.; Li, X.L.; Yuan, X.Z.; Liang, F.X.; Zhang, J. One Step Synthesis of 2,5-Furandicarboxylic Acid from Fructose Catalyzed by Ce Modified Ru/HAP. *Ranliao Huaxue Xuebao/J. Fuel Chem. Technol.* **2020**, *48*, 942–948. [CrossRef]
95. Rathod, P.V.; Jadhav, V.H. Efficient Method for Synthesis of 2,5-Furandicarboxylic Acid from 5-Hydroxymethylfurfural and Fructose Using Pd/CC Catalyst under Aqueous Conditions. *ACS Sustain. Chem. Eng.* **2018**, *6*, 5766–5771. [CrossRef]
96. Karich, A.; Kleeberg, S.; Ullrich, R.; Hofrichter, M. Enzymatic Preparation of 2,5-Furandicarboxylic Acid (FDCA)—A Substitute of Terephthalic Acid—By the Joined Action of Three Fungal Enzymes. *Microorganisms* **2018**, *6*, 5. [CrossRef] [PubMed]
97. Hayashi, E.; Yamaguchi, Y.; Kamata, K.; Tsunoda, N.; Kumagai, Y.; Oba, F.; Hara, M. Effect of MnO2 Crystal Structure on Aerobic Oxidation of 5-Hydroxymethylfurfural to 2,5-Furandicarboxylic Acid. *J. Am. Chem. Soc.* **2019**, *141*, 899–900. [CrossRef] [PubMed]
98. Casanova, O.; Iborra, S.; Corma, A. Biomass into Chemicals: Aerobic Oxidation of 5-Hydroxymethyl-2-Furfural into 2,5-Furandicarboxylic Acid with Gold Nanoparticle Catalysts. *ChemSusChem* **2009**, *2*, 1138–1144. [CrossRef]
99. Mishra, D.K.; Lee, H.J.; Kim, J.; Lee, H.S.; Cho, J.K.; Suh, Y.W.; Yi, Y.; Kim, Y.J. MnCo2O4 Spinel Supported Ruthenium Catalyst for Air-Oxidation of HMF to FDCA under Aqueous Phase and Base-Free Conditions. *Green Chem.* **2017**, *19*, 1619–1623. [CrossRef]
100. Venkateswar Rao, L.; Goli, J.K.; Gentela, J.; Koti, S. Bioconversion of Lignocellulosic Biomass to Xylitol: An Overview. *Bioresour. Technol.* **2016**, *213*, 299–310. [CrossRef]
101. Insights, G.M. Alkyl Polyglucosides (APG) Biosurfactants Biosurfactants Market Size. 2022. Available online: https://www.Gminsights.Com/Industry-Analysis/Alkyl-Polyglucosides-Apg-Biosurfactants-Market (accessed on 10 July 2016).
102. Delgado Arcaño, Y.; Valmaña García, O.D.; Mandelli, D.; Carvalho, W.A.; Magalhães Pontes, L.A. Xylitol: A Review on the Progress and Challenges of Its Production by Chemical Route. *Catal. Today* **2020**, *344*, 2–14. [CrossRef]
103. Ikeuchi, T.; Azuma, M.; Kato, J.; Ooshima, H. Screening of Microorganisms for Xylitol Production and Fermentation Behavior in High Concentrations of Xylose. *Biomass Bioenergy* **1999**, *16*, 333–339. [CrossRef]
104. Tai, C.; Li, S.; Xu, Q.; Ying, H.; Huang, H.; Ouyang, P. Chitosan Production from Hemicellulose Hydrolysate of Corn Straw: Impact of Degradation Products on Rhizopus Oryzae Growth and Chitosan Fermentation. *Lett. Appl. Microbiol.* **2010**, *51*, 278–284. [CrossRef]
105. Yang, L.; Li, X.; Lai, C.; Fan, Y.; Ouyang, J.; Yong, Q. Fungal Chitosan Production Using Xylose Rich of Corn Stover Prehydrolysate by *Rhizopus oryzae*. *Biotechnol. Biotechnol. Equip.* **2017**, *31*, 1160–1166. [CrossRef]
106. Satari, B.; Karimi, K.; Zamani, A. Oil, Chitosan, and Ethanol Production by Dimorphic Fungus *Mucor Indicus* from Different Lignocelluloses. *J. Chem. Technol. Biotechnol.* **2016**, *91*, 1835–1843. [CrossRef]

107. Omogbai, B.A.; Ikeneborneh, M. Solid-State Fermentative Production and Bioactivity of Fungal Chitosan. *J. Microbiol. Biotechnol. Food Sci.* **2013**, *3*, 172–175.
108. Cybulska, I.; Chaturvedi, T.; Thomsen, M.H. Lignocellulosic Thermochemical Pretreatment Processes. In *Biorefinery*; Bastidas-Oyanedel, J.R., Schmidt, J., Eds.; Springer: Cham, Switzerland, 2019; pp. 153–165.
