3rd Symposium of Young Researchers on Pharmacognosy

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BOOK OF ABSTRACTS

University of Szeged
Institute of Pharmacognosy

3rd Symposium of Young Researchers on Pharmacognosy

BOOK OF ABSTRACTS

(ed. Tivadar Kiss, Judit Hohmann)

Department of Pharmacognosy, University of Szeged, Szeged, Hungary

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ABSTRACTS

A1

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Antimicrobial, multidrug resistance reversal, and biofilm formation inhibitory effect of *Origanum majorana* extracts and essential oil

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Origanum majorana L. (OM) is a well-known medicinal and aromatic plant used since ancient times for the treatment of respiratory, gastrointestinal, and urinary tracts disorders [1]. In the food industry its distilled oil is frequently applied for increasing the storage stability and reducing microbial contamination. Several studies demonstrated the antimicrobial activity of the essential oil and its main constituent, terpinene-4-ol against bacterial and fungal strains [2,3].

The aim of the present study was to provide a broader insight into the effects of OM extracts, essential oil, and their constituents against human pathogen microorganisms. Essential oil was prepared by steam distillation, and extracts of different polarities by solvent-solvent partition of the MeOH extract. Six main compounds of the essential oil (terpinene-4-ol, sabinene, sabinene-hydrate, α-terpinene, γ-terpinene, and linalool) and a newly isolated non-volatile furanonaphtoquinone (OM-3) obtained from the chloroform extract were also included in the microbiological examinations. For antimicrobial evaluation disk diffusion and microdilution methods were applied. The extracts and essential oil were also investigated for multidrug resistance reversal and biofilm formation inhibitory effects by real-time ethidium bromide accumulation and crystal violet method, respectively.

The highest antibacterial effect was measured for OM-3, while essential oil and sabinene exerted pronounced inhibitory effect on efflux mechanisms. Concerning the biofilm formation, sabinene and sabinene-hydrate proved to be the most potent inhibitors.

Supervisor: Judit Hohmann

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Studies on biomimetic oxidized resveratrol metabolite mixtures

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Resveratrol, though reported for a myriad of pharmacological activities, has low systemic bioavailability due to extensive phase I and II biotransformation. While numerous reports are available on the structure and bioactivities of glucuronidated and sulfated conjugates of resveratrol [1-2], limited knowledge is available on the metabolites formed via oxidation. Although it has a minor effect on toxic reactive oxygen species levels in living systems, resveratrol can directly scavenge free radicals due to its chemical structure, resulting in the generation of new metabolites with altered bioactivities. [3]. The oxidation of resveratrol through various chemical reactions, including biomimetic approaches, resulted in several mixtures that exhibited greater bioactivities compared to the parent compound. Mixtures were tested for *in vitro* antioxidant activities (DPPH, ORAC), and inhibitory action on lipoxygenase, xanthine oxidase, and angiotensin converting enzymes. Using a multi-step chromatographic isolation procedure and spectral analysis, 20 compounds were obtained in pure form. Isolated compounds included dimers, chlorine-, iodine-, ethoxy- and benzofuran derivatives. Antioxidant and xanthine oxidase inhibitory studies show that chlorine- and iodine-substituted compounds exhibited greatest bioactivities, with molecular docking simulations showing the importance of these substituents. Dimers, ethoxy- and benzofuran- derivatives, exhibited the greatest inhibitory activity on lipoxygenase enzyme. All groups of metabolites showed enhanced activity in inhibiting angiotensin converting enzyme. Additional studies to further explore the cardioprotective and anti-inflammatory properties are currently ongoing.

Supervisor: Attila Hunyadi

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A3

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The optimization of pyrolysis reactions and GC fatty acid profiling of hemp oils

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Pyrolysis studies provide a good way of detecting newly formed chemical compounds. Recently electronic cigarettes have become popular that may contain cannabidiol (CBD). In connection with our cannabinoid research and based on our previous findings, we continued to perform pyrolysis studies on a bigger scale. In a sealed metal chamber, we established proper physico-chemical parameters for pyrolysis to be carried out. The results were then analysed by UHPLC [1].

