4th International Symposium of Young Researchers
on Medicinal Plants and Natural Product Research

Szeged, 22-24 May 2023

EUGLOH

BOOK OF ABSTRACTS

4th Symposium of Young Researchers on Pharmacognosy

BOOK OF ABSTRACTS

(ed. Judit Hohmann)

Institute of Pharmacognosy, University of Szeged, Szeged, Hungary

22–24 May 2023

Venue:
Szeged Regional Committee of Hungarian Academy of Sciences
H-6720 Szeged, Somogyi u. 7, Szeged

https://us06web.zoom.us/j/89528815637?pwd=dHk1ODcyaXFIcWpRK0xnZXk1QU9tQT09
Meeting ID: 895 2881 5637, Passcode: 227572

doi: 10.14232/syrmpnpr.2023.af

University of Szeged, Faculty of Pharmacy, Institute of Pharmacognosy
Szeged, 2023

PROGRAM

22 May 2023 (Monday)

10:00–12:50 **Presentations**
Szeged Regional Committee of Hungarian Academy of Sciences
H-6720 Szeged, Somogyi u. 7, Szeged

12:50–14:00 **Lunch**
Restaurant 'Famous'
6720 Szeged, Kelemen László u. 3.

14:30 **Social program: Crime in the city**
Meeting point: in front of the Rectorate, University of Szeged
6720 Szeged, Dugonics tér 13.

23 May 2023 (Tuesday)

10:00–11:20 **Presentations**
Szeged Regional Committee of Hungarian Academy of Sciences
H-6720 Szeged, Somogyi u. 7, Szeged

11:20–11:40 **Coffee break**

11:40–13:00 **Presentations**

14:30 **Visiting Klebelsberg Library**
Meeting point: in front of the Klebelsberg Library
6720 Szeged, Ady tér 10.

15:30–19:00 **Online exam**
For details check: https://www.coosp.etr.u-szeged.hu/Scene-721162

19:00 **Conference Dinner**
Restaurant 'Napfény Műterem'
6720 Szeged, Széchenyi tér 2.

24 May 2023 (Wednesday)

10:00–13:00 **Visiting laboratories of Faculty of Pharmacy**
Institute of Pharmaceutical Technology and Regulatory Affairs
Institute of Pharmacognosy
Institute of Pharmacodynamics and Biopharmacy
Institute of Pharmaceutical Chemistry
Institute of Pharmaceutical Analysis
Institute of Clinical Pharmacy
Meeting point: Institute of Pharmacognosy, University of Szeged,
6720 Szeged, Eötvös Str. 6.

LIST OF PRESENTATIONS

MONDAY – 23 May 2023, 10:00 AM

KEYNOTE LECTURE (10:00–10:30)

1. Maria M. M. Santos
   Indole derivatives as promising therapeutic agents for cancer and malaria

SHORT LECTURES (10:30–12:50)

2. Mária Gáborová, Máté Vágvölgyi, Attila Hunyadi, Szabolcs Béni, Renata Kubínová
   Structure elucidation of diterpenoids isolated from three *Plectranthus sensu lato* species
3. Anita Barta, Petra Petz, Dóra Stefkó, Dragica Purger, László Bakacsy, Judit Hohmann, Andrea Vasas
   Isolation and structure determination of compounds from *Juncus* species
4. Ricardo J. F. Ferreira, Francisca Lopes, Gábor Girst, Attila Hunyadi, Maria M. M. Santos
   Hybrid molecules of tryptophan derivatives and protoflavones to tackle colon cancer
5. Zsuzsanna Csilla Dávid, András Juhász, Norbert Kúsz, Judit Hohmann, Andrea Vasas
   Phytochemical investigation of a Hungarian sedge, *Carex morrowii*
6. Tasneem Abu Ghazal, Katalin Veres, Judit Hohmann
   Isolation and evaluation of antimicrobial properties of non-volatile compounds from sweet marjoram
7. Sara H. H. Ahmed, Bizhar A. Tayeb, Tímea Gonda, Gábor Girst, Kornél Szőri, Róbert Berkecz, István Zupkó, Renáta Minorics, Attila Hunyadi
   Thymoquinone-protoflavone hybrids: Studies into anticancer potentials
8. Ákos Bajtel, Péter Püski, Tivadar Kiss, Dezső Csupor
   A new validated HPLC method for cannabidiol quantification in CBD-containing food supplements

