5th Symposium of Young Researchers on Pharmacognosy

Szeged, 23 July 2024

BOOK OF ABSTRACTS

University of Szeged
Institute of Pharmacognosy

5th Symposium of Young Researchers on Pharmacognosy

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(ed. Tivadar Kiss, Judit Hohmann)

Institute of Pharmacognosy, University of Szeged, Szeged, Hungary

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LIST OF PRESENTATIONS

23 July 2024, 10:00 AM

University of Szeged, Faculty of Pharmacy, Clasroom No. 5.

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7. **Glória Papdi, Gábor Girst, Attila Hunyadi, Máté Vágvölgyi**
   Preparation and investigation of B-ring modified nitrogen-containing calonysterone derivatives
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   Isolation and structure elucidation of compounds from *Euphorbia systyloides*
9. **Anita Barta, Petra Petz, László Bakacsy, Judit Hohmann, Andrea Vasas**
   Phytochemical investigation of cultivated *Juncus hybridus*
10. **Manar Madah, Anita Barta, Judit Hohmann, Tivadar Kiss**
    Preliminary phytochemical results of *Heracleum sphondylium*

ABSTRACTS

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doi: 10.14232/syrpharmacognosy.2024.a1

Dearomatized *p*-coumaric acid derivatives: Potent *in vitro* antitumor DNA damage response inhibitors

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Our research group previously identified graviquinone as a promising antitumor metabolite, which is generated in situ when the antioxidant methyl caffeate scavenges free radicals. Graviquinone not only damaged DNA in cancer cells but also protected DNA in normal keratinocytes. To further investigate and expand the chemical space around graviquinone, we synthesized nine new alkyl-substituted derivatives and assessed their *in vitro* antitumor potential. These new compounds bypassed ABCB1-mediated multidrug resistance and exhibited distinct cell line specificity compared to graviquinone. Notably, all new derivatives were more potent against MDA-MB-231 cells than MCF-7 cells. Specifically, the *n*-butyl-substituted derivatives 2 and 8 modulated the cell cycle and inhibited ATR-mediated phosphorylation of checkpoint kinase-1 in MCF-7 cells. Building on our previous findings, these results underscore the significant antitumor potential of alkyl-substituted graviquinone derivatives.

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Ecdysteroids present in various insect meals

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Ecdysteroids are a naturally occurring, diverse group of compounds, found in animals, plants and fungi alike. Its plant-derived variants are the analogues of 20-hydroxyecdysone (20E), the hormone responsible for moulting in insects. We have selected insect meals manufactured from various grasshopper and locust species, sourced from Thailand. After an extensive extraction process targeting ecdysteroids and other relevant compounds, we synthesized multiple ecdysteroid-esters to function as reference material for the quantitative and qualitative analysis of our samples. The results of our venture will be detailed in the presentation.

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French paradox: Exploring the biological relevance of the scavengome of resveratrol

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Vast studies on low-to-moderate consumption of red wine, which contains a variety of polyphenols including resveratrol, have been linked to low incidence of coronary heart diseases despite high fat diets. This is the so-called “French paradox” [1]. Widely acknowledged for its cardiovascular protective activities, resveratrol also acts as a dietary antioxidant, reducing oxidative stress by modulating intrinsic enzymatic systems involved in redox regulation [2] and by scavenging reactive oxygen/nitrogen species (RONS) resulting in a complex mixture of chemically stable bioactive oxidized metabolites [3]. Therefore, we aimed at a performance-based diversity-oriented chemical approach to study the scavengome of resveratrol, i.e., the chemical space of metabolites formed from resveratrol oxidized by RONS.

Using a combined strategy of oxidation screening, untargeted metabolomics against a diverse resveratrol metabolite library, and bioactivity-guided isolation techniques, the chemical oxidation of resveratrol resulted in a chemically diverse group of compounds, including viniferins, and ethoxy-, chlorine- or iodine-substituted compounds. Taking the various biomimetic oxidation models into account, some of these compounds such as viniferins, ethoxy-substituted and chlorine-substituted compounds, could also be expected in aged red wines and biological environments.