Fatty acid profiles can be used as markers for the identification of vegetable oils. Products that contain oils are often adulterated by various abundantly available other sources like sunflower seed oil or olive oil. Performing GC analysis would allow us to verify product authenticity in a reliable and simple way. Commercially purchasable products would be analysed mainly focusing on CBD products. Authentic hemp oil is rich in linoleic acid and linolenic acid. It also contains a significant amount of oleic acid concentration. The aim of our project is to characterize the fatty acid profiles of commercial hemp seed oils and to identify potentially adulterated products.

Supervisors: Dezső Csupor, Tivadar Kiss

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Phytochemical investigation of *Juncus articulatus* and *J. kraussii*

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Among the secondary metabolites accumulated by species of the family Juncaceae, phenanthrenes have remarkable biological activity and structural diversity. The aim of our work is the phytochemical and pharmacological investigation of Juncaceae species. So far, the isolation of the special metabolites of *J. articulatus* was finished and started preparative work with an African species *J. kraussii*.

To obtain pure compounds from *J. articulatus*, preparative TLC and HPLC were used as final purification steps. Methanol extract of *J. kraussii* was obtained from South Africa. After evaporation, the extract was dissolved in 50% aqueous methanol, and solvent-solvent partitions were performed with *n*-hexane, chloroform, and ethyl acetate. Phenanthrenes are enriched in the chloroform phase; therefore, a rough separation was performed by column chromatography and then almost all fractions were purified by Sephadex LH-20 gel chromatography. Structure elucidation of the isolated compounds is carried out by NMR and MS spectroscopy as well as by comparison of spectroscopic data with literature values.

To date, twelve phenanthrenes, among them five new compounds were identified from *J. articulatus*. Moreover, two flavonoids, and three other compounds were also isolated from the plant. All compounds were determined for the first time from *J. articulatus*. Pharmacological investigations of the isolated compounds are in progress.

Supervisors: Andrea Vasas

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Phytochemical investigation of *Carex praecox* and *C. morrowii*

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Plant derived natural products have always played a key role in drug development [1]. Therefore, it is of crucial importance to isolate new bioactive plant special metabolites. Species belonging to the Cyperaceae family occur worldwide, also in the Carpathian Basin, and accumulate a variety of metabolites, among them biologically active phenolic compounds (flavonoids, lignans, stilbenes). *Carex* is the largest genus of the family (*n*=2000) [2].

The aim of our work is the phytochemical and pharmacological investigation of *Carex* species occurring in Hungary. Based on the results of the antibacterial screening study involving 26 Cyperaceae species, *C. praecox* was chosen for further preparative work. We previously processed the chloroformic fraction of the plant and isolated 14 compounds. Now, we report the investigation of the ethyl acetate-soluble fraction of *C. praecox* methanolic extract. By using a combination of different chromatographic methods, eight compounds, namely vanillic acid, two flavonoids (tricin and quercetin), a chalcone derivative (cilicione-B), three stilbenes (resveratrol, *cis*- and *trans*-ε-viniferin) and a new lignan (carexosin C) were isolated. The structures of the isolated compounds were determined by a combination of 1D and 2D NMR, and MS measurements.

The phytochemical investigation of *C. morrowii* has also been started. Dried and ground leaves and roots of the plant were separately extracted with methanol and after evaporation, each of the extracts were subjected to solvent-solvent partition with *n*-hexane, chloroform and ethyl acetate.

Supervisor: Andrea Vasas

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Isolation and identification of diterpenes of the genus *Plectranthus sensu lato*

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The genus *Plectranthus sensu lato* (Lamiaceae), which according to the latest systematic revision includes three separate genera (*Coleus, Plectranthus sensu stricto* and *Equilabium*), is well-known due to its medicinal properties. Many species have been used in the treatment of various ailments of gastrointestinal, genitourinary, respiratory tract and skin for centuries [1,2].
Phytochemical studies have revealed that the genus *Plectranthus s.l.* is a rich source of phenolic compounds and terpenes. Within the group of terpenoids, diterpenes have become the most studied group because of their significant structural diversity and promising biological activities such as antimicrobial, antiprotozoal, cardiotonic, hypotensive, gastroprotective, and cytotoxic activity [2,3].
Our investigation of the methanolic extracts of the aerial parts of *P. ciliatus*, *C. forsteri* 'Marginatus', and *C. comosus* led to the isolation of six abietane diterpenes and two kaurane diterpenes. Two of them were isolated from a natural source for the first time. The compounds were obtained using different chromatographic methods such as column chromatography, flash chromatography, and RP-HPLC and their structures were elucidated by high-resolution mass spectrometry and 1D and 2D NMR spectroscopy.