TUESDAY – 23 May 2023, 10:00 AM

9. Tohfa Nasibova, Judit Hohmann, Schelz Zsuzsanna, István Zupkó, Attila Horváth, Anita Barta, Hiba Faroug Muddather
   Chemical composition and antiproliferative properties of *Peganum harmala*
10. Márton B. Háznagy, Máté Vágvölgyi, Sandhya R. Krishnan, Jürg Gertsch, Attila Hunyadi
    Antitrypanosomal activity of natural and semi-synthetic ecdysteroids
11. Ching-Chia Chang, Judit Hohmann, Fang-Rong Chang
    Bioactive components from *Epicoccum sorghinum*
12. Elizabeth A. Lopes, Margarida Espadinha, Joana D. Amaral, Rebecca Piccarducci, Elisa Zappelli, Simona Daniele, Claudia Martini, Mattia Mori, Cecília M. P. Rodrigues, Daniel J. V. A. dos Santos, Maria M. M. Santos
    Designing novel spiropyrazoline oxindoles to dual target p53-MDM2/X PPIs
13. Dyke Gita Wirasisya, Anita Barta, Gabriella Spengler, Gordana Krstić, I Gde Mertha, Judit Hohmann
    Investigation of antimicrobial and antitumor properties of selected Euphorbiaceae species
14. Gábor Girst, György T. Balogh, Attila Hunyadi
    Antioxidant and pharmacokinetic studies on new semi-synthetic nitrogen-containing diarylheptanoids derived from curcumin
15. Muhammad Bello Saidu, Anita Barta, Dóra Rédei
    Phytochemical investigations of *Euphorbia desmondii*
16. Péter Püski, Tímea Körmöczi, Róbert Berkecz, Ákos Bajtel, Tivadar Kiss
    New adulteration pattern of *Boswellia* extracts

ABSTRACTS

1 – KEYNOTE LECTURE **doi: 10.14232/syrmpnpr.2023.1**

Indole derivatives as promising therapeutic agents for cancer and malaria

Maria M. M. Santos

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof Gama Pinto 1649-003, Lisbon, Portugal, mariasantos@ff.ulisboa.pt

Indole, a versatile heterocyclic moiety present in the structure of several molecules, has garnered significant attention in the realm of natural product research due to its broad range of biological activities, including anticancer and antimalarial activities. The structural diversity and pharmacological versatility of indole derivatives make them attractive candidates for drug discovery and development. This keynote aims to shed light on the design, synthesis, and evaluation of novel indole derivatives with enhanced efficacy against cancer and malaria, showcasing their potential as promising natural product-based therapeutics. Our most recent results on the development of new tryptophanol and spirooxindoles derivatives will be presented [1-2]. Moreover, we will disclose our most recent results on the development of hybrid compounds with selective toxicity against triple-negative breast cancer cells, obtained in the bilateral action in collaboration with the research group of Professor Hunyadi.

References

[1] Santos, MMM, et al. *Eur J Med Chem* **2022**, 241:114637. doi: 10.1016/j.ejmech.2022.114637
[2] Santos, MMM, et al. *Pharmaceuticals* **2023**, 16(2):146. doi: 10.3390/ph16020146

Acknowledgements

I would like to thank all co-authors whose contribution was key for the presented results, including Attila Hunyadi and Gábor Girst, and Fundação para a Ciência e Tecnologia for financial support through UIDB/04138/2020 (iMed.Ulisboa), projects PTDC/QUI-QOR/29664/2017, PTDC/QUI-QOR/1304/2020 and bilateral action FCT/NKFIH 2019/2020.

