Dietary polyacetylenes, falcarinol and falcarindiol, prevents the formation of neoplastic lesions in the colon of azoxymethaneinduced rats

Kobæk Larsen, Morten; El-Houri, Rime; Christensen, Lars Porskjær; Fretté, Xavier; Baatrup, Gunnar

Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

NPC 2017 Denmark

Odense, 18-21 June 2017

SDU UNIVERSITY OF SOUTHERN DENMARK

7th Nordic Natural Products Conference
Odense, Denmark, 18–21 June 2017
Abstract book

Edited by Rime B. El-Houri, Xavier Fretté & Lars P. Christensen

Oral Presentations

NNPC 2017

Accelerating discovery of bioactive cyanobacterial natural products through application of novel methodologies

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Traditional approaches in natural products drug discovery have been highly effective in the discovery of clinically useful agents, accounting by some estimates for up to 70% of our approved drugs, either directly or by inspiring related agents to be chemically synthesized. Nevertheless, the field continues to pioneer new advances in terms of approaches and classes of organisms under study, striving to maintain this record of success while at the same time introducing new innovations. This is especially important given the critical issues of compound dereplication and the desire to discover new natural products that represent truly novel chemotypes. Fortunately, we have many highly sophisticated techniques available in analytical chemistry, molecular pharmacology, computational chemistry, synthetic organic chemistry, and chemical and molecular biology. Simultaneous integration of advances from these various fields is both challenging and highly enriching to natural products studies. We have sought to explore the unique bioactive compounds of marine cyanobacteria with the goal of identifying those of high significance to biomedicine. This presentation will describe several recent projects that have utilized integrated approaches and thereby enriched the discovery process, including genome-based drug discovery, total synthesis of natural products, innovations in mass spectrometry applied to natural product metabolomics, and automatic recognition technologies for rapidly analyzing NMR spectra.

Accelerating natural products-based drug lead discovery by targeting HPLC-HRMS-SPE-NMR analyses with three bioactivity-correlated techniques

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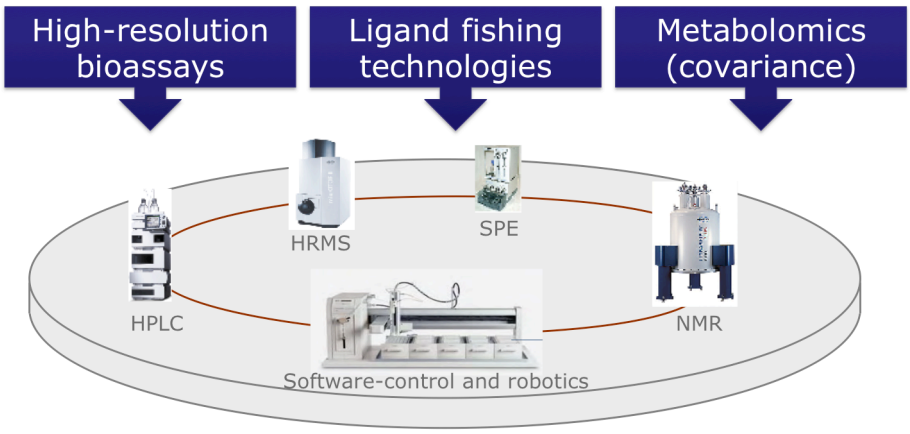
In recent years, hyphenation of analytical-scale high-performance liquid chromatography and high-resolution mass spectrometry with solid-phase extraction and nuclear magnetic resonance spectroscopy, *i.e.*, HPLC-HRMS-SPE-NMR, has proven successful for full structure elucidation directly from crude extracts without any prepurification steps.[1] This even includes acquisition of direct-detected ^{13}C NMR spectra[2] and database-assisted NMR structure elucidation.[3] However, the basic HPLC-HRMS-SPE-NMR setup does not give any information about the bioactivity of individual constituents in the crude extract. Thus, the recent development of microplate-based high-resolution bioassays,[4] ligand fishing[5] and bioactivity-correlated metabolomics[6] has allowed targeting subsequent HPLC-HRMS-SPE-NMR experiments towards the bioactive constituents only - as indicated in the figure below.



In this talk some recent examples of the successful use of high-resolution bioassays, ligand-fishing technologies and bioactivity-correlated metabolomics profiling in combination with HPLC-HRMS-SPE-NMR analysis will be given - with emphasis on key-enzymes in the management of type 2 diabetes.

Acknowledgements: The instrumental platform that made this research possible was acquired through grants from 'Apotekerfonden af 1991', The Carlsberg Foundation, The Danish Research Foundation for Strategic Research, The Danish Research Foundation for Basic Research, and the Danish Agency for Science, Technology and Innovation via the National Research Infrastructure funds. Arife Önder and numerous postdocs, PhD's and MSc students did contribute to development as well as application of the technologies.

References: 1. Vinther J.M. et al. (2015) *NMR-based metabolomics and hyphenated NMR techniques – a perfect match in natural products research* In *Ethnopharmacology – a Reader.* Heinrich, M.; Jäger, A.K. (Eds.) Wiley, Chichester, pp 63-74. 2. Wubshet S.G. et al. (2013) J. Chromatogr. A 1302:34-39. 3. Kongstad K.T. et al. (2013). Anal. Chem. 85:3183-3189. 4. Wubshet S.G. et al (2016). J. Nat. Prod. 79:1063-1072. 5. Wubshet S.G. et al. (2015) J. Nat. Prod. 78:2657-2665. 6. Liu Y. et al. (2017). Molecules 22: 41.

Raw drug trade analysis of *Garcinia* fruit in the Western Ghats of India using DNA barcoding for species identification and NMR for quantification of hydroxycitric acid content

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Garcinia fruits are widely used in traditional medicine and the fruits from several species are harvested commercially from the wild. The fruits are a rich source of (2S,3S)-hydroxycitric acid (HCA), a compound that has gained considerable attention as a promising anti-obesity agent, and commercial *Garcinia* based products are popular as dietary supplements worldwide. In this study, we assessed whether increasing demand for *Garcinia* fruit has spurred adulteration in the trade of raw herbal drugs based on *Garcinia* species in herbal markets in southern India. We assessed adulteration of the morphologically similar substrates using DNA barcoding, and NMR was used to quantify the content of (2S,3S)-HCA and its lactone (2S,3S)-HCAL in raw herbal drugs. Raw herbal drug analysis revealed that mostly *Garcinia gummi-gutta* and *Garcinia indica* were traded in the herbal markets, and DNA barcoding revealed that there was no adulteration beyond these two species. The content of (2S, 3S)-HCA in the Kodampuli and Kokum samples varied from 1.7% to 16.3% and the content of (2S,3S)-HCAL varied from 3.5% to 20.7% .

3D pharmacophore models as tools for virtual screening and target fishing

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Abstract: 3D pharmacophores have become an established and consolidated method for in-silico drug discovery as well as for the investigation of molecular mechanisms of natural products. Their main advantage is their ability to reflect the traditional way of thinking in terms of hit identification, hit expansion and lead optimization. The simplicity and descriptive character of such a 3D pharmacophore model thus enables clear communication and rapid feedback cycles between modeling and experimentalists. Despite the broad usage of the methodology, there are still several pitfalls and challenges for successful pharmacophore modeling – mainly related to the algorithmic challenge of flexibly fitting a molecule to a 3D pharmacophore model in a computationally efficient way. In this talk, several structure- and ligand-based 3D pharmacophore application studies will be presented in the context of both natural product profiling and medicinal chemistry as well as critically discussed in the context of virtual screening algorithms and overlay algorithms.

Presenting Northern African Natural Products Database (NANPDB): A web-accessible and downloadable resource for natural products from Northern Africa

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Natural products (NPs) are often regarded as sources of drugs, drug leads or simply as a "source of inspiration" for the discovery of novel drugs [1-2]. We have built the Northern African Natural Products Database (NANPDB) by collecting information on ~4,500 NPs, covering data available in the literature for the period from 1962 to 2016. The data covers compounds isolated mainly from plants, with contributions from some endophyte, animal (e.g. coral), fungal and bacterial sources. The compounds were identified from 617 source species, belonging to 146 families. Computed physicochemical properties, often used to predict drug metabolism and pharmacokinetics (DMPK) [3], as well as predicted toxicity information have been included for each compound in the dataset [4]. To the best of our knowledge, this is the largest collection of annotated natural compounds produced by native organisms from Northern Africa. While the database already includes well known drugs and drug leads [5], the medical potential of a majority of the molecules is yet to be investigated. The database could be useful for drug discovery campaigns, analysis of the bioactivity of selected compounds, or for the discovery of synthetic routes of secondary metabolites. The current version of NANPDB is available at http://african-compounds.org/nanpdb/.

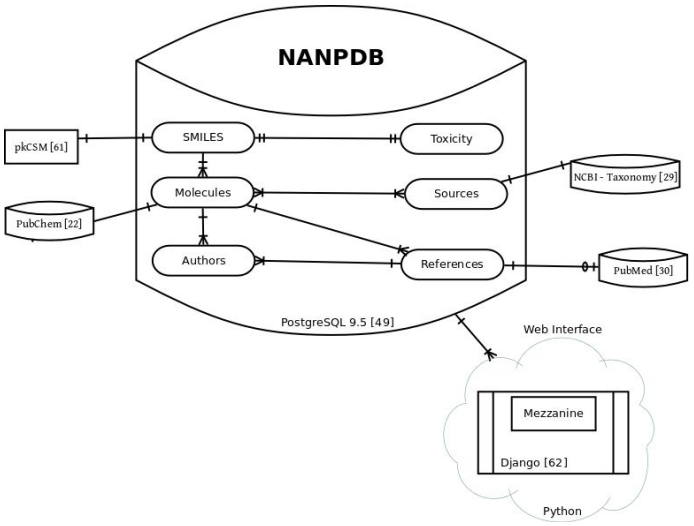

Figure 1: NANPDB database scheme.

Acknowledgements: FNK acknowledges a Georg Forster fellowship from the Alexander von Humboldt Foundation, Germany. CVS is currently a doctoral candidate financed by the German Academic Exchange Services (DAAD), Germany (Award number: 91611788). The authors also acknowledge the free academic license to use the JSDraw chemical structure editor. The authors also acknowledge the assistance of Dr. Douglas E. V. Pires for providing the toxicity predictions calculated with the pkCSM server.

References: 1. Harvey, A. L. et al. (2015) Nat. Rev. Drug Discov. 14: 111-129. 2. Rodrigues, T. et al. (2016) Nat. Chem. 8: 531-541. 3. Lipinski, C. A. et al. (1997) Adv. Drug Deliv. Rev. 23: 3-25. 4. Pires, D. E. V. et al. (2015) J. Med. Chem. 58: 4066-4072. 5. Risinger, A. L. et al. (2010) Cancer Lett. 291: 14-19.

Screening for potential α-glucosidase, α-amylase and PTP1B inhibitory constituents from selected Vietnamese plants used to treat type 2 diabetes

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Type-2 diabetes is affecting 246 million people worldwide [1]. In this study, 18 medicinal plants traditionally used in Vietnam for treatment of diabetes were investigated for inhibition of carbohydrate-hydrolysing enzymes and human recombinant protein-tyrosine phosphatase 1B enzyme (PTP1B) based on a recently developed microplate-based assay [2-4]. This enzyme is a key negative regulator of the insulin signaling pathway and is considered a promising potential therapeutic target, in particular for treatment of type-2 diabetes.

In the α-glucosidase assay, ethanol and water extracts of *Nepenthes mirabilis, Phyllanthus amarus*, *Phyllanthus urinaria*, *Lagerstroemia speciosa*, *Syzygium cumini*, *Rhizophora mucronata* and *Kandelia candel* had IC_{50} ranging from 3.3 to 39.7 µg/mL. Ethanol extracts of *K. candel* and *Ficus racemosa* inhibited α-amylase with IC_{50} 7.6 and 46.7 µg/mL. *P. amarus* and *P. urinaria* water extracts and *F. racemosa* ethanol extract were chosen for microfractionation, followed by bioassays. Only high-resolution α-glucosidase inhibition profiles of the first two extracts showed active compounds, which were isolated and identified as corilagin, repandusinic acid A, and mallotinin. IC_{50} of these compounds were 1.70 ± 0.03, 6.10 ± 0.10, and 3.76 ± 0.15 µM, respectively. Kinetics analysis revealed that corilagin displayed a mixed type mode of inhibition with K_i and $K_{i'}$ values of 2.37 ± 0.90 and 2.61 ± 0.61 µM, respectively, whereas repandusinic acid A and mallotinin competitively inhibited α-glucosidase with K_i values of 4.01 ± 0.47 and 0.65 ± 0.11 µM, respectively.

In the PTP1B assay, ethyl acetate extracts and butanol extracts of *N. mirabilis, P. amarus, P. urinaria, Ludwigia octavalvis, L. speciosa, Euphorbia hirta, S. cumini, F. racemosa, Pithecellobium ducle, Cassia fistula*, and *Pandanus odoratissimus* strongly inhibited PTP1B with IC_{50} ranging from 0.4 to 74.4 µg/mL. These active extracts were submitted to high-resolution PTP1B bioactivity profiling for identification of individual bioactive constituents. Ethyl acetate extract *F. racemosa* was chosen for further analysis and there PTP1B inhibitors have been isolated and identified as isoderrone, derrone and alpinumisoflavone using HPLC-HRMS-SPE-NMR.

Acknowledgements: Trinh B.T.D. is thankful to Vietnam Ministry of Education and Training for a scholarship and Son V. Dang for assistance in verifying collected plants. HPLC equipment used for obtaining high-resolution α-glucosidase, α-amylase and PTP1B inhibition profiles was obtained via a grant from The Carlsberg Foundation.

References: 1. Shaw J.E. et al (2010) Diabetes Res. Clin. Pract. 87: 4–14. 2. Schmidt J.S. et al (2012) Food Chem. 135: 1692-99. 3. Okutan L. et al. (2014) J. Agric. Food Chem. 62: 11465-7. 4. Tahtah Y. et al. (2016), Fitoterapia 110: 52–58.

