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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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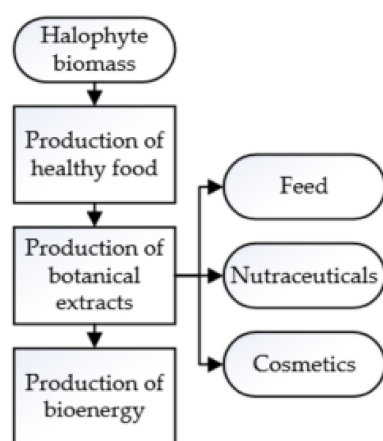
## 1. Introduction

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, whereas residual 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, neuro-protective, 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  $2.01 \pm 0.04$  mgCE/gDM [32] and  $20.3 \pm 0.98$  mgCE/gDM [33], and in *Suaeda* species between  $1.21 \pm 0.05$  mgCE/gDM [32] and  $15.76 \pm 1.43$  mgCE/g [34]. The highest concentration of proanthocyanidins ( $118.43 \pm 11.79$  mgCE/gDM), measured from water extract, was found from *Tamarix africana* [31]. For comparison,  $17.28 \pm 1.95$  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-Bechlaghem et al. [31], who used the modified method by Julkunen-Tiitto [36].

Plant Species	Solvent	Method	Concentration [mgCE/gDM]	Ref.
<i>Arthrocnemum macrostachyum</i>	Water	Vanillin assay	$7.50 \pm 0.80$	[31]
<i>Aster tripolium</i>	Acetone (80%)	DMACA-HCl	Not detected	[3]
<i>Cakile maritimum</i>	Methanol	Vanillin assay	$14.94 \pm 0.04$	[37]
<i>Carpobrotus edulis</i>	Ethanol	DMACA-HCl	$20.3 \pm 0.98$	[33]
<i>Cladium mariscus</i>	Acetone (80%)	DMACA-HCl	$38.7 \pm 2.21$	[3]

Table 1. Cont.

Plant Species	Solvent	Method	Concentration [mgCE/gDM]	Ref.
<i>Crithmum maritimum</i>	n/a	n/a	1.06 ± 0.77	[38]
	Acetone (80%)		0.63	[39]
<i>Inula crithmoides</i>	Acetone (80%)		0.95	[39]
<i>Ipomoea pes-caprae</i>	Methanol (80%)		19.67 ± 2.54	[34]
<i>Limonium delicatulum</i>	Methanol (80%)	Vanillin assay	48.38 ± 0.75	[40]
<i>Mesembryanthemum edule</i>	Methanol		14.2 ± 0.9	[28]
	Methanol		9.51 ± 1.07	[4]
	Methanol		7.16	[10]
<i>Mesembryanthemum nodiflorum</i>	Methanol	DMACA-HCl	2.01 ± 0.04	[32]
<i>Pluchea lanceolata</i>	Methanol (80%)		20.52 ± 4.32	[34]
<i>Reaumuria vermiculata</i>	Dichloromethane	Vanillin assay	27.98 ± 1.01	[41]
<i>Retama raetam</i>	Acetone (60%)		10.16	[42]
<i>Salicornia ramosissima</i>	Acetone (70%)	DMACA-HCl	32.5 ± 4.6	[5]
	Acetone (80%)		Not detected	[3]
<i>Salsola kali</i>	Methanol		2.03 ± 0.62	[43]
<i>Salsola vermiculata</i>	Acetone (80%)	Vanillin assay	Not detected	[3]
<i>Salvadora persica</i>	Methanol (80%)		22.35 ± 2.87	[34]
<i>Sarcocornia fruticosa</i>	Methanol	DMACA-HCl	0.46 ± 0.04	[32]
<i>Sarcocornia perennis</i>	Methanol		1.09	[44]
	Methanol		7.76 ± 0.28	[31]
	Methanol (80%)	Vanillin assay	15.76 ± 1.43	[34]
<i>Suaeda fruticosa</i>	Acetone		1.50 ± 0.13	[45]
	Methanol	DMACA-HCl	1.21 ± 0.05	[32]
<i>Tamarix africana</i>	Water		118.43 ± 11.79	[31]
<i>Tamarix gallica</i>	Methanol	Vanillin assay	17.28 ± 1.95	[35]
<i>Thespesia populnea</i>	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.
<i>Artemisia santonicum</i>	Methanol	Folin–Ciocalteu	212.71 ± 0.68	[49]
<i>Arthrocnemum macrostachyum</i>	Water		10.24 ± 0.01	[31]
	Ethyl acetate		29.54 ± 0.78	[52]
<i>Aster tripolium</i>	Acetone (80%)		223 ± 2.63	[3]
	Methanol		144.75 ± 0.59	[49]
<i>Cladium mariscus</i>	Acetone (80%)		254 ± 2.26	[3]
<i>Carpobrotus edulis</i>	Ethanol		272.82 ± 5.59	[33]
<i>Crithmum maritimum</i>	Acetone (80%)		7.9	[39]
	Water		35.3 ± 2.98	[51]
<i>Halimone portulacoides</i>	Ethyl acetate		14.59 ± 0.21	[52]
<i>Inula crithmoides</i>	Acetone (80%)		14.1	[39]
<i>Ipomoea pes-caprae</i>	Methanol (80%)		54.21 ± 2.31	[34]
<i>Limoniastrum monopetalum</i>	Methanol		15.85	[55]
<i>Limonium algarvense</i>	Methanol		228 ± 2	[50]
<i>Limonium delicatulum</i>	Acetone (80%)		92.9 ± 1.45	[40]
<i>Limonium densiflorum</i>	Methanol		56.18 ± 0.92	[56]
<i>Lycium shawii</i>	Ethanol (95%)		52.72 ± 3.17	[53]
	Water		212.2 ± 4.8	[28]
<i>Mesembryanthemum edule</i>	Methanol		68.75 ± 1.07	[4]
	Methanol		70.07	[10]
<i>Mesembryanthemum nodiflorum</i>	Methanol		6.75 ± 0.08	[32]
<i>Pluchea lanceolata</i>	Methanol (80%)		42.28 ± 3.58	[34]
<i>Reaumuria vermiculata</i>	Methanol		117.12 ± 3.31	[41]
<i>Retama raetam</i>	Water		137.0	[42]
<i>Rumex vesicarius</i>	Ethanol (95%)		28.54 ± 1.13	[53]
<i>Salicornia europaea</i>	Ethyl acetate		24.46 ± 0.16	[52]
	Ethanol/water		~11	[57]
<i>Salicornia ramosissima</i>	Methanol		58.20 ± 0.44	[49]
	Acetone (80%)	74.1 ± 2.49	[3]	
	Acetone (80%)	12.9 ± 1.9	[5]	
	Water	15.02 ± 2.01	[58]	
<i>Salsola kali</i>	Methanol	17.23 ± 1.0	[43]	
	Methanol	17.23	[10]	