Supervisors: Renata Kubínová, Attila Hunyadi, Máté Vágvölgyi

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6-gingerol derivatives and thymoquinone-protoflavones hybrids: natural antioxidants as building blocks of new bioactive compounds

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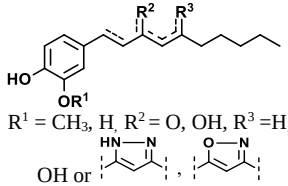
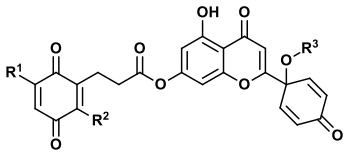
Ginger (*Zingiber officinale* Roscoe) has been well known as a spice and in traditional medicine [1]. Ginger extract has been investigated for many diseases and the activity was mainly attributed to gingerols and shogaols, the most abundant antioxidants in ginger root [2,3]. Black seed (*Nigella sativa* L.) has been widely used as a seasoning spice and in folk medicine [4]. A wide array of bioactivities was reported for the extract and for thymoquinone, a predominant active constituent in the plant [5].

Fourteen 6-gingerol derivatives were semi-synthesized, 8 of which are new. They were investigated for their *in vitro* antiplatelet activity and *in silico* ADME behavior. The most promising compound showed an IC_{50} of 2.1 µM. Eight thymoquinone-protoflavone hybrids were also synthesized and will soon be studied for their *in vitro* antitumor activity.



R^1 = CH_3, H, R^2 = O, OH, R^3 =H, OH or



R^1 = Me, *i*Pr, R^2 = *i*Pr,Me, R^3 =H, Me, Et, Pr, *i*Pr, Bu, Benzyl, Propargy, Allyl

Supervisors: Attila Hunyadi, Tímea Halasi-Gonda

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Ecdysteroid ester derivatives – preparation and investigation

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Chagas disease, a chronic infectious disease, is one of the neglected tropical diseases, and deteriorates more than 6 million patients worldwide, mainly in Latin America. The causative agent of it is *Trypanosoma cruzi*, a protozoan parasite. The transmission is occurred often by the strings of the vector Triatominae bugs [1,2].

In our previous work a preliminary screening of 52 ecdysteroid derivatives was carried out on the *T. cruzi* epimastigote stage. Parasite selective promising activity was observed at 5 µm with the 20-hydroxyecdysone (20E) 2,22- and 3,22 dicinnamic esters and 6-*E* and *Z*-tert-butyl-oximethers of 20-hydroxyecdysone diacetonide [3].

Based on this, the objective of this study was to prepare new bioactive ecdysteroid cinnamic esters of 20E-6-tert-butyl-oximethers against *T. cruzi*. 20-hydroxyecdysone-6-*E*- and *Z*-tert-butyl-oximethers were reacted with cinnamic acid, EDC-HCl and DMAP in dry dichloromethane. RP-HPLC was applied for the purification. Four ecdysteroid derivatives were separated, and their structure evaluation is currently ongoing.

Another goal was to identify ecdysteroid fatty acid esters suspected to be present in mealworm extracts. Alkaline- and enzymatic hydrolysis were carried out. The products are under analytical investigation.

Supervisors: Attila Hunyadi, Zsolt Szakonyi

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Phytochemical analysis of three food plants

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Although the nutritive value of food plants depends on primary metabolites such as carbohydrates, lipids and proteins, their physiological effects and enjoyment values largely depend on secondary metabolites. The aim of our work was to analyze the secondary metabolites of three food plants.

Capsicum annuum is currently being studied because it is a characteristic spice of Hungarian gastronomy. The rationale of the *Capsicum* experiments was to find correlation with the organoleptic characteristics to contribute to the development of quality standards based on objective data. The analysis of *Capsicum annuum* samples focused on the carotenoids content. In case of *C. annuum*, we first developed a MPLC method to separation of carotenoids in paprika.