Cultivation of *Stevia rebaudiana* in Denmark as raw material for a natural sweetener for organic food products in the EU

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Scientists from Aarhus University collaborate with several industrial partners in developing a Danish grown stevia (*Stevia rebaudiana*) [1] product that can be used as a sweetener in organic food products in the EU. Here we report the results of two years (2015-16) of cultivation experiments.1) A variety trial with seven clones/varieties selected in a screening trial in 2014. The varieties were grown at Aarslev research station (6 plants m^2) and harvested at two harvest times, after 5 and 6 month of growth, respectively. 2) An organic fertilizer experiment with four nitrogen levels (0, 80, 160 and 240 kg N ha^{-1}) and two potassium levels (122 and 246 kg K ha^{-1}). 3) An experiment with development of plant yield and steviol glycoside [2] content over the season (six harvest times from August to December). In all experiments the biomass yield parameters were recorded and content and quality of steviol glycosides were measured by HPLC [3].
Beside the cultivation experiments we are working on organic acceptable extraction and purification methods of the steviol glycosides from dried leaf material. The intention was to use membrane filtration to purify the extract but this has proven to be very difficult. We therefore have changed tactics and are now working on purification by use of other filtration techniques with active carbon, diatomaceous earth and ethanol/water eluents combined with crystallisation. The results of all the different experiments will be presented.

Acknowledgements: We gratefully acknowledge the funding from the GUDP programme under the Ministry of Environment and Food of Denmark
References: 1. Brandle et al. (1998) Can. J. Pl. Sci. 78: 527-536. 2. Ceunen & Geuns (2013). J. Nat. Prod. 76: 1201−1228. 3. Wöwer-Rieck et al. (2010) Eur. Food Res. Technol. 231: 581-588.

Production of natural blue colorants based on phycobiliproteins from *Arthrospira platensis* and stability studies

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In the recent years, there has been a growing tendency to avoid the use of artificial colorants in food products. However, the incorporation of natural colorants remains a challenge for food technologists, as these are typically less vivid and less stable than their synthetic alternatives. Phycocyanins from cyanobacteria are currently in the spotlight as promising new blue natural colorants. Their applications are, however, limited as they show poor stability in certain conditions of light, pH, and temperature. The blue colour of phycocyanins results from the presence of the chromophore phycocyanobilin (PCB). It is hypothesized that cleavage of PCB from the protein followed by careful product design is a possible approach to help overcoming the present stability issues of phycocyanin-based colorants. This research work presents a process for cleavage of PCB from phycocyanin by solvolysis as a first step towards the formulation of a new natural blue colorant. Stability studies on the free chromophore are also presented.

Acknowledgements: The financial support of the Danish Agency for Science Technology and Innovation [J nr.: 9-2013-1] is highly acknowledged.

Application of chemometric methods in processing of natural products: Case study of artemisinin from *Artemisia annua*

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Natural products, secondary metabolites produced by plants, are value added chemicals with unlimited chemical diversity and assume great significance for several industries such as food and pharmaceuticals [1]. In recent times, the demand for development of efficient process technologies for recovery of high value chemicals from plants has grown considerably due to lack of viable synthetic routes for such chemicals. In this regard, several multi-disciplinary approaches have been combined together in order to overcome challenges such as lack of information about impurities, low concentration of desired product etc. encountered during processing of natural products from plants [2,3]. One such approach reported before [4] involve extensive use of process analytical technology (PAT) tools such as HPLC-DAD, HPLC-MS/MS etc. for analysis of raw materials, in process materials and finished products. Major goal of such analysis is to define the process streams in terms of concentration of desired products and identification of impurities. However, use of these tools often generates enormous amount of data, making it difficult and time consuming to extract relevant chemical information manually. Therefore, application of chemometric methods to extract relevant chemical information from the analytical data can speed up the development of process for recovery of natural products from plants. In this work, application of chemometric method PARAFAC (parallel factorization) to extract the required chemical information from analytical data is demonstrated for recovery of an antimalarial drug, artemisinin, from leaves of plant *Artemisia annua*.

References: 1. Newman, D.J. et al. (2012) J. Nat. Prod. 75: 311-335. 2. Malwade, C.R. et al. (2013) Ind. Eng. Chem. Res. 52: 7157-7169. 3. Harjo B. (2004) Chem. Eng. Res. Des. 82: 1010-1028. 4. Malwade, C.R. et al. (2014) Ind. Eng. Chem. Res. 53: 5582-5589.

Effect of plant material, processing and storage of ramson (*Allium ursinum* L.) on allicin concentration and antibacterial effect against *L. monocytogenes, S. typhimurium* and *E. coli*.

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Ramsons *(Allium ursinum* L.*)* are known to contain compounds with antibacterial activity and may thus be of interest as an alternative preservation ingredient to protect against pathogenic bacteria in food products. In this study we investigated how the concentration of allicin varies with different plant organs, bulbs, stems, leaves, flowers and how bulb size affect the concentration. Furthermore we examined the effect of processing of bulbs into four products on allicin content. Products that potentially could be used as a preservation ingredient: freeze dried milled powder, oven dried milled powder, wet grinded pesto, wet grinded pesto with pasteurization. The four products were tested for allicin content after long term storage for up to 1.5 years at -20°C. Short term storage of dry ramson powder for 1 month at four different temperatures from -20°C to +40°C was also studied. We also investigated the effect of different plant organs, processing and long term storage of ramson on the antibacterial activity of processed bulbs against *Salmonella typhimurium, Listeria monocytogenes* and *Escherichia coli* in a BHI growth inhibition model. Results showed that bulbs and flowers have a higher concentration of allicin than leaves and stems and also display higher inhibitory activity against growth of all three bacteria strains. Allicin content on a dry weight basis was low in small bulbs and increases up to 4 g bulb size after which no further increase was seen. Allicin concentration was highest in freeze dried and oven dried milled products followed by wet grinded product and the wet grinded pasteurized product had the lowest concentration. One month storage at between -20° to +40° did not significantly alter the concentration of allicin in dry ramson powder. All processed bulb products showed strong antibacterial effects against all three bacteria strains in the BHI growth inhibition model and with little or no change over 1.5 years of storage. The effect against *Salmonella* and *E. coli* was stronger than against *Listeria*. The results suggest that ramson products may potentially be used as an alternative to preserve organic and conventional meat products. However, documentation in final meat products need to be obtained before any recommendations can be given.

Acknowledgement: We acknowledge the funding of the project BERRYMEAT from the Organic RDD Program grant no 3405-10-OP-00144 funded by the Danish AgriFish Agency, Ministry of Food, Agriculture and Fisheries and are grateful to Stiftelsen Hofmansgave, Middelfart Kommune and Aarhus Kommune for permission to collect ramson plants in their forests.

Fate of artemisinin in *Artemisia annua* infusion

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In the Chinese traditional medicine *Artemisia annua* (AA) was taken as an herbal tea in the treatment of febrile fevers, including malaria. In 1972 You You Tu and collaborators discovered that the active compound of this plant was artemisinin. Artemisinin (ART) and its derivatives are now considered as the most efficient treatments against malaria [1]. WHO recommends the use of Artemisinin Combined Therapies (ACT) to avoid the development of resistance of the parasite to these compounds [2]. However, in many African countries these treatments are neither affordable, nor readily available. Furthermore, many African communities still rely on traditional medicines and AA infusion is often used to treat malaria symptoms. According to WHO this type of treatment is to be banned as it contains inadequate amounts of artemisinin [3]. The current work investigates factors affecting the efficiency of AA tea preparations in extracting ART and its stability upon storage. Fresh AA is not always available, therefore, fresh and old dried AA (> 8 years) were used to prepare AA tea and the content of ART was found to be 6.55 mg/kg DW and 6.76 mg/kg DW, respectively. Temperature and infusion time may impact ART content, therefore teas were prepared using different water temperatures (22 ^{o}C and 90 ^{o}C) and infusion times (5 min, 15 min, 30 min, 2 h and 24 h) showing that infusing 5 min with 90 ^{o}C water gave highest content of ART (twice as much as at 22 ^{o}C) in the tea and the amount of ART decreased for the longer infusion times tested (40% loss with 30 min infusion time). The tea thus prepared can be kept at least 2 h before consumption without significant loss of ART. This study confirmed that AA infusions do contain ART and that the infusion can be kept for cooling down before consumption.

References: 1. Hsu E (2006) Trans. R. Soc. Trop. Med. Hyg. 100: 505-508. 2. WHO (2006) Guidelines for the treatment of malaria. 3rd ed. ISBN 9789241549127. 3. Ogwang P.E. et al. (2012) Trop. J. Pharm. Res. 11: 445-453

Colour quality of *Aronia* pomace–a natural resource for colour extraction depends on juice processing techniques

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Colour is an important attribute in the marketability of foods and beverages. In recent years, the utilization of natural colours in food has substantially increased following consumer demands who desire colourants from plant sources, reinforced by the belief that they are better from a health perspective. Anthocyanins–a group of phenolic compounds, with reddish-purple hues, are well known natural alternatives to synthetic colours, with potential application in colouring of food products. Berry fruits are widely recognized plant sources of anthocyanins.

For instance, black chokeberries (*Aronia melanocarpa* (Michx.) Elliot) are a rich source of anthocyanins, and its pomace – a byproduct of juice processing could be efficiently used for extraction of natural colours for colouring foodstuffs. In this study, we characterized the anthocyanin profile of *Aronia* pomace using HPLC, and evaluated the influence of processing treatments (blanching, no blanching, freezing), temperatures during maceration and enzyme treatments (2 °C, 50 °C, with enzyme, without enzyme) before and during juice pressing on the yield and quality of pomace. In addition, anthocyanin retention during processing of berries to juice and pomace was also evaluated. Pre-heating of the mash to 50 °C prior to enzyme addition increased juice yield, regardless of enzyme addition and pre-processing treatments. Anthocyanins were retained in higher concentrations in the pomace than in the juice. Enzyme treatment influenced concentration of several monomeric and total anthocyanins in pomace the most followed by maceration temperature, particularly seen during cold maceration of frozen berries. Cold maceration of frozen berries without enzyme addition gave the highest concentrations of anthocyanins (1221.1 mg/100 g FW) in the pomace, and both cold or hot maceration of fresh unblanched berries with enzyme the lowest. Cyanidin-3-galactoside and cyanidin-3-arabinoside were the most sensitive to processing treatments, but the quantitative frequency distribution of each anthocyanin did not vary.

This study supports future exploitation of pomace side streams of *Aronia* to be used as an ingredient or as colourant in food products, thus increase the total value of berry production by utilizing press-residue from juice production.

Acknowledgments: We acknowledge the funding of the project COLARO by GUDP grant No. 34009-14-0786.

Targeting Phorbols towards Tumours

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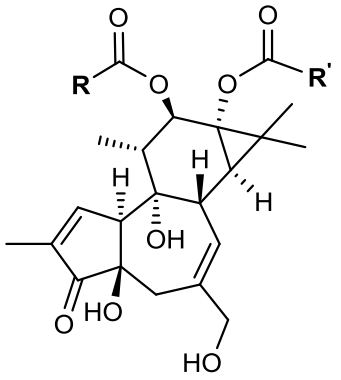
Naturally occurring polyhydroxytiglians like 12-*O*-tetradecanoyl-β-phorbol-13-*O*-acetate (**PMA ; R** = $CH_3(CH_2)_{12}CO$-, **R'** = CH_3CO-) are potent activators of Protein Kinase C and consequently potent cytotoxins. The use of phorbol derivatives as chemotherapeutics, however, is prohibited by the general toxicity, preventing their use a systematic administered drugs. In the case of thapsigargin a similar problem has been overcome by transforming the terpenoid into a prodrug, in which the active cytotoxin is only formed in neovascular tissue in tumours or in the prostate cancer tumors. Isolation of natural phorbol esters in gram scale, solvolysis to obtain β-phorbol (**R** = **R'** = -H) and synthesis of derivatives of β-phorbol which can be conjugated with the targeting peptides were carried out and will be described.



PMA

Production of Artemisinin and other sesquiterpenoids in the moss *Physcomitrella patens*.

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Direct assembly of multiple linear DNA fragments via homologous recombination, a phenomenon known as in vivo assembly was recently introduced as a technology for transformation of the moss *Physcomitrella patens* [1]. This technology has enable us to establish several sesquiterpenoid-producing lines in this green cell factory [2]. We have demonstrated that we in moss can produce up to 200 mg/L amorphadiene (the precursor for Artemisinin) and that we can achieve a production of 0.21 mg/g dry weight of Artemisinin within just a few days of cultivation [3].
Similarly we have established lines that produce between 0.2 – 1.8 mg/g dry weight of patchoulol [4], santalene [4], bisabolol, α-humulene and valencene. Altogether, this show that the moss is a very good cell factory for the production of terpenoids and in particularly sesquiterpenoids.
Our research also demonstrates that employing the same strategies as for yeast, such as upregulation of HMGR and overexpression of FPPS enhances the overall yield of terpenoids [4].
These proof-of-principle experiments have paved the way for more complex and increasingly flexible approaches for large-scale metabolic engineering in plant biotechnology. The successful integration of five active genes for Artemisinin has inspired us to perform stable integration of even longer pathways. The technology has also allowed us study *in planta* enzyme activity and interactions [5, 6] along with perform combinatorial biochemistry.

Acknowledgements: Nur Kusaira Binti Khairul Ikram was supported by a grant from the Ministry of Higher Education, Malaysia and the University of Malaya. Anantha Peramuna and Henrik Toft Simonsen was supported by The Danish Council for Independent Research (#4005-00158B).

References: 1. King, B.C., et al., In vivo assembly of DNA-fragments in the moss, *Physcomitrella patens*. Scientific Reports, 2016. 6: p. 25030. 2. Ikram, N.K.B.K., et al., Stable Heterologous Expression of Biologically Active Terpenoids in Green Plant Cells. Frontiers in Plant Science, 2015. 6: p. 129. 3. Ikram, N.K.B.K., et al., Stable production of the antimalarial drug artemisinin in the moss *Physcomitrella patens*. Nature, 2017. In review. 4. Zhan, X., et al., Metabolic engineering of the moss *Physcomitrella patens* to produce the sesquiterpenoids patchoulol and α/β-santalene. Frontiers in Plant Science, 2014. 5: p. 636. 5. Zhan, X., et al., Additional diterpenes from *Physcomitrella patens* synthesized by copalyl diphosphate/kaurene synthase (PpCPS/KS). Plant Physiology and Biochemistry, 2015. 96: p. 110-4. 6. Ikram, N.K.B.K., et al., Insights into arteannuin B and artemisinin biosynthesis heterologously expressed in *Physcomitrella patens*. Plant Journal, 2017. in review.