109. Guragain, Y.N.; Chitta, D.; Karanjikar, M.; Vadlani, P.V. Appropriate Lignocellulosic Biomass Processing Strategies for Efficient 2,3-Butanediol Production from Biomass-Derived Sugars Using Bacillus Licheniformis DSM 8785. *Food Bioprod. Process.* **2017**, *104*, 147–158. [CrossRef]
110. Cha, J.W.; Jang, S.H.; Kim, Y.J.; Chang, Y.K.; Jeong, K.J. Engineering of *Klebsiella oxytoca* for Production of 2,3-butanediol Using Mixed Sugars Derived from Lignocellulosic Hydrolysates. *GCB Bioenergy* **2020**, *12*, 275–286. [CrossRef]
111. Li, D.; Dai, J.Y.; Xiu, Z.L. A Novel Strategy for Integrated Utilization of Jerusalem Artichoke Stalk and Tuber for Production of 2,3-Butanediol by Klebsiella Pneumoniae. *Bioresour. Technol.* **2010**, *101*, 8342–8347. [CrossRef]
112. Li, L.; Li, K.; Wang, K.; Chen, C.; Gao, C.; Ma, C.; Xu, P. Efficient Production of 2,3-Butanediol from Corn Stover Hydrolysate by Using a Thermophilic Bacillus Licheniformis Strain. *Bioresour. Technol.* **2014**, *170*, 256–261. [CrossRef] [PubMed]
113. Hazeena, S.H.; Nair Salini, C.; Sindhu, R.; Pandey, A.; Binod, P. Simultaneous Saccharification and Fermentation of Oil Palm Front for the Production of 2,3-Butanediol. *Bioresour. Technol.* **2019**, *278*, 145–149. [CrossRef] [PubMed]
114. Yang, S.; Mohagheghi, A.; Franden, M.A.; Chou, Y.C.; Chen, X.; Dowe, N.; Himmel, M.E.; Zhang, M. Metabolic Engineering of Zymomonas Mobilis for 2,3-Butanediol Production from Lignocellulosic Biomass Sugars. *Biotechnol. Biofuels* **2016**, *9*, 189. [CrossRef] [PubMed]
115. Ji, X.J.; Huang, H.; Ouyang, P.K. Microbial 2,3-Butanediol Production: A State-of-the-Art Review. *Biotechnol. Adv.* **2011**, *29*, 351–364. [PubMed]
116. Joo, J.; Lee, S.J.; Yoo, H.Y.; Kim, Y.; Jang, M.; Lee, J.; Han, S.O.; Kim, S.W.; Park, C. Improved Fermentation of Lignocellulosic Hydrolysates to 2,3-Butanediol through Investigation of Effects of Inhibitory Compounds by Enterobacter Aerogenes. *Chem. Eng. J.* **2016**, *306*, 916–924. [CrossRef]
117. Quiñones, T.S.; Retter, A.; Hobbs, P.J.; Budde, J.; Heiermann, M.; Plöchl, M.; Ravella, S.R. Production of Xylooligosaccharides from Renewable Agricultural Lignocellulose Biomass. *Biofuels* **2015**, *6*, 147–155. [CrossRef]
118. Santibáñez, L.; Henríquez, C.; Corro-Tejeda, R.; Bernal, S.; Armijo, B.; Salazar, O. Xylooligosaccharides from Lignocellulosic Biomass: A Comprehensive Review. *Carbohydr. Polym.* **2021**, *251*, 117118.
119. Akpinar, O.; Erdogan, K.; Bostanci, S. Production of Xylooligosaccharides by Controlled Acid Hydrolysis of Lignocellulosic Materials. *Carbohydr. Res.* **2009**, *344*, 660–666. [CrossRef]
120. Parajó, J.C.; Garrote, G.; Cruz, J.M.; Domínguez, H. Production of Xylooligosaccharides by Autohydrolysis of Lignocellulosic Materials. *Trends Food Sci. Technol.* **2004**, *15*, 115–120.
121. Otieno, D.O.; Ahring, B.K. The Potential for Oligosaccharide Production from the Hemicellulose Fraction of Biomasses through Pretreatment Processes: Xylooligosaccharides (XOS), Arabinooligosaccharides (AOS), and Mannooligosaccharides (MOS). *Carbohydr. Res.* **2012**, *360*, 84–92.
