

**2nd Symposium of
Young Researchers on
Pharmacognosy**



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BOOK OF ABSTRACTS



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ABSTRACTS

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Antimicrobial potential of extracts and essential oils from different varieties of Marjoram

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Species belonging to the genus *Origanum* from the family Lamiaceae are used since ancient times as spices, medicinal, aromatic, and ornamental plants. *Origanum majorana* L. (syn. *Majorana hortensis* Moench), commonly known as sweet marjoram, is native to the Mediterranean region and cultivated in many countries of Asia, North Africa, and Europe. *O. majorana* is known for its antimicrobial, antioxidant, antidiabetic, hepatoprotective, antiulcer, anti-inflammatory, and antitumor activities [1]. Different types of terpenoids (mono-, di and triterpenes), and phenolic compounds (phenolic acids, hydroquinones, and flavonoids), are the most abundant constituents detected in sweet marjoram to date [2]. The present study aims to investigate marjoram, its essential oil, chemical compounds, and possible activities against bacteria, fungus, and other microorganisms, alone or synergized with other materials. Previously only the essential oil was studied in detail for these activities [3], but non-volatile compounds effective against microorganisms were not identified. Two samples of 'Hungarian' and one of 'Egyptian' varieties were involved in our investigations. Essential oils were obtained by steam distillation, and extracts of different polarities were prepared by a solvent-solvent partition of the MeOH extracts. Besides the dried leaves, stems were also extracted and subjected to essential oil distillation. The antimicrobial evaluation of 15 extracts and essential oils was made by disk diffusion and broth dilution methods against 9 bacterial and 1 fungal strains. Preliminary TLC tests showed no substantial differences in chemical profiles of the three samples of marjoram. The highest antibacterial activities were detected for the CHCl₃ fraction of a Hungarian variety against *Staphylococcus aureus*, *S. aureus* MRSA, *S. epidermidis*, and *Candida albicans*.

Supervisor: Judit Hohmann

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Studies on oxidized resveratrol metabolite mixtures

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Resveratrol is a well-known polyphenol with a plethora of pharmacological activities [1,2]. Due to its chemical structure, resveratrol may directly scavenge reactive oxygen and nitrogen species. In living systems, this can lead to the generation of a wide range of new metabolites with altered bioactivities [3].

The aim of our studies is to evaluate the potential biological relevance of this phenomenon. The oxidation of resveratrol through various chemical reactions, including biomimetic approaches, resulted in mixtures that exhibited greater bioactivities compared to the parent compound. Mixtures were tested for *in vitro* DPPH and ORAC antioxidant activities, and inhibitory action on lipoxygenase, xanthine oxidase, acetylcholinesterase and angiotensin converting enzymes. Based on chromatographic fingerprint and chemical diversity, promising mixtures were selected for activity-guided isolation.

To this end, isolation of constituents from the bioactive oxidized resveratrol mixtures afforded 31 compounds in pure form, which are currently being investigated for antioxidant and enzyme inhibitory bioactivities. Their structure elucidation is mostly ongoing, and several resveratrol dimers are assumed based on preliminary data.

In conclusion, the oxidation of resveratrol led to the formation of new metabolites that showed greater inhibitory activities to several enzymes of significant clinical importance when compared to the parent compound.

Supervisor: Attila Hunyadi

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Cannabinoids: an interesting and promising, but challenging field

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The issue of *Cannabis sativa* has affected numerous fields of research and has provided space for valuable research possibilities. Its importance as a remedy is unquestionably getting popular nowadays. Due to the unclear legislation, consumers have access to cannabis-containing food supplements in shops and through online websites. Uncontrolled authorisation procedures contribute to the potential health risk factors of these products.

The main goal of our work was to investigate cannabis oil food supplements available on the online market. We developed several separation methods to accomplish full resolution of 14 cannabinoid markers in fifteen commercially available *Cannabis* products. We carried out a meta-analysis on the safety evaluation of two cannabinoid, nabilone and dronabinol, which are the active substances of prescription drugs approved in several countries. We managed to expand our research to pharmacological and pyrolysis studies of cannabidiol (CBD) in co-operation. We examined the *in vitro* and *in vivo* cardiovascular effects of cannabidiol and analysed the pyrolysis products of CBD using standard heating conditions applied in electronic cigarettes.