Pair correlation analysis of Fixed PALM and Analysis of Live PALM applied on the Water Channel AQP3

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Water transport across the plasma membrane is mediated by aquaporin (AQP) water channels. The drug forskolin regulates this process and can be purified from the plant Coleus Foskohlii.
We previously found that stimulation with forskolin, which leads to increase in cAMP also leads to an increase in lateral diffusion of AQP3, revealing short-term

regulation. To further study if AQP3 is regulated at the nanoscale, we applied pair Correlation in combination with PALM. This showed that AQP3 organize in nano-domains smaller than 60 nm and upon cAMP stimulation, changed organization to 60 – 200 nm sized nano-domains. Thus, PC-PALM revealed regulation at the nanometer resolution.

Furthermore, we performed live-PALM of AQP3 upon cAMP stimulation. Power-spectral analysis of the single-molecule trajectories revealed that the molecules were not freely diffusing. Rather, data were consistent with a simple model for 2D diffusion in confinement. While the measured diffusion coefficients of AQP3 were identical between control and cAMP stimulated cells, the confinement radius increased significantly.

Thus fixed and live PALM measurements both revealed a change of AQP3 nano-organization in the plasma membrane upon cAMP stimulation, indicating short-term hormone regulation of AQP3 at the nanoscale level which has so far been undetectable.

Investigating the uptake and response of hMSC cells exposed to Falcarindiol

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Falcarindiol is a dietary polyacetylene that is found in many food plants of the Apiaceae family and is typically isolated from carrots. Falcarindiol have shown anti-cancer effects e.g. reducing formation of neoplastic lesions in rats[1]. Falcarindiol have also been shown to have a positive effect on diabetes where falcarindiol increased peroxisome proliferator-activated receptor (PPAR)γ-mediated transactivation signi-ficantly[2]. The biological mechanisms on a single cell level is however unknown.

Experimental:
To investigate the uptake mechanism of falcarindiol we have designed a Raman imaging experiment of hMSC cells at different time points of exposure to 10 µm falcarindiol in the cell culture medium. Cells were cultured on glass and Exposed (FC) and control cells were fixed at 0h, 1h, 5h and 24h in three biological replicates.

Raman imaging were performed using a in house build Raman imaging setup utilizing Laser Quantum Ventus 532nm Laser (Stockport, UK). The laser is coupled via free space optics into an Olympus BX60 microscope (Hamburg, Germany) that are fiber coupled using a 105µm fiber to an Acton SpectraPro 2500i f/6.5 spectrograph, using a 600l/mm with a Princeton Instruments PIXIS 400F 1340 × 400 pixel CCD camera (Trenton, NJ) operating at −75°C. The microscope is coupled to a Ludl motorized stage (Hawthorn, NY). For imaging a 100x NA=1 water immersion objective was applied resulting in an effective spatial resolution around 800nm. Images were collected with 60mW at the sample and integration times of 1s per spectrum. For each control and FC a minimum of three cells were imaged for each time point and biological replicate.

Data Analysis

As cells were measured on glass, the glass background was a major issue. This was corrected using EMSC-SIS with references extracted from each individual dataset. The images where then analyzed using the N-FINDR spectral unmixing algorithm using three to five endmembers per image. For each image a combined false color image was constructed based on abundance values. The method is described in further detail by Hedegaard et al[3].

Results

For the time points 0h and 1h there were in general no change in cell composition. The same was the case for the 24h control cells which confirms that no greater changes are occurring during the 24h for our control group. After 5 and 24h exposure to falciariol we do significant changes by the formation of cholesteryl linoleate (CLA) droplets in the cytoplasm. In most 5h we see this trend and

after 24h all cells show CLA droplets as seen in figure 1. These results will be confirmed by Nile Red staining.

Conclusions:
We can conclude that after 24h falcarindiol have a significant impact on hMSC cells, resulting in a major increase CLA production. This indicates potential impact on sterol pathways and possible indirect mechanisms for the effects shown on cancer and diabetes.

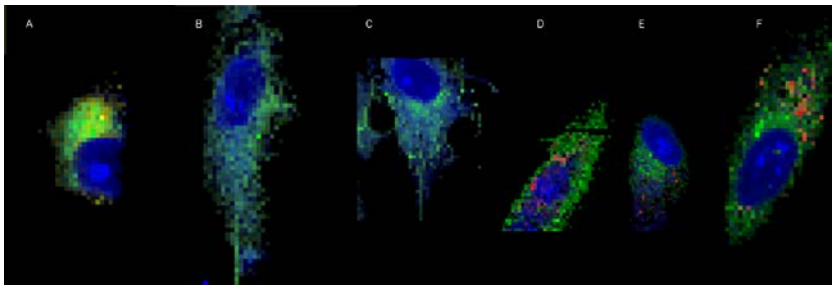

Figure 1. Images of A: 0h Control, B:24h Control, C:0h FC, D:1h FC, E:5h FC and F:24h FC. Blue represents nucleus like spectra, Green and yellow represents normal cytoplasm and red CLA. Each pixel corresponds to 1µm.

[1] Kobaek-Larsen, M., El-Houri, R. B., Christensen, L. P., Al-Najami, I., Fretté X., Baatrup G., (2017) *Food & Function*. DOI: 10.1039/c7fo00110j.
[2] El-Houri,R. B., Kotowska, D., Christensen, K. B.,
Bhattachary, S., Oksbjerg, N., Wolber, G., Kristiansen K., Christensen, L.P. (2015), *Food & Function* , 54, (6), 2135–2144.
[3] Hedegaard, M. A. B., Bergholt, M. S., Stevens, M. M., (2016), *J. Biophotonics (9), No. 5, 542–550*

Discovery and Engineering of Fungal Natural Products

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At the Department of Biotechnology and Biomedicine, at DTU, we have a unique collection of filamentous fungi of more than 35.000 strains. We have strong tradition for using chemotaxonomy as part of identification and classification of among others the ca. 340 species known in genus *Aspergillus* [1], which has been the fundament for selection of representative strains for a current genus wide sequencing project. This presentation will illustrate how we use bioassays and LC-DAD-MS for metabolite profiling and dereplication of individual compounds of interest, in combination with in-house spectral libraries and bioassays for discovery of novel bioactive compounds [2,3]. Furthermore examples from our recent research will illustrate how we use bioinformatics, gene targeting and gene shuffling, for genomics driven discovery, characterization and engineering of polyketide and non-ribosomal peptide biosynthetic pathways [4-6].

References: 1. Frisvad, J.C. & Larsen, T.O. (2015). Applied Microbiology & Biotechnology, 99:7859-7877. 2. Nielsen, K.F. et al. (2011). Journal of Natural Products, 74:2338-2348. 3. Kildgaard, S. et al. (2014). *Marine Drugs*, 12:3681-3705. 4. Andersen, M.R. et al. (2013). PNAS, 110, 99-107. 5. Frandsen, R.J.N. Scientific Reports, DOI: 10.1038/srep26206. 6. Nielsen et al. (2016). *PLoS ONE*, DOI: 10.1371/journal pone.0161199.

Antidiabetic Effects of polyacetylenes and related compounds

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Type 2 diabetes (T2D) is a metabolic disorder that is characterized by hyperglycemia in the context of insulin resistance and relative lack of insulin. T2D is currently treated with a combination of diet restriction and oral drugs. In a previous screening study a dichloromethane (DCM) extract of carrot and Echinacea roots were found to stimulate insulin-dependent glucose uptake (GU) in adipocytes in a dose-dependent manner [1]. Bioassay-guided fractionation of the DCM extracts resulted in the isolation of different secondary metabolites (polyacetylenes and alkamides), which significantly stimulated basal and/or insulin-dependent GU in 3T3-L1 adipocytes and porcine myotubes in a dose-dependent manner. Insulin sensitizing drugs such thiazolidinediones (TZDs) may cause severe side effects, which have been linked to their behaviour as full agonists of peroxisome proliferator-activated receptor (PPAR)γ [2]. Partial PPARγ agonists are associated with fewer side effects, although they maintain their effect on insulin resistance. All the isolated compounds from both roots increased PPARγ-mediated transactivation significantly at different concentrations but partially compared to the TZD rosiglitazone. Docking studies accordingly indicated that all compounds exhibit characteristics of PPARγ partial agonists. The results of the present study suggest that polyacetylenes and other rlated compounds may represent scaffold for novel partial PPARγ agonist with possible antidiabetic properties.

References: 1. El-Houri, R. B. et al. (2013) Evid. Based Complement. Alternat. Med., 2014, Article ID 156398. 2. Berger, J. P. et al. (2003) Mol. Endocrinol., 17, 662−676.

Fungal Polysaccharides & Health: Has the increasing popularity of the fungus *Inonotus obliquus* (Chaga) any basis in science, or is it just hype?

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Inonotus obliquus (Chaga) – a white rot fungus found on birch trees in the northern hemisphere – has been used in traditional medicine in Europe and Asia for centuries (1). Native peoples have made use of Chaga by brewing it as a tea to treat gastro-intestinal problems, to heal wounds and even to treat cancer. The last few decades, studies have found Chaga to contain biologically active substances such as polysaccharides, triterpenoids, polyphenols and melanin (2). *In vivo* effects such as tumor growth inhibition have been observed in mice receiving various Chaga extracts (2,3). The main hypothesis behind the tumor inhibiting effect is two-fold: i) fungal polysaccharides may inhibit tumor growth indirectly by activating certain immune cells such as macrophages and ii) triterpenoids and other steroids from Chaga may give a direct cytotoxic effect against cancer cells (3,4). While triterpenoids from Chaga have been extensively characterized, detailed analysis of the polysaccharides is lacking. The present work has aimed to isolate and characterize the polysaccharides in Chaga, by e.g. column chromatography (ion-exchange/gel filtration), GC-MS and extensive NMR analysis. The water-soluble polysaccharides were found to be complex hetero-polysaccharides, with a structure dominated by (1→3/1→6)-β-glucan and (1→6)-α-galactan, with β-xylose, α-mannose and α-galacturonic acid present in significant amounts. 3-O-methyl α-galactose was reported in Chaga for the first time. The polysaccharide fractions obtained were screened in *in vitro* bioassays for their potential as immunomodulators. Several of the fractions showed promising results by activating primary macrophages (BL6 BMDM) to inhibit the growth of Lewis lung carcinoma cells *in vitro*. The results suggest further studies to be conducted on immune cell activation and *in vivo* tumor growth inhibition.

References: 1. Babitskaya V et al. (2002) Int J Med Mushrooms. 4: 139-145. 2. Zheng W. et al. (2010) Appl Microbiol Biotechnol. 87: 1237-1254. 3. Chen Y. et al. (2015) Int J Biol Macromol. 79: 587-594. 4) Zhao F. et al. (2015) Fitoterapia. 101: 34-40.

In vitro activities of an Alzheimer's disease drug, is galanthamine a dual-active medication?

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Galanthamine, a plant alkaloid isolated from snowdrop (*Galanthus* sp.) which is approved as a drug for treatment of mild-to-moderate Alzheimer's disease. Galanthamine works primarily as an acetylcholinesterase inhibitor but it is also commonly referred to as a positive allosteric modulator (PAM) of neuronal α7 and α4β2 subtypes of the nicotinic acetylcholine receptor (nAChR). Previous experiments reported to show nAChR PAM activity were primarily conducted on rat hippocampal neurons[1], PC12 cells[2] naturally expressing different nAChRs and on the receptors expressed in HEK cells[3]. Data available from receptors expressed in *Xenopus* oocytes are limited and show questionable PAM activity. In our hands, galanthamine was unable neither to activate any of the four receptors subtypes tested α7, $(\alpha4)_3(\beta2)_2$, $(\alpha4)_2(\beta2)_3$ and 4β4 nAChRs expressed in *Xenopus* oocytes, nor positively modulate their responses to acetylcholine (ACh). However, in agreement with the literature we observed inhibition of ACh-evoked responses at high concentrations (10 – 100 µM range) which showed to be a result of an open channel block. Our results therefore question the perception of galanthamine as a nAChR positive modulator.

1. Pereira, E. F.; Alkondon, M.; Tano, T.; Castro, N. G.; Froes-Ferrao, M. M.; Rozental, R.; Aronstam, R. S.; Schrattenholz, A.; Maelicke, A.; Albuquerque, E. X., A novel agonist binding site on nicotinic acetylcholine receptors. *Journal of receptor research* **1993,** *13* (1-4), 413-36.

2. Storch, A.; Schrattenholz, A.; Cooper, J. C.; Abdel Ghani, E. M.; Gutbrod, O.; Weber, K. H.; Reinhardt, S.; Lobron, C.; Hermsen, B.; Soskic, V.; et al., Physostigmine, galanthamine and codeine act as 'noncompetitive nicotinic receptor agonists' on clonal rat pheochromocytoma cells. *European journal of pharmacology* **1995,** *290* (3), 207-19.

3. (a) Samochocki, M.; Zerlin, M.; Jostock, R.; Groot Kormelink, P. J.; Luyten, W. H. M. L.; Albuquerque, E. X.; Maelicke, A., Galantamine is an allosterically potentiating ligand of the human α4/β2 nAChR. *Acta Neurologica Scandinavica* **2000,** *102*, 68-73; (b) Samochocki, M.; Hoffle, A.; Fehrenbacher, A.; Jostock, R.; Ludwig, J.; Christner, C.; Radina, M.; Zerlin, M.; Ullmer, C.; Pereira, E. F.; Lubbert, H.; Albuquerque, E. X.; Maelicke, A., Galantamine is an allosterically potentiating ligand of neuronal nicotinic but not of muscarinic acetylcholine receptors. *The Journal of pharmacology and experimental therapeutics* **2003,** *305* (3), 1024-36.