Subsequently, a reversed-phase HPLC method was developed to examine the carotenoid fingerprint of paprika powders. In this case, we examined the proportion of carotenoids described in the literature [1] in the extracts of good quality as well as a poor-quality peppers. This method allows the determination of carotenoid composition in the analysis of samples of different qualities.

Supervisor: Dezső Csupor

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A10

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Dangerous and potentially dangerous components of weight loss products

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Based on the signals recorded in the RASFF (Rapid Alert System for Food and Feed*), Acacia rigidula* is a repeatedly emerging unauthorized ingredient in weight loss dietary supplements in the European Union [1,2]. The safety and potential efficacy of this plant has not been assessed scientifically [3]. In our work we presented the first systematic overview of the phytochemical and pharmacological data reported on safety and efficacy of *A. rigidula*, and the major issues which resulted in unauthorization of this taxon.

The analysis of RASFF data also revealed that DNP (2,4-dinitrophenol) was one of the major adulterants of weight loss products in the United Kingdom [1]. Our goal was to examine supplements potentially containing DNP marketed in Hungary. After a risk assessment process, we analyzed 17 samples of highly suspicious weight loss dietary supplements with HPLC, focusing on DNP content. Based on our observations there were no detectable DNP in the suspected 17 samples. However, a still unidentified compound was found in one sample, which will be further analyzed by NMR-spectroscopy.

As part of our comprehensive analysis on weight loss products, we aimed to prepare a systematic review and meta-analysis of synephrine, a common component of slimming products [4]. Synephrine is a pharmacologically similar substance to ephedrine, with less side effects, but there is no proper knowledge on its efficacy and safety based on human clinical studies [5,6]. We aimed to analyze the most relevant outcomes of efficacy and safety in a meta-analysis. The analyzed outcomes include cardiovascular effects, mean VO_2, energy expenditure, Respiratory Exchange Ratio (RER), weight loss, changes in fat oxidation and carbohydrate utilization and ratings of perceived exertion.

Supervisors: Dezső Csupor, Barbara Tóth

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B1

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Sesquiterpene lactones of *Ambrosia artemisiifolia*

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Ambrosia artemisiifolia L. is an invasive species in Europe, containing sesquiterpene lactones as characteristic secondary metabolites. These compounds possess numerous biological activities, including allergic, allelopathic, anti-inflammatory, antitumor and antimicrobial activities [1]. Although several phytochemical and -pharmacological studies have been carried out with *Ambrosia* species, the secondary metabolites of the plant have not explored completely. As part of our work, we isolated eight sesquiterpene lactones from the aerial parts of *A. artemisiifolia*, and performed cytotoxic, antiproliferative and combination assays with the isolated compounds.

In our ongoing project the first aim is to measure the sesquiterpene lactone content by using HPLC from plant samples collected from May to October near Szeged, Tiszacsermely, and Nyíri. Our hypothesis is that the sesquiterpene lactone content of the plant may vary depending on the geographical distribution and vegetation period. Eight compounds previously isolated from the plant and will be used as reference compounds for the analytical methods. Secondly, three compounds, psilostachyin, peruvin, and acetoxydihydrodamsin isolated from the aerial parts of the plant will undergo some semisynthetic modification to enhance the antiproliferative, cytotoxic and especially the selectivity of the compounds toward normal cells. Ultimately, we would like to evaluate the action of a selection of sesquiterpenoids from *A. artemisiifolia* on TRPA1 ion channels, whether these compounds are potent activators of these kind of receptors, which are involved in allergen-induced airways neurogenic inflammatory responses. The possible activation of TRPA1 receptors by sesquiterpene lactones promotes the allergic and inflammatory reactions induced by the plant.

Supervisor: Dezső Csupor

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B2

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Centrifugal partition chromatography in the separation of ecdysteroids: challenges and opportunities

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Ecdysteroids are the analogues of insect's molting hormone; they are bioactive, non-toxic compounds for mammals and are widely consumed as dietary supplements for their beneficial biological effects. Some derivatives were shown to have cholesterol-lowering [1], antioxidant and neuroprotective [2] effects, among others, and a recent in silico study suggests a probable anti-COVID-19 activity of an ecdysteroid (calonysterone) [3]. The extreme growth in consumer and research use requires the development of efficient and large-scale preparative purification methods.