Supervisors: Dezső Csupor, Tivadar Kiss

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Isolation and structure determination of compounds from *Juncus articulatus*

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Juncaceae species are characterized by the synthesis and accumulation of phenanthrenes. These compounds are of great interest from structural, pharmacological, semisynthetic and chemotaxonomical points of view. The aim of our work is the phytochemical and pharmacological investigation of Juncaceae species occurring in the Carpathian Basin. In the course of this work, the investigation of the secondary metabolites of *J. articulatus*, a perennial plant grows in moist areas, such as wet sand, is performed [1,2].

The dried and ground plant material was extracted with methanol. After concentration, the extract was dissolved in 50% aqueous methanol, and solvent-solvent partitions were performed with *n*-hexane, chloroform and ethyl acetate. Based on the preliminary studies, it was concluded that phenanthrenes are enriched in the chloroform phase. Therefore, at first this fraction was separated by column chromatography and then further purified by Sephadex LH-20 gel chromatography. As a final purification, preparative thin-layer chromatography and high-performance liquid chromatography are used. Structure elucidation of the isolated compounds is carried out by NMR and MS spectroscopy as well as by comparison with literature data. To date, three compounds were identified, two phenanthrenes and one flavonoid.

Supervisor: Andrea Vasas

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Phytochemical investigation of *Carex praecox*

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Cyperaceae species are common members of the flora of the Carpathian Basin. In recent years, species belonging to the genus *Carex* (Cyperaceae) have attracted attention due to their chemical composition; flavonoids and stilbenoids were identified from some of the species of this genus [1,2].

The aim of our work is the isolation and structure determination of bioactive compounds of Cyperaceae species native to the Carpathian Basin. In the course of this project, *Carex praecox* Schreb., a small perennial plant native to Europe and western Asia, was investigated.

During the preparative work, the dried and ground whole plant was extracted with methanol and after evaporation it was subjected to solvent-solvent partition with *n*-hexane, chloroform and ethyl acetate. The chloroform fraction seemed to be the most promising based on the preliminary antibacterial tests and TLC investigation. Therefore, it was purified by diverse chromatographic methods, including VLC, MPLC, preparative TLC and HPLC. The structure elucidation of the isolated compounds was carried out by NMR and MS spectroscopic methods. Altogether seven compounds have been isolated from the plant so far, among them two novel flavonoids, two novel and one known lignan derivative, an aldehyde and a chromene type metabolite. All compounds have been isolated for the first time from the plant.

Supervisor: Andrea Vasas

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Study on peroxyxynitrite-induced oxidation of hydrocurcumins

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Hydrocurcumins (tetrahydrocurcumin, hexahydrocurcumin and octahydrocurcumin) are major phase I, bioreduced metabolites of curcumin, the main bioactive compounds of Turmeric (*Curcuma longa*). These compounds exhibit similar or, in some cases, even better bioactivities *in vitro* and *in vivo* compared to curcumin itself (e.g. antioxidant, anti-inflammatory or cardiovascular protective activity) [1]. Further, unlike curcumin, hydrocurcumins are much more stable in the human body, have a slower metabolism, and better bioavailability.

The reaction between antioxidants and scavenging reactive oxygen or nitrogen species often results in chemically stable, bioactive metabolites. These new metabolites could have altered bioactivity compared to the parent compound, and this activity can be directly translated from oxidative stress. It is reasonable to extend the chemical space by utilizing RONS-mediated oxidation [2,3].

In this work we aimed to study the reaction between peroxyxynitrite, a biorelevant reactive nitrogen species, and hydrocurcumins to extend the chemical space, and to better understand the mechanism of such antioxidant activity. So far, we isolated 14 different compounds from the reaction mixtures by using RP-HPLC. Among these compounds 4 have been confirmed as fragments of the starting materials.