2 – SHORT LECTURE

doi: 10.14232/syrmpnpr.2023.2

Structure elucidation of diterpenoids isolated from three *Plectranthus sensu lato* species

Mária Gáborová,[1] Máté Vágvölgyi,[2] Attila Hunyadi,[2] Szabolcs Béni,[3] Renata Kubínová[1]

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Plectranthus sensu lato (Lamiaceae) represents a large and widespread genus which comprises more than 400 semi-succulent to succulent herbs and shrubs with a significant diversity of its use in traditional medicine including mainly the treatment of various diseases of digestive tract, respiratory tract and skin [1, 2]. The genus is well-known as a source of diterpenoids. Until now, more than 350 highly oxygenated diterpenoids from abietane, beyerene, cembrane, clerodane, halimane, icetexane, kaurane, labdane, pimarane, and phyllocladane classes have been reported within the genus *Plectranthus s.l.*, with almost 70% of them belonging to the abietane class [3].

Our phytochemical study aiming to acquire bioactive substances from the methanolic extracts of the aerial parts of *Coleus forsteri* 'Marginatus', *P. ciliatus*, and *C. comosus* led to the isolation of 14 diterpenoids from abietane, *ent*-kaurane and *ent*-clerodane classes. Three compounds were isolated from a natural source for the first time and the structure of one known compound was revised. The structures of isolated diterpenoids were elucidated by extensive analysis of mass spectrometric and nuclear magnetic resonance (NMR) spectroscopic data. The relative configurations were inferred from ^{1}H - ^{1}H *J*-values and NOESY correlations. Circular dichroism spectroscopy was used to determine the absolute configuration.

References

[1] Paton, A, et al. *PhytoKeys* **2019,** 129:1–158. doi: 10.3897/phytokeys.129.34988
[2] Lukhoba, CW, et al. *J Ethnopharmacol* **2006,** 103(1):1–24. doi: 10.1016/j.jep.2005.09.011
[3] Gáborová, M, et al. *Molecules* **2021,** 27(1):166. doi: 10.3390/molecules27010166

Acknowledgements

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3 – SHORT LECTURE

doi: 10.14232/syrmpnpr.2023.3

Isolation and structure determination of compounds from *Juncus* species

Anita Barta[1], Petra Petz[1], Dóra Stefkó[1], Dragica Purger[2], László Bakacsy[3], Judit Hohmann[1,4], Andrea Vasas[1,4]

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In addition to flavonoids, coumarins, and triterpenes, plants belong to the family Juncaceae contain also phenanthrenes, which are a promising group of natural small molecules, possessing noteworthy pharmacological (e.g., antiproliferative, antibacterial, anti-inflammatory, and sedative) activities [1,2]. The aim of our work was to continue the isolation of phenanthrenes from Juncaceae species, namely *Juncus tenuis* and *J. kraussii* occurring in the Carpathian Basin.

The isolation was started by the extraction of the dried and ground plant materials with methanol. After evaporation, the extracts were dissolved in 50% aqueous methanol, and solvent-solvent partitions were performed with *n*-hexane, chloroform, and ethyl acetate. Phenanthrenes are enriched in the chloroform phases; therefore, these phases were fractionated by column chromatography and the eluates obtained were further purified by gel filtration, and high-performance liquid chromatography. The structure elucidation of the compounds was carried out by NMR and HRMS experiments as well as by comparison of spectroscopic data with literature values.

To date, twelve phenanthrenes, among them two new dimers, and flavonoids were identified from the two investigated plants. Our plans include the isolation and structure elucidation of additional compounds and their pharmacological investigation on different human tumour cell lines.

References

[1] Tóth B, et al. *J. Nat. Prod*. **2018**, 81(3): 661–678. doi: 10.1021/acs.jnatprod.7b00619
[2] Bús C, et al. *Phytochem. Rev*. **2018**, 17: 833–851. doi: 10.1007/s11101-018-9561-5

Acknowledgements

This research was funded by the UNKP-22-3 New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund, and the National Research, Development and Innovation Office, Hungary (NKFIH; K128963).