Forty Five years of Natural products Chemistry Concluding in Transformation of Trilobolide into Thapsigargin

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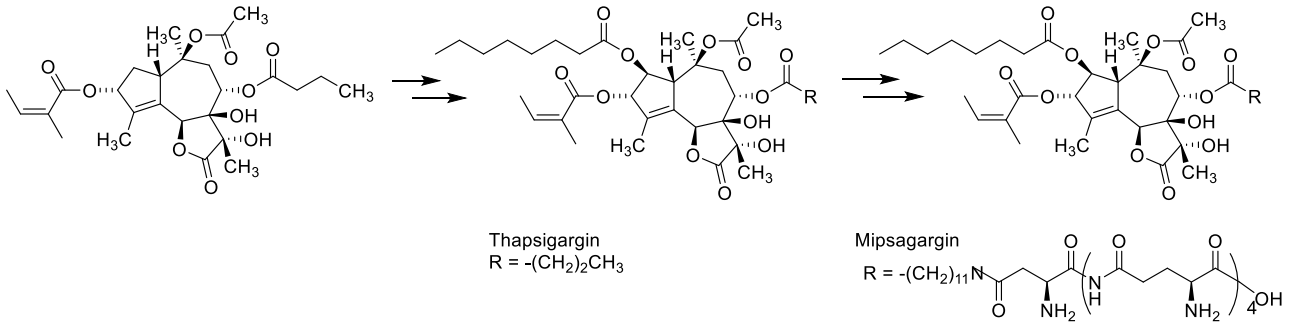
A prodrug of thapsigargin, mipsagargin, may be used for treatment of some cancer diseases. Consequently an interest for a sustainable supply of compounds, which may be converted into mipsagargin, is developing. At the present three total syntheses of thapsigargin with a limited number of steps have been published. Two of these are claimed to be scalable. An alternative would be to convert trilobolide into thapsigargin. A four step procedure for converting trilobolide into thapsigargin will be presented.

In addition some thoughts on natural products chemistry and pharmacognosy will be presented based on 45 years of experience.



Polyacetylenes of the falcarinol type: A promising new class of neutraceuticals and lead compounds for the development of anticancer drugs

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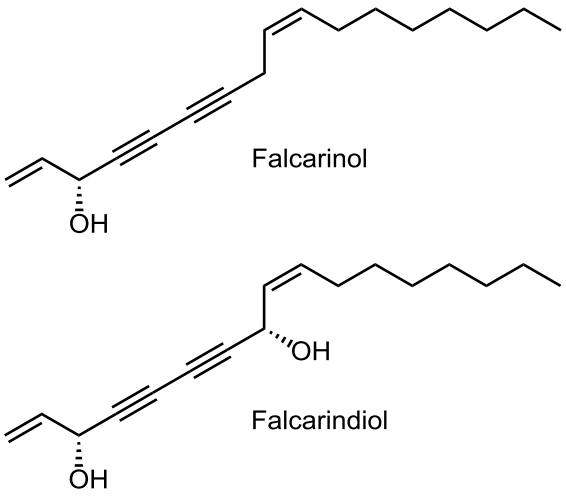
Aliphatic C_{17}-polyacetylenes of the falcarinol type such as falcarinol (FaOH) and falcarindiol (FaDOH) are found in many food and/or medicinal plants of the Apiaceae and Araliaceae families [1]. Several *in vitro* studies have shown that falcarinol type polyacetylenes are highly cytotoxic and possess anti-inflammatory activity [1]. In addition, it has been shown that that synergistic interaction between bioactive polyacetylenes of the falcarinol type such as FaOH and FaDOH may be important for their bioactivity [2]. The anticancer activity of FaOH and FaDOH isolated from carrots have recently been demonstrated in an azoxymethane (AOM)-induced rat model where dietary amounts of FaOH and FaDOH in the rat diet reduced the number of neoplastic lesions with up to 83% as well as the growth rate of the polyps suggesting a preventive effect of FaOH and FaDOH on the development of colorectal cancer [3]. The molecular mechanism of falcarinol type polyacetylenes underlying their anticancer activity is still not known but is most likely related to their alkylating properties, leading to the inhibition of proteins such as COX and NF-κB that plays a central role in the development of cancers [1]. Based on the present available *in vitro* and *in vivo* data of polyacetylenes of the falcarinol type it appears that these natural products are a new promising class of neutraceuticals and lead compounds for the development of anticancer drugs.



References: 1. Christensen LP, Brandt K. (2006) J. Pharm. Biomed. Anal. 41: 683-693. 2. Purup S, Larsen E, Christensen LP. (2009) J. Agric. Food Chem. 57: 8290-8296. 3. Kobaek-Larsen M et al. (2017) Food Funct. 8: 964-974.

Poster Presentations

NNPC 2017

Simple multipurpose apparatus for solubility measurement of solid natural products and other solutes in liquids: Artemisinin as an example

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Solubility is one of the most basic chemical phenomena. It has a key role in understanding of unit operation such as solid–liquid extraction, which is commonly employed for recovery of natural products from plants. In addition, solubility data is crucial for design, development, and operation of extraction processes as it decides the choice and amount of solvent required for complete extraction of natural product. Therefore, solubility measurement of solid natural products in different solvents or solvent mixtures as a function of temperature can be of great significance to the commercial extraction processes. In this work, we demonstrate a comparatively inexpensive apparatus for solubility measurement of solid natural products in pure or mixture of solvents, which can be easily assembled by using readily available equipment in the laboratory. The proposed apparatus shown in Fig. 1 uses classical isothermal technique for measurement of solubility. In this technique, excess amount of solute is suspended in a fixed volume of solvent contained in a glass vial and then the sealed glass vials are maintained at constant temperature for 24 h under constant magnetic stirring. In addition, the jacketed beakers of the apparatus can be used for performing small-scale extraction experiments where different solvents as well as extraction temperature can be employed to find out the optimal one. Reliability of the proposed apparatus was demonstrated by measuring solubility of the naturally occurring antimalarial drug artemisinin in *n*-hexane-ethyl acetate mixtures of varying composition at different temperatures. Extraction of artemisinin from dried leaves of plant *Artemisia annua* was also performed with different solvents.

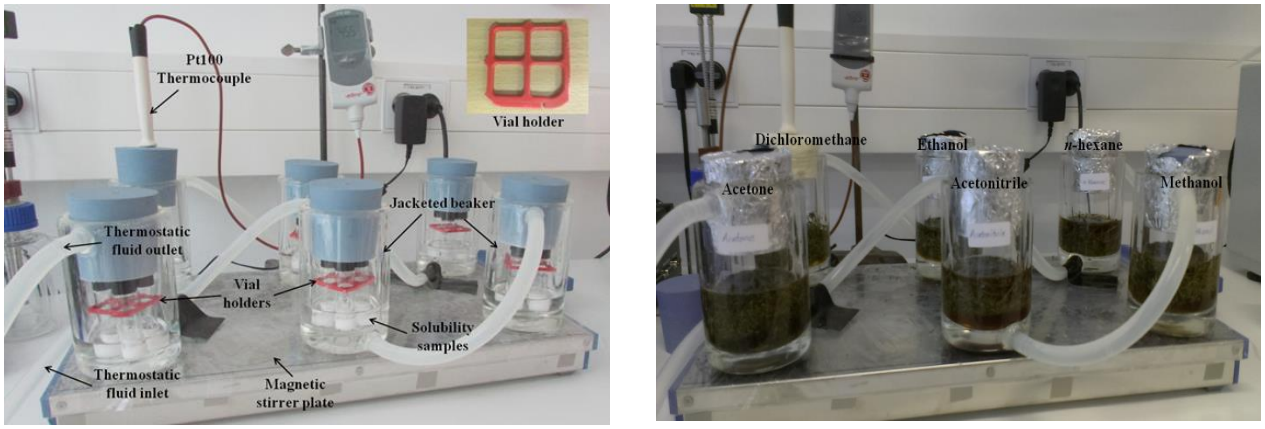

Figure 1. The proposed multipurpose apparatus for solubility measurement of natural products. Left: solubility measurement samples; right: extraction of artemisinin from *Artemisia annua* leaves.

References: 1. Malwade, C.R, Christensen LP (2016) Educ. Chem. Eng. 16: 29-38.

DTU Bioengineering Metabolomics Platform: What We Can Do For You

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The DTU Metabolomics Platform specialises in the analysis and discovery of biologically active metabolites and industrially relevant compounds using advanced mass spectrometry combined with chromatography. We have a long tradition of work with secondary metabolite profiling from micro-organisms especially filamentous fungi and possess an in-house MS/MS library of 1500 compounds, for fast and automated de-replication[1]. We have a collection of more than 1500 unique secondary metabolite and mycotoxin reference standards which can be used to verification and authentication. Our primary expertise lies in chromatography, structural elucidation, synthetic biology and stable isotope labelling for the characterisation of biosynthetic pathways from a range of microbes including fungi, bacteria and algae. We work in close collaboration with biologists from growth, through extraction to analysis to ensure the highest quality data is obtained.

1. Nielsen, K. F.; Månsson, M.; Rank, C.; Frisvad, J.C.; Larsen, T. O. *J. Nat. Prod*., 2011, *74* (11), pp 2338–2348

Identification of α-glucosidase and PTP1B inhibitors from an endophytic fungus by a combination of the One Strain MAny Compounds (OSMAC) approach and HR-bioassays/HPLC-HRMS-SPE-NMR

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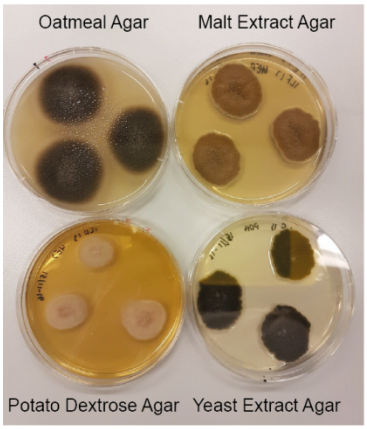
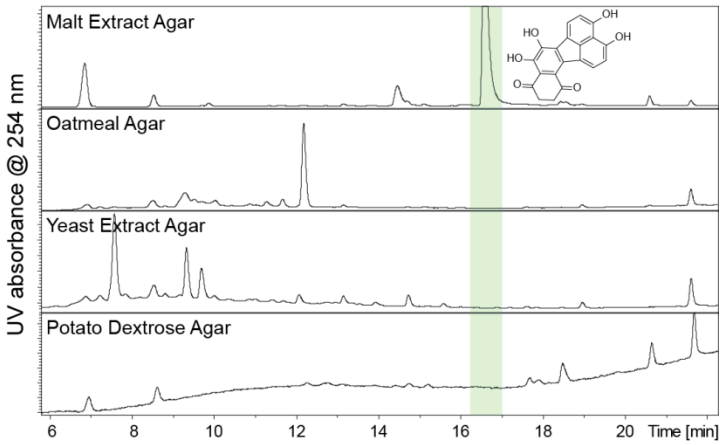
The prevalence of type 2 diabetes is steadily increasing worldwide, leading to increased risk of complications and lower quality of life for patients.[1] Endophytes are microorganisms living in the intercellular space of plants in symbiosis with the host - providing a potential source of novel bioactive compounds.[2] In this study, the One Strain MAny Compounds (OSMAC) approach[3] was utilized to access a larger part of the biosynthetic potential of the endophytic fungi than what is observed under standard laboratory conditions.[4] The endophytic fungus, *Ulocladium spp.* was isolated from the *Juniperus oxycedrus*, a traditional medicinal plant endemic to Iran, known to be effective against several diseases, including diabetes.[5] The fungus was cultivated on 4 different solid media including Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Yeast Extract Agar (YES), and Oatmeal Agar (OA). Morphology and the chemical profiles, analyzed by HPLC-HRMS, confirmed that the different growth conditions activated otherwise silent biosynthetic pathways.





By *in vitro* testing of the crude extracts against pharmacologically relevant targets related to T2D, it was found that only when cultivated on MEA, the fungus possessed inhibitory activities with IC_{50} values of 220 and 59 µg/mL against PTP1B and α-glucosidase, respectively. The crude extract from cultivation on MEA was investigated using the hyphenated technique HR-Bioassay/HPLC-HRMS-SPE-NMR.[6,7] This allowed identification of the active constituent against α-glucosidase, absent from cultivations on the 3 other media, as hortein,[8] having an unusual acenaphtho[1',2':7,8]naphthalene ring system. To our knowledge, this is the first report of α-glucosidase inhibitory activity of hortein and its isolation from an endophytic fungus.

Acknowledgements: HPLC equipment used for high-resolution inhibition profiling was obtained via a grant from The Carlsberg Foundation, and the 600 MHz HPLC-HRMS-SPE-NMR system was acquired through a grant from 'Apotekerfonden af 1991', The Carlsberg Foundation, and the Danish Agency for Science, Technology and Innovation via the National Research Infrastructure funds. Katrine Krydsfeldt and Arife Önder are acknowledged for technical assistance.

References: 1. Deshpande A.D. et al. (2008) Phys. Ther. 88:1254-64. 2. Hawksworth D.L. et al. (1997) Phytopathology 87:888-891. 3. Bode H.B. et al. (2002) ChemBioChem 3:619-27. 4. Scherlach K. et al. (2009) Org. Biomol. Chem. 7:1753-60. 5. Tuzlacı E. et al. (1999) Fitoterapia 70:593-610. 6. Johansen K.T. et al. (2011) J. Nat. Prod. 74:2454-2461. 7. Schmidt J.S. et al. (2002) Food Chem. 135:1692-1699. 8. Brauers G. et al. (2001) J. Nat. Prod. 64:651-652.

Antimalarial activities of *Zanthoxylum zanthoxyloides*

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Background: Malaria is a serious tropical disease and an important cause of death among African children. *Zanthoxylum zanthoxyloides* is a West African tree that is used against malaria and other diseases by traditional healers. Usually, the bark is used, but it is not well known which constituents are responsible for the anti-malaria effect. The anti-malaria effects of substances isolated from the root bark and stem bark extracts of this tree were tested.

Results: Twenty substances were isolated and identified. Seven of these have not been reported previously from this plant: The alkaloids buesgenine, 6-hydroxydihydrochelerythrine, bis-dihydrochelerythrinyl ether, dictamnine and (-)-*p*-synephrine, the coumarin 6,7,8-trimethoxycoumarin, and neochlorogenic acid. The dichloromethane extracts of the root bark and stem bark and the methanol extract of the stem bark killed the protozoa *Plasmodium falciparum*, the organism that gives malaria, with IC_{50} values between 1 and 10 µg/ml. This effect was observed both towards chloroquine sensitive and chloroquine resistant protozoa. Among the isolated substances, skimmianine, γ-fagarine and bis-dihydrochelerythrinyl ether showed good antiplasmodial effect.

