Natural blue food color from cyanobacteria Spirulina platensis

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Visby, Gotland, Sweden, 15 - 16 June 2015
Abstract book
Edited by Anders Backlund

Welcome to NNPC 2015 in Visby!

On behalf of the Organizing Committée, we have the honour to welcome you to the 6th Nordic Natural Products Conference in Visby, and a joint venture between the Division of Pharmacognosy at Uppsala University and the Swedish University of Agricultural Studies.

This conference is presented under the sub-heading of "Natural Products Research – Past Present and Future" in honour of Prof. Lars Bohlin, who has for many year been a driving force in the development of natural products research in the Nordic countries.

The series NNPC was initiated by Prof. Bohlin in 2005, and the first meeting was pursued exactly ten years ago, 13-15 June, at Tjärnö Marinbiologiska Station outside the city of Strömstad on the Swedish west-coast.

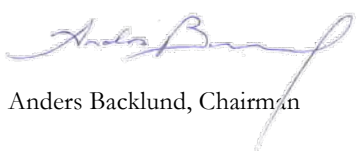
Among the 70 participants at NNPC 2005, we find several contributors of this years meeting, including (but not limited to): A.-K. Borg-Karlsson, A. Brogren, H. R. El-Seedi, A.K. Jäger, S. Larsson, K.E. Malterud, T. Ostenfeld Larsen, R. Riccio, K. J. Rosengren, B. Smestad Paulsen, D. Stærk, and H. Wangensteen.

With 87 registered participants from California to Taiwan, and Iceland to Sri-Lanka, this meeting at Uppsala University Campus Gotland on the Baltic island of Gotland shows that NNPC has become established as an important meeting-place for natural products researchers not only in the Nordic countries, but also worldwide.

The broad scope of the meeting, open for all aspects of studies in natural products, is in this years program pronounced by mingling presentations from different disciplines. It is our hope that this will even further foster interdisciplinary research and intefacing idéas for the years to come.

Again, welcome to Visby!

10th of June 2015



Anders Backlund, Chairman

Ulf Göransson, Vice Chairman

PROGRAMME for NNPC 2015

Nordic Natural Products Conference

(14-) 15-16 June 2015, Uppsala University Campus Gotland, Visby

SUNDAY 2015.06.14

20.05-23.20 **Registration** and **Welcoming Reception** at M/S Visby.

MONDAY 2011.08.15

09.30-09.45 **Opening Ceremony:**
By 7th ECMNP Chairman Prof. Anders Backlund and the Governor of Gotland: Cecilia Schelin-Seidegård

09.45-10.45 SESSION I CHAIRMAN: **Anders Backlund**

09.45-10.15 **Lars Bohlin** **L01**
Modern pharmacognosy – 40 years of experience in natural products research

10.15-10.45 **Raffaele Riccio** **L02**
Disclosing biological targets of bioactive natural products by chemical proteomics

10.45-11.15 MORNING COFFEE

11.15-12.00 SESSION II CHAIRMAN: **Christina Wedén**

11.15-11.30 **Adyary Fallarero** **L03s**
Strategies to protect medical devices from biofilm infections

11.30-11.45 **Joakim Bjerketorp** **L04s**
New antibiotics from nature: SLU-Medivir collaboration

11.45-12.00 **Erik Jacobsson** **L05s**
Peptide toxins from L. longissimus*: extraction, biological activity, structure and production*

12.00-13.30 LUNCH supported by Medivir

MONDAY 2015.06.15

13.30-15.00 SESSION III CHAIRMAN: **Anders Broberg**

13.30-14.00 **Lena Gerwick** **L06**
Honaucin A, mechanism of action and role as a cancer prevention agent

14.00-14.15 **Sesselja Omarsdottir** **L07s**
Immunomodulatory effects of novel N-acyl dopamine derivatives, myxillin A, B and C from the sponge Myxilla incrustans *collected at submarine geothermal chimneys in Northern Iceland*

14.15-14.30 **Håkan Andersson** **L08s**
Discovery of peptide toxins in the world's longest animal (the bootlace worm; Lineus longissimus*): challenging claims of tetrodotoxin production*

14.30-14.45 **Anna Jäger** **L09s**
Finding the world's best plants for type-2 diabetes targets

14.45-15.00 **Sonny Larsson** **L10s**
Life-and-death pharmacognosy – or: What did we have for dinner?

15.00-15.30 AFTERNOON TEA

15.30-17.30 SESSION IV CHAIRMAN: **Ulf Göransson**

15.30-16.00 **Marcel Jaspars** **L11**
Extreme structure determination

16.00-16.15 **Henrik Toft-Simonsen** **L12s**
The quest for large amounts of Thapsigargin

16.15-16.30 **Anna Koptina** **L13s**
Sialidase enzyme as a novel drug target against trichomoniasis

16.30-17.00 **Nina Rønsted** **L14**
Phylogenetic exploration of medicinal plant diversity. Can the evolutionary history of plants help guide better healthcare, new leads, and sustainable use of medicinal plants?

17.00-17.30 **Anders Backlund**
Information regarding poster session

17.30–21.00 POSTER SESSION

EVENING MEAL supported by Region Gotland

TUESDAY 2015.06.16

08.30-10.00 SESSION V CHAIR: **Jolanta Levenfors**

08.30-09.00 **Johan Rosengren** **L15**
PawS-derived peptides: A diverse family of cyclic plant peptides from sunflowers

09.00-09.15 **Paco Cárdenas** **L16s**
Sponge taxonomy 2.0 meets pharmacognosy

09.15-09.30 **Malena Skogman** **L17s**
Cinchona *alkaloid derivatives as antibacterial and anti-biofilm chemotypes*

09.30-09.45 **Taj Muhammad** **L18s**
A new biological tool for disulfide-rich cyclic peptide synthesis: Application of PatG macrocyclase enzyme in the synthesis of cyclotides and sunflower trypsin inhibitor 1

09.45-10.00 **Madeleine Ernst** **L19s**
From ethnomedicine to phylogenetic prediction in drug discovery in the genus Euphorbia*: Suggestions for a new classification system of diseases*

10.00 - 10.30 MORNING COFFEE

10.30-12.00 SESSION VI CHAIR: **Corine Sandström**

10.30-11.00 **Dan Staerk** **L20**
High resolution bioactivity profiling combined with hyphenated HPLC-HRMS-SPE-NMR for accelerated identification of bioactive natural products directly from crude extracts

11.00-11.15 **Rime El-Houri** **L21s**
*Polyacetyenes from carrots (*Daucus carota*) with potential antidiabetic effects*

11.15-11.30 **Muaaz Al-Ajlani** **L22s**
Notes on chemical property position of bedaquiline constructed by the chemical global positioning system ChemGPS-NP

11.30-11.45 **Blazej Slazak** **L23s**
Immunolocalization of cyclotides in plant cell, tissue and organs support their role in plant defence

11.45-12.00 **Karl-Egil Malterud** **L24s**
Zanthoxylum heitzii*, a possible anti-malaria medicine*

12.00-13.30 LUNCH supported by Galderma

TUESDAY 2015.06.16

13.30-15.00 SESSION VII CHAIR: **Adam Strömstedt**

13.30-14.00 **Gerhard Ecker** **L25**
Open innovation and data integration – from docking poses to prediction of side effects

14.00-14.15 **Anders Broberg** **L26s**
Sesquiterpenes from the saprotrophic fungus Granulobasidium vellereum

14.15-14.30 **Xia-Xia Di** **L27s**
Bio-guided fractionation of immunomodulatory compounds from the marine sponge Halicondria sitiens

14.30-14.45 **Shaikh Jamal Uddina** **L28s**
Central-stimulating and analgesic activity of the ethanolic extract of Alternanthera sessilis *in mice*

14.45-15.00 **Sileshi Wubshet** **L29s**
Ligand fishing using functionalized beads combined with HPLC-HRMS-SPE-NMR: Analysis of α-glucosidase inhibitors in a crude plant extract

15.00-15.30 AFTERNOON TEA

15.30-17.00 SESSION VIII CHAIR: **Lars Bohlin**

15.30-16.00 **Anna-Karin Borg-Karlsson** **L30**
Chemical ecology

16.00-16.15 **Sunithi Gunasekera** **L31s**
Circular disulfide-rich peptide scaffolds as anti-citrullinated peptide antibody inhibitors

16.15-16.30 **Thomas Ostenfeld-Larsen** **L32s**
Fungal natural product chemistry in the genomic era

16.30-17.00 **William Gerwick** **L33**
Interdisciplinary approaches to marine natural products drug discovery

17.00-17.30 Closing ceremony

19.00– CONFERENCE BANQUET
at Hotel Visby, supported by BergmanLabora

BERGMAN LABORA

REGISTRATION and RECEPTION

The registration and welcoming reception will start in the conference room onboard M/S Visby after departure from Nynäshamn on Sunday 2015.06.14.

Registration will continue at Scandic Hotel Visby in the evening 2015.06.14, and at the congress venue from 08.00 on the 2015.06.15.

POSTER SESSION

The posters are mounted upon arrival and are displayed adjacent to the lecture hall throughout the conference. The poster session is set for Monday evening starting at 17.30.

There will be a wine and beer bar opening at 18.00, and all participants are provided with tickets for a complimentary drink and a hamburger served by restaurant Smakrike and supported by Region Gotland. Soft drinks and snacks will be served for free.

VENUE

The conference venue is Almedalsbiblioteket, lecture hall E22.

Address:
Almedalsbiblioteket E22
Cramérgatan 5
621 22 Visby
Tel: +46 (0)498-29 90 00

http://www.almedalsbiblioteket.se/english/Welcome.html

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Speakers in chronological order

Contributors to poster session, by poster first author

L01 – 2015.06.15, at 09.45-10.15

Modern pharmacognosy – 40 years of experience in natural product research

L. Bohlin

Div. of Pharmacognosy, Dept. of Medicinal Chemistry, Uppsala Univ., Sweden

The subject Pharmacognosy at Uppsala University, with a long history and connected to the famous Uppsala scientist Carl Linnaeus, is responsible for teaching and research in the area of bioactive novel molecules with medicinal potential from natural sources. The subject combines chemistry with biology in a multidisciplinary way. This type of approach is necessary in order to discover, describe, and communicate the richness of nature to overcome threats against biodiversity and the future sustainable use of natural resources. The ultimate purpose being to secure molecules with natural origin as potential leads in drug development and also to reveal potential new targets. Discovery of novel structure-activity relationships in nature has also an increased importance for inspiration of synthesis of natural product like compounds resulting in greater diversity but with less complexity and increased understanding of biological processes. Identifying bioactive molecules from complex biomasses requires careful selection and execution of relevant bioassays in the various stages of the discovery process of potential leads and targets. The aim of this lecture is to share our long-term experience in bioassay-guided isolation, and mechanistic studies, of bioactive compounds from different organisms in nature. A long-term research has provided experience of selection and combination of bioassay models, which has led to an increased understanding of ethnopharmacological and ecological observations, together with in depth knowledge of mode of action of isolated compounds

References:

1. Bohlin, L., Alsmark, C., Göransson, U., Klum, M., Wedén, C. and Backlund, A. Strategies and Methods for a Sustainable Search for Bioactive Compounds, Chapter 1, pp. 1-36, (2011)
2. Strömstedt, A.A., Felth, J. and Bohlin, L. (2014). Phytochemical Analysis, 25(1), 13-28.

Disclosing biological targets of bioactive natural products by chemical proteomics

R. Riccio, A. Casapullo, M.C. Monti, C. Cassiano, and L. Margarucci

Department of Pharmacy, University of Salerno, Fisciano, Italy,

The identification of the cellular targets of bioactive small-molecules is often a crucial step during the investigation of natural products. This aspect is particularly relevant in pharmaceutical research, where the identification of target proteins and investigation of ligand-receptor interactions are recognized as essential requirements in the process of drug D&D. Owing to our involvement in the field of bioactive natural products, we are investigating different strategies toward target identification, based on computational approaches (1-3) or on chemical proteomics (4-10). Both protocols appear to be attractive alternatives to in vitro binding assays, since they can be easily and extensively applied in the early stages of the process of drug D&D from bioactive small-molecules of synthetic or natural origin. Indeed, target identification is, in principle, feasible through a chemical proteomics protocol based on a combination of affinity purification (pulldown) using suitable small-molecule probes that have been incubated in a cell culture or in a cell lysate and of mass spectrometric identification of the set of captured proteins. The molecular probe (the bait) is made up by anchoring the compound under investigation onto a solid support through a flexible spacer arm. This matrix is then incubated with an opportune lysate, from cells or tissues, to promote the interaction between the bait and its specific protein targets. The fished proteins are eventually isolated and identified through MS methodologies. In this communication we will present the in vitro and in cell chemical proteomics approaches used in our lab, through the most recent results obtained on a set of bioactive marine natural products: Heteronemin (11), Theonellasterone (12) and Scalaradial (13).

References:

1. Lauro, G. et al. (2011) J. Nat. Prod. 74 (6), 1401–1407. **2.** Lauro, G. et al. (2012) Bioorg. Med. Chem. 20 (11), 3596–3602. **3.** Jun Gong, et al. (2014) Org. Lett. 16(8), 2224-2227. **4.** Margarucci, L. et al. (2010) Angew. Chem. Int. Ed. 49, 3960–3963. **5.** Margarucci, L. et al. (2011) Mol. BioSyst. 7, 480–485. **6.** Margarucci, L. et al. (2012) Mol Biosyst 8, 1412-1417. **7.** Cassiano, C. et al. (2012) ChemBioChem 13 (13), 1953–1958. **8.** Vilasi, A. et al. (2013) Mar. Drugs 11, 1288-1299. **9.** Margarucci, L. et al. (2013) Chem. Commun. 49, 5844-5846. **10.** Cassiano, C. et al. (2015) Natural Product Communications, in press. **11.** Cassiano, C. et al. (2014) Chem. Commun., 50, 406-408. **12.** Margarucci, L. et al (2015) Chem. Commun., 51(9), 1591-1593. **13.** Cassiano, C. et al. (2014) Chem. Commun., 50 (45), 6043-6045.