4 – SHORT LECTURE

doi: 10.14232/syrmpnpr.2023.4

Hybrid molecules of tryptophan derivatives and protoflavones to tackle colon cancer

Ricardo J. F. Ferreira[1,*], *Francisca Lopes*[1], *Gábor Girst*[2], *Attila Hunyadi*[2], *Maria M. M. Santos*[1,*]

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The tumor suppressor protein p53 is responsible for the genome integrity of cells, controlling apoptosis, cell cycle arrest and several other functions in response to stress signals. In human cancers, this protein is inactivated either by mutation or by negative regulators. For this reason, there is a high interest to discover new molecules able to reactivate the p53 tumor suppressor function. In the last years, our research group has been involved in the development of tryptophan derivatives to reactivate wild-type and mutant p53 [1].

The ataxia telangiectasia and Rad3 related protein (ATR) is another important target on cancer. ATR plays a central role in DNA damage response, and ATR inhibitors kill p53-deficient cancer cells [2]. To develop new drugs to tackle colon cancer, we decided to combine in one molecule two distinct pharmacophores (indole p53 activators and protoflavone ATR inhibitors). The rational is that ATR inhibitors will complement p53-targeted therapies. In this oral communication, we will show our most recent advances on the synthesis of these hybrid molecules.

References

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[2] Mei, L, et al. *J Hetamol Oncol* **2019,** 12(1):43. doi: 10.1186/s13045-019-0733-6

Acknowledgements

This research was funded by the bilateral action FCT/NKFIH 2019/2020 with references 2018-2.1.15-TÉT-PT-2018-00016-(Hungary) and 5183/2019 (Portugal). We also acknowledge FCT (Fundação para a Ciência e a Tecnologia, I.P.) through iMed.ULisboa (UID/DTP/04138/2019), project PTDC/QUI-QOR/1304/2020, and PhD fellowship 2022.11539.BD (R Ferreira).

5 – SHORT LECTURE **doi: 10.14232/syrmpnpr.2023.5**

Phytochemical investigation of a Hungarian sedge, *Carex morrowii*

Zsuzsanna Csilla Dávid[1], András Juhász[1], Norbert Kúsz[1], Judit Hohmann[1,2], Andrea Vasas[1,2]

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Cyperaceae is the third largest plant family among the monocotyledon plants [1]. Cyperaceae species (or sedges) occur worldwide and accumulate a large variety of secondary metabolites (e.g., flavonoids, lignans and stilbenes) with noteworthy biological activities [2,3].

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, 41 sedges were collected and the preliminary phytochemical and pharmacological (antioxidant, antibacterial) investigations of different extracts of the plants were carried out. Based on the results of the pharmacological screening studies, *C. morrowii* was chosen for further preparative work.

Dried, ground whole plant was extracted with methanol and after evaporation, the extract was subjected to solvent-solvent partition with *n*-hexane, chloroform ($CHCl_3$) and ethyl-acetate (EtOAc). The $CHCl_3$ and EtOAc fractions were further purified by multistep chromatographic methods, including VLC, MPLC, RPC, preparative TLC and HPLC. The structures of the isolated compounds were determined by NMR and MS measurements.

To date, two compounds from the $CHCl_3$ fraction, and six components from the EtOAc fraction, among them two new natural stereoisomer cinnamic acid derivatives have been identified. All compounds have been isolated for the first time from the plant.

References

[1] Dávid, CZ et al. *Molecules* **2021**, 26:2794. doi: 10.3390/molecules26092794
[2] Fiorentino, A et al. *Biochem Syst Ecol* **2008**, 36(9):691–698. doi: 10.1016/j.bse.2008.07.002
[3] Li L et al. *J Agric Food Chem* **2009**, 57(16):7282–7287. doi: 10.1021/jf901716j

6 – SHORT LECTURE

doi: 10.14232/syrmpnpr.2023.6

Isolation and evaluation of antimicrobial properties of non-volatile compounds from sweet marjoram

Tasneem Abu Ghazal,[1] *Katalin Veres*,[1] *Judit Hohmann* [1,2]