Conclusion: From our results, it would seem possible that *Z. zanthoxyloides* may have effect against malaria, and that the use by traditional healers may be a rational one.

Production of Artemisinin and other sesquiterpenoids in the moss *Physcomitrella patens*.

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Direct assembly of multiple linear DNA fragments via homologous recombination, a phenomenon known as in vivo assembly was recently introduced as a technology for transformation of the moss *Physcomitrella patens* [1]. This technology has enable us to establish several sesquiterpenoid-producing lines in this green cell factory [2]. We have demonstrated that we in moss can produce up to 200 mg/L amorphadiene (the precursor for Artemisinin) and that we can achieve a production of 0.21 mg/g dry weight of Artemisinin within just a few days of cultivation [3].
Similarly we have established lines that produce between 0.2 – 1.8 mg/g dry weight of patchoulol [4], santalene [4], bisabolol, α-humulene and valencene. Altogether, this show that the moss is a very good cell factory for the production of terpenoids and in particularly sesquiterpenoids.
Our research also demonstrates that employing the same strategies as for yeast, such as upregulation of HMGR and overexpression of FPPS enhances the overall yield of terpenoids [4].
These proof-of-principle experiments have paved the way for more complex and increasingly flexible approaches for large-scale metabolic engineering in plant biotechnology. The successful integration of five active genes for Artemisinin has inspired us to perform stable integration of even longer pathways. The technology has also allowed us study *in planta* enzyme activity and interactions [5, 6] along with perform combinatorial biochemistry.

Acknowledgements: Nur Kusaira Binti Khairul Ikram was supported by a grant from the Ministry of Higher Education, Malaysia and the University of Malaya. Anantha Peramuna and Henrik Toft Simonsen was supported by The Danish Council for Independent Research (#4005-00158B).

References: 1. King, B.C., et al., In vivo assembly of DNA-fragments in the moss, *Physcomitrella patens*. Scientific Reports, 2016. 6: p. 25030. 2. Ikram, N.K.B.K., et al., Stable Heterologous Expression of Biologically Active Terpenoids in Green Plant Cells. Frontiers in Plant Science, 2015. 6: p. 129. 3. Ikram, N.K.B.K., et al., Stable production of the antimalarial drug artemisinin in the moss *Physcomitrella patens*. Nature, 2017. In review. 4. Zhan, X., et al., Metabolic engineering of the moss *Physcomitrella patens* to produce the sesquiterpenoids patchoulol and α/β-santalene. Frontiers in Plant Science, 2014. 5: p. 636. 5. Zhan, X., et al., Additional diterpenes from *Physcomitrella patens* synthesized by copalyl diphosphate/kaurene synthase (PpCPS/KS). Plant Physiology and Biochemistry, 2015. 96: p. 110-4. 6. Ikram, N.K.B.K., et al., Insights into arteannuin B and artemisinin biosynthesis heterologously expressed in *Physcomitrella patens*. Plant Journal, 2017. in review.

Gallic acid affects the profile of proanthocyanidins in cell cultures

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The response of a *Rubus arcticus* cell culture [1] to the addition of gallic acid, the precursor to the hydrolyzable tannin biosynthesis pathway, was studied. The cell culture was sampled immediately before and 5, 10, and 13 days after the gallic acid addition, with two different concentrations of gallic acid being used. The quantities of proanthocyanidins, along with several other polyphenolic compound groups, were analyzed from each time point using rapid and sensitive UHPLC–MS/MS methods [2,3].

It was observed that the total concentration of proanthocyanidins decreased considerably with the addition of a certain gallic acid concentration, but the composition of the proanthocyanidins changed significantly as well as evidenced by their chromatographic fingerprint. Both the decrease in concentration and the change in the profile held true for both procyanidin and prodelphinidin subunits of proanthocyanidins. A similar decrease in the concentrations of kaempferol- and quercetin-based flavonols, derived from the same biosynthetic pathways as proanthocyanidins, was observed as well.

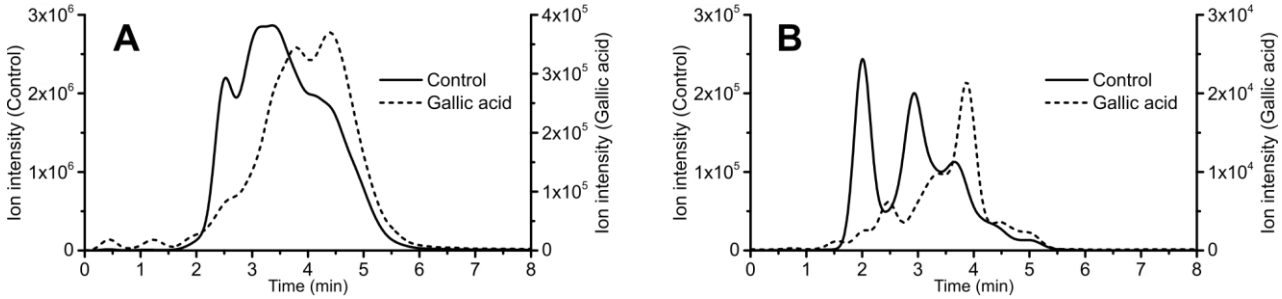

Figure 1. The procyanidin (A) and prodelphinidin (B) UHPLC–MS/MS profiles of the control and gallic acid fed cell cultures.

From a biosynthetic viewpoint, the effect was curious, as gallic acid is not a precursor to proanthocyanidins; both the acetate/malonate pathway and the shikimic acid pathway are used for the biosynthesis of proanthocyanidins, but gallic acid is an independent branch of the shikimic acid pathway leading almost exclusively to hydrolyzable tannins. The decrease in proanthocyanidins and flavonols could simply be derived from gallic acid's known cytotoxicity, caused by the generation of H_2O_2. The change in the profile could therefore be part of this same stress response.

Acknowledgements: Academy of Finland ("Novel approach to modulate and characterize ellagitannin biodiversity in Rubus cell and organ cultures", project 276527 to RP-P and "Evolution and global distribution of plant polyphenol-based pro-oxidant defenses", project 258992 to J-PS) is acknowledged for financial support.

References: 1. Suvanto J. et al. (2017) Planta doi:10.1007/s00425-017-2686-8. 2. Engström M.T. et al. (2014) J. Agric. Food Chem. 62: 3390–3399. 3. Engström M.T. et al. (2015) J. Agric. Food Chem. 63:4068–4079.

Discovery of novel Ilicicolin H analogs from *Stilbella sp.* by UHPLC-DAD-MS/HRMS

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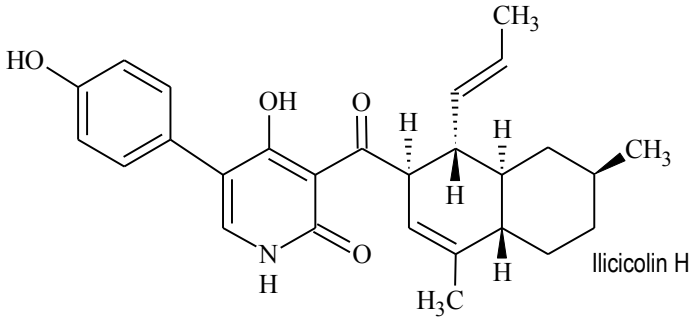
With marine environments offering a great diversity of microorganisms with unique chemical and biological properties, dereplication, along with bioactivity screening, is a key step in the initial chemical profiling of new strains. *Stilbella fimetaria* (IBT 28361) was isolated from the sea water sample off the coast of Fanoe island, Denmark, and screened using combined bio-guided and analytical approach incl. a number of bioassays and UHPLC-DAD-MS/HRMS dereplication based on manual and automated Natural Product libraries search [1,2]. Prefractionation prior biological and chemical analysis was found to be a great aid in the dereplication process, since anticancer, antifungal and antibacterial activities were observed in separate fractions.



The key compound responsible for antifungal bioactivity against A. fumigatus was found to be Ilicicolin H (pictured above). Following media optimisation, a number of novel hits were detected based on UV and daughter ion scan, and further search in our in-house MS/HRMS library [2] revealed at least a 90% similarity in MS/HRMS fragmentation patterns to that of Ilicicolin H. To our best knowledge no other natural Ilicicolin H analogs, apart from synthetically derivatized ones [3], were reported up to date.

Acknowledgements: Support from EU for the FP7 PharmaSea project (2009-2013) and Fundación Medina for biological assay experiments.
References: 1. Nielsen K.F. et al. (2011) J. Nat. Prod. 74:2338-2348. 2. Kildgaard S. (2014) Mar. Drugs 12:3681-3705. 3. Singh S.B. et al. (2011) Tetrahedron Lett. 52:6190-6191.

HPLC-HRMS-SPE-NMR combined with high-resolution bioactivity profiling against *Staphylococcus aureus* for identification of active constituents in endophytic fungi

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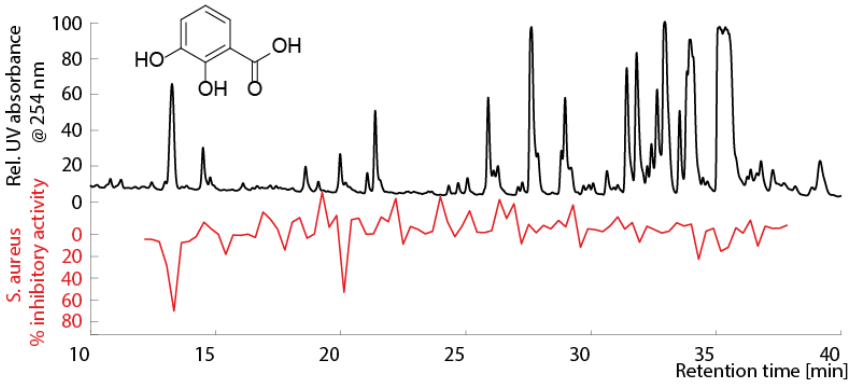
Infections caused by multi-resistant pathogenic bacteria are, besides being a threat on the individual level, a major burden on the society and health care system. Endophytic fungi, non-pathogenic fungi living in the inter-cellular space within plants, are a promising source of novel antibiotics with new modes of action.[1] Not only do they constitute a largely unexplored biosynthetic gene pool, but some endophytic fungi have even been suggested to act as the host plants defense mechanism against pathogenic microorganisms.[2] For identification of bioactive metabolites directly from crude extracts, the bioanalytical platform High-Resolution bioassay/HPLC-HRMS-SPE-NMR has in recent years been shown to be one of the most efficient techniques.[3-5] Herein is reported the development of such a bioanalytical platform for rapid identification of active metabolites against *Staphylococcus aureus* and the application on endophytic fungi isolated from *Juniperus oxycedrus*, a traditional Iranian medicinal plant. Screening of crude extracts of cultivations on Potato Dextrose Agar (PDA) identified a promising *Aspergillus* spp. with an IC_{50} value of 40 µg/mL. Subsequent HR Bioassay/HPLC-HRMS-SPE-NMR analysis allowed for identification of constituents inhibiting *S. aureus*, including 2,3 dihydroxybenzoic acid which has previously been reported to possess antibiotic activity against a range of human pathogenic bacteria.[6]

Isolation of endophytic fungi

High Resolution *Staphylococcus aureus* inhibitory screening



Acknowledgements: INEF (Iran's National Elites Foundation) is acknowledged for a research fellowship to Hamidreza Ardalani (1286782590). HPLC equipment used for high-resolution inhibition profiling was obtained via a grant from The Carlsberg Foundation, and the 600 MHz HPLC-HRMS-SPE-NMR system was acquired through a grant from 'Apotekerfonden af 1991', The Carlsberg Foundation, and the Danish Agency for Science, Technology and Innovation via the National Research Infrastructure funds. Katrine Krydsfeldt and Arife Önder are acknowledged for technical assistance.

References: 1. Hawksworth D.L. et al. (1997) Phytopathology 87:888-891. 2. Hardiom P.R. et al. (2015) Microbiol. Mol. Biol. Rev. 79:293-320. 3. Kongstad K.T. et al. (2015) Fitoterapia 105:102-106. 4. Liu B. et al (2016) Food. Chem. 203:16-22. 5. Wubshet S.G. et al. (2016) J. Nat. Prod. 79:1063-1072. 6. George S. et al (2011) Asian J. Pharm. Clin. Res. 4:126-130.

Dietary polyacetylenes, falcarinol and falcarindiol, prevents the formation of neoplastic lesions in the colon of azoxymethane-induced rats

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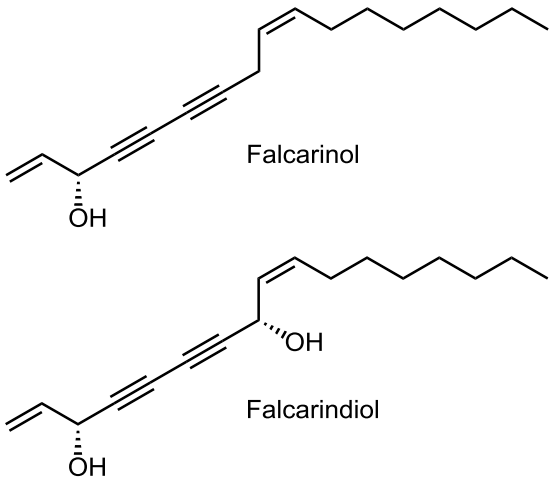
The polyacetylenes falcarinol (FaOH) and falcarindiol (FaDOH) are found in many food plants of the Apiaceae family [1]. Carrots are a major dietary source of these polyacetylenes. Feeding azoxymethane (AOM)-induced rats with carrots and purified FaOH have previously been shown to inhibit neoplastic transformations in the colon [2]. FaOH and FaDOH have also shown to have a synergistic effect *in vitro*, resulting in a significant increased cytotoxic activity [3]. Based on these findings the antineoplastic effect of FaOH and FaDOH purified from carrots (purity > 99%) was investigated in the AOM-induced rat model [4]. Twenty rats received rat diet containing 7 µg FaOH per g feed and 7 µg FaDOH per g feed and 20 rats were controls receiving only rat diet. Then carcinogenesis was induced in all 40 rats with the carcinogen AOM. All animals received the designated diet for 2 weeks before AOM induction and continued on the designated diet throughout the experiment. Rats were euthanized 18 weeks after the first AOM injection and macroscopic polyp/cancers were measured, harvested and stained for histology. The number of small ACF clusters was reduced by 26.6% ($P < 0.001$), number of large ACF clusters reduced by 56.7% and ($P = 0.027$), and finally the number of tumors larger than 3 mm were reduced by 83.3% ($P = 0.032$) in treated rats compared to controls. In conclusion dietary supplements with FaOH and FaDOH reduced the number of neoplastic lesions as well as the growth rate of the polyps suggesting a preventive effect of FaOH and FaDOH on the development of colorectal cancer.