L03s – 2015.06.15, at 11.15-11.30

Strategies to protect medical devices from biofilm infections

A. Fallarero[a], T. Oja[b], M. Skogman[a], S. Manner[b], D. Goeres[c], N. Sandler[b] and P. Vuorela[a]

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[c]*Center for Biofilm Engineering, Montana State University, Bozeman, USA.*

Biofilm-related infections (BRIs) are chronic, resilient and highly tolerant infections that differ from the acute ones caused by single-cell microbes. An estimated 65-80% of all infections are thought to be biofilm-related, particularly those occurring in indwelling medical devices [1], such as prosthetic joints, fracture-fixation as well as devices used in plastic surgery, urology and neurosurgery. For orthopaedic implants alone, biofilm infections cause up to 25% of surgical failures, costing € 800 million/year. We propose here an innovative strategy to protect medical devices involving the use of printing technologies to produce individualized implants containing effective antimicrobials that can prevent bacterial colonization and minimize tissue-remodelling needs. Three key challenges were identified and addressed on, during this project. First: assays to measure the anti-biofilm activity in printed materials were needed. This we tackled by optimizing the so-called Static Biofilm Assay, which serves as a model for the testing of nearly any kind of fabricated materials against bacterial biofilms grown in the absence of shear flow [2]. Second: effective anti-biofilms were needed. In this case, we took advantage of the collection of natural and naturally inspired biofilm inhibitors identified by our lab, which are highly potent even against pre-formed biofilms [3]. Third: proofs of the technological feasibility of this approach were needed. Indeed, we showed that it is technically feasible to incorporate antimicrobials into the carrier polymer used for 3D printing (using hot melt extrusion), which results in a dramatic inhibition of biofilm formation in vitro when compared to a coated surface [4]. These findings pave the way for new natural products applications to better protect medical devices against biofilms.

Acknowledgements: Academy of Finland grants 272266 (ArtFilm) and 282981 (WoodyFilm) are acknowledged.

References:

1. Beloin, C. et al. (2014) Curr Opin Pharmacol. 18:61-8.
2. Oja, T. et al. (2014) J Microbiol Methods.107:157-60.
3. Manner, S. et al. (2013) Int J Mol Sci. 14(10):19434-51.
4. Sandler, N. et al. (2014) Int J. Pharm. 459:62-64.

L04s – 2015.06.15, at 11.30-11.45

New antibiotics from nature: SLU-Medivir collaboration

J. Bjerketorp[a], J.J. Levenfors[a], B. Guss[a], J. Schnürer[a], P. Andersson[b], A. Broberg[b], C. Nord[b], K. Benkestock[c], K. Göhlin[c], C. Sahlberg[c], L. Vrang[c], F. Öberg[c], B. Öberg[c], and R. Bethell[c]

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The increase of antibiotic resistance is recognized as one of mankind's greatest challenges – new antibiotics are urgently needed [1, 2]. The challenges to antibacterial discovery have kept the output of novel antibacterial drug classes at extraordinarily low levels over the past 50 years and rational drug design of new compounds has failed.
International expert organizations, such as Infectious Diseases Society of America (IDSA) and British Society for Antimicrobial Chemotherapy (BSAC) proposes a return to nature as a place to find new antibiotics [3].
Collaboration between SLU and Medivir started in August 2012. It has a primary objective to discover and develop new antibiotics that are active against bacterial pathogens resistant to present therapies. Bacterial and fungal isolates from the existing SLU-collection as well as microorganisms that are isolated from new environmental samples are used as a source for isolation of secondary metabolites with antibacterial activity.
Since then, over three hundred antibacterial natural products were isolated and at least partially characterized, and many of these were novel compounds. The discovered molecules have varying antimicrobial profiles and capacity to inhibit the growth of bacterial pathogens, some of them resistant to existing drug therapies.

References:

1. The Review on Antimicrobial Resistance (11th December 2014) Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations. UK Government and Wellcome Trust.
2. National action plan for combating antibiotic-resistant bacteria (27th March 2015) The White House.
3. Infectious Diseases Society of America, 2010: The 10 × '20 Initiative: Pursuing a Global Commitment to Develop 10 New Antibacterial Drugs by 2020. Clinical Infectious Diseases 50, 1081-1083.

L05s – 2015.06.15, at 11.45-12.00

Peptide toxins from *L. longissimus*: extraction, biological activity, structure and production

E. Jacobsson[a], H.S. Andersson[b], M. Strand[c],
C. Eriksson[a], and U. Göransson[a]

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One of the more spectacular marine invertebrates found in Swedish waters is the nemertean worm *Lineus longissimus*. *L. longissimus* may reach a length of up to 30-50 m [1,2], and is considered to be the longest animal on earth. It predates on small crustaceans and polychaetes and uses, in analogy to *Conus* spp., a proboscis to catch its pray. The worm is covered in mucus, which it secretes to facilitate movement and to deter predators. The constituent responsible for *L. longissimus* mucus toxicity was generally believed to be TTX, or analogues thereof [3]; however, we recently determined that TTX is not present in *L. longissimus* mucus. In this study we show that the crustacean toxicity is instead due (at least partly) to peptide toxins present in the mucus. We also give an estimation of the effective dose in the green shore crab *Carcinus maenas*, the preliminary structure of the most abundant peptide toxin and bioinformatic insights into the putative toxin content of the *L. longissimus* transcriptome.

Mucus from *L. longissimus* was lyophilized and major peptide components were isolated using SEC, HPLC-UV and sequenced by means of UPLC-MS/MS with aid of transcriptomic data obtained by NGS (Illumina HiSeq2000 platform). The most abundant peptide was synthesized using SPPS to yield sufficient amounts for *C. maenas* bioassay [4] and NMR-based structural elucidation.

References:

1. Andrade, S. C. S. et al. (2014) Mol. Biol. Evol. 31: 3206–15.
2. Strand, M., Sundberg, P. (2010) Nationalnyckeln till Sveriges flora och fauna – stjärnmaskar – slemmaskar. Artdatabanken, SLU, Uppsala.
3. Carroll, S. et al. (2003) J. Exp. Mar. Bio. Ecol. 288: 51–63.
4. Kem, W. R. (1971) Toxicon 9: 23–32.

L06 – 2015.06.15, at 13.30-14.00

Honaucin A, mechanism of action and role as a cancer prevention agent.

L. Gerwick, S. J. Mascuch, G. Navarro, P. Boudreau, and W. H. Gerwick

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Three related natural products, the honaucins A-C, were isolated from a cyanobacterium overgrowing a coral reef in Hawaii. Subsequent biological investigations revealed that these molecules inhibit both prokaryotic quorum sensing and eukaryotic inflammation. The honaucins were originally identified as molecules of interest in an in vitro assay that quantified its ability to attenuate nitric oxide production in LPS-stimulate macrophages. Continued experiments using honaucin A displayed a transcriptional down-regulation of IL-6, TNFα, IL-1β, and iNOS in these cells. Additionally, in vivo anti-inflammatory activity in a murine model of ear edema was demonstrated. In order to uncover the mechanism of action of honaucin RNA deep sequencing was performed using RNA from honaucin A-treated macrophages. Analysis of differentially-regulated transcripts strongly suggested that honaucin A is an activator of a pathway which results in the transcription of cytoprotective genes. This signaling pathway has recently drawn interest for its potential application to the treatment of neurodegenerative and autoimmune diseases, as well as cancer. Experiments involving reporter assays and protein pull down using a biotinylated probe to validate the proposed target will be discussed.

L07s – 2015.06.15, at 14.00-14.15

Immunomodulatory effects of novel N-acyl dopamine derivatives, myxillin A, B and C from the sponge *Myxilla incrustans* collected at submarine geothermal chimneys in Northern Iceland

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In this project sponges collected at unique submarine shallow water geothermal chimneys, north of Iceland, were investigated for their potential for anti-inflammatory drug discovery. The purpose of this study was to utilize UPLC-QTOF for bio-guided profiling of new compounds in sponges.

Thirtyfive sponge specimens were collected at the hydrothermal vent site by scuba diving. Extracts were prepared by CH2Cl2:MeOH (1:1) solvent extraction and further fractionated with a modified Kupchan solvent partition, giving four fractions of different polarity. The fractions were analyzed with UPLC-QTOF assay and data evaluation performed with MarkerLynx and EZinfo.

The sponge, *Myxilla incrustans* was found to have novel chemistry and pure compounds were isolated by prepartive-HPLC. The structures of three new N-acyl dopamine derivatives named myxillin A,B and C, were elucidated by 1D and 2D NMR spectroscopy and HR-MS data. The immunomodulatory effects of myxillin A was tested in a human monocyte-derived dendritic cells (DCs) model (1) and preliminary results indicate a 30% reduction in IL-12p40 secretion by treated DCs compared to untreated cells and no effects on IL-10 secretion at 10µg/ml. None of the compounds were cytotoxic at 100µg/ml. In conclusion, three new N-acyl dopamine derivatives were isolated from *M. Incrustans* and myxcillin A has a moderate immunomodulatory effects in vitro.

Acknowledgements: Doctoral grant and project grant of The University of Iceland Research Fund; Icelandic Research Fund

References:

1. Freysdottir, J. et al. (2011) Immunol Lett.: 136(1); 90-6.

L08s – 2015.06.15, at 14.15-14.30

Discovery of peptide toxins in the world's longest animal (The bootlace worm; *Lineus longissimus*): challenging claims of tetrodotoxin production

H.S. Andersson*[a], E. Jacobsson[b], C. Eriksson[b], M. Hedström[c], H. Seth[d], E.G. McEvoy[e], P. Sundberg[d], M. Strand[d,f], and U. Göransson[b]

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[c]Dept. of Biotechnology, Lund University, Sweden.
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[e]School of Natural Sciences and Psychology, Liverpool John Moores Univ., UK.
[f]Swedish Species Information Centre, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Approximately 1300 species of ribbon worms (Nemertea) have been identified worldwide. Most species are predators living in marine environments, which hunt their prey by everting a proboscis containing a mixture of toxins which brings on rapid paralysis [1]. Another characteristic of ribbon worms is a thick layer of mucus on the epidermis, seemingly constituted by a similar mixture of toxins. Among these toxins, the highly potent neurotoxin tetrodotoxin (TTX) has been identified [2-5]. The toxicity of TTX (lethal by ingestion of 0.5-2 mg [6]) stems from its ability to block voltage-gated sodium channels [7]. Although several bacterial species, among these Vibrio sp. have been linked to its synthesis, the biogenic origin and biosynthesis of TTX is unclear [8]. One hypothesis is that TTX production occurs In a symbiotic relationship with its host, which in this case would be a ribbon worm [5].

Inspired by earlier observations, we developed a setup for TTX production where *Vibrio alginolyticus* was cultivated in nutrient broth infused with mucus from bootlace worm, *Lineus longissimus*. Injections of fractions into shore crabs were used as an indicator of toxicity. A compound of near identical molecular weight to TTX was observed, but LC retention and MS fragment ions showed that this compound is unrelated to TTX. These observations highlight the risk for erroneous assessment in studies of TTX production.

In addition, it could be shown that toxicity was not a product of the *Vibrio* culture, but that it rather originates from the bootlace worm mucus. LC-MS analyses indicated the presence of peptides in the toxic mucus fractions, which could be linked to the toxic effect. We suggest that the paralytic effect of *L. longissimus* mucus can be explained by the presence of toxic peptides, and that our result may point at the need to reinvestigate some published claims of TTX production.

References:

1. Kem, W.R. (1985) Integrative and Comparative Biology, 25, 99-111; **2.** Miyazawa, K., et al. (1988) Toxicon, 26, 867-74; **3.** Asakawa, M., K. Ito, and H. Kajihara. (2013) 5, 376-95; **4.** Magarlamov, T.Y., et al. (2014) Toxicon 85, 46-51; **5.** Carroll, S., E.G. McEvoy, and R. Gibson. (2003) Journal of Experimental Marine Biology and Ecology, 288, 51-63; **6.** Klaassen, C.D., L.J. Casarett, and J. Doull, Casarett and Doull's toxicology : the basic science of poisons. 8th ed. 2013, New York: McGraw-Hill Education. xiii, 1454 p; **7.** Lee, C.H. and P.C. Ruben. (2008) Channels (Austin), 2008. 2, 407-12.; **8.** Chau, R., J.A. Kalaitzis, and B.A. Neilan. (2011) Aquat Toxicol. 104, 61-72.

L09s – 2015.06.15, at 14.30-14.45

Finding the world's best plants for type-2 diabetes targets

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The number of people afflicted by type-2 diabetes is rapidly increasing. 382 million people in the world are estimated to have type-2 diabetes today. In Denmark, more than 20 % of the population is afflicted, meaning that one in four adults are affected. Currently, there are very few herbal products on the marked in Denmark for management of type-2 diabetes. This prompted the aim of our new project: identifying the best plants in the world for the various targets involved in type-2 diabetes. Our research takes its starting point in ethnopharmacological research already performed, mining results, selecting the most promising candidates, then evaluating them in our lab, to find the truly promising plants. Preclinical data are required if herbal products are to be registered with the health authorities, and thereby becoming available for the patients.

L10s – 2015.06.15, at 14.45-15.00

Life-and-death pharmacognosy — or: What did we have for dinner?

S. Larsson

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In the Western world human poisonings with plants or fungi are uncommon. Based on phone calls to Poison Information Centres they constitute 1-13% of inquiries depending on age group. The higher numbers are seen among young children, and the vast majority of cases are completely benign requiring no specific treatment.

Severe cases of poisoning occur for three reasons: 1) intentional self-harm, 2) drug-seeking behaviour, or 3) lack of knowledge of how to correctly identify edible species when foraging for mushrooms or plants for food. All of these reasons are usually associated with older teenagers to young adults, though the third reason is common in all adult age groups especially when it comes to fungi.

In Sweden there is a small number of species of wild plants and fungi capable of causing deadly poisonings unless eaten as a meal, and only a few are added even if eaten in substantial quantities. Due to the difficulties of identifying mushrooms the Swedish Poison Information Centre has a collaboration with mycologists, but no such provision is found when it comes to plant ingestions. Thus for the most part putative plant identities are deduced from the presented symptoms, and advice on treatment and possible progression of the poisoning become rather uncertain.

Here I will report on a poisoning which started out as family fun on a spring holiday, continued on three hospitals and ended up putting new twists on a classic poisonous plant's pharmacognosy and clinical toxicology.

Acknowledgements: Javid Hussain and Ulf Göransson, Division of Pharmacognosy, Uppsala University, performed the chemical analyses and Ulla Westberg, Swedish Poison Information Center, did the follow-up with the poisoned family.

L11 – 2015.06.15, at 15.30-16.00

Extreme structure determination

M. Jaspars

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The process of structure determination of complex natural products faces a number of possible challenges. In this presentation, a number of examples will be discussed with the natural products in question having been derived from different extreme environments such as deep oceans, cold seas and arid environments. The structures of these molecules are also extreme in different ways and require tailored solutions to define their structures. The examples discussed present difficulties in defining their stereochemistry, have too few hydrogens compared to the number of heavy atoms, or simply be very large (3600 MW). Strategies that will be presented to solve such problems include the use of computer simulations, atomic force microscopy and the combined use of genetics and spectroscopic techniques.