Table 2. Cont.

Plant Species	Solvent	Method	Concentration [mgCAE/gDM]	Ref.
<i>Salsola vermiculata</i>	Acetone (80%)		133 ± 2.62	[3]
<i>Salvadora persica</i>	Methanol (80%)		58.23 ± 3.54	[34]
<i>Sarcocornia fruticosa</i>	Methanol		5.69 ± 0.06	[32]
	Methanol		11.09 ± 0.08	[59]
<i>Suaeda fruticosa</i>	Acetone		31.7 ± 0.51	[45]
	Methanol/water		47.73 ± 1.17	[31]
	Methanol (80%)	Folin–Ciocalteu	46.54 ± 4.32	[34]
<i>Suaeda maritima</i>	Methanol		16.6 ± 0.1	[32]
	Methanol		62.88 ± 0.88	[49]
<i>Tamarix africana</i>	Water		61.06 ± 0.40	[31]
<i>Tamarix gallica</i>	Methanol		135.35 ± 7.70	[35]
	Methanol		70.56	[10]
<i>Thespesia populnea</i>	Methanol (80%)		63.91 ± 5.28	[34]
<i>Zaleya pentandra</i>	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 fruticosa*, 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.
<i>Arthrocnemum macrostachyum</i>	Acetone/hexane		28.3 ± 7.1	mg/100 gDM	[6]
<i>Aster tripolium</i>	Ethanol		~51	mg/cm <sup>2</sup>	[68]
<i>Halimione portulacoides</i>	Acetone		95.04 ± 23.47	µg/gFW	[67]
<i>Mesembryanthemum nodiflorum</i>	Methanol	Spectrophotometry	~280	mg/100 gDM	[32]
<i>Salicornia bigelovii</i>	Acetone		569.1 ± 9.10	mg/kgFW	[22]
<i>Salicornia brachiata</i>	Acetone		746.5 ± 88.2	µg/gDM	[77]
<i>Salicornia europaea</i>	Acetone		~1.25	mg/gFW	[73]
<i>Salicornia neei</i>	Methanol	HPLC	233.3 ± 42.5	µg/gDM	[78]
<i>Salicornia persica</i>	Acetone		~1.21	mg/gFW	[73]
	Acetone		~325	µg/gFW	[63]
<i>Salicornia prostrata</i>	Acetone		~0.14	mg/gFW	[79]
<i>Salicornia ramosissima</i>	Acetone/hexane		21.56 ± 3.45	mg/100 gDM	[6]
	Methanol		~290	mg/100 gDM	[32]
<i>Sarcocornia fruticosa</i>	Acetone	Spectrophotometry	102.01 ± 18.23	µg/gFW	[67]
	Acetone		~350	µg/gFW	[63]
<i>Sarcocornia perennis</i>	Acetone/hexane		14.78 ± 2.33	mg/100 gDM	[6]
<i>Suaeda maritima</i>	Methanol		~280	mg/100 gDM	[32]
<i>Suaeda prostrata</i>	Acetone		~0.08	mg/gFW	[79]
<i>Tamarix gallica</i>	Acetone		~180	mg	[70]