References: 1. Christensen LP, Brandt K (2006) J. Pharm. Biomed. Anal. 41: 683-693. 2. Kobaek-Larsen et al. (2005) J. Agric. Food Chem. 53: 1823-1827. 3. Purup S, Larsen E, Christensen LP (2009) J. Agric. Food Chem. 57: 8290-8296. 4. Kobaek-Larsen M et al. (2017) Food Funct. 8: 964-974.

Cytotoxic and allergenic sesquiterpene lactones from cushion bush (*Leucophyta brownii* Cass.)

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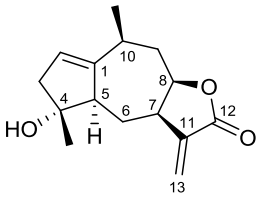
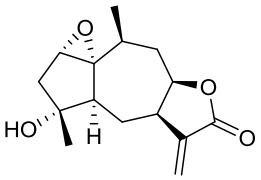
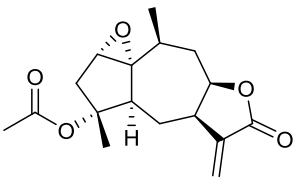
Cushion bush (*Leucophyta brownii* Cass., Asteraceae) has become a popular pot and outdoor container plant in some Nordic countries. Several cases of allergic contact dermatitis caused by cushion bush have been reported [1, 2]. Cushion bush is rich in sesquiterpene lactones containing an α,β-unsaturated γ-lactone moiety that are known for their anti-inflammatory and cytotoxic activity due to reactions with sulfhydryl groups of functional proteins via a Michael-type reaction. This also makes this type of sesquiterpene lactones potential allergenic [1, 3]. Seven sesquiterpene lactones (**1**–**7**) containing an α,β-unsaturated γ-lactone moiety were isolated from cushion bush and identified by LC-MS and 1D and 2D NMR spectroscopy as described previously [3]. Compounds **1**–**7** were investigated for their cytotoxic activity towards human breast cancer (MCF-7) and colon cancer (HT-29) cells as well as their allergenicity. Compounds **2**, **3**, **5** and **6** reduced proliferation of HT-29 and MCF-7 cells with IC_{50} values < 10 µM, whereas compounds **1**, **4** and **7** showed less cytotoxicity with an IC_{50} value of > 20 µM for both cell lines. Six of seven sesquiterpene lactones elicited positive reactions in 4 of 11 patients. The sesquiterpene lactones **3** and **5**–**7**, were confirmed to be sensitizers, whereas leucophytalin A (**4**) and 4α-hydroxy-5αH,10αH-1,11(13)-guaidien-8β,12-olide (**1**) were shown to be allergenic for the first time. No clear correlation between the cytotoxic activity and allergenicity of the tested compounds could be established. However, the present investigation confirmed a connection between type IV allergenicity and cytotoxicity of sesquiterpene lactones containing an α,β-unsaturated γ-lactone moiety.



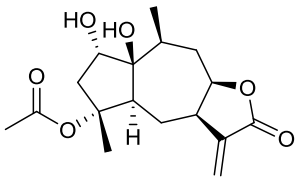
4α-Hydroxy-5αH,10αH-1,11(13)-guaidien-8β,12-olide (**1**)



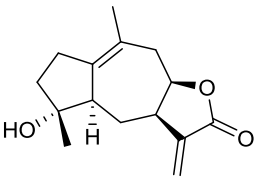
4α-Hydroxy-1α,2α-epoxy-5αH,10αH-11(13)-guaien-8β,12-olide (**2**)



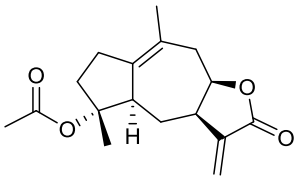
Calocephalin (**3**)



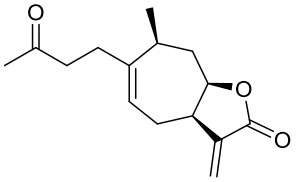
Leucophytalin A (**4**)



Pseudoivalin (**5**)



Pseudoivalin acetate (**6**)



Tomentosin (**7**)

References: 1. Paulsen et al. (2013) Contact Dermatitis 69: 303-310. 2. Paulsen et al. (2017) Contact Dermatitis 76: 280-286. 3. Hyldgaard MG et al. (2015) J. Nat. Prod. 78: 1877-1885.

Harvest strategies of aerial parts and roots of *Echinacea purpurea* for high content of bioactive compounds

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Different herbal preparations of *Echinacea purpurea* L. (purple coneflower) are widely used in North America and Europe for the prevention or treatment of infectious diseases and enhancement of the immune system [1]. The therapeutic compounds of interest in *E. purpurea* are believed to be alkamides, caffeic acid derivatives and polysaccharides [2]. Aerial parts and roots of *E. purpurea* were harvested consecutively in order to find the best strategy for harvest of both types of plant material in relation to high content of bioactive alkamides and caffeic acid derivatives. The hypothesis of the present investigation was that the developmental stage at harvest of aerial parts affects the content of alkamides and caffeic acid derivatives of both aerial parts and the subsequent harvested root parts. The aerial parts were harvested in bud, bloom and wilting stage and the roots were harvested one week, one month and three months after each harvest of aerial parts. Four caffeic acid derivatives (caftaric acid, chlorogenic acid, cichoric acid and echinacoside) and 15 alkamides were identified in plant samples by LC–MS and quantified by reverse-phase HPLC-DAD [3]. The concentration of caffeic acid derivatives decreased with later harvest stages of aerial parts whereas concentration of most alkamides increased. The concentration of caffeic acid derivatives showed a significant interaction between harvest stage of aerial parts and subsequent harvest time of roots. Furthermore, it was found that the concentration of alkamides in roots is higher if the aerial parts are not harvested prior to root harvest. It is therefore not recommendable to harvest the aerial parts the same year as the roots are harvested. If the aerial parts must be harvested the roots should be harvested one week after in order to secure the highest content of bioactive compounds in the roots.

Acknowledgement: Financial support from the The Danish Counsil for Strategic Research (Project “Health promoting effects of bioactive compounds in plants” 2101-07-006) is greatly acknowledged.

References: 1. Barrett B (2003) Phytomedicine 10: 66-86. 2. Bauer R. (1999) In: Immunomodulatory Agents from Plants, Wagner H, Ed., 41-88. 3. Thomsen MO et al. (2012) J. Agric. Food Chem. 60: 12131-12141.

High-resolution α-glucosidase inhibition profiling and HPLC-HRMS-SPE-NMR for phytochemical investigation and identification of antidiabetic constituents in *Picramnia glazioviana*

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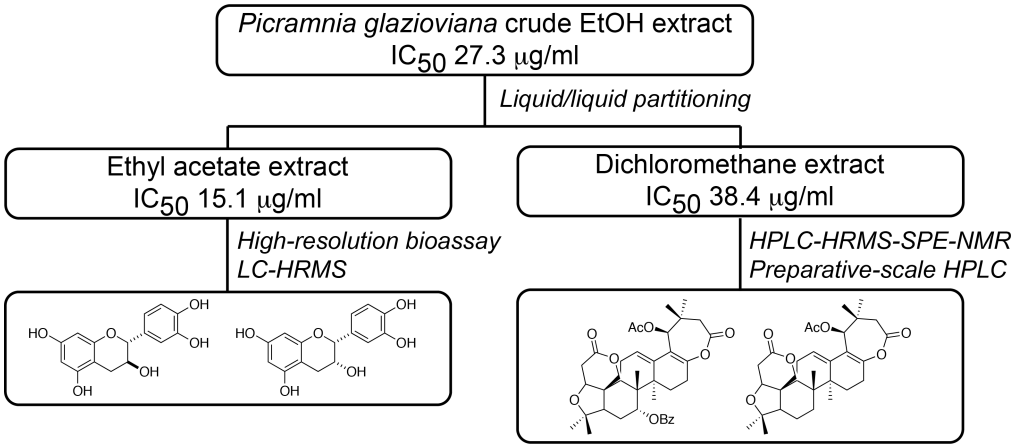
[1]Chemistry Department – Federal University of São Carlos, São Carlos, SP, Brazil. [2]Department of Drug Design and Pharmacology, University of Copenhagen, Copenhagen, Denmark

The chemistry of natural products has remained an important source for discovery of new drug leads. Due to the enormous diversity of secondary metabolites and biological activities that can be found in nature, a large proportion of the currently available drugs are of natural origin or derivatives thereof[1]. Type 2 diabetes (T2D) is a chronic metabolic disorder, and the incidence of T2D increases at an alarming rate and constitutes a global health problem[2]. In this context, the use of plants as a source for inhibitors of α-glucosidase, an enzyme involved in carbohydrate metabolism in the brush border of the small intestines, seems as an attractive approach.



The genus *Picramnia* shows high diversity of secondary metabolites, such as anthrones, oxanthrones, anthraquinones, coumarins, and triterpenes[3]. In our on-going screening programme for antidiabetic compounds from plants, the ethanolic extract of *P. glazioviana* showed an inhibitory concentration (IC_{50}) of 27.31 μg/mL towards α-glucosidase, and was therefore selected for further investigation. This extract was subjected to liquid-liquid partition, and the ethyl acetate and dichloromethane fractions showed an IC_{50} value of 15.11 μg/mL and 38.44 μg/mL, respectively. The ethyl acetate fraction was investigated with high-resolution α-glucosidase inhibition profiling combined with HPLC-HRMS-SPE-NMR. This disclosed two flavonoids, (+)catechin and (-)epicatechin which has previously been reported to have α-glucosidase inhibitory activity.[4,5] The dichloromethane fraction was investigated with a combination of HPLC-HRMS-SPE-NMR and preparative-scale HPLC. This disclosed six new nortriterpenes named picravianes A-F. The results show that plants of the genus *Picramnia* are a valuable source of new and bioactive compounds.

Acknowledgements: FAPESP is acknowledged for a sandwich PhD scholarship to Leila Gimenes HPLC equipment used for high-resolution inhibition profiling was obtained via a grant from The Carlsberg Foundation, and the 600 MHz HPLC-HRMS-SPE-NMR system was acquired through a grant from 'Apotekerfonden af 1991', The Carlsberg Foundation, and the Danish Agency for Science, Technology and Innovation via the National Research Infrastructure funds. Arife Önder is thanked for technical assistance.

References: 1. Kingston D.G.I. et al. (2011) J. Nat. Prod. 74:496-511. 2. Silva E.L. et al. (2016) Molecules 21:782-793. 3. Rodríguez-Gamboa T. et al. (2001) J. Braz. Chem. Soc. 12:386-390. 4. Zhang Y. et al. (2013) Nat. Prod. Res. 27:1513-1520. 5. Kim T.H. (2016) J. Nat. Med. 70:811-815.

Secondary metabolite production of cereal associated *Penicillia* and their potential interaction with oxidative exo-enzymes

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Stored cereals are vulnerable to mycotoxin contamination by the major fungal genus *Penicillium* and especially the species from the series *Viridicata* which is constituted by the 7 species: *P. aurantiogriseum*, *P. cyclopium*, *P. freii*, *P. neoechinulatum*, *P. polonicum*, *P. tricolor*, and *P. viridicatum*, but also known cereal associated species such as *P. verrucosum* and *P. hordei* [1]. With base in the mentioned species, we will here present a thorough investigation on the secondary metabolome, performed on a powerful UHPLC-HRQTOFMS platform. This platform, coupled together with our in-house MSMS library and our extensive knowledge on fungal secondary metabolites [2], allowed us to identify several known, but new to these species, secondary metabolites like e.g. the ergot alkaloid pathway found in *Claviceps purpurea* and *Penicillium commune* [3] or Chrysogine, which is produced by various phylogenetically unrelated ascomycetous genera, were found in three species from the series *Viridicata*. We also looked at the secretion of these metabolites into the media and their potential for interacting with co-secreted oxidative enzymes, like LPMOs.

Acknowledgements: We would like to thank the Novo Nordisk Foundation grant #NNF 130 C0005201 for the funding of this study. We are grateful to Agilent Technologies for the Thought Leader Donation of the UHPLC-MS systems.
References: 1. Mills JT, Seifert KA, Frisvad JC, Abramson D. Nephrotoxigenic *Penicillium* species occurring on farm-stored cereal grains in western Canada. Mycopathologia 1995; 130:23–28. 2. Frisvad JC, Smedsgaard J, Larsen TO, Samson RA. Mycotoxins, drugs and other extrolites produced by species in Penicillium subgenus Penicillium. Stud Mycol 2004; 49:201–241. 3. Gerhards, N, Neubauer L, Tudzynski P, Shu-Ming L. Biosynthetic Pathways of Ergot Alkaloids. Toxins 2014; 6: 3281-3281.