L12s – 2015.06.15, at 16.00-16.15

The quest for large amounts of Thapsigargin

T. B. Andersen, C. Q. López, K. Martinez, and **H. Toft Simonsen**

Department of Plant and Environmental Sciences, Copenhagen Plant Science Centre, University of Copenhagen, Denmark

Since 1978 when Ulla Wagner Smitt together with Søren Brøgger Christensen discovered Thapsigargin in the roots of *Thapsia garganica* L. the tale has moved forward [1]. Today a cancer drug based on Thapsigargin is progressing through clinical trials and GenSpera Ltd will market the drug under the generic name Mipsagargin [1]. Few have attempted to cultivate *Thapsia garganica* and currently only the company ThapsIbiza is working on this. With some success though it remains to be shown whether these cultivated species contain Thapsigargin. This is ongoing in our laboratory in collaboration with ThapsIbiza and as part of the EU project MedPlant (www.medplant.eu). Secondly, in the EU project DrugTissueCult (www.drugtissuecult.eu) we will attempt to use tissue culture systems to produce biomass of *Thapsia garganica*; this is performed in collaboration with Alkion Biopharma in France.

The third approach is the elucidation of the biosynthesis of Thapsigargin (www.spotlight.ku.dk) and the product in a heterologous host. Here we published the first step of the pathway in 2012 [2], and can now present the second step. The first step was biosynthesized by a terpene synthase to yield kunzeaol, whereas the following step is mediated by a cytochrome P450. This step establish the lactone ring, and provide the backbone for further modifications. Further steps are currently being elucidated, and we will try to express these in yeast and the moss *Physcomitrella patens*.

Acknowledgements: The work was supported by: SpotLight, a grant from the Danish Council for Strategic Research; DrugTissueCult, a Marie-Curie ITN-EID project funded from European Union's Seventh Framework Programme FP7/2007-2013/ under REA grant agreement n° [607011]; MEDPLANT, a Marie Curie Actions Initial Training Network (ITN), funded by the European Union under the Seventh Framework Programme FP7/2007-2013.

References:

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2. Pickel, B., et al., Identification and characterization of a kunzeaol synthase from *Thapsia garganica*: implications for the biosynthesis of the pharmaceutical thapsigargin. Biochemical Journal, 2012. 448(2): p. 261-271.

L13s – 2015.06.15, at 16.15-16.30

Sialidase enzyme as a novel drug target against trichomoniasis

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Trichomoniasis is one of the most common sexually transmitted diseases with the prevalence rates of 15 % or higher among women in many developing countries [1]. It is caused by unicellular protozoan parasite *Trichomonas vaginalis*. Our current project is devoted to the investigation of one sialidase enzyme, acquired by *T. vaginalis* via lateral gene transfer from bacteria [2], as a target for drugs against trichomoniasis and to the discovery of natural compounds with inhibitory activity.

The sialidase encoding gene from *T. vaginalis* was cloned into pLEXY-invitro2 plasmid and translated into GFP-coupled protein using cell-free protein expression kit based on *Leishmania tarentolae* (Jena Bioscience GmbH, Jena, Germany). Expression of the protein and following enzymatic activity confirmation was monitored using fluorescence correlation spectroscopy.

We have confirmed that the LEXY cell-free protein expression system functions for sialidase enzyme expression and that sialidase-GFP has a reduced diffusion coefficient compared to GFP alone, as expected due to the larger size of Sialidase-GFP. In the next step, we have analysed the enzymatic activity of sialidase by monitoring the catalysis of the substrate provided in the Neuraminidase Activity Assay Kit (Sigma-Aldrich, St. Louis, MO) into a fluorescent product. Potential inhibitors of expressed trichomonas specific sialidase enzyme among natural products will be further investigated.

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L14 – 2015.06.15, at 16.30-17.00

Phylogenetic exploration of medicinal plant diversity. Can the evolutionary history of plants help guide better healthcare, new leads, and sustainable use of medicinal plants?

N. Rønsted

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The use of plants for medicine is closely associated with human culture and has provided both local healthcare and new leads. Through evolution plants have developed sophisticated chemical defenses, which may explain their bioactivity in humans. Intuitively, the evolutionary history of plants may enable predictive approaches allowing systematic evaluation of current and potential medicinal value as well as help guide safety, conservation policies and agriculture. Recent studies by our group [1-3] and others [4], have highlighted that medicinal use, plant defensive compounds and bioactivity are correlated with phylogeny, but the predictive power of phylogenies is yet unknown. Developing new systematic and integrative approaches and tools to synthesize and take advantage of phylogeny, bioinformatics, ethnobotany, natural products, chemistry and bioactivity studies could supplement traditional selection approaches with the ultimate aim of providing better healthcare. This presentation will summarize recent studies, current efforts, and future directions as well as introduce the MedPlant International Training Network (www.MedPlant.eu) educating 15 young scientists in phylogenetic exploration of medicinal plant diversity.

Acknowledgements: The research leading to these results has received funding from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme FP7/2007-2013/ under REA grant agreement n° 317184.

References:

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PawS-derived peptides: A diverse family of cyclic plant peptides from sunflowers

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[b]Univ. of Queensland, Institute for Molecular Bioscience, Brisbane, Australia.
[c]School of Chemistry and Biochemistry & ARC Centre of Excellence in Plant Energy Biology, Univ. of Western Australia, Perth, Australia.

The peptide SunFlower Trypsin Inhibitor-1 (SFTI-1) is produced in the seeds of sunflower (*Helianthus annuus*). It comprises 14 amino acids and is characterized by a bicyclic structure due to the presence of a head-to-tail cyclized backbone and one disulfide bond, which together provide a remarkably stable structure [1]. SFTI-1 is as the name suggests a potent inhibitor of trypsin and in addition has been shown to be a promising scaffold for drug design in that the sequence can be modified to incorporate and stabilise other bioactive epitopes. Biosynthetically SFTI-1 is produced from a precursor for an albumin seed storage protein named PawS1. PawS1 is post-translationally processed by an aspariginyl endopeptidase into a mature two-chain albumin, and during this process SFTI-1 is cleaved out and cyclized [2].

Recent work has provided insights into the evolution of SFTI-1. Genetic and liquid chromatography mass spectrometry based screening approaches of an additional 273 species in the family Asteraceae have identified a number of cyclic and acyclic peptides, with structural and sequence homology to SFTI-1. These are referred to as PawS-Derived Peptides (PDPs) [3]. Here we have used solution NMR spectroscopy to characterize the 3D structures of a number of PDPs showing that in general they form well-defined, rigid structures with a diverse range of folds as a result of their different sequences. The closest relatives of SFTI-1 are able to inhibit trypsin, however, no biological significance has yet been described for the more structurally diverse peptides.

References:

1. Luckett, S., et al. J Mol Biol. 1999 290:525-33.
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L16s – 2015.06.16, at 09.00-09.15

Sponge taxonomy 2.0 meets pharmacognosy

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Proper species identification and classification is crucial to any scientific study. Naming a species and using a proper classification is the only way to make sure that scientists are studying the same entity, and that all the data linked to conspecific specimens but produced by different researchers can be understood, associated and compared. Linking biological data (molecular, morphological, biochemical, ecological) to an incorrect species name or to no species name will result in these data losing tremendous value. This is an important issue in the field of sponge natural products since 1) the biodiversity of sponges is still poorly known and since 2) sponge taxonomy has been very unstable in the last decades. In the past 13 years, the classical taxonomy has been considerably overturned by an increasing number of molecular phylogenetic studies, with numerous polyphyletic groups revealed or confirmed and new clades discovered. Based on these results, Morrow & Cárdenas [1] now propose a revised classification of the Demospongiae, hoping to convince end-users to 1) abandon the use of artificial groups, and to 2) use the new/resurrected names proposed here when referring to the new Demospongiae clades. This updated classification will undoubtedly facilitate communication between end-users, reduce taxonomically biased results, and ultimately provide a better understanding of Demospongiae biochemistry and evolution.
In this talk I will illustrate with previous studies and others from my current research how this new revised classification can shed a new light on previous and current sponge natural product and chemical ecology studies.

References:
Morrow C. and Cárdenas P. (2015) Proposal for a revised classification of the Demospongiae (Porifera). Frontiers in Zoology, 12:7.

L17s – 2015.06.16, at 09.15-09.30

Cinchona alkaloid derivatives as antibacterial and anti-biofilm chemotypes

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[b]Pharmaceutical Sciences Laboratory, Åbo Akademi University, Turku, Finland.
[c]Laboratory of Organic Chemistry, Åbo Akademi University, Turku, Finland.

Bacterial biofilms are highly tolerant to most available antibacterial treatments, including various kinds of chemotherapy and host cell responses. A challenging problem is caused by biofilm involvement in nosocomial infections, which will continue rising due to the increasing use of indwelling medical devices [1]- Thus, an enormous need exists to meet the demands of novel effective anti-biofilm therapy.

In this study, a library of *Cinchona*-alkaloids, including the natural compounds cinchonidine and cinchonine, as well as various synthetic derivatives was screened for antibacterial and anti-biofilm activity against *Staphylococcus aureus*. To evaluate the anti-biofilm activity of the compounds an assay platform including two staining methods, resazurin and crystal violet, was used to measure biofilm viability and total biomass, respectively. Also, the effect on planktonic bacteria was measured. Cinchonidine was found to be inactive, whereas interestingly, a synthetic derivative, 11-TPSCD (structure depicted), was effective against planktonic bacteria as well as in preventing biofilm formation at low micromolar concentrations [2]. Higher concentrations were required to eradicate pre-formed biofilms. The chemical space of these derivatives was also mapped using ChemGPS-NP [3,4] together with chemically-related quinolones antibiotics. The results show another ”priviledged” region for quinine-related molecules outside of the one populated by the quinolones antibiotics, which is worth exploring in future drug discovery strategies.

Acknowledgements: This contribution is dedicated to the memory of Dr. Igor Busygin (1980-2015). Academy of Finland (Projects WoodyFilm and ArtFilm) is acknowledged.

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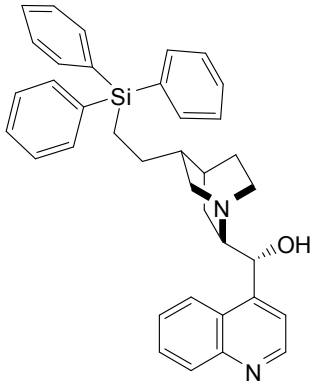
Si
N
OH
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L18s – 2015.06.16, at 09.30-09.45

A new biological tool for disulfide-rich cyclic peptide synthesis: Application of PatG macrocyclase enzyme in the synthesis of cyclotides and sunflower trypsin Inhibitor-1

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[b]Dept. of Chemistry, Marine Biodiscovery Centre, University of Aberdeen, UK.

Circular peptides with disulfid bonds (cyclotides and SFTI-1) have created a great interest in the development of peptide-based drugs due to their improved physicochemical properties [1, 2, 3]. However, the most commonly used method for the head-to-tail cyclization of peptides has been native chemical ligation, [4] providing peptides with considerable purity and yields. However, the exposure to chemicals and costs associated with chemical synthesis drive the continuous search for novel methods for circular peptide synthesis [5]. A subtilisin-like macrocyclase called PatGmac produced by the cyanobacteria Prochloron sp. that are symbionts of the sea squirt *Lissoclinum patella* was identified in a recent study [6]. The PatGmac recognizes the C-terminal macrcyclization signature (positions P1'-P4') AYDG, which is subsequently cleaved off to form a head-to-tail cyclized peptide [6]. Here, we demonstrated that linear SFTI-1 precursor with a C-terminal AYDG enzyme recognition sequence could be efficiently cyclized by recombinant PatGmac macrocyclase mediated cyclization. Our findings provide proof-of-concept of the potential broad applicability of PatG enzymatic cyclization of disulfide rich peptides in drug development.

References:

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2. Luckett, S., et al. (1999) J. Mol. Biol. 290, 525-533.
3. Craik, D. J., et al. (1999) J. Mol. Biol. 294, 1327-1336.
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L19s – 2015.06.16, at 09.45-10.00

From ethnomedicine to phylogenetic prediction in drug discovery in the genus *Euphorbia*: Suggestions for a new classification system of diseases

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It has long been hypothesized that plant-derived natural products, responsible for many pharmacological effects in humans, form a mirror image of evolutionary relationships of plants producing them [1]. Several studies [2–5] have thus proposed a systematic approach for drug discovery from natural products inferring bioactivity due to reported uses in ethomedicine and making predictions about bioactivity of ethnomedicinally unknown species based on their phylogenetic relationships. To allow statistical analysis, described ethnomedicinal uses are classified into disease categories (often describing diseases associated with certain body parts), a common practice in ethnomedicinal research [6]. In the present study we propose an alternative classification system of ethnomedicinal uses based on molecular disease mechanisms as understood by most recent medical research and suggest that those are more closely related to the chemical nature and thus bioactivity of the natural products contained in plants and are therefore more appropriate for studies using phylogeny as a predictive tool in drug discovery. Using the extremely species-rich (~2000) [7] and almost cosmopolitan genus Euphorbia, known for its immunomodulatory diterpenes [8], we illustrate the impact different classification systems have on subsequent data interpretation. Our study shows that a classification system based on molecular disease mechanism providing a link between pharmacological properties and medicinal effects of plant-derived natural products, allows a more targeted approach for future biodiversity-driven drug lead discovery.

Acknowledgements: The research leading to these results has received funding from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme FP7/2007-2013/ under REA grant agreement n° 317184.

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L20 – 2015.06.16, at 10.30-11.00

High-resolution bioactivity profiling combined with hyphenated HPLC-HRMS-SPE-NMR for accelerated identification of bioactive natural products directly from crude extracts

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Nature is a rich source of bioactive constituents, but traditional bioactivity-guided fractionation is a time-consuming and laborious task involving several preparative-scale chromatographic steps. In recent years, the 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, database-assisted NMR structure elucidation and off-line assessment of circular dichroism spectra for assignment of absolute configuration. 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 coupling of microplate-based high-resolution bioassays with HPLC-HRMS-SPE-NMR, i.e., HR-bioassay/HPLC-HRMS-SPE-NMR, represents one of the most promising new developments for advancing research in bioactive constituents from natural sources like food [2], traditional medicine [3], plants [4], and microorganisms [5].

A schematic illustration of the workflow in the HR-bioassay/HPLC-HRMS-SPE-NMR analysis is given overleaf - and involves **i:** analytical-scale separation(s), **ii:** micro-fractionation into 96-well microplates, **iii:** bioassaying (e.g., aldose reductase, α-glucosidase, and ABTS•+ reduction), **iv:** results from bioassays plotted against their respective retention time to produce high-resolution biochromatogram(s), **v:** identification of bioactive analytes from biochromatogram, **vi:** HPLC-HRMS-SPE-NMR analysis targeting bioactive constituents, and **vii:** structural identification of bioactive constituents. In this talk some recent examples of the successful use of high-resolution bioactivity profiling in combination with HPLC-HRMS-SPE-NMR analysis will be given.