Firstly, we analyzed large quantities of *Cyanotis arachnoidea* extracts purchased from Chinese sources using LC-MS. In addition to the major component (20-hydroxyecdysone; 20E), 5 minor components were selected, and their partitioning properties were investigated in nearly 30 two-phase liquid-liquid chromatographic solvent systems. Based on these tests, the fractionation of the extract was attempted in three ternary systems in ascending mode using a laboratory-scale centrifugal partition chromatograph (CPC) (250 ml rotor volume), and one system was selected for scale-up (10 g of injected volume per run in a 2100 ml rotor). The purity of the fractions was monitored by HPLC. The autoxidation of purified 20E yielded calonysterone, which was the first to be purified up to industrial scale-up using our CPC method.

Our optimized method can be used to produce not only 20E, but also several valuable bioactive minor ecdysteroids in a cost-effective and scalable manner. Further studies of these may well lead to the discovery of additional compounds with pharmacological potential.

Supervisors: Árpád Könczöl, Attila Hunyadi, Máté Vágvölgyi

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Neuroprotective potentiel of polymethoxylated flavones isolated from *Citrus reticulata*

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Neurodegenerative disorders affect a large and increasing population, and there is a growing interest to find treatment opportunities in nature. *Citrus reticulata* contains a variety of compounds that display antioxidant, antibacterial, anticancer activities [1]. This plant is particularly rich in polymethoxylated flavones (PMFs) [2]. This class is known by its neuro-protective activity [3]. In the present investigation, we aim to study this group of compounds from this perspective, and to evaluate the effect of environmental conditions on the PMFs content and neuroprotective potential of *C. reticulata* samples collected from various regions of Morocco. Ultrasonic extraction was used to prepare a crude methanol extract. Then, liquid–liquid extraction was performed using an eluotropic series of solvents. Analytical HPLC with reverse phase system was used to evaluate all the fractions obtained, and the chloroform phase was selected to isolate major PMFs constituents. Analytical TLC was assessed to determine the solvent system appropriate for flash column chromatography. Ethyl acetate-dichloromethane (EtOAC-DCM) with gradient system of 0–50% DCM was found suitable to this purpose. Preparative HPLC was subsequently carried out to the subfractions to obtain 4 compounds that are likely PMFs based on their characteristic UV spectra. After structure elucidation by NMR, these compounds will serve as analytical standards to a quantitative evaluation of the available samples.

Supervisors: Bellaouchou Abdelkbir, Boudalia Maria, Hunyadi Attila

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B4

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Chemical-pharmacological analysis of *Peganum harmala*

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Peganum harmala is one of the valuable medicinal plants due to its chemical and pharmacological properties and wide distribution area around the world [1]. The plant is rich in alkaloids, and also contains other groups of plant metabolites [2,3].

In the first stage of our experiment harmine, harmaline and vasicine alkaloids were isolated from *P. harmala* seeds. Later harmine, harmaline and β-sitosterol were also isolated from the roots of this plant.

In addition, the micro- and macroelements, group B vitamins, amino acids [4], fatty acids, phytosterols, total phenol and flavonoid content of various organs (seeds, leaves, stems, roots, capsules, and flowers) of *P. harmala* were analyzed. The physical properties of the seed oil, such as color, refraction, iodine, and soap number were determined. Also, the anatomical-morphological features of *P. harmala* organs were studied by modern microscopy methods.

In the next stage anti-Alzheimer research (acetylcholinesterase and butyrylcholinesterase enzyme inhibitor activity); antioxidant assays such as DPPH, NOSA, FRAP, PRAP, MCA and CUPRAC; antibacterial and antifungal evaluation; anticancer: cytotoxic (HepG2, FaDu) and antiproliferative tests (SiHa, HeLa, A2780, MCF-7, MDA-MB-231) of organs of *P. harmala* were studied. The root extract has shown particularly high results in anticancer tests.

Finally, *in silico* studies were performed on the interaction of *P. harmala* substances with dopamine receptors [5], COVID [6] and various molecular targets [7].