Supervisor: Attila Hunyadi

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Isolation and structure determination of pregnane glycosides and lignans from an African species

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Africa has a rich diversity of plants. Recent statistics show that about 25% of the total number of higher plants in the world is found in Africa [1]. A large amount of the plants is regularly used in traditional medicine; the best-known ones are *Aloe ferox*, *Centella asiatica*, *Cyclopia genistoides*, *Harpagophytum procumbens*, *Hoodia gordonii*, *Pelargonium sidoides*, *Prunus africana*, and *Withania somnifera*, among others [1]. The diversity of plants represents a very valuable resource, not only for commercial but also for basic scientific points of view. The aim of our work was the phytochemical and pharmacological investigation of selected African plants. In this experiment, the chloroform extract of the dried plant material (exact botanical identification of the plant is in progress) was separated by the combination of different chromatographic techniques including OCC, VLC, TLC, and HPLC. The structures of the isolated compounds were determined by NMR and MS spectroscopy and comparison with literature data. To date, twelve components, among them eight pregnane glycosides, three lignans and a flavanone were identified. Eight compounds are new natural products. Pregnane glycosides are substituted with three or four sugar moieties (glucose, tevetose, digitoxose and cymarose), joining at C-3 to the pregnane skeleton with *O*-glycosidic bond. If a glucose molecule occurs in the sugar chain, it can be found at the end of the chain, otherwise tevetose is the last sugar in the chain.

Supervisor: Andrea Vasas

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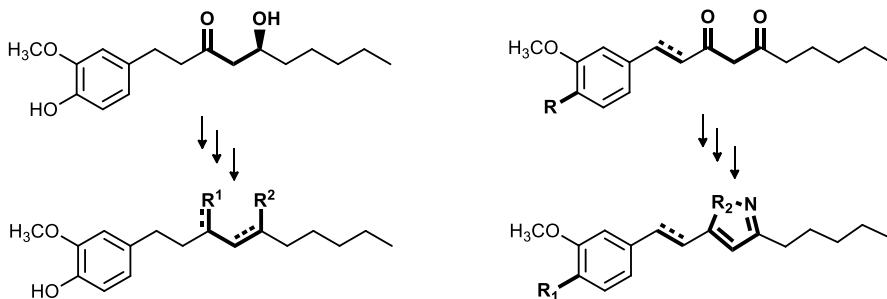
Isolation and synthesis of 6-gingerol and 6-gingerdione derivatives

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Ginger (*Zingiber officinale* Roscoe) has been used as a spice and a traditional remedy since ancient times, especially in Chinese herbal medicine [1,2]. A wide array of bioactivities were reported regarding ginger extract, e.g. antioxidant, anti-inflammatory, antiemetic, anticancer activity [3]. Most of the bioactivities had been correlated to gingerols and shogaols, the most abundant secondary metabolites in ginger [4].

In our work, we aimed to investigate the chemical space around 6-gingerol by introducing different changes in the skeleton and investigating the bioactivity of the resulting derivatives. The current work also focuses on the total synthesis of 6-gingerdione, another important compound in ginger root, and its heterocyclic analogues. So far, six molecules have been synthesized and characterized; the final purification and structure elucidation of further derivatives are currently in progress.



Supervisors: Attila Hunyadi, Tímea Halasi-Gonda

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Preparation and investigation of ecdysteroid – cinnamic acid hybrid compounds

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During the COVID lock-down, the chemical structures of more than 170 semi-synthetic ecdysteroid derivatives were collected from 21 articles since 2012, when the latest semi-synthetic reviews were published. These ecdysteroids, along with the semi-synthetic ecdysteroid library of our group, were processed into SMILES strings and organized according to their available bioactivities. Altogether, more than 300 ecdysteroids and, where available, their biological data were collected into a database in a way that is appropriate for an in-depth *in silico* evaluation. Further investigations on this database are planned with the ChemGPS-NP [1] software in collaboration.

During the literature survey, some reports on ecdysteroid cinnamic derivatives have been found. These esters were isolated from the fronds of *Microsorium membranifolium* [2]. This inspired us to prepare related new hybrid compounds through semi-synthesis.