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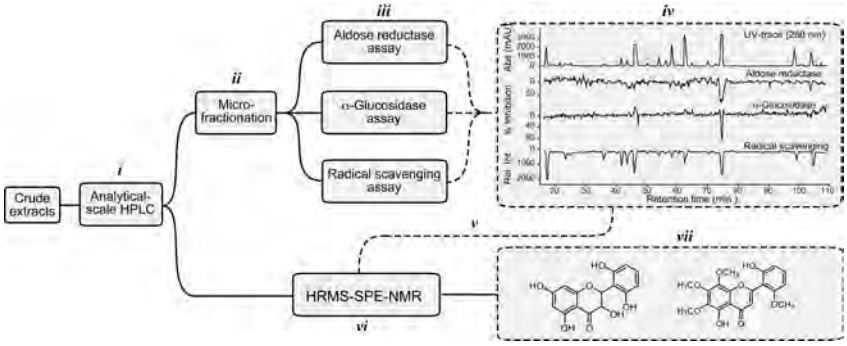
Crude extracts
Analytical-scale HPLC
Micro-fractionation
Aldose reductase assay
α-Glucosidase assay
Radical scavenging assay
HRMS-SPE-NMR
UV-trace (298 nm)
Aldose reductase
α-Glucosidase
Radical scavenging
Retention time (min)
i
ii
iii
iv
v
vi
vii

L21s – 2015.06.16, at 11.00-11.15

Polyacetylenes from carrots (*Daucus carota*) with potential antidiabetic effects

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[b]Dept. of Biology, Univ. of Copenhagen, Denmark,
[c]Dept. of Food Science, Aarhus Univ., Tjele, Denmark,
[d]Computer-Aided Drug Design, Institute of Pharmacy, Medicinal and Pharmaceutical Chemistry, Freie Universität Berlin, Germany.

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 roots was found to stimulate insulin-dependent glucose uptake (GU) in adipocytes in a dose-dependent manner [1]. Bioassay-guided fractionation of the DCM extract resulted in the isolation of the polyacetylenes falcarinol and falcarindiol, which both 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. Falcarindiol increased PPARγ-mediated transactivation significantly at concentrations of 3, 10 and 30 μM, while PPARγ-mediated transactivation by falcarinol was only observed at 10 μM but partially for both compounds compared to the TZD rosiglitazone. Docking studies accordingly indicated that both polyacetylenes exhibit characteristics of PPARγ partial agonists. Falcarinol was shown to inhibit adipocyte differentiation as evident by gene expression studies and Oil Red O staining, whereas falcarindiol did not inhibit adipocyte differentiation, which indicates that these polyacetylenes have distinct modes of action. The results of the present study suggest that falcarinol and falcarindiol may represent scaffold for novel partial PPARγ agonist with possible antidiabetic properties.

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L22s – 2015.06.16, at 11.15-11.30

Notes on chemical property position of bedaquiline construed by chemical global positioning system-natural product (ChemGPS-NP).

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BMC - Biomedical Centre, Box 574, S-751 23 UPPSALA, Sweden.

Bedaquiline represents both a novel chemical class of diarylquinolines and a novel mechanism of action against tuberculosis. It is one of the rare discoveries that have many clinically desirable properties. Thus quickly establishing a new paradigm for treatment of tuberculosis. Ranking of similarity and dissimilarity is of particular interest in lead discovery and compound optimization. Discrimination of bedaquiline is critical due to the fact that bedaquiline has a different mechanism of action from the relatively similar chemical structures.
A new approach using eight scores of principle component analysis (PCA) provided by ChemGPS-NP has been employed to study the chemical property position of bedaquiline. The results were refereed as accumulative Euclidian distance for different antituberculosis sets and ChemGPS-NP was compared with extended connective finger-prints and Molecular ACCess System showing 30% and 182 % increase in accumulative Euclidian distance respectively. Potentially similar compounds from publically available anti-tuberculosis compounds and Maybridge sets based on bedaquilines eight dimensional similarity and different filtrations, were determined and provided as a contribution towards the challenging anti-tuberculosis drug discovery research.

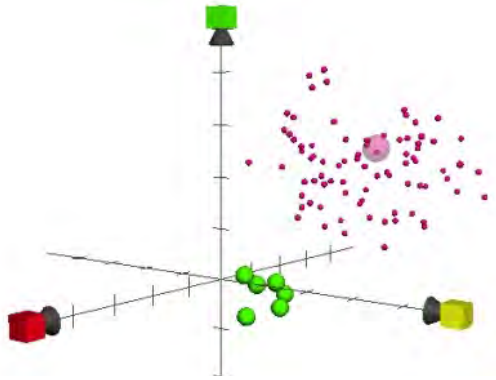

Figure 1. Position of bedaquiline (large pink sphere), 100 closest antituberculosis compounds (small spheres) and quinoline compounds (green spheres) by ChemGPS-NP where PC1 (x=red), PC2 (y=yellow) and PC3 (z=green).

L23s – 2015.06.16, at 11.30-11.45

Immunolocalization of cyclotides in plant cell, tissue and organs supports their role in plant defense

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[b]Div. of Pharmacognosy, Dept. of Medicinal Chemistry, Uppsala Univ., Sweden.
[c]Dept. of Plant Cytology and Embryology, Faculty of Biology, Univ. of Gdańsk, 59 Wita Stwosza St., 80-308 Gdańsk, Poland.
[d]Dept. of Plant Cytology and Embryology, Inst. of Botany, Jagiellonian Univ., Cracow, Poland.
[e]Faculty of Biochemistry, Biophysics and Biotechnology; Jagiellonian Univ., Cracow, Poland

Premise of the study Cyclotides are cyclic plant peptides with a wide range of reported biological activities. Their role in plant defense has been suggested on the basis of insecticidal properties [1]. The tissue distribution of a defense compound may suggest type of pathogen it protects a plant from [2].

Methods Antibodies were raised in rabbit using the bracelet cyclotide cycloviolacin O2. Slides for immunohistochemistry were prepared from leaf, petiole and root fragments of *Viola odorata, V. uliginosa* and from plants known to not produce cyclotides (*Arabidopsis thaliana* and *Nicotiana benthamiana*) using procedure described before [3]. Visualization of specimens was performed using indirect epi-fluorescence microscopy.

Antibodies were proven to be specific against bracelet cyclotides, abundant in *Viola odorata*. High relative amounts of cyclotides were found in leaf epidermis, and in the epidermis of petioles, in both *Viola* species. Cyclotides were also found in vasculature tissue (phloem) in all the assessed plant organs. Double immunostaining clearly indicated vacuolar storage of cyclotides.

Acknowledgements: This work was funded in part by Ahlquists Stiftelse (Ahlquist Foundation) grant for research exchange at the Faculty of Pharmacy, Uppsala University; the Swedish Research Council (2012-5063); the Swedish Foundation for Strategic Research (F06-0058) (Ulf Göransson)

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L24s – 2015.06.16, at 11.45-12.00

Zanthoxylum heitzii, a possible anti-malaria medicine

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The Central African plant *Zanthoxylum heitzii* is used by traditional healers against malaria. We have found that the hexane bark extract is active against adults of the mosquito *Anopheles gambiae*, a vector for malaria transmission [1], and that this is mainly due to its content of the amide pellitorine. Admixture with other, inactive constituents of the extract increases the activity of the amide. Pellitorine also kills *A. gambiae* larvae. In addition, the extract is toxic to the malaria protozoa *Plasmodium falciparum*. In this case, the alkaloid dihydronitidine seems to be the main factor for toxicity. Neither of these compounds have been reported from this plant previously. Eight other substances have been isolated and identified. Seven of these are new to the plant, and one, the alkaloid heitziquinone, is a new natural product. From these investigations, it would seem possible that the traditional use of *Z. heitzii* against malaria may have a rational basis.

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L25 – 2015.06.16, at 13.30-14.00

Open Innovation and Data Integration - from docking poses to prediction of side effects

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Within the past decade the way which computational drug design is conducted changed dramatically. With the availability of ChEMBL, PubChem, and ChemSpider, cheminformatics gradually moves to open access. One of the current major challenges is the integration of all these public data sources in order to allow targeting complex research questions, such as “give me all oxidoreductase inhibitors in humans an mice with an IC50 value <100 nM”. Semantic data integration platforms such as the Open PHACTS Discovery Platform not only enable to query across multiple data domains, they also open hitherto unreached possibilities for in silico model generation and model validation [1].

Within this talk we will present case studies for validating docking poses by ligand-based structure-activity relationships [2,3], for creating predictive classification models for transporters by mining public data bases [4], and for predicting side effects based on ligand-receptor interaction profiles [5].

Acknowledgements: We acknowledge financial support provided by the Austrian Science Fund (F3502) and the Innovative Medicines Initiative (Open PHACTS, 115191)

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L26s – 2015.06.16, at 14.00-14.15

Sesquiterpenes from the saprotrophic fungus *Granulobasidium vellereum*.

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Granulobasidium vellereum is a wood-decomposing basidiomycete fungus that arrives at dead trunks of fallen hard wood trees at a late stage of the decomposition process. Thus, the fungus needs to out-compete the organisms already colonizing the site. It was hypothesized that part of the fungus competitive ability was based on the production of secondary metabolites. Consequently, the production of the secondary metabolites by this fungus was investigated.

In total, 33 secondary metabolites, all of sesquiterpenoid origin, were isolated from fungal cultures by chromatographic methods and characterized by spectroscopic techniques [1-3]. Twenty-two of these compounds were previously not described, and two were new as natural products. Only one of the compounds displayed potent antifungal activity, whereas five of the isolated compounds were highly cytotoxic.

When cultivated together with other wood-decay fungi, the production of 19 metabolites from *G. vellereum* were up-regulated in all studied interactions, whereas several other compounds were up-regulated in individual interaction cultures [4].

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L27s – 2015.06.16, at 14.15-14.30

Bio-guided fractionation of immunomodulatory compounds from the marine sponge *Halicondria sitiens.*

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Iceland has a unique position in the North Atlantic Ocean where geothermal heat and cold water masses meet. Despite this unique environment, marine natural products have only been investigated to a limited extent with regard to chemical constituents of pharmacological interest. The aim of this study was to collect sponges at marine hydrothermal vent sites, prepare extracts and test their immunomodulatory effects in a human monocyte-derived dendritic cell (DC) model (1) and to use bio-guided isolation to identify new compounds with anti-inflammatory potential. The maturation of DCs in the presence of an organic extract of the sponge *Halicondria sitiens* at 100 µg/ml resulted in lower secretion of the cytokines IL-10 and IL-12p40 and a lower ratio of IL-12p40/IL-10 in comparison with DCs matured without the extract. This pattern of response indicated reduced inflammatory capacity of the DCs. The extract was separated into fractions using various chromatographic methods (i.e. CC, VLC, SPE and preparative HPLC) followed by TLC, 1H-NMR and LC-MS/MS. At each step in the bio-guided isolation procedure the fractions were tested for activity in the DC model. Eight of the fractions showed significant immunomodulatory effects and two of them almost completely hindered IL-12p40 secretion. The two fractions with the most immuno-modulatory effects also caused morphological changes of the DCs. None of the active fractions were cytotoxic. The final steps of the isolation of pure compounds and their structure elucidation (NMR, MS/MS) are in progress. These results indicate the presence of new immuno-modulating compound(s) in *H. sitiens* but to our knowledge no compounds have been described from this species.

Acknowledgements: Doctoral grant and project grant of The University of Iceland Research Fund; Project grant from the Ministry of Fisheries and AVS R&D Fund of Ministry of Fisheries and Agriculture in Iceland; Project grant from Landspitali University Hospital Research Fund.

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L28s – 2015.06.16, at 14.30-14.45

Central-stimulating and analgesic activity of the ethanolic extract of *Alternanthera sessilis* in mice

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Alternanthera sessilis is a popular vegetable and used in traditional medicinal practice of Bangladesh and other parts of Asia to relive tiredness, laziness, and sleeps as well as pain and inflammation [1]. However, no report was found on the neuropharmacological and analgesic activity of this plant to-date. Present study was undertaken to evaluate the neuropharmacological and analgesic activity of the ethanol extract of *A. sessilis* whole plant (ETAS) in mice models. Central stimulating activity was investigated by pentobarbitone induced sleeping time, open field, and hole cross tests. Analgesic activity was evaluated by acetic acid induced writhing and hot-plate methods. The tests were performed at 250 and 500 mg/kg body weight dose levels. In sleeping time test, ETAS significantly ($p < 0.001$) increased the onset of sleep, and decreased the duration of sleep. In open field and hole cross tests, ETAS significantly ($p < 0.001$) increased the movements of mice which persisted throughout the study period. In writhing test, ETAS showed, significant ($p < 0.001$) inhibition of writhing reflex. In hot plate test, ETAS significantly ($p < 0.001$) raised the pain threshold. In HPLC analysis for polyphenols, (+)-catechin, rutin, ellagic acid, and quercetin were detected in ETAS (117.72, 490.74, 3007.26, and 13.85 mg/100 g of dry extract, respectively). Present study supported the traditional uses of *A. sessilis* and indicated that the plant can be a potential source of bioactive molecules.

Acknowledgements: Khulna University Research Cell, Khulna, Bangladesh

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L29s – 2015.06.16, at 14.45-15.00

Ligand fishing using functionalized magnetic beads combined with HPLC-HRMS-SPE-NMR: Analysis of α-glucosidase inhibitors in a crude plant extract

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Screening complex plant extracts for new drug leads is a challenging task, and therefore advanced alternatives to classical bioassay-guided fractionation are needed. Among the advanced technologies introduced in recent years is ligand fishing, which is a technique where a selected therapeutic target (a receptors or an enzyme) is immobilized on a solid support. The solid support with the immobilized enzymes/receptors is then used to selectively fish out potential ligands from complex mixtures. In natural products, this technique has been used as a pharmacological profiling tool to discover biologically active metabolites in crude extracts. Ligand fishing has been utilized in combination with mass spectrometry - either by desorption ionization directly off the fishing probe [1] or through LC-MS after releasing the bound ligands into appropriate solvent [2]. Despite the successful implementation of mass spectrometry for analysis of biological concentrates prepared by ligand fishing, the limited structural information can hamper elucidation of novel ligands. Therefore, we present a combination of the ligand fishing technology with a one of the most powerful structural analysis tool, i.e. high-performance liquid chromatography - high-resolution mass spectrometry - solid-phase extraction - nuclear magnetic resonance (HPLC-HRMS-SPE-NMR) [3]. In the current work, functionalized magnetic beads with the N-terminus bound α-glucosidase were synthesized and used as a ligand fishing tool to fish out potential anti-diabetic compounds from a crude plant extract. The HPLC-HRMS profile of the 'fished-out' concentrate was used to pinpoint the high-affinity ligands, which afterwards, were subjected to a targeted HPLC-HRMS-SPE-NMR analysis directly from the crude extract.