Can molecular phylogeny predict phytochemical diversity? An example of Icelandic cetrarioid lichens

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Nature-derived drugs as well as selection of medicinal plants have been shown to cluster in certain microbial and plant phylogenetic groups [1,2]. In light of ethnic uses of cetrarioid lichens in traditional medicines and foods [3], in particular the lichen *Cetraria islandica*, this study aimed to investigate the correlation between the phylogeny of lichenized fungi and profiles of secondary metablites. In total 88 Icelandic cetrarioid lichen specimens representing 13 lichen taxa of 6 genera (i.e. *Cetraria*, *Tuckermannopsis*, *Vulpicida*, *Cetrariella*, *Flavocetraria*, *Melanelia*) were incorporated into phylogenetic analysis, which was based on 4 genetic markers (i.e. fungal nr ITS, MCM7, mtSSU and algal nrITS). Chemical profiling was carried out using UPLC-QTOF-MS/MS analysis of lichen acetone extracts. Most lichen taxa were recognized by phylogeny except for the complex of *Cetraria islandica* and *Cetraria ericetorum*. Major lichen compounds were identified from their MS data or authentic standards, belonging to the classes of depsidones, depsides, pulvinic acid derivatives, dibenzofurans and aliphatic lactones. Our results have shown that chemical profiles reflect strong phylogenetic signals along the fungal phylogeny only in the genus *Cetraria* (5 species containing aliphatic lactones), but weak correlations in the genus *Melanelia* and *Flavocetraria*. As sister taxa, the genus *Vulpicida* and *Cetrariella* displayed distinct chemical profiles, dominated by pulvinic acid derivatives and depsides, respectively. Interestingly, chemistry seems to correlate well with habitats. For example, the genus *Cetraria*, except for *C. sepincola*, contains species who prefer growing on dry and sunny ground or soil over rocks although *C. islandica* can also be found in more shady habitats, while genera with distinct chemistry can be more specific concerning habitat requirements, such as humid bogs (*Cetrariella*) or tree twigs (*Vulpicida*). Our study demonstrated that the predictive power of phylogeny to chemistry does rather restrict to very closely related taxa with similar habitats, and that habitat type or ecology could play a more important role in lichen chemistry than phylogenetic relatedness.

Acknowledgements: The study was financially supported by the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme FP7/2007-2013/ under REA grant agreement No. 606895. In addition, the contribution from Bergthora and Thorsteinn Scheving Thorsteinsson Fund is gratefully acknowledged.

References: 1. Zhu F. et al. (2011) PNAS. 108:12943-12948. 2. Saslis-Lagoudakis CH. et al. (2012) PNAS. 109: 15835-15840. 3. Xu M. et al. (2016) Phytomedicine 23:441-459.

Discovery and structure elucidation of natural pigments from Aspergillus amylovorus

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The industry of natural food colorants is dependent on a steady and consistent supply of seasonal raw materials for production with minimal batch-to-batch variations. This dependency has produced a growing interest in exploring fungal metabolite pigments as an alternative. Fungal cell factories will be independent of seasonal supply and could produce more cost-competitive pigments. [1]

To this purpose, metabolites of the filamentous fungus Aspergillus amylovorus have been profiled using LC-DAD-MS, with a focus on metabolites with strong chromophores resulting in red and yellow pigments. The metabolites were dereplicated using in-house spectral libraries, resulting in a several potential candidates for novel compounds.

Full structural elucidation of the candidates using NMR spectroscopy will be implemented for further exploration of the pigment candidates.

References: 1. Mapari S.A., et al. (2010). Trends Biotechnol. 28:300-307.

Dual high-resolution α-glucosidase and PTP1B bioassays coupled with HPLC-HRMS-SPE-NMR for investigation of Brazilian *Myrcia* species: 'Insulin plants' as new medicines for type 2 diabetes

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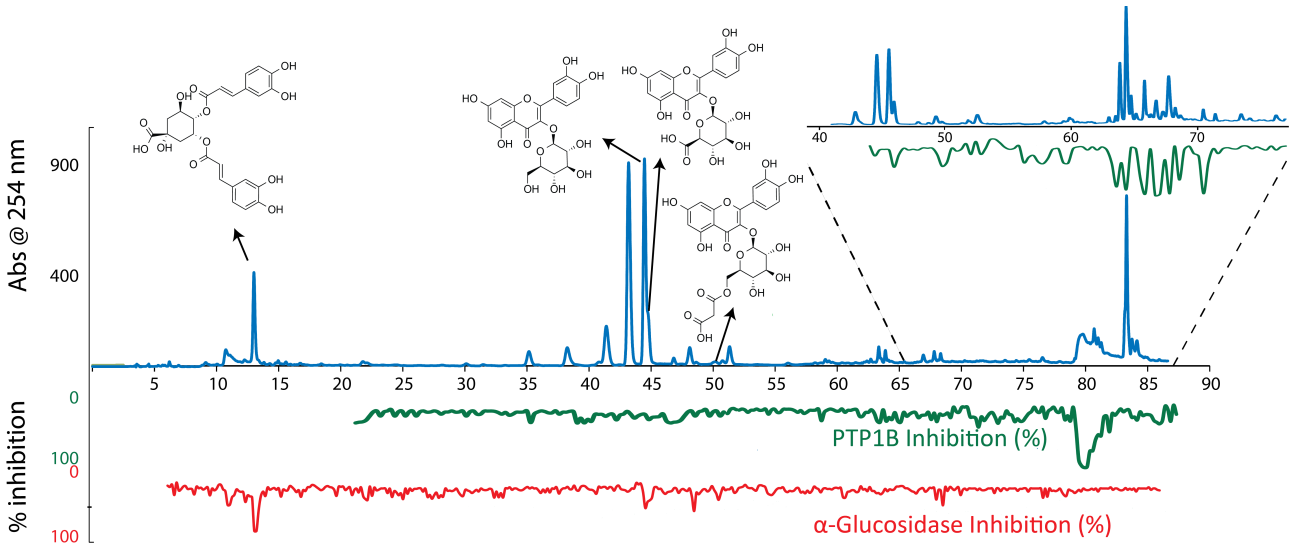
Myrcia species are commonly found on the Brazilian savannah (Cerrado), and they are named 'insulin plants' by indigenous communities in Brazil due to their effectiveness in the treatment of type 2 diabetes.[1] T2D is a metabolic disease characterized by chronic hyperglycemia due to unregulated metabolism of carbohydrates, lipids and proteins, and therapeutic targets for treatment of T2D include carbohydrate-metabolizing enzymes such as α-amylase and α-glucosidase.[2] Protein tyrosine phosphatase 1B (PTP1B) downregulates the effect of insulin, and PTP1B inhibitors are therefore a new important class of antidiabetic drugs.[3] Herein, we report the screening for α-glucosidase and PTP1B inhibitors from six *Myrcia* species - *M. guianensis* (IC_{50AG}= 7.80 µg/mL; $IC_{50PTP1B}$= 3.26 µg/mL); *M. rubella* (IC_{50AG}= 4.36 µg/mL; $IC_{50PTP1B}$= 9.84 µg/mL); *M. torta* (IC_{50AG}= 5.30 µg/mL; $IC_{50PTP1B}$= 27.00 µg/mL); *M. variabilis* (IC_{50AG}= 3.17 µg/mL; $IC_{50PTP1B}$= 1.10 µg/mL); *M. vestita* (IC_{50AG}= 13.60 µg/mL; $IC_{50PTP1B}$= 2.83 µg/mL), and *M. virgata* (IC_{50AG}= 6.30 µg/mL; $IC_{50PTP1B}$= 3.00 µg/mL). *Myrcia rubella* was chosen for further analysis, and the crude ethyl acetate extract was submitted to dual high-resolution α-glucosidase and PTP1B bioactivity profiling[4,5] for identification of individual bioactive constituents.



The crude extract was subsequently analyzed using HPLC-HRMS-SPE-NMR,[4,5] and the HRMS and NMR analyses led to identification of four α-glucosidase inhibitors - 4,5-caffeoylquinic acid, isoquercitrin, quercetin-3-*O*-β-D-glucuronide, and quercetin-3-*O*-6-malonyl-glucoside, and quercetin. PTP1B inhibition was associated with triterpenes such as arjunolic acid and 3-4-3'-tri-*O*-methylellagic acid. This is the first report on biological and chemical investigation of *Myrcia rubella*, and extensive chemical profiling resulted in identification of 27 additional compounds in the crude extract.

Acknowledgements: CAPES/Science without Borders is acknowledged for a Ph.D. scholarship to Rita Lima (BEX12010/13-8). HPLC equipment used for high-resolution inhibition profiling was obtained via a grant from The Carlsberg Foundation, and the 600 MHz HPLC-HRMS-SPE-NMR system was acquired through a grant from 'Apotekerfonden af 1991', The Carlsberg Foundation, and the Danish Agency for Science, Technology and Innovation via the National Research Infrastructure funds. Arife Önder is thanked for technical assistance

References: 1. Cascaes M.M. et al. (2015) Int. J. of Mol. Sci. 16:23881-23904. 2. Lebovitz H.E. (2011) Nat. Rev. Endocrin. 7:408-419. 3. Zhang S. and Zhang Z.-Y. (2007) Drug Disc. Today 12:373-381. 4. Wubshet S.G. et al. (2016) J. Nat. Prod. 79:1063-1072, 5. Tahtah Y. et al. (2015) J. Chrom. A 1408:125-132.

Isolation and Characterization of the Fish-Killing Toxin from the Marine Dinoflagellate *Karlodinium Armiger*.

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Marine fish farming has the potential to become an economically important industry worldwide. However, blooms of ichthyotoxic (fish-killing) harmful microalgae are a recurring phenomenon in coastal marine waters with some times huge impacts on wild fish stocks as well as caged fish. The genus Karlodinium is known to produce a suite of fish-killing and hemolytic polyhydroxy polyene compounds, named karlotoxins.[1] Recently, a new fish-killing mixotrophic *Karlodinium* species was discovered in the Mediterranean Sea, named *K. armiger*.[2] We have isolated and structurally elucidated a new karlotoxin congener that we thus have named karmitoxin.[3] Using ^{13}C enrichment and high-field 2D NMR spectroscopy the structure of karmitoxin was elucidated. This compound interestingly differs from all other isolated karlotoxins and the related amphidinols from *Amphidinium* sp. by containing a primary amine group. The isolated compound was tested towards the marine copepod, *Acartia tonsa* and using a rainbow trout gill cell (RTgill-W1) assay as a proxy for fish toxicity. This showed that karmitoxin was able to immobilize and kill *A. tonsa* (LC_{50}: 400±100 nM) and lyse RTgill-W1 (LC_{50}: 125±1 nM) cells in the mid nanomolar range, in agreement with the observed concentration in culture.

References:

1. Mooney et al. (2009) J. Phycol. 45:164–175.
2. Bergholtz et al. (2006) J. Phycol. 42:170–193.
3. Rasmussen et al. (2017). J. Nat. Prod. (DOI: 10.1021/acs.jnatprod.6b00860)

Prediction of secondary metabolite encoding genes based on chemical structure analysis

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Dereplication of the secondary metabolite profile from the filamentous fungus *Aspergillus brasiliensis*, by High Performance Liquid Chromatography coupled with Diode Array Detection and High Resolution Mass Spectrometry[1], lead to the discovery of a novel biomarker having a unique UV spectrum and elemental composition. Structural elucidation based on Nuclear Magnetic Resonance spectroscopy of the pure compound revealed an apolar polyketide or fatty acid derived secondary metabolite, possibly assembled from a C8 and a C12 entity, fused via a Claisen-like condensation and subsequent cyclisation to form a core lactone ring structure. The compound has been named brasenol (figure 1). To our knowledge, only a few other compunds contain a similar ring structure, with examples being alternaric acid from *Alternaria solani*[2] and fujikurin D found in *Fusarium fujikuroi*[3]. Despite the apolar nature of the compound initial bioassay investigation have demonstrated antibacterial activity (MIC=28.44 µg/mL) against methicillin-resistant *Staphylococcus aureus* MB5393.

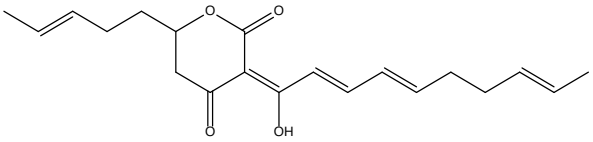

Figure 1. Structure of brasenol

Investigation of other closely related black Aspergilli in section nigri revealed *A. carbonarius* to also be a brasenol producer. Using comparative bioinformatics, a series of five polyketide synthases (PKSs) candidates were selected for deletion in *A. brasiliensis*. Metabolite profile analysis of the mutants showed one of the genes to be involved in the biosynthesis of brasenol as the production of the compound stopped upon deletion of the gene. Additionally, a putative esterase was found close to the PKS. Deletion of this gene also ceased the production. Following the successful deletion of the two genes in *A. brasiliensis*, they were heterologously expressed in *A. nidulans*. When either gene was expressed individually no new metabolites were observed. However, when the two genes were expressed together, a new major peak corresponding to brasenol was detected. This poster will demonstrate how, using the information obtained from the deletion and expression mutants, we were able to suggest the biosynthetic pathway of brasenol.

Acknowledgements: We thank Fundación Medina, Granada, Spain for bioactivity assessment.
References: 1. Nielsen, K. F. et al. (2011) J. Nat. Prod. 2338:2348-74. 2. Brian, P. W. et al. (1949) Nature. 534:534-164. 3. von Bargen, K. W. et al. (2015). Nat. Prod. 1809:1815-78.

Screening for potential α-glucosidase, α-amylase and PTP1B inhibitory constituents from selected Vietnamese plants used to treat type 2 diabetes

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Type-2 diabetes is affecting 246 million people worldwide.[1] In this study, 18 medicinal plants traditionally used in Vietnam for treatment of diabetes were investigated for inhibition of a-glucosidase and α-amylase (two carbohydrate-hydrolysing enzymes) as well as protein-tyrosine Phosphatase 1b (PTP1B). PTP1B is a negative regulator of the insulin-signalling pathway, and PTP1B inhibitors therefore prolong the effect of insulin. Results from crude extract screening using recently developed microplate-based assays[2-4] are shown in table 1.