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Sweet marjoram (*Origanum majorana* L.) is a perennial plant that is extensively utilized in the traditional and modern therapy, and as a spice and condiment in many cuisines to add flavour to dishes. In the folk medicine, sweet marjoram was used for the treatment of respiratory or gastrointestinal disorders and urinary tract infection and also as a spasmolytic, antirheumatic, diuretic and antiasthmatic remedy. *O. majorana* is cultivated in the regions of Central Europe, Egypt and Morocco. In many studies, EO and its constituents, were investigated for antibacterial and antifungal effects on a variety of bacterial and fungal strains, including some drug-resistant clinical isolates [1,2]. Because there is no reference in the literature regarding the antibacterial and antifungal effects of the compounds of *O. majorana* outside EO and its constituents, the present study was designed to examine the antimicrobial activity of non-volatile compounds.

In the present study, the chloroform extract of sweet marjoram was subjected to a bioactivity-guided isolation process, including OCC, VLC, RPC and HPLC methods, resulting eight pure compounds. Their structures were determined by 1D and 2D NMR and HRESIMS experiments. The compounds belong to the groups of phenolic compounds and terpenoids, among them four previously undescribed ones. The isolated compounds were investigated for antibacterial, antifungal, biofilm formation inhibitory and bacterial efflux pump inhibitory activities.

References

[1] Abu Ghazal, TS, et al. *Plants* **2022**, 27;11:1432. doi: 10.3390/plants11111432
[2] Bina, F, et al. *J. Evid. Based Complementary Altern. Med.* **2017**, 22(1):175–185. doi: 10.1177/2156587216650793

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7 – SHORT LECTURE

doi: 10.14232/syrmpnpr.2023.7

Thymoquinone-protoflavone hybrids: Studies into anticancer potentials

Sara H. H. Ahmed[1], Bizhar A. Tayeb[2], Tímea Gonda[1], Gábor Girst[1], Kornél Szőri[1], Róbert Berkecz[3], István Zupkó[2], Renáta Minorics[2], Attila Hunyadi[1,4]

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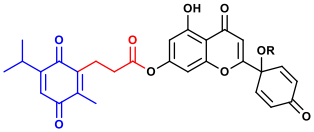
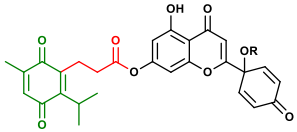
Cancer represents the second leading cause of death worldwide [1]. Among its types; breast and cervical cancer are classified among the leading causes of death among women [2], another very common and aggressive tumour type is glioblastoma multiforme [3].

Protoapigenone; a rare flavonoid from *Thelypteris torresiana* Gaud., and its semisynthetic protoflavone derivatives demonstrated promising activity against multiple cancer cell lines. Thymoquinone; a monoterpene from the seeds of *Nigella sativa* L., is another molecule described as a promising lead for cancer therapy, acting through multiple mechanisms of action [4–7].

Our work aimed to combine these two compounds into potentially antitumour hybrid molecules. Eight ester-coupled hybrids were prepared and tested on a cancer cell line panel in comparison with their fragments alone and in combination. Among the new hybrids, compound **5** showed the most promising result against HeLa, MDA-MD-231, MCF-7, and U87 cell lines with IC_{50} values of 1.06, 0.52, 1.2, and 1.16, respectively. Compound **5** was more potent than the combination of its thymoquinone and protoflavone fragments and the positive controls (17.05, 20.65, and 5.78 µM for cisplatin against MDA-MB-231, HeLa and MCF-7 cell lines, respectively, and 388.2 µM for temozolomide against U87 cell line). Kinetic studies on the ester-coupled hybrids showed their susceptibility to hydrolysis. To overcome this problem, the synthesis of more stable thymoquinone-protoflavone hybrids is currently in progress.