122. da Silva Menezes, B.; Rossi, D.M.; Squina, F.; Ayub, M.A.Z. Xylooligosaccharides Production by Fungi Cultivations in Rice Husk and Their Application as Substrate for Lactic Acid Bacteria Growth. *Bioresour. Technol. Reports* **2018**, *2*, 100–106. [CrossRef]
123. Reddy, S.S.; Krishnan, C. Production of High-Pure Xylooligosaccharides from Sugarcane Bagasse Using Crude β-Xylosidase-Free Xylanase of Bacillus Subtilis KCX006 and Their Bifidogenic Function. *LWT—Food Sci. Technol.* **2016**, *65*, 237–245. [CrossRef]
124. Chapla, D.; Pandit, P.; Shah, A. Production of Xylooligosaccharides from Corncob Xylan by Fungal Xylanase and Their Utilization by Probiotics. *Bioresour. Technol.* **2012**, *115*, 215–221. [CrossRef]
125. Aachary, A.A.; Prapulla, S.G. Value Addition to Corncob: Production and Characterization of Xylooligosaccharides from Alkali Pretreated Lignin-Saccharide Complex Using Aspergillus Oryzae MTCC 5154. *Bioresour. Technol.* **2009**, *100*, 991–995. [CrossRef] [PubMed]
126. Vázquez, M.J.; Alonso, J.L.; Domínguez, H.; Parajó, J.C. Enhancing the Potential of Oligosaccharides from Corncob Autohydrolysis as Prebiotic Food Ingredients. *Ind. Crops Prod.* **2006**, *24*, 152–159. [CrossRef]
127. Yang, R.; Xu, S.; Wang, Z.; Yang, W. Aqueous Extraction of Corncob Xylan and Production of Xylooligosaccharides. *LWT—Food Sci. Technol.* **2005**, *38*, 677–682. [CrossRef]
128. Misra, S.; Raghuwanshi, S.; Saxena, R.K. Evaluation of Corncob Hemicellulosic Hydrolysate for Xylitol Production by Adapted Strain of Candida Tropicalis. *Carbohydr. Polym.* **2013**, *92*, 1596–1601. [CrossRef]
129. Li, M.; Meng, X.; Diao, E.; Du, F. Xylitol Production by Candida Tropicalis from Corn Cob Hemicellulose Hydrolysate in a Two-Stage Fed-Batch Fermentation Process. *J. Chem. Technol. Biotechnol.* **2012**, *87*, 387–392. [CrossRef]
130. Ping, Y.; Ling, H.Z.; Song, G.; Ge, J.P. Xylitol Production from Non-Detoxified Corncob Hemicellulose Acid Hydrolysate by Candida Tropicalis. *Biochem. Eng. J.* **2013**, *75*, 86–91. [CrossRef]
131. Cheng, K.K.; Zhang, J.A.; Ling, H.Z.; Ping, W.X.; Huang, W.; Ge, J.P.; Xu, J.M. Optimization of PH and Acetic Acid Concentration for Bioconversion of Hemicellulose from Corncobs to Xylitol by Candida Tropicalis. *Biochem. Eng. J.* **2009**, *43*, 203–207. [CrossRef]
132. Tada, K.; Horiuchi, J.I.; Kanno, T.; Kobayashi, M. Microbial Xylitol Production from Corn Cobs Using Candida Magnoliae. *J. Biosci. Bioeng.* **2004**, *98*, 228–230. [CrossRef]

133. Prakash, G.; Varma, A.J.; Prabhune, A.; Shouche, Y.; Rao, M. Microbial Production of Xylitol from D-Xylose and Sugarcane Bagasse Hemicellulose Using Newly Isolated Thermotolerant Yeast Debaryomyces Hansenii. *Bioresour. Technol.* **2011**, *102*, 3304–3308. [CrossRef]
134. de Arruda, P.V.; de Cássia Lacerda Brambilla Rodrigues, R.; da Silva, D.D.V.; das Graças de Almeida Felipe, M. Evaluation of Hexose and Pentose in Pre-Cultivation of Candida Guilliermondii on the Key Enzymes for Xylitol Production in Sugarcane Hemicellulosic Hydrolysate. *Biodegradation* **2010**, *22*, 815–822. [CrossRef]
135. da Cunha, M.A.A.; Converti, A.; Santos, J.C.; Ferreira, S.T.S.; da Silva, S.S. PVA-Hydrogel Entrapped Candida Guilliermondii for Xylitol Production from Sugarcane Hemicellulose Hydrolysate. *Appl. Biochem. Biotechnol.* **2008**, *157*, 527–537. [CrossRef] [PubMed]
136. Rao, R.S.; Jyothi, C.P.; Prakasham, R.S.; Sarma, P.N.; Rao, L.V. Xylitol Production from Corn Fiber and Sugarcane Bagasse Hydrolysates by Candida Tropicalis. *Bioresour. Technol.* **2006**, *97*, 1974–1978. [CrossRef] [PubMed]
137. Asachi, R.; Karimi, K. Enhanced Ethanol and Chitosan Production from Wheat Straw by Mucor Indicus with Minimal Nutrient Consumption. *Process Biochem.* **2013**, *48*, 1524–1531. [CrossRef]
138. Ling, H.Z.; Cheng, K.K.; Ge, J.P.; Ping, W.X. Corncob Mild Alkaline Pretreatment for High 2,3-Butanediol Production by Spent Liquor Recycle Process. *Bioenergy Res.* **2017**, *10*, 566–574. [CrossRef]