## 6. Carotenoids

Carotenoids, which can be divided into carotenes (e.g.,  $\beta$ -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.	
<i>Arthrocnemum macrostachyum</i>	Acetone	Spectrophotometry	210 ± 10	mg/100 gDM	[6]	
<i>Aster tripolium</i>	Ethanol		~8	mg/cm <sup>2</sup>	[68]	
<i>Halimione portulacoides</i>	Acetone		51.47 ± 17.76	µg/gFW	[67]	
<i>Ipomoea pes-caprae</i>	Acetone (90%)		0.61 ± 0.01	mg/g	[34]	
<i>Mesembryanthemum nodiflorum</i>	Methanol		~21	mg/100 gDM	[32]	
<i>Pluchea lanceolata</i>	Acetone (90%)		0.07 ± 0.02	mg/g	[34]	
<i>Salicornia bigelovii</i>	Hexane		159.0 ± 5.74	mg/kgFW	[22]	
<i>Salicornia brachiata</i>	Acetone		433.8 ± 46.0	µg/gDM	[77]	
<i>Salicornia europaea</i>	Acetone		~0.43	mg/gFW	[73]	
<i>Salicornia neei</i>	Methanol		HPLC	28.71 ± 7.52	µg/gDM	[78]
<i>Salicornia persica</i>	Acetone	Spectrophotometry	~0.44	mg/gFW	[73]	
	Acetone		54.5	µg/gFW	[63]	
<i>Salicornia prostrata</i>	Acetone		~0.25	mg/gFW	[79]	
<i>Salicornia ramosissima</i>	Ethyl acetate		HPLC	3.49	mg/100 gFW	[5]
	Acetone		290 ± 20	mg/100 gDM	[6]	
<i>Salvadora persica</i>	Acetone (90%)		0.84 ± 0.02	mg/g	[34]	
	DMF		11.4 ± 2.5	µg/100 gDM	[84]	
<i>Sarcocornia fruticosa</i>	Methanol		~16	mg/100 gDM	[32]	
	Acetone		56.6	µg/gFW	[63]	
	Acetone		34.76 ± 9.03	µg/gFW	[67]	
<i>Sarcocornia perennis</i>	Acetone	Spectrophotometry	280 ± 10	mg/100 gDM	[6]	
<i>Suaeda fruticosa</i>	Acetone (90%)	0.56 ± 0.01	mg/g	[34]		
<i>Suaeda maritima</i>	Methanol	~12	mg/100 gDM	[32]		
<i>Suaeda prostrata</i>	Acetone	~0.19	mg/gFW	[79]		
<i>Tamarix gallica</i>	Acetone	~70	mg	[70]		
<i>Thespesia populnea</i>	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  $\beta$ -carotene and the total xanthophyll content of *Salicornia neei* biomass to be  $0.99 \pm 0.41 \mu\text{g/gDM}$  and  $27.72 \pm 7.12 \mu\text{g/gDM}$ , 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  $2.37 \pm 0.12 \text{ mg/100gFW}$  and  $1.12 \pm 0.05 \text{ mg/100gFW}$  of  $\beta$ -carotene and lutein, respectively. Alongside measuring the total carotenoids using a spectrophotometric method, Castañeda-Loaiza et al. [32] determined the amount of carotenenes 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  $\beta$ -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  $\beta$ -carotene ( $7.60 \pm 2.06 \mu\text{g/gFW}$  and  $5.53 \pm 0.94 \mu\text{g/gFW}$ , respectively), lutein ( $17.09 \pm 6.04 \mu\text{g/gFW}$  and  $14.93 \pm 3.91 \mu\text{g/gFW}$ , respectively) and other xanthophylls ( $26.78 \pm 9.66 \mu\text{g/gFW}$  and  $14.09 \pm 4.18 \mu\text{g/gFW}$ , 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.
<i>Arthrocnemum indicum</i>	m-Phosphoric acid (4.5%)	HPLC-UV	19.17 ± 0.50 (C)	mg/100 gFW	[90]
<i>Halocnemum strobilaceum</i>	m-Phosphoric acid (4.5%)		7.38 ± 0.54 (C)	mg/100 gFW	
<i>Mesembryanthemum nodiflorum</i>	Ammonium acetate/methanol (50:50)	HPLC-PDA	~0.5 (C)	g/100 gDM	[32]
			~24 (B6)	mg/100 gDM	
<i>Salicornia bigelovii</i>	Oxalic acid solution	Indophenol titration	58.4 ± 1.39 (C)	mg/kgFW	[22]
<i>Salicornia ramosissima</i>	Ammonium acetate/methanol (50:50)	HPLC-PDA	30.4 ± 2.0 (B1)	µg/100 gFW	[5]
			2.6 ± 0.1 (B6)	µg/100 gFW	
<i>Salvadora persica</i>	Trichloroacetic acid (6%)	Spectrophotometry	68.0 ± 15.9 (C)	mg/100 gDM	[84]
<i>Sarcocornia fruticosa</i>	Ammonium acetate/methanol (50:50)	HPLC-PDA	~1.0 (C)	g/100 gDM	[32]
			~10 (B6)	mg/100 gDM	
<i>Suaeda aegyptiaca</i>	Methanol	HPLC-DAD	181 ± 2.3 (B6)	mg/kg	[89]
			118 ± 2.2 (B9)	mg/kg	
			466 ± 2.5 (B12)	mg/kg	
<i>Suaeda fruticosa</i>	m-Phosphoric acid (4.5%)	HPLC-UV	2.46 ± 0.07 (C)	mg/100 gFW	[90]
<i>Suaeda matritima</i>	Ammonium acetate/methanol (50:50)	HPLC-PDA	~3 (C)	g/100 gDM	[32]
			~9.5 (B6)	mg/100 gDM	
<i>Suaeda vera</i>	Methanol	HPLC-DAD	102 ± 1.4 (B6)	mg/kg	[89]
			118 ± 2.2 (B9)	mg/kg	
			271 ± 1.7 (B12)	mg/kg	
<i>Thespesia populnea</i>	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,  $\alpha$ -tocopherol being reported as the most abundant in halophyte biomasses [11].