Acknowledgements: The ligand fishing project is funded by The Danish Council for Independent Research | Technology and Production Sciences

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L30 – 2015.06.16, at 15.30-16.00

Chemical ecology.

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Research in chemical ecology gives us knowledge about the fascinating diverse chemical communication that exists among organisms, including humans. The aim is to develop chemical tools for controlling insects and other organisms preventing diseases in animals and plants. Examples from our research of how we can utilize butterfly pheromones, fungi volatiles and conifer resin constituents for organic cropping and sustainable agriculture and forestry will be given.

L31s – 2015.06.16, at 16.00-16.15

Circular disulfide-rich peptide scaffolds as anti-citrullinated peptide antibody inhibitors.

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Circular proteins are an important class of natural products, now described from all sources of life, including plants, animals, bacteria and fungi [1]. Inspired from nature's repertoire of circular proteins, the concept of backbone cyclization is utilized to improve stability of many pharmaceutically important peptides [2]. The current work describes the use of sunflower trypsin inhibitor 1 (SFTI-1) scaffold, a 14 residues disulfide stabilized circular peptide for incorporating bioactive epitopes that are relevant in the therapeutic context rheumatoid arthritis (RA) [2]. Autoantibodies highly specific for RA, known as anti-Citrullinated Peptide Antibodies (ACPAs), are not only prognostic markers of RA, but appear to play a key pathogenic role in RA disease progression itself [3]. Citrullination has been observed in different synovial proteins of RA patients including type II collagen, fibrinogen, α-enolase and vimentin. Although synthetic peptides that mimic the natural antigens of ACPA have the potential to block ACPA, the stability of such peptides is a major concern in their ultimate use in a biological context. By incorporating the citrullinated peptide epitopes within the stable SFTI-1 scaffold we have provided proof of concept that ACPA can be inhibited in vitro by stable peptides that mimic their natural antigen. Currently, the isolation of specific subtypes of ACPA and development of a patient tailored ELISA system to facilitate diagnosis of specific subtypes of ACPA unique to each patient profile is underway.

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Fungal natural product chemistry in the genomic era.

T. Ostenfeld Larsen

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With the entering into the Genomic Era the field of microbial natural product chemistry has changed dramatically in recent years. The number of available genomes are expanding exponentially. AT DTU we are currently involved in a genome sequencing project targeting all > 340 known species in genus *Aspergillus*. Fortunately the genes involved in biosynthesis of fungal secondary metabolites are located in clusters, setting the scene for bioinformatics driven analysis of the chemical potential of a given species. Surprisingly these types of analyses have shown that the number of clusters encoding secondary metabolism is much higher than the number of known compounds even in well-studied model organisms. Consequently the potential for discovery of novel compounds from such cryptic gene cluster is still vast.
This presentation will highlight the different approaches that we are undertaking at DTU to discover, characterize and engineer fungal biosynthetic pathways. This will include examples of: **i)** Genome wide deletion of all polyketide synthase genes in *A. nidulans* to clarify the biosynthesis of meroterpenoids; **ii)** Development of a novel algorithm for analysis of transcription data to link distantly located but co-expressed genes to discover novel prenylated non-ribosomal peptides; **iii)** How deletion of all genes involved in the 6-methyl-salicylic acid derived biosynthetic pathway has led to many novel yanuthones; **iv)** How induction of sclerotium formation stimulated expression of previously silenced genes and led to novel chemistry; **v)** Examples of how overexpression of transcription factors can boost biosynthetic pathways and even stimulate chemistry across different pathways.

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L33 – 2015.06.16, at 16.30-17.00

Interdisciplinary approaches to marine natural products drug discovery

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The unique organisms living in the world's oceans are an inspiring source of new pharmaceutical leads. To date, some 13 drugs of marine derivation or inspiration have reached the clinic in the US, Europe or Asia, and many more are on the horizon. In this regard, we have focused our investigations on the bioactive metabolites available from marine cyanobacteria and algae, both groups of which are extraordinarily rich in structurally diverse natural products. Moreover, we are exploring the integrated use of several different technologies, such as genome-based information, mass spectrometry-based Molecular Networks, and synthetic medicinal chemistry, to innovatively discover and develop new drug leads from these marine organisms. For example, a Curaçao collection of a tuft-forming cyanobacterium, *Symploca* sp., possessed an extract that was highly cytotoxic to several different cancer cell lines. A combination of a bioassay and NMR guided isolation process yielded two compounds, named carmaphycin A and B, which were responsible for the potent activity. We are exploring their utility as potential anticancer agents through a variety of orthogonal approaches. Another recent project involves a new approach that integrates genomic and metabolomic information to identify structurally novel and biologically active metabolites. This latter work identified a series of novel chlorinated acyl amides that have potent binding properties to cannabinoid receptors. By integrating several different and contemporary approaches, new vistas in the natural products sciences are being revealed.

P01

Identification of bioactive peptides from enzymatic hydrolysis of royal jelly

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Royal jelly (RJ) has been widely used as a popular and traditional food for health promotion. RJ is rich with bioactive constituents such as 10-hydroxy-2-decenoic acid, jelleines, and major royal jelly proteins (MRJPs) considered as anti-cancer and anti-bacterial agents [1,2] as well as royalisin, well-known as anti-bacterial agent against gram-positive bacteria [3,4]. The complete identification of RJ complex composition is a research issue that remains quite open, and the development of new characterization techniques is allowing the discovery of new compounds.

The enzymatic hydrolysis method is one of the most preferred methods in the food and pharmaceutical industries over the other methods, which could leave the organic residues or toxic chemicals in the products. The released peptides from enzymatic hydrolysis are exert various physiological as antioxidant [5] and anti-hypertensive [6]. For the first time we identified the antimicrobial peptides from RJ hydrolyzate using mass spectrometry analysis with aid of Mascot, NCBI databases. The peptides were screened against four pathogens including; *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* [6].

Taken together, Bioassay guided fractionation for RJ hydrolyzate using RP-HPLC lead to isolate for three peptides with promising antimicrobial activity. More detailed studies are required to explore their possible future applications in functional food and/or pharmaceuticals industry [7].

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*Dedicated with regards and respect to Prof. Lars Bohlin on the occasion of his 67 birth day and retirement

P02

Isolation, characterization and structure elucidation of new secondary metabolites from *Bacillus subtilis* A-4

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Antibacterial activities were detected in six bacteria strains obtained from soil samples. One strain designated as A-4 produced an inhibitory substance, which was active against number of Gram-positive bacteria as well as clinical isolates. The strain was identified as *Bacillus subtilis* by standard microbiological and biochemical tests. Total secondary metabolites compounds isolation was performed. A total of 16 secondary metabolites were identified along with lipopeptide. Identified as hexa-lipopeptide with a mass of 1230 Da. The later did not lose its activity on treatment at different pH values, temperatures and enzymes. The pronounce activity against multidrug resistant clinical isolates and the favorable biochemical properties of this peptide from the newly isolate strain suggest a new and effective agent against resistance in pathogenic microbes

P03

Common deviation screening: A targeted approach for novel metabolite discovery using common molecular formula deviations and high resolution mass spectrometry.

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The discovery of novel and/or bioactive natural products is often a difficult and time-consuming process. In some cases, where chemistry of an organism is previously known, dereplicating known compounds is vital for highlighting potentially unknown metabolites [1]. In some instances little is known of the chemistry of a target species, and the vast majority of the chemical features observed in a chromatogram are unknown. A chromatogram produced by high resolution mass spectrometry of a crude algae extract can easily contain over 1000 unknown molecular features making the identification of a metabolite target difficult. This is particularly evident in algae, which produce chlorophyll metabolites that, along with their degradation by-products, complicate spectra.
Although there may be little known about a particular species of algae, when considering algae as a whole many toxins have been characterised [2]. From certain species, classes of toxins have been characterised, such as the karlotoxins from *Karlodinium veneficum* [3]. One thing that many algae metabolite classes have in common is that they are typically large polyketides, often containing multiple ether rings. Due to structural similarities between metabolite classes, structural differences within classes can be summarised and applied to metabolites of other species to generate a list of over 1000 hypothetical molecular formulas within seconds. This list can then be used to screen a chromatogram, identifying a potential target metabolite within a few minutes.

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P04

How to navigate the chemical space using ChemGPS-NP: validation study and application to Natural Products

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Natural Products (NPs) are "privileged structures" that have inspired the design of many approved drugs. In the light of their importance, *in silico* tools aimed to explore the chemical space defined by NPs have been developed.
ChemGPS-NP is a global chemical positioning system for the exploration of biologically relevant chemical space and represents an extension of the previous ChemGPS model by addition of Natural Products [1,2]. This global space map is based on 35 physico-chemical properties; the resulting multidimensional data is simplified through a Principal Component Analysis (PCA) in 8 dimensions describing the variance of the physico-chemical properties. Molecules with known chemical structures can be projected in the ChemGPS map and resulting properties can be compared with other molecules. An important concept in medicinal chemistry is the relationship between similarity of structural features, physico-chemical properties and biological activity. In other words, similar molecules are likely to present similar biological properties [3]. In this perspective, the location of a molecule in the ChemGPS-NP space can be used to predict its biological profiles by analyzing the biological activities of the surrounding molecules.
In this work, we challenged ChemGPS-NP for the ability to retrieve the bioactivity of candidate compounds, when compared to reference sets gathered from ChEMBL collection [4]. We also analyzed the complementarities between the description of molecular similarity using ChemGPS-NP and a fingerprint-based method (based on ECFP_4) [5]. Finally, we applied these approaches to investigate the similarity of Natural Products to known bioactive compounds.

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P05

Alkaloids and their proposed biosynthesis from *Pandanus amaryllifolius*

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Pandanus amaryllifolius Roxb. (Pandanaceae) is a tropical shrub distributed in Southeast Asia and used as a flavor and folk medicine as a remedy for the treatment of hyperglycemia, liver protection, and inhibition of tumor growth. The ethanolic crude extract of *P. amaryllifolius* aerial part showed potential antioxidant, anti-biofilm, and anti-inflammatory activities. The purification of the ethanolic extract yielded nine new compounds, including two norpandamarilactonines, norpandamarilactonine-C (1) and -D (2), two indolizinones, pandalizine-A (3) and -B (4), a pandanamine, pandanamine-A (5), three pandamarilactones, pandamarilactone-2 (6), -3 (7), and -4 (8), and an unusual pandamarilactonine, pandalatonine (9).
In the current study, the isolated alkaloids, with either γ-alkylidene-α,β-unsaturated-γ-lactone or γ-alkylidene-α,β-unsaturated-γ-lactam system, can be classified into five skeletons of *Pandanus* alkaloids, including norpandamarilactonine, indolizinone, pandanamine, pandamarilactones, and pandamarilactonine. The structural elucidation and absolute configuration of these compounds are revealed in this investigation. Moreover, a plausible biogenetic pathway of *Pandanus* alkaloids 1–5, 7, and 9 is proposed.

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P06

Isolation, structure elucidation and synthesis of bioactive peptides from bee products*

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Honeybee products have been used in traditional medicine for thousands of years, and there is an increasing interest in the applications in modern medicine. Bee products can have diverse biological activities as anticancer [1], antimicrobial [2], anti-oxidant [3], anti-inflammatory [4], antiviral and hepatoprotective [5]. Todays, there is an urgent call to find anticancer and antimicrobial agents from the natural products with less ecological damage and minimum health and environmental hazards. The nature, geography and weather of Egypt provide possibly a rich source of bee products and with the help of the scientific facilities in Sweden, diverse pharmaceutical leads can be discovered. Our main aim is to identify and characterize the bioactive peptides from bee products. These peptides have been poorly characterized, partly because they are generally present in trace quantities. Isolated active peptides from the bee products have been identified using techniques including High Performance Liquid Chromatography (HPLC), Mass Spectrometry (MS, LC/MS, MS/MS), Amino Acid Analysis (AAA) and 2D-Nuclear Magnetic Resonance Spectroscopy (2D-NMR). Polar fractionation prior to screening of anticancer and antimicrobial activities has been done.

Acknowledgements: Swedish International Development Agency - Middle East and North Africa (SIDA-MENA).

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*Dedicated with regards and respect to Prof. Lars Bohlin on the occasion of his 67 birth day and retirement

P07

The traditional medical uses and cytotoxic activities of sixty-one Egyptian plants: Discovery of an active cardiac glycoside from *Urginea maritima* and *Adenium obesum**

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61 species from 29 families of Egyptian medicinal plants were collected from the Sinai desert and documented. The native Bedouins relied completely on the natural products for their food and medication usage. The practice of traditional folk medication is as old as the community itself and the documentation is kept by local herbalists. The traditional uses of the Egyptian plants cover a wide range of medical conditions including cancer. The anti-cancer biological activity was investigated using a cytotoxicity assay against human lymphoma U-937 GTB. The most potent extracts were those from *Asclepias sinaica*, *U. maritima*, *Nerium oleander* and *Catharanthus roseus*. Literature reports indicate that several of these plants produce cardiac glycosides. Bioassay-guided fractionation of alcoholic *U. maritima* extracts led to the isolation of a bioactive bufadienolide that was subsequently shown to be proscillaridin A, as determined by 1D and 2D-NMR spectroscopy [1]. Another important Egyptian medicinal plant is *Adenium obesum*. Bioassay-guided fractionation of steam extract leads to isolation of a novel cardiac glycoside compound named oleandrigenin-β-D-glucosyl-2`-O-hydroxy-β-D-thevetoside (2`-O-hydroxy-obebioside B) with strong cytotoxic activity against human histiocytic lymphoma cell-line, U937-GTB. The structure was elucidated using intensive spectroscopic tools of UV, IR, 1D and 2D NMR in details, including TOCSY, HSQC-DEPT, and HMBC, and MS^2. This result demonstrates the value of plants used in traditional medicine as sources of medicinally interesting cytotoxic compounds. The presentation concludes with a brief discussion on the integration of traditional medical knowledge into the existing literature as well as discovery of some classes of natural products in plant families.