139. Ma, K.; He, M.; You, H.; Pan, L.; Wang, Z.; Wang, Y.; Hu, G.; Cui, Y.; Maeda, T. Improvement of (R,R)-2,3-Butanediol Production from Corn Stover Hydrolysate by Cell Recycling Continuous Fermentation. *Chem. Eng. J.* **2018**, *332*, 361–369. [CrossRef]
140. Wong, C.L.; Huang, C.C.; Lu, W.B.; Chen, W.M.; Chang, J.S. Producing 2,3-Butanediol from Agricultural Waste Using an Indigenous Klebsiella Sp. Zmd30 Strain. *Biochem. Eng. J.* **2012**, *69*, 32–40. [CrossRef]
141. Saratale, G.D.; Jung, M.Y.; Oh, M.K. Reutilization of Green Liquor Chemicals for Pretreatment of Whole Rice Waste Biomass and Its Application to 2,3-Butanediol Production. *Bioresour. Technol.* **2016**, *205*, 90–96. [CrossRef]
142. Zhao, X.; Song, Y.; Liu, D. Enzymatic Hydrolysis and Simultaneous Saccharification and Fermentation of Alkali/Peracetic Acid-Pretreated Sugarcane Bagasse for Ethanol and 2,3-Butanediol Production. *Enzyme Microb. Technol.* **2011**, *49*, 413–419. [CrossRef]
143. Um, J.; Kim, D.G.; Jung, M.Y.; Saratale, G.D.; Oh, M.K. Metabolic Engineering of Enterobacter Aerogenes for 2,3-Butanediol Production from Sugarcane Bagasse Hydrolysate. *Bioresour. Technol.* **2017**, *245*, 1567–1574. [CrossRef]
144. Samanta, A.K.; Senani, S.; Kolte, A.P.; Sridhar, M.; Sampath, K.T.; Jayapal, N.; Devi, A. Production and in Vitro Evaluation of Xylooligosaccharides Generated from Corn Cobs. *Food Bioprod. Process.* **2012**, *90*, 466–474. [CrossRef]
145. Samanta, A.K.; Jayapal, N.; Kolte, A.P.; Senani, S.; Sridhar, M.; Dhali, A.; Suresh, K.P.; Jayaram, C.; Prasad, C.S. Process for Enzymatic Production of Xylooligosaccharides from the Xylan of Corn Cobs. *J. Food Process. Preserv.* **2015**, *39*, 729–736. [CrossRef]
146. Sabiha-Hanim, S.; Noor, M.A.M.; Rosma, A. Effect of Autohydrolysis and Enzymatic Treatment on Oil Palm (Elaeis Guineensis Jacq.) Frond Fibres for Xylose and Xylooligosaccharides Production. *Bioresour. Technol.* **2011**, *102*, 1234–1239. [CrossRef]
147. Chen, M.; Lu, J.; Cheng, Y.; Li, Q.; Wang, H. Novel Process for the Coproduction of Xylo-Oligosaccharide and Glucose from Reed Scraps of Reed Pulp Mill. *Carbohydr. Polym.* **2019**, *215*, 82–89. [CrossRef]
148. Khat-udomkiri, N.; Sivamaruthi, B.S.; Sirilun, S.; Lailerd, N.; Peerajan, S.; Chaiyasut, C. Optimization of Alkaline Pretreatment and Enzymatic Hydrolysis for the Extraction of Xylooligosaccharide from Rice Husk. *AMB Express* **2018**, *8*, 115. [CrossRef] [PubMed]
149. Brienzo, M.; Carvalho, W.; Milagres, A.M.F. Xylooligosaccharides Production from Alkali-Pretreated Sugarcane Bagasse Using Xylanases from Thermoascus Aurantiacus. *Appl. Biochem. Biotechnol.* **2010**, *162*, 1195–1205. [CrossRef]
150. Zhou, X.; Xu, Y. Integrative Process for Sugarcane Bagasse Biorefinery to Co-Produce Xylooligosaccharides and Gluconic Acid. *Bioresour. Technol.* **2019**, *282*, 81–87. [CrossRef] [PubMed]
151. Jayapal, N.; Samanta, A.K.; Kolte, A.P.; Senani, S.; Sridhar, M.; Suresh, K.P.; Sampath, K.T. Value Addition to Sugarcane Bagasse: Xylan Extraction and Its Process Optimization for Xylooligosaccharides Production. *Ind. Crops Prod.* **2013**, *42*, 14–24. [CrossRef]
152. Akpinar, O.; Erdogan, K.; Bostanci, S. Enzymatic Production of Xylooligosaccharide from Selected Agricultural Wastes. *Food Bioprod. Process.* **2009**, *87*, 145–151. [CrossRef]
153. Faryar, R.; Linares-Pastén, J.A.; Immerzeel, P.; Mamo, G.; Andersson, M.; Stålbrand, H.; Mattiasson, B.; Karlsson, E.N. Production of Prebiotic Xylooligosaccharides from Alkaline Extracted Wheat Straw Using the K80R-Variant of a Thermostable Alkali-Tolerant Xylanase. *Food Bioprod. Process.* **2015**, *93*, 1–10. [CrossRef]
154. Ruiz, H.A.; Thomsen, M.H.; Trajano, H.L. *Hydrothermal Processing in Biorefineries*; Ruiz, H.A., Thomsen, M.H., Trajano, H.L., Eds.; Springer: Cham, Switzerland, 2017; ISBN 9783319564562.