20-Hydroxyecdysone (20E) and cinnamic acid were reacted with EDCI.HCl and DMAP in mol. sieve DCM for 4.5 day in room temperature [3]. The crude products were purified via flash chromatography and HPLC. Four ecdysteroid derivatives including two new compounds were obtained, and their structures were confirmed via NMR as the 2-, 2,3-, 2,22, and the 3,22-cinnamates of 20E. The compounds exhibited moderate antioxidant activities on DPPH and ORAC assays. Synthesis of the caffeate and ferulate derivatives of 20E are currently ongoing.

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 analyse the secondary metabolites of three food plants. The examined species included *Camellia sinensis*, the source of the most widely consumed herbal beverage, *Coffea* sp., that are used not only to prepare coffee but also to produce weight loss supplements, and *Capsicum annuum*, a characteristic spice of Hungarian gastronomy.

In case of *C. sinensis*, we first developed a densitometric method to measure the amount of theanine in tea. The advantage of this method is that it is fast, reliable and does not require special instrumentation [1]. Subsequently, a reversed-phase HPLC method was developed to examine the theanine content of tea leaves [2]. Moreover, this robust method is suitable for the simultaneous determination of the stimulant caffeine and the sedative theanine [3].

The analysis of coffee samples focused on the chlorogenic acid content, a group of compounds that have confirmed weight reducing effect. Our method allows the simultaneous determination of caffeine and chlorogenic acid. Further, a reversed-phase HPLC method was developed for the quantitative measurement of capsaicin analogues in peppers.

We have shown that the theanine content of tea does not always correlate with the degree of fermentation, since the theanine content of some green tea samples were below the levels of black teas [3]. In case of green coffee samples, we identified varieties with high chlorogenic acid content and developed a method that allows the removal of caffeine to enrich chlorogenic acids. 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.

Supervisor: Dezső Csupor

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Phytochemical and pharmacological study of furocoumarins in *Citrus trifoliata* L.

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Furocoumarins are heterocyclic aromatic compounds, in which a furan ring is fused with a coumarin skeleton. Basically there are two types of furocoumarins – angular and linear furocoumarins – depending on the position of the furan ring [1,2].

Furocoumarins have already been reported to exert several beneficial pharmacological effects, like antifungal, antibacterial activities [3]. Some of them were shown to have analgesic and anti-inflammatory effects [4], some others have been shown some interesting enzyme inhibitory effects [5]. In spite of these effects, furocoumarins can be dangerous/unsafe. In the literature many of their harmful effects have already been demonstrated such as photosensitizing effect [6].

Coumarins and furocoumarins are found in large quantities in citrus species. The peel contains higher concentrations of the furocoumarins than the pulp.

In my research work different coumarins and furocoumarins were isolated from *Citrus trifoliata* L. by various separation techniques such as flash chromatography, high performance liquid chromatography and rotation planar chromatography. Six coumarins and furocoumarins – auraptene, triphasiol, imperatorin, myrsellin, phellopterin, 7-methoxycoupoletin – were identified by NMR spectroscopy.

The antiproliferative and cytotoxic activity of the isolated compounds were tested *in vitro*. The bioactivities of certain compounds were examined for the first time. The presented results contribute to a better understanding of risks and beneficial effects of *C. trifoliata* secondary metabolites.

Supervisor: Dezső Csupor

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Reports of illegal food supplements for weight loss in the European Rapid Alert System for Food and Feed (RASFF)