The anti-inflammatory effect associated with the marked inhibitory activity of angiotensin converting enzyme of these compounds compared to resveratrol, along with their stronger scavenging activity against DPPH and AAPH radicals [4] demonstrate a multiple-faceted approach to their cardiovascular protective effects. Using this evidence as proof of concept, we suggest that a diversity-oriented exploration of the antioxidant scavengome can be used as a high hit-rate strategy for drug discovery.

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Preliminary Phytochemical and Pharmacological Investigation of *Homalanthus giganteus*

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Homalanthus giganteus, a species within the Euphorbiaceae family, thrives in wet tropical regions of Southeast Asia and the Pacific, particularly in the Lesser Sunda Islands, including Bali, Nusa Tenggara, and Timor within the Indonesian archipelago. Traditionally, *H. giganteus* has been used in Indonesia to treat fever [1]. Our research group has published the only available data on this species, specifically regarding its antimicrobial and antiproliferative properties. Our findings demonstrated that *H. giganteus* extracts exhibit significant potential against diverse microbes, with minimum inhibitory concentrations (MIC) ranging from 12.5 to 500 µg/mL and show remarkable activity (IC_{50} 0.92 µg/mL) against the COLO 205 cell line [2]. However, the specific phytochemicals responsible for these activities have not yet been identified.

To address this gap, our research aims to isolate and identify the bioactive phytochemicals in *H. giganteus*. This involves separating the plant extracts using High-Performance Liquid Chromatography (HPLC) and characterizing the isolated compounds through Nuclear Magnetic Resonance (NMR) spectroscopy. A total 4 compounds have been isolated and characterized, which include one fatty acid, one triterpene and two diterpenes. Identifying these phytochemicals is crucial for understanding the mechanisms underlying the medicinal properties of *H. giganteus* and could lead to the development of new therapeutic agents. This study represents a significant step toward the pharmacological exploitation of *H. giganteus*, providing insights that could enhance its application in drug development.

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Synthesis of bioactive evodiamine and rutaecarpine analogues under ball milling conditions

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Mechanochemical reactions achieved by processes such as milling and grinding are the future of chemistry. This approach not only eliminates the need for large amounts of solvents, thereby reducing waste generation, but also finds applications in chemical and materials synthesis.

This study focuses on the synthesis of quinazolinone derivatives by ball milling, in particular evodiamine and rutaecarpine analogues. These compounds are of interest due to their diverse bioactivities, including potential anticancer properties. The study examines the reactions carried out under ball milling conditions and demonstrates their efficiency in terms of shorter reaction times and reduced environmental impact compared to conventional methods. The ball milling reaction of evodiamine and rutaecarpine analogues resulted in yields of 63–78% and 22–61%, respectively. Furthermore, these compounds were tested for their cytotoxic activity, with evodiamine exhibiting an IC_{50} of 0.75 ± 0.04 μg mL^{-1} against the Ca9-22 cell line. This research represents a new and effective means to synthesise these compounds, providing a more environmentally friendly and sustainable alternative to traditional approaches.

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Acknowledgements

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Composition decipherment of *Ficus pumila* var. *awkeotsang* and its potential on COVID-19 symptom amelioration and in silico prediction of SARS-CoV-2 interference