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P08

Citrullinated peptide analogues as antibody-neutralizers in Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is a systemic inflammatory autoimmune disease primarily affecting synovial joints. Studies indicate that citrullination (the posttranslational deimination of arginine to citrulline) of peptides and proteins in synovial tissue play an important role in initiation of RA. The thus modifed proteins are recognized by pathogenic anti-citrullinated protein/peptide antibodies, so called ACPAs1. ACPAs are highly specific for RA patients with a sensitivity of 76% and specificity of 96%2 and their presence in RA is associated with more aggressive disease. Citrullination have also been observed in various inflammatory diseases such as SLE, Sjögrens syndrome and fibrosis.
The best characterized citrulline-containing peptides in RA are fibrin/fibrinogen, α-enolase, type-II collagen, vimentin and filaggrin, but selectivity and degree of citrullination differ between individuals. A set of linear truncated citrullinated peptide analogues based on the sequences above, have been synthesized for determination of ACPA selectivity. Inspired by stable cyclic peptides from natural origin and their potential to stabilize bioactive peptides3 we aim to develop selective ACPA-binding peptides for use as diagnostic tests or as ACPA-neutralizing compounds. To that end, peptides will be selected for cyclization and insertion into scaffold sequences based on the results of affinity studies,.

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P09

Anti-biofilm polyketides against *Staphylococcus aureus* from the pyranonaphthoquinone biosynthetic pathways of *Streptomyces* species

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Soil bacteria of the genus *Streptomyces* are renowned for their ability to produce various antibiotics and other bioactive secondary metabolites. The pyranonaphthoquinone (PNQ) type II polyketide class contains *Streptomyces* metabolites with a common biosynthetic origin and a fused three-ring aglycone unit consisting of a pyran, a quinone and a benzene ring.

Some PNQ polyketides possess reported antibacterial activity against Gram-positive species, including methicillin-resistant strains of the important pathogen *Staphylococcus aureus*, but the bioactivities of several recently isolated PNQs have not been previously explored. Furthermore, none of the PNQs have been specifically investigated for their anti-biofilm activity. In this study, the antimicrobial potency of the PNQs against both planktonic and biofilm cells was evaluated using redox dye-based viability staining, and the anti-biofilm efficacy was confirmed by viable counting.

Unexpectedly, one of the most potent anti-biofilm PNQs was a pathway shunt product, which was more active against both planktonic cells and pre-formed biofilms than the pathway end product. The most active anti-biofilms were found to share several structural features related to glycosylation, charge and the oxygenation pattern of the aglycone unit.

The findings suggest that effective anti-biofilms with optimized chemical structures may have co-evolved in *Streptomyces* spp. along with antibiotics.

P10

Tracing evolution of sesquiterpene lactones in chemical property space

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Natural compounds, selected by evolutionary forces, provide an enormous reservoir of chemical diversity and a corresponding diversity of biological activities. Among them, sesquiterpene lactones (STLs) represent a large and diverse group of secondary plant metabolites with a wide range of biological activities. Mainly produced in Asteraceae, STLs are also found in other families of the angiosperm such as Magnoliaceae or Apiaceae [1].

A long-standing hypothesis in phytochemistry states that among more advanced groups of angiosperm one would find more advanced chemistry. To address this question, we investigated the correspondence between phylogenetic relationships and chemical diversity of a comprehensive 'in-house' dataset of STLs.

In summary, we collected over 5300 compounds and analysed them through ChemGPS-NP, a tool to navigate chemical property space of natural products [2]. Based on Principal Component Analysis (PCA) it allows an efficient analysis and comparison of compounds based on their physical-chemical properties in 8 dimensions. Through visualisation in the ChemGPS-NP global map, clusters and trends can easily be identified.

By evaluating the distribution of STLs in chemical space, we identified different volumes and trends mirroring how STLs evolve during evolution, and analysed changes in physico-chemical properties in more evolved families. The results of this study support the above-mentioned hypotheses and lay the foundation for quantification and further exploration of the correlation between phylogenetic advancement of a taxon and chemical complexity by taking advantage of ChemGPS-NP visualisation.

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P11

Straight from the horse's mouth: Thin Layer Chromatography versus DNA barcoding identification of common horsetail (Equisetum arvense L.; Equisetaceae)

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The global herbal products market has grown in recent years, making regulation of these products paramount for public healthcare [1,2,3]. For instance, the common horsetail (*Equisetum arvense* L.) is used in numerous herbal products, but it can be adulterated with closely related species, especially *E. palustre* L. that can produce toxic alkaloids [4]. As morphology-based identification of these products is often difficult or impossible, the identification of processed material can be aided by molecular techniques [5,6].
In this study, we explored two molecular identification techniques ability to test the purity of these products: a Thin Layer Chromatography approach (TLC-test) included in the European Pharmacopoeia and a DNA barcoding approach, used in recent years to identify material in herbal products. We tested the potential of these methods for distinguishing and identifying these two species using material from herbarium collections and commercial herbal products.
We found that both methods could discriminate between the two species and positively identify *E. arvense*. The TLC-test is more cost- and time-efficient, but DNA barcoding is more powerful in determining the identity of adulterant species. Our study shows that, although DNA barcoding presents certain advantages, other established laboratory methods can perform as well or even better in confirming species' identity in herbal products.

Acknowledgements: Charlotte Hansen, Corrie Madsen, Cæcilie Ryhl Olsson and Mirnesa Rizvanovic (Natural History Museum of Denmark) for DNA sequences and Katrine Krydsfeldt (Department of Drug Design and Pharmacology, University of Copenhagen) for TLC analysis.

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P12

Plantago major L. Travel tales of a worldwide weed. Predicting chemotypes from migration routes and habitats.

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The common or broad-leaved plantain, *Plantago major* L., is a cosmopolitan weed known from every continent except Antarctica. The species was named "white man's footprint" by the American natives because it flourished everywhere white people had been [1]. Despite its reputation as a worldwide weed, *P. major* has had a long history of medicinal use, primarily for its wound-healing properties. It continues to be used as a herbal remedy today, including in the introduced parts of its range [2,3].

Caffeic acid esters, iridoid glycosides and polysaccharides are some of the compounds in the plant responsible for its anti-bacterial and anti-microbial activity [1,4,5]. The activity and uses of *P. major* have been well-studied, yet the role that geographic location or environment play on the types of chemicals produced by the species has not been thoroughly investigated, though there is some evidence that the chemical compounds produced by the plants vary geographically [4,5]. Due to its worldwide distribution and ability to grow in such a wide range of environmental conditions, *P. major* is a model species for exploring whether leaf chemical profiles are more restricted by genotype than by environment.

Genetic and chemical fingerprinting, and habitat (soil type) parameters are being sampled from over 50 localities across the world to investigate patterns of chemotype diversity at a global scale. In addition, a phlyogeographic approach is being taken to map the migration routes of *Plantago major* from its putatively native origins in Europe and Asia to its current worldwide distribution.

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P13

High-resolution α-glucosidase and radical scavenging profiling with HPLC-HRMS-SPE-NMR for identification of bioactive constituents in crude extract of *Pueraria lobata*

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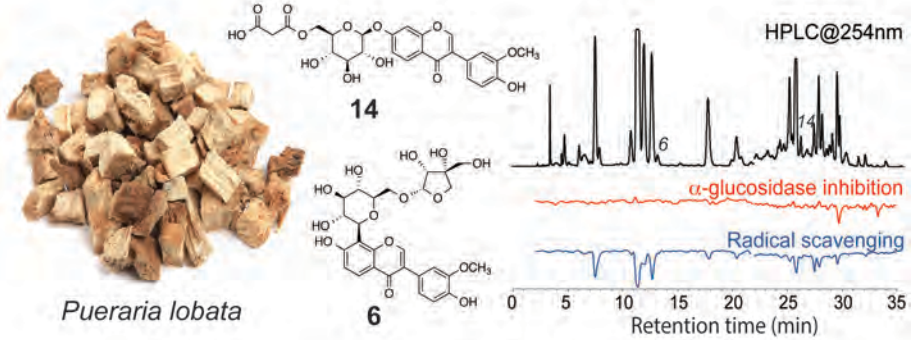
Pueraria lobata is a perennial leguminous vine, which is widely distributed in China where it is used as a dietary supplement and herbal medicine due to its profound pharmacological functions [1]. *P. lobata* extract has previously shown antioxidant activity – one of the most important effects of functional food, dietary supplements and anticancer natural products – as well as α-glucosidase inhibitory activity – an important effect for managing blood glucose for type 2 diabetics.

The crude methanol extract of *P. lobata* was investigated by dual high-resolution α-glucosidase inhibition [2] and radical scavenging [3] profiling combined with hyphenated HPLC-HRMS-SPE-NMR [4]. Direct analysis of both major and minor constituents in the crude extract without preceding purification was facilitated by combining chromatograms from two analytical-scale HPLC separations of 120 and 600μg on-column, respectively. High-resolution α-glucosidase and radical scavenging profiles were obtained after microfractionation of the eluate in 96-well microplates. This allowed full bioactivity profiling of individual peaks in the HPLC chromatogram. Subsequent HPLC-HRMS-SPE-NMR analysis allowed identification of 24 compounds of which several showed radical scavenging activity while two isoflavonoids showed α-glucosidase inhibitory activity.



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P14

Antileukemic lanostanoids from *Poria cocos*

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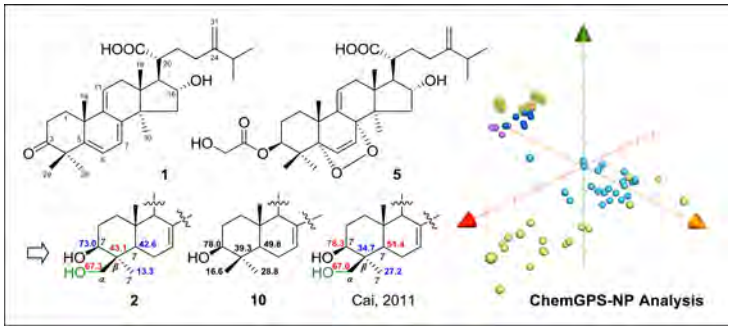
Seven new lanostanoids, isolated from the sclerotia of *Poria cocos*, were elucidated to be: (20ξ)-16α-hydroxy-3-oxo-24-methyllanosta-5,7,9(11),24(31)-tetraen-21-oic acid (1), (20ξ)-3β,16α,29-trihydroxy-24-methyllanosta-7,9(11),24(31)-trien-21-oic acid (2), (20ξ)-3β,16α,30-trihydroxy-24-methyllanosta-7,9(11),24(31)-trien-21-oic acid (3), (20ξ)-3β-acetyloxy-16α,24α-dihydroxy-lanosta-7,9(11),25-trien-21-oic acid (4), (20ξ)-5α,8α-epidioxy-3-O-hydroxyacetoxy-3β,16α-dihydroxy-24-methyllanosta-6,9(11),24(31)-trien-21-oic acid (5), (20ξ)-3β,16α-dihydroxy-7-oxo-24-methyllanosta-8,24(31)-dien-21-oic acid (6) and (20ξ)-3α,16α-dihydroxy-7-oxo-24-methyllanosta-8,24(31)-dien-21-oic acid (7), based on extensive spectroscopic analyses. The antileukemic activity of the new compounds (except 3 and 4), along with the fifteen known lanostane-type triterpenoids, was evaluated against four leukemic cell lines (Molt 4, CCRF-CEM, HL 60 and K562). Dehydropachymic acid (9), dehydroeburicoic acid (12), pachymic acid (14) and lanosta-7,9(11),24-trien-21-oic acid (20) exhibited cytotoxic effect on CCRF-CEM cancer cell line with IC50 values of 1.43, 2.96, 2.61 and 5.96 μg/mL, respectively. Both dehydropachymic acid (9) and dehydroeburicoic acid (12) showed cytotoxicity against Molt 4 (IC50 7.26 and 6.67 μg/mL) and HL 60 (IC50 3.84 and 2.79 μg/mL) leukemic cell lines. ChemGPS-NP analysis on the active lanostanoids from *P. cocos* suggested that targets other than topoisomerases may be involved in the cytotoxic effect.



P15

Developing a QSAR model for hepatotoxicity screening of the active compounds in traditional Chinese medicines

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Traditional Chinese medicines (TCMs) are widely used in the ethnic Chinese population. Recently, it has also been found that more Asian and non-Asian consumers and patients are using TCMs in the United States. The perception that natural substances are deemed safe has made TCM popular in the treatment and prevention of disease globally. However, such an assumption is often misleading owing to a lack of scientific validation.

To assess the safety of TCM, *in silico* screening provides major advantages over the classical laboratory approaches in terms of resource- and time-saving and full reproducibility. To screen the hepatotoxicity of the active compounds of TCMs, a quantitative structure-activity relationship (QSAR) model was firstly established by utilizing drugs from the Liver Toxicity Knowledge Base. These drugs were annotated with drug-induced liver injury information obtained from clinical trials and post-marketing surveillance.

The performance of the model after nested 10-fold cross-validation was 79.1%, 91.2%, 53.8% for accuracy, sensitivity, and specificity, respectively. The external validation of 91 well-known ingredients of common herbal medicines yielded a high accuracy (87%). After screening the TCM Database@Taiwan, the world's largest TCM database, a total of 6853 (74.8%) ingredients were predicted to have hepatotoxic potential. The one-hundred chemical ingredients predicted to have the highest hepatotoxic potential by our model were further verified by published literatures. Our study indicated that this model can serve as a complementary tool to evaluate the safety of TCM.

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P16

Resistance development to cycloviolacin O2

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Cyclotides are a family of plant proteins with a cyclic backbone and three disulfide bonds that tie them into the so-called cyclic cystine knot. The extreme stability of cyclotides to chemical, thermal and enzymatic degradation makes them a promising scaffold for drug design applications [1]. We have previously shown that the cyclotide, cycloviolacin O2 (cyO2) has a killing effect on Gram-negative bacteria in low micro-molar concentrations [2]. In the present study, we have explored the mechanisms of resistance development to cyO2.
For this purpose, 14 independent lineages of *Salmonella typhimurium* and 4 independent lineages of *Escherichia coli* were cycled in increasing concentrations of cyO2 for 150 cycles (900-1050 generations). Clones were isolated from the populations evolving under this selective pressure. Mutations identified by whole genome sequencing were involved in controlling diverse biochemical pathways. Genetic analysis of individual mutations confirmed the proposed mechanisms of resistance. Phenotypic characterization of these effects may hint about the mechanism(s) of action of cyO2. In addition, studies of fitness costs may provide data based on which rate and trajectory of evolution and spread of resistance can be predicted.

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P17

Natural blue food color from cyanobacteria *Spirulina platensis*

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The use of colors is an important tool to increase the attractiveness of the food products. Among different colors used in food products, blue color is gaining more importance due to its unique appeal and psychological perception as trustworthiness of the product. Currently available blue colors on the market are produced by chemical synthesis, and recent reports have suggested that these colors induce allergic reactions among children [1]. In addition, there is growing perception among the consumers that natural colors are safe as compared to the synthetic ones. Therefore, there is a need for development of natural blue food color as an alternative to synthetic blue colors.