Supervisors: Eldar Garaev, Ilkay Erdogan Orhan, Judit Hohmann

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Limitations of pharmacopoeial herbal monographs as source of risk

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Herbal monographs in European Pharmacopoeias contain definition of drugs, which includes biological identification and chemical tests. Herbal material by meeting pharmacopeial criteria is considered as good quality and safe drug. However, quality control case reports point on limitations of Pharmacopoeia definitions and demonstrate how are these limitations leading to usage of low-quality drug material or adulterations.

In our work the herbal monographs of European Pharmacopoeia (10.5) were reviewed and assessed. During assessment the reliability, accuracy and safety of chemical procedures were estimated, and four-level scale was set up: **1**–chemical investigation is missing or with major limitations; **2**–chemical investigation does not include LC or GC analysis; **3**–chemical investigation is based mainly on spectrophotometry or titration; **4**–chemical investigation is satisfactory. According to our assessment out of 313 monographs are classified (level 1: 10 monographs, level 2: 30 monographs; level 3: 92 monographs; level 4: 181 monographs).

Assessment allowed us to create database in which the monographs and their limitations were organised. This database made possible the selection of several drugs for further chemical studies with limitations in order to elaborate safer chemical procedures, and by this, to eliminate possible safety risks.

Supervisor: Tivadar Kiss

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Phytochemical and pharmacological studies of *Centrapalus pauciflorus*

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Background: *Centrapalus pauciflorus* (Willd.) H. Rob. (syn: *Vernonia pauciflora,* Astereaceae [1,2]) is native to Africa. It is an herbaceous annual plant growing up to 5 m in height [3, 4]. It is used for medicinal purposes in the treatment of diabetes, chest pain, external injury, and stomach problems [5].
Aims: To evaluate anti-cancer potentials and isolate compounds from *C. pauciflorus* using bioassay guided fractionation.
Methods: Powdered plant material (548 g) was extracted with methanol using percolation method. Solvent-solvent fractionation of water-chloroform mixture gave organic (chloroform) portion. The organic portion was subjected to OCC on polyamide using step gradient of methanol-water to yield five fractions: 20%, 40%, 60%, 80%, and 100% MeOH, respectively. All five fractions were evaluated on human ovarian (A2780), cervical (HeLa) and breast (MCF-7, 231) cancer cell lines for their antiproliferative activities. 60% MeOH fraction was subjected to normal and reverse phase VLC, normal and reverse phase HPLC and preparative TLC. Structures were established using NMR and HRMS data.
Results: 60% MeOH fraction of *C. pauciflorus* showed tremendous antiproliferative activity on all cancer cell lines evaluated. Phytochemical investigation of the 60% MeOH fraction led to the isolation of 35 compounds, mainly coumarins and chromones but also flavonoids and others. 22 of the isolated compounds are new natural products.

Supervisor: Dóra Rédei

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Examination of *Artemisia annua* for the prevention and adjunctive treatment of West Nile virus infection in horses

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Artemisia annua (sweet wormwood) has been used in traditional Chinese medicine for centuries to treat fever, treat infections transmitted by mosquitoes and other infectious vectors. Studies on the analytical and antioxidant capacity of previously published articles have demonstrated that *Artemisia annua* is capable of producing large amounts of phenolic compounds that have shown highly active antioxidant capacity. Complementary treatment for malaria infection registered by the WHO since 2004. Like the malaria virus, mosquitoes are the transmitter and carrier of West Nile virus. West Nile virus (WNV) is a zoonotic virus belonging to the genus *Flaviviruses* that causes nervous system diseases in birds, humans and horses. After an incubation period of 3 to 15 days after infection, clinical symptoms suddenly appear, occurring in a variety of ways, to varying degrees and duration. In horses that have survived the disease, there may be residual symptoms that may improve over time, often resulting in a deterioration in the horse's quality of life, or death due to residual symptoms.

The treatment of West Nile virus disease is symptomatic, as there is currently no active ingredient for specific therapy. The vaccine is available, but its protection is uncertain.

The goal of my work is to create a dietary supplement that can provide protection against WNV infection, and it can also be used as an adjunct treatment for an existing infection. I grow the plants needed for the study, so I am also involved in the creation of the cultivation technology.