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Obesity is considered as an epidemic; thus the treatment is of major importance. Many customers are using dietary supplements to induce weight loss; however, the efficacy and safety of these products are often doubtful [1]. In the European Union, the Rapid Alert System for Food and Feed (RASFF) was put in place to provide food and feed control authorities with an effective tool to information exchange on measures taken responding to serious risks detected in relation to food or feed [2]. The reports of RASFF confirmed that adulterated products are on the market. These products often contain sympathomimetic drugs or herbal substances to enhance efficacy; therefore, the use of these supplements might pose a serious health risk for consumers. The aim of our work was to summarize the trends concerning adulterated food supplements associated with a warning released by the RASFF between 1988 and 2019, focusing on products with intended use as slimming agent. Data were extracted from the RASFF portal. In total, 299 (11.7%) records of food supplements with quality problems were identified in the RASFF, and several of these products were marketed to facilitate weight loss. 182 (60.8%) of these contained synthetic drug ingredients. Significant number of weight loss supplements is adulterated to satisfy patients' expectations. Hence, these products may cause serious adverse effects depending on the adulterants in it. The data presented here can be used as a basis for a suitable prediction model for the quality control of slimming products.

Supervisors: Dezső Csupor, Barbara Tóth

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Cytotoxic and antiproliferative activity of sesquiterpene lactones isolated from the aerial parts of *Ambrosia artemisiifolia*

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Ambrosia artemisiifolia (common ragweed) is an invasive weed in Europe, well-known for the strong allergenic effect of its pollen, as well as for its invasiveness and impact in crop fields. This species produces a broad range of sesquiterpenoids. These bioactive molecules possess allergic, allelopathic [1], anti-inflammatory [2], antitumor [3] and antimicrobial activities [4]. From a phytochemical view the secondary metabolites of the plant have not explored completely. The primary aim of our work was to isolate the major phytochemicals from the aerial parts of *A. artemisiifolia*, focusing on the sesquiterpene lactone content and to elucidate their chemical structure. Subsequently, we performed cytotoxic, antiproliferative and combination assays with the isolated compounds. Also the genus *Ambrosia* was reviewed focusing especially on the sesquiterpene lactone compounds and their pharmacological effects.

The methanolic extract of the plant was separated by several chromatographic techniques, including preparative TLC and HPLC. Eight compounds were isolated. Their structure was identified by ¹H NMR, ¹³C NMR and mass spectrometry. Two new seco-pseudogaianolides 1'-noraltamisin and 1,10-dihydro-1'-noraltamisin were reported for the first time from this plant. Their cytotoxic and antiproliferative effect were measured on different human cancer cell lines and also on a normal cell line. A combination assay was applied to study the effect of drug interactions between the compounds and doxorubicin.

Supervisor: Csupor Dezső

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Semi-synthetic preparation and investigation of new, potentially bioactive ecdysteroid derivatives

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Ecdysteroids are a group of bioactive, non-toxic compounds known as analogues of the insect molting hormone. Some of their derivatives have been shown to have antidiabetic [1], cholesterol-lowering [2], immunomodulatory [3], antioxidant and neuroprotective [4] effects, and a recent in silico study suggested anti-COVID19 activity of an ecdysteroid [5]. Further, the tumor resistance-reducing effect of ecdysteroids was discovered by the research group that hosts my work [6].

The aim of our present research is to identify new bioactive ecdysteroid derivatives. Our experimental efforts include the methodological implementation of large-scale semi-synthetic production and purification of calonysterone that has a diverse pharmacological potential but limited availability [7]. It is also our objective to prepare further derivatives of this compound.

To this, end, we have performed the functionalization of calonysterone through an oxime functional group that is a valuable building block in organic syntheses, and its presence can directly affect the bioactivity of compounds. Eleven new ecdysteroid derivatives were prepared so far. Their bioactivity studies are carried out in collaboration, and they are currently being tested for neuroprotective, anti-*Trypanosoma cruzi*, antidiabetic and antihyperlipidemic activities.

Supervisors: Attila Hunyadi, Máté Vágvolgyi

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Isolation and anti-HSV2 studies of compounds from *Euphorbia deightonii*

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Background: Euphorbiaceae family has 275 genera and 7,500 species, found in tropical and temperate regions [1,2]. *Euphorbia deightonii* Croizat, native to West Africa is a cactus-like shrub which grows to 6 m tall. Historically, it served ornamental, medical, and military purposes [3].

Aims: To isolate compounds from *E. deightonii* and evaluation of anti-HSV activities of selected compounds.