Spirulina platensis is a cyanobacteria containing blue light harvesting proteins (phycocyanins) for photosynthesis. Recently, USFDA has approved the use of spirulina extract as a source of blue color; however, only for use in gums and candy. In addition to the limited use, the extract does not offer stable blue color shades comparable to the synthetic ones [2]. Aim of this work is to cleave and purify the chromophore, phycocyanobilin, from the phycocyanins present in the *Spirulina* extract. Different cleavage processes such as acid hydrolysis and methanolysis will be investigated and process parameters will be optimized. Intensification and stabilization of phycocyanobilin color is envisaged through co-pigmentation. Naturally occurring non-colored compounds such as amino acids, phenolic acids, flavonols and dihydroflavonols will be screened as cofactors in co-pigmentation experiments. The possibilities of phycocyanobilin forming co-pigment complexes with metal ions will also be investigated. Selected results of the work will be presented.

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P18

Multidisciplinary approach towards an economically viable isolation of natural products from plants: Example of artemisinin from *Artemisia annua*

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Several secondary metabolites from medicinal plants have been recognized as leading drug molecules. Traditional medicinal systems still continue to serve as an important guide in the search for lead molecules for the pharmaceutical industry. However, recovery of such molecules from plants remains a challenging task due to the intricacies involved in the process. The boundaries of the problem extend to multiple disciplines such as chemical engineering, natural product chemistry, and analytical chemistry. Therefore, a multidisciplinary approach involving coordinated efforts from all disciplines is required in order to ensure an economically viable isolation of important drugs from plants.

Artemisinin, an antimalarial drug, from the plant *Artemisia annua* is a very good example of such a drug molecule. We have developed a process to isolate and purify artemisinin from dried leaves of *A. annua* by using a multidisciplinary approach. At first, artemisinin is extracted from the leaves with dichloromethane by using maceration technique followed by partial purification of crude extract with flash column chromatography (flash CC). Finally, pure artemisinin is crystallized from flash CC fractions. Chemical engineering principles combined with knowledge from other research disciplines have been used to identify the suitable separation techniques and optimize the process parameters. Extensive analysis of the process streams has been carried out with high performance liquid chromatography and mass spectrometry for identification and quantification of impurities. Chemometric methods have been employed to extract useful process information from the vast analytical data obtained [1].

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P19

Identification of amino acid derivatives of dehydroabietic acid (DHA) as potent anti-biofilm compounds against *Staphylococcus aureus*

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Biofilms, defined as structured communities of bacteria embedded in a matrix of extracellular polymeric substances (EPS), represent the predominant lifestyle of bacteria. Biofilms are involved up to 80% of all human bacterial infections and characterized as highly resistant to antimicrobial therapy and host immune system [1]. Moreover, the selection of existing anti-biofilm agents is extremely limited, thus the demands for research in this area are enormous.

In this work, a compound collection consisting of amino acid derivatives of Dehydroabietic acid (DHA), a naturally occurring resin acid from conifers, was synthesized, inspired by the recent identification of DHA as a potent inhibitor of *Staphylococcus aureus* biofilms as well as the reports of D-amino acids as biofilm dispersal agents [2, 3]. The derivatives were screened for anti-biofilm activity and effects on planktonic bacteria were investigated in two exposure conditions, prior-to and post biofilm formation using resazurin staining and turbidity measurements, respectively. Further, mode of action studies including kinetic studies, were performed to assess the changes in membrane depolarization and ATP efflux caused by the most active compounds.

To the best of our knowledge, the identified lead compounds here, a D-tryptophan derivative 9b and a cyclohexyl-L-alanine derivative 11, are the most potent abietane-type biocides reported so far. Each of the compounds displays anti-biofilm activity with potency (IC50) values in the low micromolar range (33.2 and 9.4 μM) prior-to and (86.1 and 27.9 μM) post biofilm formation, in addition to antibacterial activity on planktonic bacteria.

Acknowledgements: Academy of Finland project 282981 and National Doctoral Programme in Informational and Structural Biology (ISB) are acknowledged.

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P20

Assessment of antidiabetic potential of Nigerian plants used in traditional treatment

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Nigeria is rich in plants used for treatment of diabetes and its complications by traditional herbalists. Therefore, documentation of their knowledge as well as scientific validation of their claims is needed. This study aims at evaluating the antidiabetic potential of nine ethanolic extracts from seven plants used traditionally to treat diabetes. The postprandial effects were evaluated in albino rats divided into three groups of 5 each. Each rat received peroral gavage, 1mL/100g body weight of 80% w/v glucose. 30 min later, extracts were administered perorally in 0.5 mL 2% w/v acacia gum to two groups using doses of 250 and 500 mg/kg, respectively. The control group received 0.5 mL 2% w/v acacia gum. Blood glucose levels were monitored at 30, 60 and 120 min [1]. There were significant reductions ($P < 0.05$) in blood glucose levels after 30 mins in groups treated with *Funtumia africana* root and *Diodia scandens* stem compared to the control. Both extracts were assessed for a-glucosidase and a-amylase inhibitory activity in vitro [2,3]. Results showed that the two extracts could not significantly inhibit these enzymes. Ability of the extracts to reduce the stable DPPH was also evaluated for all samples [4]. *Dialium guineense, F. africana* and *Dalbergiella welwitchii* reduced the stable DPPH by 72%, 68% and 55% respectively. *F. africana* root seemed to contain little or no tannin and was selected for further study based on the HPLC fingerprints.The present study suggests that there may be other explanations for including the tested plants in the antidiabetic recipes. Further work is ongoing to investigate other possible mechanisms of action and to identify the compound(s) responsible for the radical scavenging activity.

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P21

NMR-based metabolomics for identification of α-amylase inhibitors in rowan berries (*Sorbus* spp.)

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Type 2 diabetes is a metabolic disorder estimated to affect millions of people all over the world [1]. One way of reducing diabetes-related complications is to control postprandial glucose [2]. Inhibition of the carbohydrate digestive enzyme α-amylase is a therapeutic target for maintaining low blood glucose levels. A study from 2011 shows that berries from *Sorbus* spp. (rowan berries) effectively inhibits α-amylase activity and suggests that the active compounds are proanthocyanidins [3]. The aim of this project is to identify the rowanberry species with highest α-amylase inhibitory activity - and to find a 1H-NMR method suitable for NMR-based metabolomics.

Acetone extracts of 16 species of rowan berries collected in the botanical garden of Copenhagen were prepared - and IC50 values were assessed using a newly developed microplate-based α-amylase inhibition assay [4]. This showed a large variation in α-amylase inhibitory activity of the 16 Sorbus spp. in the in-vitro α-amylase inhibition assay. 1H NMR spectra of all extracts were subsequently acquired, and the multivariate data analysis score-plot revealed a correlation between the α-amylase inhibitory effect and the fingerprint of the extracts. This information will be used in our future experiments aiming at identification of components responsible for the α-amylase inhibitory activity in rowanberries.

Figure 1. Selection of collected Sorbus species.

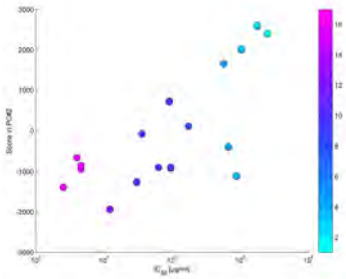

Figure 2. PCA score of comp. 2 versus IC50 values. Light pink = most active, while light blue = the least active extracts

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P22

Sesquiterpene lactones and their bioactivities in the family Chloranthaceae

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Chloranthaceae is a plant family consisting of four genera: *Ascarina, Chloranthus, Hedyosmum* and *Sarcandra* and ca. 70 species, occurring in tropical Asia. The whole plants or roots of plants have been used in traditional Chinese medicine to treat e.g. bone fractures, inflammation, cancer, and high blood pressure. Previous studies of this family have resulted in the isolation of various compounds and elucidation of some bioactivities [1]. Among these compounds are the sesquiterpene lactones (STLs), a group of secondary plant metabolites. Extracts from plants rich in STLs have gained considerable interest for treating human diseases since STLs hold features that make them appealing compounds in clinical trials [2].

The aim of this study was to chart the bioactivities of STLs from the family of Chloranthaceae in chemical property space. Over 300 STLs have been collected of which about 50 had reported bioactivity. Using ChemGPS-NP, a tool based on principal component analysis (PCA) to navigate chemical property space in 8 dimensions, we calculated the physicochemical property of the analyzed STLs [3]. Thought visualization in the ChemGPS-NP global map we could describe the large diversity found in these compounds and highlight similarities and differences in their physico-chemical properties. We could identify STLs that form cluster and occupy defined volumes in chemical property space, and correlating this to a particular biological activity.

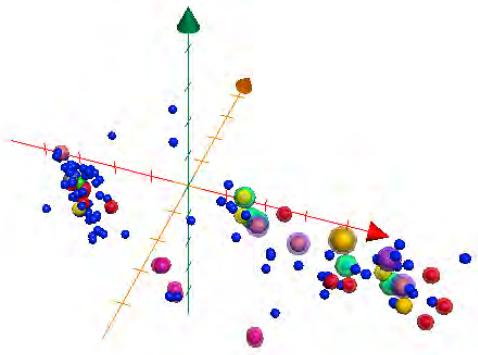

Figure 1: ChemGPS-NP mapping (three first dimensions, PC1-PC3). Blue compounds represent all STLs found in the Chlorantaceae, biological activities are displayed in various colors.

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P23

Cyclotide structure–activity relationships: Qualitative and quantitative approaches linking cytotoxic and anthelmintic activity to the clustering of physicochemical force

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Cyclotides are a family of plant-derived proteins that are characterized by a cyclic backbone and a knotted disulfide topology [1,2]. Their cyclic cystine knot (CCK) motif makes them exceptionally resistant to thermal, chemical, and enzymatic degradation [3]. Cyclotides exert much of their biological activity via interactions with cell membranes. In this work, we qualitatively and quantitatively analyze the cytotoxic and anthelmintic membrane activities of cyclotides. The qualitative and quantitative models describe the potency of cyclotides using four simple physicochemical terms relevant to membrane contact. Specifically, surface areas of the cyclotides representing lipophilic and hydrogen bond donating properties were quantified and their distribution across the molecular surface was determined. The resulting quantitative structure-activity relation (QSAR) models [4] suggest that the activity of the cyclotides is proportional to their lipophilic and positively charged surface areas, provided that the distribution of these surfaces is asymmetric. In addition, we qualitatively analyzed the physicochemical differences between the various cyclotide subfamilies and their effects on the cyclotides' orientation on the membrane and membrane activity.

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P24

UHPLC-QTOF and MALDI-TOF analysis of novel antifungal natural products

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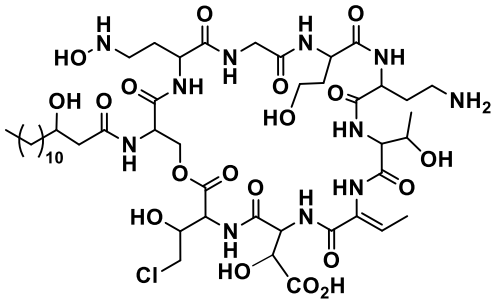
Greenlandic potato farmers do not practice crop rotation or utilise fallow seasons due to the very limited area of arable land available. They also do not apply pesticides or herbicides to their crops due to the high cost of these products in these isolated communities. Despite the continued cultivation of a single crop, no fungal infections occur. Given this unusual occurrence we hypothesised that the lack infection was due to the presence of beneficial biocontrol organism in the Greenlandic potato soils. This guided approach led to the discovery of a *Pseudomonas* species which produces two classes of novel anti-fungal cyclic depsipeptides; the nunamycins and nunapeptins. Current work, in collaboration with Copenhagen University, is focused on determination the conditions which promote the production of these natural products and structural refinement of the nunapeptins. We are using MALDI-TOF and high resolution LC-MS to quickly analyse samples in conjunction with genetic manipulation of the biocontrol bacteria. We will also soon perform MALDI-TOF imaging of the bacteria in situ to gather further detail about the nature of the bacterial-fungal interaction.



Nunamycin

P25

The search for new antimicrobial compounds from Sri Lankan medicinal plants

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Sri Lanka is a unique island, which possesses high degree of biodiversity. There are about 3,154 indigenous flowering plant species of flora and about quarter (894) of that is endemic to the country. Natural products compounds have recently gained popularity and scientific interest. Researchers have been interested in bioactive compounds from plant species for the treatment of infectious diseases caused by microorganisms. According to the World Health Organization (WHO), more than 80% of the world's population depends on traditional medicine for their primary health care needs. In recent years there has been an emerging interest in the discovery of plant peptides with medicinal properties [1]. The current project aims to establish the scientific basis of plant-derived medicinal compounds in Sri Lankan plants following anecdotal evidence for their use in Ayurvedic medical practice.

In the present study, the aqueous extracts of 17 plants belonging to the family Rubiaceae were tested for their antimicrobial activity. Arial part of the plants were extracted into a mixture of CH2Cl2: MeOH=1:1 and concentrated under reduced pressure to obtain the crude extracts. Organic crude extracts were dissolved in the above solvent mixture and partitioned with MQ-water. The aqueous extracts (4 mg/ml) were tested against *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213) and *Candida albicans* (ATCC 90028); minimum inhibitory concentration (MIC) assay was performed using a final concentration of microbes, 1×106 CFU/ml. The MIC value reported is for 100% of microbial death and the results showed that all extracts are active against *S. aureus*; all the extracts except *Wendlandia bicuspidata* were found to be inactive against gram negative bacteria and the fungal strain *Candida albicans*. Bioassay guided fractionation for the extracts with low MIC values is in progress. In addition, the plant extracts will be screened for cytotoxic activity using a fluorometric microculture cytotoxic assay (FCMA).

Acknowledgements: Financial assistance by the Swedish Research Council's International Collaborative Research Grant 2014.

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P26

Discovery of fish killing toxins from the microalgae *Prymnesium parvum*

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Harmful algae blooms are a reoccurring phenomenon that cause huge economic losses for the farmed fish industry. This paper will describe results obtained in the collaborative Danish Strategic Research project: "HABFISH - Harmful algae and fish kills" [1].

We used *Prymnesium parvum*, a harmful algae that has caused massive blooms in Scandinavian waters, in our search for new bioactivities. We found huge ichyotoxic difference among 5 strains of *P. parvum* when tested on rainbow trout [2] Chemical analysis based on LC-DAD-HRMS showed that all tested strains produced GAT and oleamide – two compounds that recently have been suggested as the ichyotoxic component of *P. parvum* [3]. Although, we found no evidence that these compounds are ichyotoxic at ecological relevant concentrations. In addition, ^{13}C feeding experiments suggested that oleamide is a contaminant derived from plastics, potentially extracted in sample preparation.