155. Ruiz, H.A.; Conrad, M.; Sun, S.N.; Sanchez, A.; Rocha, G.J.M.; Romaní, A.; Castro, E.; Torres, A.; Rodríguez-Jasso, R.M.; Andrade, L.P.; et al. Engineering Aspects of Hydrothermal Pretreatment: From Batch to Continuous Operation, Scale-up and Pilot Reactor under Biorefinery Concept. *Bioresour. Technol.* **2020**, *299*, 122685. [CrossRef]
156. Camel, V. Microwave-Assisted Solvent Extraction of Environmental Samples. *TrAC—Trends Anal. Chem.* **2000**, *19*, 229–248. [CrossRef]
157. He, B.; Zhang, L.L.; Yue, X.Y.; Liang, J.; Jiang, J.; Gao, X.L.; Yue, P.X. Optimization of Ultrasound-Assisted Extraction of Phenolic Compounds and Anthocyanins from Blueberry (Vaccinium Ashei) Wine Pomace. *Food Chem.* **2016**, *204*, 70–76. [CrossRef] [PubMed]

158. Dai, J.; Mumper, R.J. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. *Molecules* **2010**, *15*, 7313–7352. [CrossRef] [PubMed]
159. Pompeu, D.; Silva, E.; Rogez, H. Optimisation of the Solvent Extraction of Phenolic Antioxidants from Fruits of Euterpe Oleracea Using Response Surface Methodology. *Bioresour. Technol.* **2009**, *100*, 6076–6082. [CrossRef] [PubMed]
160. Monroy, Y.M.; Rodrigues, R.A.; Sartoratto, A.; Cabral, F.A. Optimization of the Extraction of Phenolic Compounds from Purple Corn Cob (*Zea mays* L.) by Sequential Extraction Using Supercritical Carbon Dioxide, Ethanol and Water as Solvents. *J. Supercrit. Fluids* **2013**, *116*, 10–19. [CrossRef]
161. Zhu, Y.; Li, T.; Fu, X.; Abbasi, A.M.; Zheng, B.; Liu, R.H. Phenolics Content, Antioxidant and Antiproliferative Activities of Dehulled Highland Barley (*Hordeum vulgare* L.). *J. Funct. Foods* **2015**, *19*, 439–450. [CrossRef]
162. Zhu, Y.; Li, T.; Fu, X.; Brennan, M.; Abbasi, A.M.; Zheng, B.; Liu, R.H. The Use of an Enzymatic Extraction Procedure for the Enhancement of Highland Barley (*Hordeum Vulgare* L.) Phenolic and Antioxidant Compounds. *Int. J. Food Sci. Technol.* **2016**, *51*, 1916–1924. [CrossRef]
163. Varun, T.K.; Senani, S.; Jayapal, N.; Chikkerur, J.; Roy, S.; Tekulapally, V.B.; Gautam, M.; Kumar, N. Extraction of Chitosan and Its Oligomers from Shrimp Shell Waste, Their Characterization and Antimicrobial Effect. *Vet. World* **2017**, *10*, 170–175. [CrossRef]
164. Riveiro, E.; González, B.; Domínguez, Á. Extraction of Adipic, Levulinic and Succinic Acids from Water Using TOPO-Based Deep Eutectic Solvents. *Sep. Purif. Technol.* **2020**, *241*, 116692. [CrossRef]
165. Marták, J.; Schlosser, Š. Extraction of Lactic Acid by Phosphonium Ionic Liquids. *Sep. Purif. Technol.* **2007**, *57*, 483–494. [CrossRef]
166. Slak, J.; Pomeroy, B.; Kostyniuk, A.; Grilc, M.; Likozar, B. A Review of Bio-Refining Process Intensification in Catalytic Conversion Reactions, Separations and Purifications of Hydroxymethylfurfural (HMF) and Furfural. *Chem. Eng. J.* **2022**, *429*, 132325. [CrossRef]
167. Galanakis, C.; Goulas, V.; Tsakona, S.; Manganaris, G.; Gekas, V. A Knowledge Base for The Recovery of Natural Phenols with Different Solvents. *Int. J. food Prop.* **2013**, *16*, 382–396. [CrossRef]
168. Hansen, C.M. *The Three Dimensional Solubility Parameter*; Danish Technical Press: Copenhagen, Denmark, 1967.
169. Hansen, C.M. Methods of Characterization—Surfaces. In *Hansen Solubility Parameters: A Users Handbook*, 2nd ed.; CRC Press: Raton, FL, USA, 2007; pp. 113–123. ISBN 9781420006834.