Supervisor: Hohmann Judit

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In vitro antimicrobial screening for bioactive secondary metabolites of selected Euphorbiaceae species from West Nusa Tenggara, Indonesia

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In our days, new and re-emerging infectious diseases are rising very rapidly. Therefore, there is an urgent need to discover newer antimicrobial compounds having diverse chemical structures and novel mechanisms of action. The emergence of multiple drug-resistant strains in human pathogenic organisms has further necessitated searching for new antimicrobial substances from natural sources [1]. The family Euphorbiaceae has attracted great interest from many researchers due to its significant antibacterial, antifungal and antiviral activities [2]. The present study is aimed at the investigation of eight species, *Macaranga tanarius*, *Mallotus mollissimus*, *M. rufidulus*, *Homalanthus giganteus*, *Shirakiopsis indica*, *Euphorbia sp.*, *E. atoto*, and *E. hypericifolia*, belonging to the Euphorbiaceae family for bioactive secondary metabolites with antimicrobial activity. In this experiment, the dried and grounded plant materials were percolated using methanol and, after evaporation, subjected to solvent-solvent partition resulting in four fractions of different polarity (*n*-hexane, chloroform, ethyl acetate, and aqueous-methanol). The antimicrobial activity of the 32 fractions was evaluated using disc diffusion method against thirteen bacterial and four fungal strains. The highest antimicrobial activity was detected in chloroform and ethyl acetate fractions from *Shirakiopsis indica* against *Candida glabrata* ATCC 2001. The most sensitive strain was *Candida glabrata* and *Candida parapsilosis*; at least one fraction of all species showed any activity against these fungi.

Supervisor: Judit Hohmann

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Isolation and identification of triterpenes from *Pholiota populnea*: new members of the Pholiol series

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Strophariaceae family has 18 genera and 1.316 species, found in northern temperature zones [1,2]. *Pholiota populnea* (syn. *Pholiota destruens*, *Hemipholiota populnea*) is a saprophytic or sometimes parasitic mushroom species, which plays a considerable role in decomposing the deadwood of cottonwoods. The aim of our work was the isolation and structure determination of bioactive compounds of *P. populnea*. The mushroom material (4.2 kg) was extracted with methanol by percolation. After concentration, the methanolic extract was subjected to solvent-solvent partition using *n*-hexane, chloroform, and ethyl acetate, respectively. The chloroform and ethyl acetate phases were separated by flash chromatography on normal (NP) and reversed phase (RP) column, by NP- and RP-HPLC and gel filtration on Sephadex LH-20. The structures were elucidated using extensive spectroscopic analyses, including 1D and 2D NMR and HRMS measurements. The detailed phytochemical analysis of the chloroform and ethyl acetate phases of *P. populnea* led to the isolation of six new lanostane diesters, named pholiols E-J, together with (+)-clavaric acid and two known ribonucleosides. All compounds were isolated for the first time from this mushroom. Our results indicate that *P. populnea* is a promising source for finding new triterpenes. We plan to investigate the anti-inflammatory activity of the compounds in the future.

Supervisors: Judit Hohmann, Attila Ványolós

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Structural modification of hydrocurcumins in the aim of increased antioxidant activity

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Tetrahydrocurcumin, hexahydrocurcumin and octahydrocurcumin are some of the major metabolites of curcumin. Like curcumin they exhibit various bioactivities such as antioxidant, anti-inflammatory or cardiovascular protective activity, but unlike curcumin, hydrocurcumins have much better bioavailability and are not considered pan-assay interference compounds (PAINS) [1,2]. In our earlier work we had concluded that based on the relationship between the pharmacokinetic properties and the antioxidant activities hexahydrocurcumin is the most promising lead compound of the three main curcumin metabolites [3].

In our present study we aimed to modify hexahydrocurcumin in a way to increase its pharmacokinetic and antioxidant potential. It has been found that the introduction of nitrone group to the vitamin E analogue Trolox has significantly increased its antioxidant activity [4]. In our attempts to introduce the nitrone functional groups to hexahydrocurcumin we have encountered unexpected outcomes and managed to isolate several new interesting hydrocurcumin derivatives using RP- and NP-HPLC methods. Among some metabolites of the starting material, four new, ring-closed derivatives have been identified. The structural conformation was done by NMR and HRMS measurements.

Supervisor: Attila Hunyadi

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