Excitingly, we found that different strains of *P. parvum* produce at least two types of prymnesin-like molecules. One strain produced prymnesin 2,4 whereas several Scandinavian strains produce a novel type of prymnesin. Extraction of >100 litres of algal culture has allowed us to isolate enough material of a novel prymnesin-like molecule, including a ^{13}C enriched version. This paper will report the status of our efforts towards structural elucidation of this large and complex polyketide derived polyether, based on various 2- and 3-D NMR experiments.

Acknowledgement: We acknowledge support from the Programme Committee for Food, Health and Welfare under the Danish Strategic Research Council (http://www.habfish.dk).

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P27

A complement fixing polysaccharide from root bark of *Terminalia macroptera*

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T. macroptera is a tree which grows in West Africa. In traditional medicine, The roots are used against hepatitis, gonorrhea and various infectious diseases. Various reports have shown that polysaccharides from different plants can be responsible for the effects associated with the healing of wounds.
Polysaccharides of certain structures modulate the immune system and intensify its defence mechanism.
Pectic polysaccharides containing rhamnogalacturonan I backbone with arabinogalactans type I and II structures have shown effects in a number of biological assays. Endo-α-D-(1-4)-polygalacturonase was performed for production of "hairy" or ramified regions of the polymers.
50WTRBH-II-I, a complement fixing pectic polysaccharide, isolated from root bark of *T. macroptera*. After enzymatic treatment, the "hairy" regions were purified by BioGel P30 gel filtration. The main sub-fraction, 50WTRBH-II-Ia, showed higher activity compared to its native fraction. The methylation result indicated the presence of AG-I and AG-II structures in 50WTRBH-II-Ia. The proposed structure of 50WTRB-II-I is long homogalacturonan and short RG-I backbone with AG-I and AG-II branched. The results suggest that the complement fixation activity of this pectin is expressed mainly by its ramified regions.

P28

Cyclotides as stable and target specific membrane disruptors

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The cyclotide family of plant-derived peptides is defined by a cyclic peptide backbone and three disulfide bonds forming a knot in its core. This cyclic and knotted structure renders them exceptionally stable against proteases, making them for example ideal grafting templates for bioactive epitopes. Many cyclotides have in our hands shown potent bactericidal activity. The bactericidal effect is derived from membrane permeabilization in a phospholipid specific manner. Besides the typical selectivity factors for membrane active antimicrobial peptides, i.e. the presence of anionic head groups and the absence of cholesterol, cyclotides frequently also have phosphatidylethanolamine (PE) affinity. We have studied the structural prerequisites for the PE-binding pocket, which alone increases the membrane disruption potential more than 700-fold on bacteria modelled liposomes. Mutations that distort the PE-binding pocket results in substantial loss of antimicrobial activity. Dividing bacteria, as well as tumour cells, present PE on the outside of their cytoplasmic membrane. The PE-affinity of cyclotides therefore represents a useful trait in terms of selectivity and broadening their potential therapeutic window as membrane active antimicrobial peptides.

P29

Marine bioprospecting of marine microalgae

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The marine environment has produced a large number of biologically active and structurally diverse natural products [1]. In this study, 11 microalgae from the Oslo fjord in Norway were cultivated and investigated for their biological activity against cancer cells. The algae were cultivated at 18 ºC in IMR 1/2 medium. The cultures were filtered and the filter discs and filtrate were frozen. The frozen filter discs were cut in small pieces and ASE (Accelerated Solvent Extraction) extraction was performed yielding hexane, ethyl acetate, ethanol and water extracts. Diaion HP20 resin was added to the filtrate and left overnight. The mixture was filtered and the Diaion HP20 resin was extracted with methanol resulting in a lipophilic fraction. The filtrate was concentrated and applied on a Biogel column for desalting resulting in a hydrophilic fraction. All extracts were screened for viability and apoptose activity against Jurkat cells. The ethyl acetate extracts of several algae exhibited activity against the cell line. *Prymnesium polylepis*, *Becheleria* aff. *cincta*, *Dunaliella tertiolecta* and *Micromonas pusilla* were most active. Preliminary NMR studies of extracts of the most active species show the presence of lipophilic constituents.

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P30

Investigation of antiradical and anticholinesterase effects of Truffles

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The name truffles/terfezia comes from the word "*terfass*," which is an Arabic word describing hypogenous desert fungus. Truffles are known as fungus growing underground. In general, most of desert truffles species belong to genera such as *Terfezia*, *Picoa*, *Tirmania*, and *Tuber* [1]. Truffle mushrooms due to their special flavour and organoleptic properties are increased to commercial importance and they are more expensive than other mushroom species. In addition, Truffles are consumed as a functional food to be healthy and to protect against disease. Up to date, Truffles have been shown several biological activities i.e. antioxidant, anti-inflammatory, cytotoxic [2], and antimicrobial [3].

In this study, *Terfezia olbiensis* Tul. & C. Tul. (AT-1905) and *Terfezia claveryi* Chatin (AT-2063) collected from Uşak and Aksaray in Turkey extracted with methanol at room conditions. The methanol extracts were dissolved in water and re-extracted with n-hexane and ethyl acetate. The antioxidant activity of extracts of truffle mushrooms was performed by DPPH free radical and ABTS cation radical scavenging assays and the anticholinesterase activity, however, against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes.

In DPPH• assay, ethyl acetate extract of *T. olbiensis* showed 92.47±0.22% inhibition activity at 800 µg/mL concentration whereas in ABTS•+ assay, the methanol extract exhibited 92.47±0.22% inhibition at same concentration. The hexane extract of *T. olbiensis* demonstrated the best inhibition activity against AChE and BChE enzymes, indicating 63.99±1.14% and 51.44±0.29%, at 200 µg/mL concentration, respectively.

Acknowledgements: Authors would like to thank The Scientific and Technological Research Council of Turkey (TUBITAK-114Z644) for financial support.

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P31

Phenolic profiles of Truffles from Anatolia

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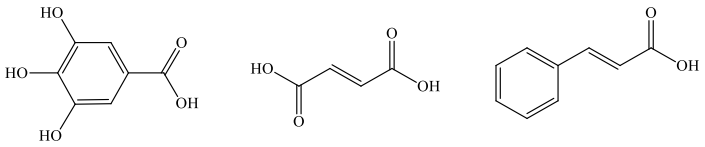
Phenolic compounds, a large class of phytochemicals, are interesting biological properties. They are available mostly in vegetables, fruits, seeds, herbs, and grains, as well as in juices, oils, and wines. Several phenolic compounds indicating antimicrobial [1], antiviral [2] cytotoxic, antioxidant [3] activities have been identified from mushroom species.

In this study, phenolic profile of *Hysterangium inflatum* Rodway, *Reddellomyces westraliensis* (G.W. Beaton & Malajczuk) Trappe, Castellano & Malajczuk, and *Terfezia leptoderma* Tul. & C. Tul. was identified by HPLC-DAD. The phenolic profile was determined according to the method of Barros et al. [4] with slight modification. Sixteen phenolic and organic acids i.e. gallic acid, fumaric acid, protocatecheuic acid, catechin hydrate, p-hydroxybenzoic acid, 6,7-dihydroxy coumarin, caffeic acid, vanillin, 2,4-dihydroxy benzoic acid, p-coumaric acid, ferulic acid, coumarin, trans-2-hydroxycinnamic acid, ellagic acid, rosmarinic acid and trans-cinnamic acid were analysed. Fumaric acid was found as major organic acid in *T. leptoderma* (6.57 µg/g) and *R. westraliensis* (27.2 µg/g) while catechin hydrate (1.76 µg/g) was found in *H. inflatum*. Gallic acid, Fumaric acid, Catechin hydrate, and trans-cinnamic acid were identified for all studied mushrooms.



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P32

Evaluation of nutritional properties of truffles associated with eucalyptus in Turkey

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Truffles have been used as food and medicine due to their unique taste and flavor as well as their chemical and nutritional properties.
Their nutritional properties are due to high protein, fiber, vitamin and mineral contents and a low-fat level [1]. In this study, four ectomycorrhizal fungus specimens; namely, *Hysterangium inflatum* Rodway, *Reddellomyces westraliensis* Trappe, Castellano & Malajczuk, *Reddellomyces parvulosporus* Trappe, Castellano & Malajczuk, *Setchelligaster tenuipes* (Setch.) Pauzar collected from different localities of Muğla (Ula, Köyceğiz, Marmaris, Dalaman, and Fethiye) in which the spread of eucalyptus trees.
The nutritional properties (moisture, ash, protein, fat, carbonhydrate, energy) of mushrooms was determined by AOAC procedures [2]. The protein content (N×4.38) was determined by the macro-Kjeldahl method; fat was estimated by extracting hexane, using a Soxhlet apparatus; ash content was calculated by incineration at 600±15 °C. The moisture contents of studied mushrooms were found to be between 66.9-81.0 g/100 g of fresh weight while the ash contents were between 0.05-0.48 g/100 g of dry weight. The protein and fat contents found in the studied mushrooms ranged 9.19-23.91 g/100 g of dry weight, and 0.09-3.25 g/100 g of dry weight, respectively. The carbohydrate values of mushroom samples were calculated between 72.78-90.21 g/100 g of dry weight whereas energy values were between 401.11-503.67 kcal/100 g of dry weight. As a consequence, studied ectomycorrhizal mushrooms are rich in moisture, protein and energy and poor in fat.

Acknowledgements: This study was financed by The Scientific and Technological Research Council of Turkey (TUBITAK-111T530).

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P33

Structure-activity relationship of immunomodulating pectins from elderberries.

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The berries of *Sambucus nigra* have traditionally been used and are still used to treat respiratory illnesses such as cold and flu in Europe, Asia and America. Structures and immunomodulating properties of the pectic polymers from elderberries were elucidated. All the purified fractions obtained from the 50% ethanol, 50 °C water and 100 °C water extracts showed potent dose-dependent complement fixating activity and macrophage stimulating activity. The isolated fractions consisted of long homogalacturonan regions, in addition to arabinogalactan-I and arabinogalactan-II probably linked to a rhamnogalaturonan backbone. Reduced bioactivity was observed after reduction of Araf residues and 1→3,6 Gal by weak acid hydrolysis. Rhamnogalacturonan regions were isolated after enzymatic degradation with endo-α-D-(1→4)-polygalacturonase and showed higher activity compared to the native polymer. The results from the linkage analysis and bioactivity tests led to the assumption that the branched moieties of the arabinogalactans, linked to rhamnogalacturonan region are important for the immunomodulating activity seen in elderberries. Immunomodulating polysaccharides might be responsible for the claimed effect of berry extracts on cold and influenza, but the ability of these polysaccharides to contribute to immunomodulating activity in humans remains to be fully investigated.

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Flavonoids and hydrolysable tannins from the African medicinal plant *Syzygium guineense*

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Syzygium guineense is a small tree with edible fruits widespread in Sub-Saharan Africa. Leaves are traditionally used to treat diarrhea, infections and stomachache. The phytochemistry of the leaves is not very well characterized; triterpenoids, sesquiterpenoids and polysaccharides are reported.
In our study, the leaves of *S. guineense* were extracted by accelerated solvent extraction with dichloromethane and methanol. The methanol extract was fractionated by different chromatographic methods (Diaion HP-20, Sephadex LH 20, Versaflash RP-18, and preparative HPLC C18) to yield flavonols (myricetin and myricetin derivatives), gallotannins and ellagitannins. NMR spectroscopy was employed for structure elucidation.
The crude extracts and pure substances were investigated for radical scavenging properties using the diphenylpicrylhydrazyl assay, and for inhibition of the enzymes 15-lipoxygenase and xanthine oxidase, both of which are involved in peroxidative processes. The methanol extract showed high radical scavenging activity and was a good inhibitor of 15-lipoxygenase and xanthine oxidase. The activity can be explained by its high content of polyphenols.

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Phytochemistry and phylogeny of Icelandic cetrarioid lichens

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Lichen is a symbiotic association between fungi and photobiotic microorganisms (algae and/or cyanobacteria), which presents a vast diversity of secondary metabolites (*i.e.* lichen acids) with marked pharmaceutical potentials [1]. Some lichen acids, such as usnic acid and protolichesterinic acid, have displayed antimicrobial and anti-proliferative activities [2,3]. Cetrarioid lichens comprise a morphological group in the Parmeliaceae (lichen-forming ascomycetes) family. Among the group members, is the lichen species *Cetraria islandica* (L.) Ach, also known as Iceland moss. It has been used traditionally to treat lung diseases and inflammation of oral and pharyngeal mucosa [4]. The aim of the study was to investigate if phylogeny can be used to predict phytochemical diversity of Icelandic cetrarioid lichens (ICLs) and to test if chemical profiles could be used as an aid to delimit closely related lichen taxa. A total of 73 samples representing 8 species from 3 genera were included in phylogenetic analyses based on fungal nuclear ribosomal internal transcribed spacer (nrITS) and small subunit (nrSSU) DNA regions as well as algal nrITS sequences. Each specimen was extracted with acetone and the chemical profiles of crude extracts from ICLs were obtained with UPLC-Q-TOF-assay, and the evaluation of the data was performed with MarkerLynx and EZinfo. The metabolomic approach has shown great potential in resolving closely related species where DNA sequence failed to give sufficient taxonomic information.

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Pharmacognostic studies on *Aloes* of Ethiopia

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The genus *Aloe* belongs to the sub- family Alooideae (Aloaceae) of the family Asphodelaceae. It is represented by 600 species and subspecies, which are native to sub- Saharan Africa, the Saudi Arabian Peninsula, and to many islands of the western Indian Ocean, including Madagascar [1]. Ethiopia is one of the *Aloe*-rich countries where 40 species, of which 20 are endemic, are so far known [1]. Aloes are also widely employed in the Ethiopian traditional medicine for the treatment of various ailments such as malaria, gastric problems, inflammation, wounds, infections, snakebite and diabetes. There is an ongoing work in our group to investigate the claimed ethnobotanical uses and phytochemistry of aloes in the country. More than 10 species have been screened thus far. The aloes were collected from their growing sites and the latex of each aloe was obtained by making transverse cuts on the leaves. Results of in vivo and in vitro biological tests showed that the aloes possess antimicrobial, antioxidant, antileshmanial and antimalarial activities [2-8]. On the other hand, bioassay guided fractionation assisted with spectroscopic techniques led to the isolation of secondary metabolites, mostly anthrones. The findings corroborate the claimed traditional uses of the plants and present potential natural products for further investigation.

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