170. Moulik, S.; Bukke, V.; Sajja, S.C.; Sridhar, S. Chitosan-Polytetrafluoroethylene Composite Membranes for Separation of Methanol and Toluene by Pervaporation. *Carbohydr. Polym.* **2018**, *193*, 28–38. [CrossRef] [PubMed]
171. Huang, K.; Mohan, M.; George, A.; Simmons, B.A.; Xu, Y.; Gladden, J.M. Integration of Acetic Acid Catalysis with One-Pot Protic Ionic Liquid Configuration to Achieve High-Efficient Biorefinery of Poplar Biomass. *Green Chem.* **2021**, *23*, 6036–6049. [CrossRef]
172. Qin, C.; Li, H.; Xiao, Q.; Liu, Y.; Zhu, J.; Du, Y. Water-Solubility of Chitosan and Its Antimicrobial Activity. *Carbohydr. Polym.* **2006**, *63*, 367–374. [CrossRef]
173. Yan, X.; Zhang, G.; Zhao, J.; Ma, M.; Bao, X.; Zeng, Z.; Gong, X.; Yu, P.; Wen, X.; Gong, D. Influence of Phenolic Compounds on the Structural Characteristics, Functional Properties and Antioxidant Activities of Alcalase-Hydrolyzed Protein Isolate from Cinnamomum Camphora Seed Kernel. *LWT* **2021**, *148*, 111799. [CrossRef]
174. Zuorro, A.; Iannone, A.; Lavecchia, R. Water–Organic Solvent Extraction of Phenolic Antioxidants from Brewers' Spent Grain. *Processes* **2019**, *7*, 126. [CrossRef]
175. Sacchi, K.L.; Bisson, L.F.; Adams, D.O. A Review of the Effect of Winemaking Techniques on Phenolic Extraction in Red Wines. *Am. J. Enol. Vitic.* **2005**, *56*, 197–206.
176. Silva, A.M.; Lago, J.P.; Pinto, D.; Moreira, M.M.; Grosso, C.; Cruz Fernandes, V.; Delerue-Matos, C.; Rodrigues, F. Salicornia Ramosissima Bioactive Composition and Safety: Eco-Friendly Extractions Approach (Microwave-Assisted Extraction vs. Conventional Maceration). *Appl. Sci.* **2021**, *11*, 4744. [CrossRef]
177. Luque de Castro, M.D.; Priego-Capote, F. Soxhlet Extraction: Past and Present Panacea. *J. Chromatogr. A* **2010**, *1217*, 2383–2389. [CrossRef]
178. Medini, F.; Bourgou, S.; Lalancette, K.G.; Snoussi, M.; Mkadmini, K.; Coté, I.; Abdelly, C.; Legault, J.; Ksouri, R. Phytochemical Analysis, Antioxidant, Anti-Inflammatory, and Anticancer Activities of the Halophyte Limonium Densiflorum Extracts on Human Cell Lines and Murine Macrophages. *S. Afr. J. Bot.* **2015**, *99*, 158–164. [CrossRef]
179. Fredsgaard, M.; Hulkko, L.S.S.; Chaturvedi, T.; Thomsen, M.H. Process Simulation and Techno-Economic Assessment of Salicornia Sp. Based Jet Fuel Refinery through Hermetia Illucens Sugars-to-Lipids Conversion and HEFA Route. *Biomass Bioenergy* **2021**, *150*, 106142. [CrossRef]
180. Dalmeida Daffodil, E.; Rajalakshmi, K.; Mohan, V.R. Antioxidant Activity, Total Phenolics and Flavonoids of Salicornia Brachiata Rox. Leaf Extracts (Chenopodiaceae). *World J. Pharm. Pharm. Sci.* **2014**, *2*, 352–366.
181. Zhang, J.; Wen, C.; Zhang, H.; Duan, Y.; Ma, H. Recent Advances in the Extraction of Bioactive Compounds with Subcritical Water: A Review. *Trends Food Sci. Technol.* **2020**, *95*, 183–195. [CrossRef]
182. Yu, X.-M.; Zhu, P.; Zhong, Q.-P.; Li, M.-Y.; Ma, H.-R. Subcritical Water Extraction of Antioxidant Phenolic Compounds from XiLan Olive Fruit Dreg. *J. Food Sci. Technol.* **2015**, *52*, 5012–5020. [CrossRef]
183. Aliakbarian, B.; Fathi, A.; Perego, P.; Dehghani, F. Extraction of Antioxidants from Winery Wastes Using Subcritical Water. *J. Supercrit. Fluids* **2012**, *65*, 18–24. [CrossRef]
184. Duba, K.S.; Casazza, A.A.; Mohamed, H.B.; Perego, P.; Fiori, L. Extraction of Polyphenols from Grape Skins and Defatted Grape Seeds Using Subcritical Water: Experiments and Modeling. *Food Bioprod. Process.* **2015**, *94*, 29–38. [CrossRef]

185. Mazzutti, S.; Salvador Ferreira, S.R.; Herrero, M.; Ibañez, E. Intensified Aqueous-Based Processes to Obtain Bioactive Extracts from Plantago Major and Plantago Lanceolata. *J. Supercrit. Fluids* **2017**, *119*, 64–71. [CrossRef]
186. Pourali, O.; Asghari, F.S.; Yoshida, H. Production of Phenolic Compounds from Rice Bran Biomass under Subcritical Water Conditions. *Chem. Eng. J.* **2010**, *160*, 259–266. [CrossRef]