	α-glucosidase (μM)		α-amylase (μM)		PTP1B (μM)	
	Ethanol	Water	Ethanol	Water	Ethyl acetate	Butanol
Nepenthes mirabilis	32.7 ± 6.3	3.3 ± 0.8			1.4 ± 0.05	0.4 ± 0.1
Phyllanthus amarus		34.9 ± 1.5			16.9 ± 3.2	
Phyllanthus urinaria	39.7 ± 9.7	14.6 ± 4.6			74.4 ± 3.9	3.3 ± 0.3
Kandelia candel	35.4 ± 13.9	4.0 ± 0.8	7.6 ± 0.9			
Lagerstroemia speciosa		5.4 ± 0.5				19.6 ± 5.1
Syzygium cumini		20.9 ± 1.8			27.5 ± 7.8	
Rhizophora mucronata		3.3 ± 0.6				
Ficus racemosa			46.7 ± 23.6		29.2 ± 6.2	1.8 ± 1.4
Ludwigia octavalvis					14.0 ± 0.8	10.8 ± 0.9
Cassia fistula					12.9 ± 2.2	
Euphorbia hirta					24.1 ± 8.4	38.2 ± 1.9
Pithecellobium ducle					17.2 ± 1.2	
Pandanus odoratissimus					20.8 ± 5.6	0.02 ± 0.01

Having low tannin constituents, *P. amarus* and *P. urinaria* water extracts and *F. racemosa* ethanol extract were subjected to microfractionation, followed by α-glucosidase inhibition bioassaying. Only high-resolution α-glucosidase inhibition profiles of the first two extracts showed active compounds, which were isolated and identified as corilagin, repandusinic acid A, and mallotinin. IC_{50} of these compounds were 1.70 ± 0.03, 6.10 ± 0.10, and 3.76 ± 0.15 μM, respectively. Kinetics analysis revealed that corilagin displayed a mixed type mode of inhibition with K_i and $K_{i'}$ values of 2.37 ± 0.90 and 2.61 ± 0.61 μM, respectively, whereas repandusinic acid A and mallotinin competitively inhibited α-glucosidase with K_i values of 4.01 ± 0.47 and 0.65 ± 0.11 μM, respectively.

For the PTP1B assay, butanol extracts of *N. mirabilis, P. urinaria, L. octavalvis, L. speciosa, E. hirta, F. racemosa*, and *P. odoratissimus* were subjected to analytical-scale HPLC and fractionated into 96-well microplates, followed by PTP1B assaying. Only the biochromatogram of the ethyl acetate extracts of *F. racemosa* were absent of tannins and showed distinct peaks correlated with PTP1B inhibitory activity; and isoderrone, derrone and alpinumisoflavone were identified as PTP1B inhibitors

Acknowledgements: HPLC equipment used for high-resolution inhibition profiling was obtained via a grant from The Carlsberg Foundation, and the 600 MHz NMR system used for structure elucidation was acquired through a grant from 'Apotekerfonden af 1991', The Carlsberg Foundation, and the Danish Agency for Science, Technology and Innovation via the National Research Infrastructure funds.

References: 1. Shaw J.E. et al. (2010). Diabetes Res. Clin. Pract. 87:4-14. 2. Schmidt J.S. et al. (2012) Food Chem. 135:1692-1699. 3. Okutan L. et al. (2014) J. Agric. Food Chem. 62: 11465-11467. 4. Tahtah Y. et al. (2016) Fitoterapia 110:52-58.

High-resolution α-glucosidase inhibition profiling combined with HPLC-HRMS-SPE-NMR for identification of α-glucosidase inhibitors in *Machilus litseifolia*

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Type 2 diabetes is a chronic multifactorial disease affecting millions of people worldwide, and new drug leads with selective α-glucosidase inhibitory activity are urgently needed.[1] In this study, the crude ethyl acetate extract of *Machilus litseifolia* branches showed inhibitory activity against α-glucosidase (IC_{50} = 8.0 ± 1.0 µg/ml), and was therefore investigated using high-resolution α-glucosidase inhibition profiling combined with high-performance liquid chromatography–high-resolution mass spectrometry–solid-phase extraction–nuclear magnetic resonance spectroscopy (HR-bioassay/HPLC-HRMS-SPE-NMR).[2,3] Results from the high-resolution α-glucosidase inhibition profile of the crude extract were used to guide preparative-scale HPLC towards five fractions correlated with bioactivity (see figure 1). Fraction 1, 4 and 5 were subjected to HR-bioassay/HPLC-HRMS-SPE-NMR using complementary analytical-scale C_{18} and pentafluorophenyl columns for separation as previously shown by Lima and coworkers.[4] This led to trapping of 17 compounds correlated with peaks in the biochromatogram of fraction 1, of which the material eluted with peaks 7 and 8 were identified as the new compounds tamarixetin-3-*O*-α-L-(2'',4''-di-*O*-*cis*-coumaroyl)-rhamnopyranoside and tamarixetin-3-*O*-α-L-(2''-*O*-*cis*-coumaroyl-4''-*O*-*trans*-coumaroyl)-rhamnopyranoside, respectively. Structural identification of the remaining bioactive constituents from fraction 1, 4 and 5 using HPLC-HRMS-SPE-NMR is in progress.

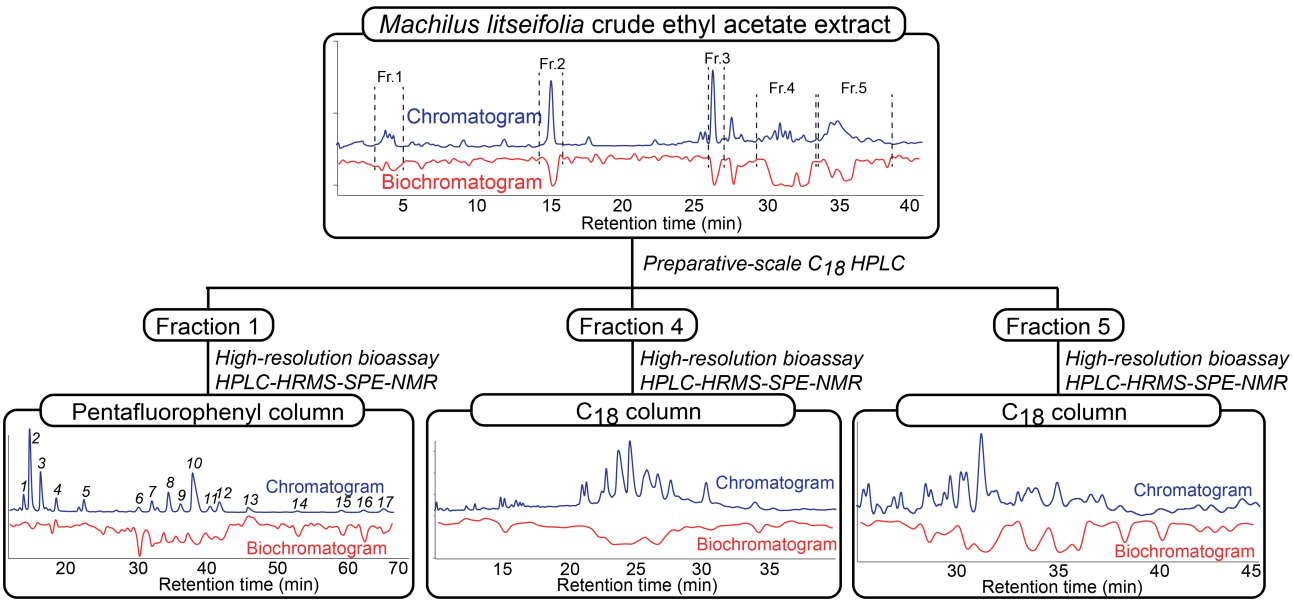

Fig 1. Schematic overview of high-resolution α-glucosidase inhibition profiles of crude *Machilus litseifolia* extract and three fractions obtained by preparative-scale HPLC.

Acknowledgements: Tuo Li acknowledges the Chinese Scholarship Council for a scholarship. HPLC equipment used for high-resolution bioassay profiles was obtained via a grant from The Carlsberg Foundation, and the 600 MHz HPLC-HRMS-SPE-NMR system was acquired through a grant from 'Apotekerfonden af 1991', The Carlsberg Foundation, and the Danish Agency for Science, Technology and Innovation via the National Research Infrastructure funds. Arife Önder is thanked for technical assistance.

References: 1. International Diabetes Federation, IDF Diabetes Atlas, 7th ed., 2015, ISBN: 978-2-930229-81-2. http://www.diabetesatlas.org/. 2. Wubshet S.G. et al. (2016) J. Nat. Prod. 79:1063-1072. 3. Liu B. et al. (2016) Food Chem. 203: 16-22. 4. Lima R.C. et al. (2017) J. Nat. Prod. 80:1020–1027.

Triple high-resolution α-glucosidase/α-amylase/PTP1B inhibition profiling combined with HPLC-HRMS-SPE-NMR for identification of anti-diabetic constituents from *Morus alba* L.

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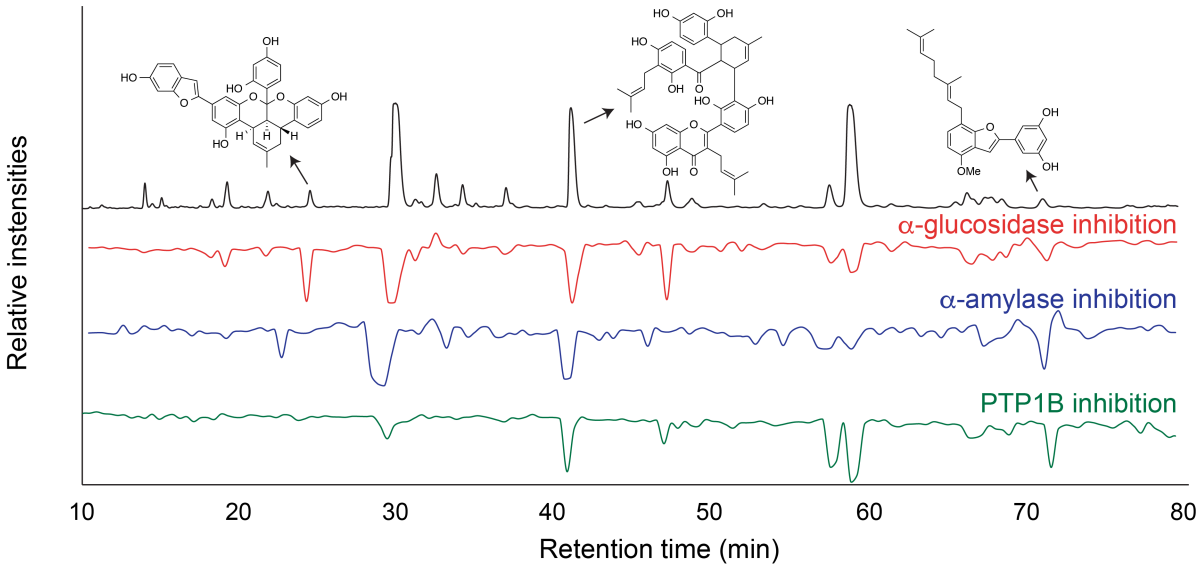
Medicinal plants are used for treatment of type 2 diabetes in many traditional medicine systems around the World, and this practice provides important information that can aid anti-diabetic drug lead discovery. *Morus alba* L. is one of the most well-known and widely distributed trees of the family Moraceae, and many parts of this plant are used for anti-diabetic purposes[1]. In our ongoing research for anti-diabetic constituents from natural sources, the crude EtOAc extract of *M. alba* root bark was found to give 97.2 % inhibition of α-glucosidase, 96.3 % inhibition of α-amylase, and 113.9 % inhibition of protein-tyrosine phosphatase 1B (PTP1B) at a concentration of 10 μM.



Here we report for the first time the combined use of triple high-resolution of α-glucosidase/α-amylase/PTP1B inhibition profiling, and combines this with HPLC-HRMS-SPE-NMR analysis[2,3] for identification of individual anti-diabetic constituents from the crude extract of *M. alba*. This lead to identification of mulberrofuran G, moracenin B, and moracenin A, kuwanon T as potent α-glucosidase inhibitors, whereas mulberrofuran G, moracenin B, moracenin A, and mulberrofuran B were identified as potent α-amylase inhibitors. In addition, moracenin A, kuwanon F, kuwanon M, morusin, mulberrofuran B, cyclomorusin, and sanggenofuran A were found to exhibit potent PTP1B inhibition. This is the first report of all these compounds as α-glucosidase, α-amylase and/or PTP1B inhibitors. These findings provide the scientific rationale for developing *M. alba* into a polypharmacological herbal remedy.

Acknowledgements: Yong Zhao acknowledges the Chinese Scholarship Council for a PhD scholarship. HPLC equipment used for high-resolution inhibition profiling was obtained via a grant from The Carlsberg Foundation, and the 600 MHz HPLC-HRMS-SPE-NMR system was acquired through a grant from 'Apotekerfonden af 1991', The Carlsberg Foundation, and the Danish Agency for Science, Technology and Innovation via the National Research Infrastructure funds. Arife Önder is thanked for technical assistance.

References: 1. Grajek K. et al. (2015). Int J. Pharm. Sci. Res. 6:3110-3122. 2. Wubshet S.G. et al. (2016) J. Nat. Prod. 79:1063-1072, 3. Tahtah Y. et al. (2015) J. Chrom. A 1408:125-132.

Nitrogen Incorporation into 3-Methyl-Orsellinic Acid Derived Natural Products in *Aspergillus nidulans*

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The filamentous fungus *Aspergillus nidulans* produces 3-methylorsellinic acid (3-MOA) and cichorine in a process, which have recently been linked to the polyketide gene *pkbA* [1-3]. Here we analyze the entire *pkb* gene cluster and account for all its activities. Initially we demonstrated that AN6446 is a transcriptional activator of *pkbA*, and used this fact to activate the cluster and facilitate pathway elucidation. In addition to 3-MOA we find that PkbA not only produces aromatic compounds, but also 4-hydroxy-3, 6-dimethyl-2-pyrone showing that this polyketide synthase can produce different C6 and C8 polyketide scaffolds. Importantly, the NRPS-like enzyme AN6444, was found to be responsible for incorporation of nitrogen into cichorine and related novel compounds via e.g. arginine. Overexpression of the gene cluster also lead to the production of two likely non-enzymatically derived dimeric compounds with a novel carbon skeleton, which we named cichonidulols. Altogether this work provides new genetic and biochemical insights for understanding the structural diversity of this important family of non-reduced polyketides including a proposed pathway for all identified compounds.

References: 1. Nielsen, M.L. et al. (2011) FEMS Microbiol. Lett. 321:157-166. 2. Hansen, B.G. et al. (2011) Appl. Environ. Microbiol. 77: 3044-3051. 3. Andersen, M.R. et al. (2013) PNAS.110: 99-107.