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The jelly derived from the achenes of *Ficus pumila* var. *awkeotsang* (FPAA) is a renowned beverage ingredient in Taiwan. In this study, the new compound ficumarin (**1**), derived from the twigs of *Ficus pumila* var. *awkeotsang* (FPAT), was elucidated with comprehensive spectroscopic data. It was proposed that the biosynthetic origin of the compound in question is the *p*-coumaroyl-CoA pathway. Alloxanthoxyletin, betulinic acid, and catechin were identified as the major and active constituents responsible for relieving neutrophilic inflammation by FPAT. Among the identified compounds, alloxanthoxyletin was found to interact with PRO350 and GLU377 of human INOSOX, exhibiting the highest potency. Furthermore, the capacity of the FPAT fraction and its coumarins to activate the Nrf2 pathway was confirmed. The analysis of LC-MS/MS data and feature-based molecular networking revealed that coumarins were the dominant and responsible components. Notably, alloxanthoxyletin was found to increase Nrf2 expression by up to 816.8 ± 58% due to its interaction with the VAL561, THR560 and VAL420 residues of the 5FNQ protein. The results of the simulation conducted using the COVID-19 Docking Server indicated that pyranocoumarins have the potential to interfere with the life cycle of SARS-CoV-2. Moreover, the findings indicated that FPAT exerts anti-inflammatory activity on neutrophils and activates Nrf2, suggesting that it may be developed as a complementary supplement for the treatment of patients with COVID-19.

References

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Preparation and investigation of B-ring modified nitrogen-containing calonysterone derivatives

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The non-toxic, bioactive 20-hydroxyecdysone (20E) is the most abundant ecdysteroid derivative in nature, whose base-catalysed autooxidation yields calonysterone, another natural ecdysteroid, possessing a dienone type B-ring [1]. Previously, we identified calonysterone as a potent cytoprotective agent, however, until now, the low reactivity of its B-ring stood as an obstacle to carry out transformations on the pharmacophore site of the molecule [2].
Therefore, in our current work, we set out to perform the oximation of the C-7 carbonyl group on the B-ring of calonysterone. Interestingly, our transformation resulted in a new, C-6 oxime derivative with an intact C-7 carbonyl moiety using hydroxylamine hydrochloride reagent in ethanol. The obtained oxime product was purified by preparative HPLC on a C-18 reversed-phase column. To enhance the chemical diversity towards additional bioactive products, we modified our reaction conditions by replacing ethanol with methanol. As a result, we observed increased by-product formation, and 3 additional compounds were isolated from the reaction by chromatography. Furthermore, we performed experiments to accomplish the Beckmann rearrangement of our new oxime derivative in hand, with the aim to convert the starting material's B-ring into a 7-membered lactam ring. The chromatographic purification and structural elucidation of the products available are currently in progress. All our isolated products are planned to be tested for their cytoprotective properties *in vitro*.

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Isolation and structure elucidation of compounds from *Euphorbia systyloides*

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Euphorbiaceae, a diverse plant family, includes large desert succulents, trees, and small herbaceous types. Numerous species produce toxic milky juice and exhibit significant medicinal properties [1]. Euphorbia species are notable for their anti-inflammatory and anticancer active constituents, particularly diterpenes and triterpenes [2].

This work aims at the isolation, structure elucidation, and pharmacological investigation of specialized plant metabolites, especially diterpenes and triterpenes from *Euphorbia* species.

Euphorbia systyloides is a toxic, tropical plant that has never been investigated from phytochemical or pharmacological points of view. The methanol extract obtained from the aerial parts of the plant was evaporated in vacuo, dissolved in 50% methanol, and then subjected to solvent–solvent partition with *n*-hexane, chloroform, and ethyl acetate, respectively. The antiproliferative activity of the three fractions was tested *in vitro* against COLO 205, COLO 320, and HeLa cell lines. The chloroform phase was then further purified by different chromatographic methods, vacuum column chromatography (VCC), flash chromatography (FC), and finally reversed-phase high-performance liquid chromatography (RP-HPLC). 1D and 2D NMR spectra were recorded in methanol-d4 on a Bruker Avance DRX 500 spectrometer at 500 MHz (^{1}H) and 125 MHz JMOD (^{13}C).

So far, three tirucallane triterpenes have been isolated, among them two novel compounds. Furthermore, one gallic acid and a megastigmane derivative were also isolated from the plant.