187. Friedman, M.; Jürgens, H.S. Effect of PH on the Stability of Plant Phenolic Compounds. *J. Agric. Food Chem.* **2000**, *48*, 2101–2110. [CrossRef]
188. Peanparkdee, M.; Patrawart, J.; Iwamoto, S. Physicochemical Stability and in Vitro Bioaccessibility of Phenolic Compounds and Anthocyanins from Thai Rice Bran Extracts. *Food Chem.* **2020**, *329*, 127157. [CrossRef] [PubMed]
189. Dawidowicz, A.L.; Typek, R. The Influence of PH on the Thermal Stability of 5-O-Caffeoylquinic Acids in Aqueous Solutions. *Eur. Food Res. Technol.* **2011**, *233*, 223–232. [CrossRef]
190. Wang, D.; Liu, J.; Qiu, S.; Wang, J.; Song, G.; Chu, B.; Li, L.; Xiao, G.; Gong, J.; Zheng, F. Ultrasonic Degradation Kinetics and Isomerization of 3- and 4-O-Caffeoylquinic Acid at Various PH: The Protective Effects of Ascorbic Acid and Epigallocatechin Gallate on Their Stability. *Ultrason. Sonochem.* **2021**, *80*, 105812. [CrossRef] [PubMed]
191. Zhao, Y.K.; Cao, Q.E.; Liu, H.T.; Wang, K.T.; Yan, A.X.; Hu, Z.D. Determination of Baicalin, Chlorogenic Acid and Caffeic Acid in Traditional Chinese Medicinal Preparations by Capillary Zone Electrophoresis. *Chromatographia* **2000**, *51*, 483–486. [CrossRef]
192. Narita, Y.; Inouye, K. Degradation Kinetics of Chlorogenic Acid at Various PH Values and Effects of Ascorbic Acid and Epigallocatechin Gallate on Its Stability under Alkaline Conditions. *J. Agric. Food Chem.* **2013**, *61*, 966–972. [CrossRef]
193. Bussemaker, M.J.; Zhang, D. Effect of Ultrasound on Lignocellulosic Biomass as a Pretreatment for Biorefinery and Biofuel Applications. *Ind. Eng. Chem. Res.* **2013**, *52*, 3563–3580. [CrossRef]
194. Ríos-González, L.J.; Medina-Morales, M.A.; Rodríguez-De la Garza, J.A.; Romero-Galarza, A.; Medina, D.D.; Morales-Martínez, T.K. Comparison of Dilute Acid Pretreatment of Agave Assisted by Microwave versus Ultrasound to Enhance Enzymatic Hydrolysis. *Bioresour. Technol.* **2021**, *319*, 124099. [CrossRef]
195. Bussemaker, M.J.; Xu, F.; Zhang, D. Manipulation of Ultrasonic Effects on Lignocellulose by Varying the Frequency, Particle Size, Loading and Stirring. *Bioresour. Technol.* **2013**, *148*, 15–23. [CrossRef]
196. Wang, Z.; Hou, X.; Sun, J.; Li, M.; Chen, Z.; Gao, Z. Comparison of Ultrasound-Assisted Ionic Liquid and Alkaline Pretreatment of Eucalyptus for Enhancing Enzymatic Saccharification. *Bioresour. Technol.* **2018**, *254*, 145–150. [CrossRef]
197. Fang, Z.; Richard, L.; Smith, J.; Qi, X. Production of Biofuels and Chemicals with Ultrasound. In *Biofuels and Biorefineries*; Fang, Z., Smith, R.L., Qi, X., Eds.; Springer: Dordrecht, The Netherlands, 2015; Volume 4.
198. Corbin, C.; Fidel, T.; Leclerc, E.A.; Barakzoy, E.; Sagot, N.; Falguiéres, A.; Renouard, S.; Blondeau, J.P.; Ferroud, C.; Doussot, J.; et al. Development and Validation of an Efficient Ultrasound Assisted Extraction of Phenolic Compounds from Flax (*Linum usitatissimum* L.) Seeds. *Ultrason. Sonochem.* **2015**, *26*, 176–185. [CrossRef]
199. Padalino, L.; Costa, C.; Del Nobile, M.A.; Conte, A. Extract of Salicornia Europaea in Fresh Pasta to Enhance Phenolic Compounds and Antioxidant Activity. *Int. J. Food Sci. Technol.* **2019**, *54*, 3051–3057. [CrossRef]
200. Zikeli, F.; Ters, T.; Fackler, K.; Srebotnik, E.; Li, J. Wheat Straw Lignin Fractionation and Characterization as Lignin-Carbohydrate Complexes. *Ind. Crop. Prod.* **2016**, *85*, 309–317. [CrossRef]
201. Bartolomé, B.; Gómez-Cordovés, C. Barley Spent Grain: Release of Hydroxycinnamic Acids (Ferulic Andp-Coumaric Acids) by Commercial Enzyme Preparations. *J. Sci. Food Agric.* **1999**, *79*, 435–439. [CrossRef]
202. Torres-Mancera, M.T.; Cordova-Lopez, J.; Rodriguez-Serrano, G.; Roussos, S.; Ramirez-Coronel, M.A.; Favela-Torres, E.; Saucedo-Castaneda, G. Enzymatic Extraction of Hydroxycinnamic Acids from Coffee Pulp. *Food Technol. Biotechnol.* **2011**, *49*, 369–373.