Methods: Dried plant material (1.2 kg) was extracted with methanol via percolation. Solvent-solvent fractionation with chloroform yielded chloroform portion which then undergo open column chromatography on polyamide using a step gradient of methanol-water to yield four fractions; 20%, 60%, 80% and 100% methanol, respectively. Compounds of the 60% fraction was purified using NP and RP-VLC, NP and RP HPLC, PLC. Structures were established using NMR and HRMS data.

Results: 38 compounds (**1–38**) were isolated which are 30 diterpenes (i.e. 27 ingol, 2 *ent*-atisane and 1 stachane types), 3 triterpenes, 2 lignans, 1 phenyl propanoid, 1 coumarin and 1 ellagic acid derivative. The research also yielded 11 new compounds: 9 ingol diterpenes, 1 triterpene and 1 lignan. All 8 non-diterpenoids were evaluated for anti-HSV2 activity with acyclovir as positive control. Two new compounds (**33** and **34**) and two known compounds (**32** and **37**) showed activity with IC₅₀ of 7.05, 11.73, 2.42, μM, and 32.09 nM, respectively. Interestingly, coumarin **37** has comparable activity to acyclovir.

Supervisor: Dóra Rédei

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Ensifolins A-M, new phenanthrenes from *Juncus ensifolius*

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Phenanthrenes form an increasing group of aromatic plant secondary metabolites. These compounds are derived mainly from stilbene precursors and can be divided into three major groups: mono-, di-, and triphenanthrenes. In many cases, phenanthrenes are substituted with special functional groups, therefore, they are characteristic to certain plant families; e.g. stilbene-substituted phenanthrenes were isolated only from Orchidaceae species, while vinyl-substituted phenanthrenes were found exclusively in Juncaceae species. These compounds can serve as chemotaxonomic markers. Phenanthrenes are representative metabolites of Juncaceae species [1].

In continuation of our work dealing with the isolation of phenanthrenes from Juncaceae species, *Juncus ensifolius* Wikstr. was investigated. 17 Phenanthrenes were isolated from the methanol extract of the plant by using different chromatographic methods (VLC, MPLC, Sephadex LH-20 gel chromatography and HPLC). The structure elucidation of the compounds was carried out by extensive NMR and HRMS experiments.

14 Compounds (ensifolins A-M), from which 8 monomers and 4 dimers are new natural products. Some compounds are unique as they are substituted with flavonoid or benzaldehyde. All compounds were isolated for the first time from the plant.

Supervisor: Andrea Vasas

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Isolation and characterization of chemical constituents from *Hemipholiota populnea* and their bioactivities

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Hemipholiota populnea (Pers.) Kühner ex Bon [syn. *Pholiota populnea* (Pers.) Kuyper & Tjall.-Beuk.] known as a member of Strophariaceae family and distributed worldwide wherever cottonwoods occur. Previously the chemistry and pharmacology of this mushroom was not investigated. Detailed phytochemical analysis of the *n*-hexane and ethyl acetate extracts of *H. populnea* led to the isolation of four lanostane derivatives (**1-4**) with specific ester functionality and a linear triterpene tetraol (**5**). Compounds **1-5** were identified as new natural products, in addition the known ergostane derivative (**6**) and two nucleosides (**7-8**) were obtained. All compounds were isolated for the first time from this mushroom. **1-3**, **5** and **6** were investigated for antimicrobial activity against different bacterial strains (Gram-negative, Gram-positive, methicillin and ofloxacin resistant strains), and for cytotoxic activity against sensitive and resistant Colo 205 and Colo 320 cell lines, respectively, and against non-tumoral MRC-5 cell line. Combination with doxorubicin and efflux pump inhibitory activity of **1-3**, **5** and **6** on Colo 320 cells were also assayed. No antibacterial activity could be detected, however, some of the triterpenes demonstrated remarkable cytotoxic and multidrug resistance reversal activity. Very strong synergism with doxorubicin was observed for compound **5**. Our results indicate that *H. populnea* is a promising source of new terpene metabolites with significant bioactivities.

Supervisors: Attila Ványolós, Judit Hohmann

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