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Phytochemical investigation of cultivated Juncus hybridus

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Plants belonging to the family Juncaceae accumulate different types of secondary metabolites, e.g. phenanthrenes, flavonoids, coumarins, triterpenes, steroids, and phenolic acid derivatives. Among them, phenanthrenes are a promising group of natural small molecules, possessing noteworthy pharmacological activities, like antiproliferative, antibacterial, anti-inflammatory, and sedative effects. According to previous studies oxidative stress and fungal infection induce the biosynthesis of these compounds in plants [1,2]. Our work aimed to analyze the chemical composition of a *Juncus* species, *Juncus hybridus*, cultivated under controlled conditions.

The isolation was started by extraction of the dried and ground whole plant with methanol. After evaporation, the extract was dissolved in 50% aqueous methanol, and solvent-solvent partitions were performed with *n*-hexane, chloroform, and ethyl acetate. The rough separation of the components of the chloroform and ethyl acetate phases was performed by column chromatography, and then further purifications were made by gel filtration and preparative thin-layer chromatography. As a final purification step, we used high-performance liquid chromatography. The structure elucidation of the compounds was carried out by NMR and HRMS experiments and by comparison of spectroscopic data with literature values.

The results allowed the identification of phenanthrenes, flavonoids, truxinic acid, and megastigmane derivatives, among them one new compound from the plant. This is the first time that truxinic acid derivatives have been isolated from a Juncaceae species. The antibacterial investigations of these compounds are in progress.

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Isolation of furanocoumarin compounds from the aerial parts of *Heracleum sphondylium*

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Heracleum sphondylium, commonly known as hogweed, belongs to Apiaceae family which is known by furanocoumarins as its specialized compounds [1, 2]. In Eastern European countries like Romania, many dietary supplements in the market promote one of *Heracleum sphondylium* ethnobotanical uses in enhancing fertility in both genders despite the lack of scientific background regarding this indication [1]. In addition, furanocoumarins have a wide range of biological effects including anticancer effect. Few studies have investigated the activity of essential oil on cancer cell lines with little attention to the possible anticancer effect of furanocoumarins in *Heracleum sphondylium* [3]. Our objective was to develop preparative isolation method in order to produce new furanocoumarins from aerial parts of *H. sphondylium* for subsequent anti-proliferative effect investigation. Moreover, investigating the extracts effect in enhancing fertility. Aerial part of H. *sphondylium* was extracted with methanol using maceration. The extract was partitioned successively with *n*-hexane, chloroform, and ethyl acetate. Further chromatography purification *n*-hexane fraction was achieved using open column chromatography with polyamide stationary phase and methanol-water gradient elution starting with 20% MeOH, 40%, 60% and 80%, respectively. Four fractions (H20, H40, H60, H80). H60 and H80 were containing the compounds of interest in considerable amount based on the TLC. Therefore, H60 and H80 were subjected to further chromatographic steps (Open Column Chromatography, Centrifugal Preparative Thin Layer Chromatography, Flash Chromatography) resulting in isolation of pure furanocoumarins. The structures of the compounds were determined by, 1D (^{1}H, ^{13}C JMOD) and 2D NMR (HSQC, HMBC, ^{1}H-^{1}H COSY, NOESY) spectroscopic analysis. Our work resulting in isolation of six pure furanocoumarin compounds. Among these six pure furanocoumarins, 8-geranoloxypsoralen was isolated for the first time form *Heracleum sphondylium* and psoralen, 8-geranyl 5-methoxy is isolated for the first time form *Heracleum* as a genus. In conclusion, *Heracleum sphondylium* aerial parts are a good source for furanocoumarin compounds which might have a promising anti-cancer effect. Furthermore, our work might provide more reliable and scientific-proved data about the claims of using *Heracleum sphondylium* supplements in fertility domain.

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The picture on the front-page shows *Mentha x piperita.*

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