203. Faulds, C.B.; Robertson, J.A.; Waldron, K.W. Effect of PH on the Solubilization of Brewers' Spent Grain by Microbial Carbohydrases and Proteases. *J. Agric. Food Chem.* **2008**, *56*, 7038–7043. [CrossRef]
204. Cristina, C.; Lucia, P.; Sara, S.; Francesco, S.; Nobile, D.; Alessandro, M.; Conte, A.; Amalia, C. Study of the Efficacy of Two Extraction Techniques from Crithmum Maritimum and Salicornia Europaea. *J. Food Nutr. Res.* **2018**, *6*, 456–463. [CrossRef]
205. Huang, H.J.; Ramaswamy, S.; Tschirner, U.W.; Ramarao, B.V. A Review of Separation Technologies in Current and Future Biorefineries. *Sep. Purif. Technol.* **2008**, *62*, 1–21. [CrossRef]
206. Galanakis, C.M.; Tornberg, E.; Gekas, V. Clarification of High-Added Value Products from Olive Mill Wastewater. *J. Food Eng.* **2010**, *99*, 190–197. [CrossRef]
207. Galanakis, C.M. Separation of Functional Macromolecules and Micromolecules: From Ultrafiltration to the Border of Nanofiltration. *Trends Food Sci. Technol.* **2015**, *42*, 44–63. [CrossRef]
208. Castro-Muñoz, R.; Yáñez-Fernández, J.; Fíla, V. Phenolic Compounds Recovered from Agro-Food by-Products Using Membrane Technologies: An Overview. *Food Chem.* **2016**, *213*, 753–762. [CrossRef]
209. Stiger-Pouvreau, V.; Jégou, C.; Cérantola, S.; Guérard, F.; Lann, K. Le Phlorotannins in Sargassaceae Species from Brittany (France): Interesting Molecules for Ecophysiological and Valorisation Purposes. In *Advances in Botanical Research*; Sibout, R., Ed.; Academic Press Inc.: Cambridge, MA, USA, 2014; Volume 71, pp. 379–411.
210. Kim, J.Y.; Cho, J.Y.; Ma, Y.K.; Park, K.Y.; Lee, S.H.; Ham, K.S.; Lee, H.J.; Park, K.H.; Moon, J.H. Dicaffeoylquinic Acid Derivatives and Flavonoid Glucosides from Glasswort (*Salicornia herbacea* L.) and Their Antioxidative Activity. *Food Chem.* **2011**, *125*, 55–62. [CrossRef]
211. Xavier, L.; Freire, M.S.; Vidal-Tato, I.; González-Álvarez, J. Aqueous Two-Phase Systems for the Extraction of Phenolic Compounds from Eucalyptus (*Eucalyptus globulus*) Wood Industrial Wastes. *J. Chem. Technol. Biotechnol.* **2014**, *89*, 1772–1778. [CrossRef]

212. Bokhary, A.; Leitch, M.; Liao, B.Q. Liquid–Liquid Extraction Technology for Resource Recovery: Applications, Potential, and Perspectives. *J. Water Process Eng.* **2021**, *40*, 101762. [CrossRef]
213. Meyer, V.R. *Practical High-Performance Liquid Chromatography*, 3rd ed.; John Wiley & Sons: Hoboken, NJ, USA, 1999.
214. Hanai, T. HPLC A Practical Guide. In *Chromatography Monographs*; Smith, R.M., Ed.; Royal Society of Chemistry: Cambridge, UK, 1999.
215. Snyder, L.R.; Kirkland, J.J.; Glajch, J.L.. *Practical HPLC Method Development*, 2nd ed.; Wiley-Interscience: Hoboken, NJ, USA, 1997.
216. Teigiserova, D.A.; Hamelin, L.; Thomsen, M. Review of High-Value Food Waste and Food Residues Biorefineries with Focus on Unavoidable Wastes from Processing. *Resour. Conserv. Recycl.* **2019**, *149*, 413–426. [CrossRef]
217. Valanciene, E.; Jonuskiene, I.; Syrpas, M.; Augustiniene, E.; Matulis, P.; Simonavicius, A.; Malys, N. Advances and Prospects of Phenolic Acids Production, Biorefinery and Analysis. *Biomolecules* **2020**, *10*, 874. [CrossRef] [PubMed]
218. Latif, Z.; Sarker, S.D. Isolation of Natural Products by Preparative High Performance Liquid Chromatography (Prep-HPLC). *Methods Mol. Biol.* **2012**, *864*, 255–274. [CrossRef]

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