Ellouzi et al. [93] studied the effect of salt stress on the  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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.**  $\alpha$ -Tocopherol (vitamin E) concentrations measured from halophyte biomasses.

Plant Species	Solvent	Method	Concentration	Unit	Ref.
<i>Arthrocnemum indicum</i>	Hexane	HPLC-FL	2.12 ± 0.01	mg/100 gFW	[39]
<i>Arthrocnemum macrostachyum</i>	Hexane		8.74 ± 0.19	mg/100 gDM	[6]
<i>Cakile maritima</i>	Methanol		~200	µg/gDM	[93]
<i>Halocnemum strobilaceum</i>	Hexane	HPLC-PDA	3.35 ± 0.08	mg/100 gFW	[90]
<i>Mesembryanthemum nodiflorum</i>	Ethyl acetate		Not detected		[32]
<i>Salicornia ramosissima</i>	Ethyl acetate		241 ± 10	µg/100 gFW	[5]
	Hexane	HPLC-FL	1.14 ± 0.07	mg/100 gDM	[6]
<i>Sarcocornia fruticosa</i>	Ethyl acetate	HPLC-PDA	~18	mg/100 gDM	[32]
<i>Sarcocornia perennis</i>	Hexane	HPLC-FL	1.11 ± 0.10	mg/100 gDM	[6]
<i>Suaeda fruticosa</i>	Hexane		11.42 ± 0.14	mg/100 gFW	[90]
<i>Suaeda matritima</i>	Ethyl acetate	HPLC-PDA	~12.5	mg/100 gDM	[32]
<i>Teucrium alopecurus</i>	Hexane	HPLC-FL	316.25	mg/kg	[94]
<i>Teucrium polium</i>	Hexane		277.25	mg/kg	[94]
<i>Teucrium nabli</i>	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  $\beta$ -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  $\alpha$ -tocopherol content of *Suaeda fruticosa* and *Arthrocnemum 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  $\alpha$ -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, utilizing 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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