1; R = H, **2**; R = Me, **3**; R = Et, **4**; R = Bu



5; R = H, **6**; R = Me, **7**; R = Et, **8**; R = Bu

References

[1] WHO. Cancer. **2022**; Available from: https://www.who.int/health-topics/cancer#tab=tab_1; [2] Giaquinto, AN, et al. *CA Cancer J Clin* **2022**, 72(6):524–541. doi: 10.3322/caac.21754; [3] Taylor, OG, et al. *Front Oncol* **2019**, 9:963. doi: 10.3389/fonc.2019.00963; [4] Homayoonfal, M, et al. *Cell Mol Biol Lett* **2021**, 26:43. doi: 10.1186/s11658-021-00286-5; [5] Homayoonfal, M, et al. *Cell Mol Biol Lett* **2022**, 27:21. doi: 10.1186/s11658-022-00320-0; [6] Karim, S, et al. *Int J Mol Sci* **2022**, 23(4):2305. doi: 10.3390/ijms23042305; [7] Al-Rawashde, FA, et al. *Asian Pac J Cancer Prev* **2021**, 22(12):3959–3965. doi: 10.31557/APJCP.2021.22.12.3959

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8 – SHORT LECTURE

doi: 10.14232/syrmpnpr.2023.8

A new validated HPLC method for cannabidiol quantification in CBD-containing food supplements

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Genuine cannabis compounds and their derivatives are popular chemicals used for medical and recreative purposes. Due to diverse regulation in EU member states a lot of cannabinoid-containing products can be found mainly in foods, food supplements, and e-cigarette liquids. The loose regulation of these products results in uncontrolled quality and content.

In Hungary, the use of cannabidiol (CBD) is prohibited in food supplements. No products with CBD that are intended for human consumption should be on the Hungarian market, but medicines. Despite of regulations, the CBD-containing products are easily available online and in stores as well. Since these products are out of the focus of authorities, their quality is not known.

The aim of the present work was to set up a new and simple method for CBD content determination. An HPLC method was elaborated and validated with simple sample preparation. This analysis included twenty food supplements. CBD could be identified in all the products. In eleven products, the amount of the active substance was less than 25% of the declared amount, whereas in eight products the amount of the CBD was 19%–60% less than displayed on the label. In one product the CBD-content was 36% higher than declared.

The findings of this analysis demonstrate that almost 50% of the products did not contain the amount of CBD stated on the label. Besides being illegal, CBD content deviation might suggest that these products were not subjected to extensive quality control, thus their administration might be unsafe.

Acknowledgements
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9 – SHORT LECTURE **doi: 10.14232/syrmpnpr.2023.9**

Chemical composition and antiproliferative properties of *Peganum harmala*

Tohfa Nasibova,[1,2] Judit Hohmann,[2] Schelz Zsuzsanna,[3] István Zupkó,[3] Attila Horváth,[2] Anita Barta, [2]Hiba Faroug Muddather[3]

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Peganum harmala (Nitrariaceae) is a perennial shrub native to the Middle East, Central Asia, and Northern Africa, has a long history of traditional use for therapeutic and spiritual purposes [1]. This plant contains fatty acids [2], macro- and microelements [3], amino acids [4], and essential oil [1]. The present work deals with β-carboline alkaloid and triterpenoid content, and antiproliferative effects of *P. harmala*.

As a result of the analyses, two triterpenoids, which are new in this species and harmine, harmaline, harmol, vasicinone alkaloids were isolated from *P. harmala* roots. The quantitative parameters of harmine, harmaline and harmol were determined in the ethanolic and aqueous extracts of different organs of the plant, such as root, stem, flower, seed and capsules. At the same time, antiproliferative effects of the aqueous extracts and alkaloids were studied using HeLa, SiHa, C33-A, MCF-7, MDA-MB-231, A2780, NIH/3T3, UPCI-SCC-131 and UPCI-SCC-154 cell lines.

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10 – SHORT LECTURE **doi: 10.14232/syrmpnpr.2023.10**

Antitrypanosomal activity of natural and semi-synthetic ecdysteroids

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A neglected tropical disease, called Chagas disease, is caused by *Trypanosoma cruzi (T. cruzi*), and it affects the lives of several millions of patients, predominantly in Latin America but also in non-endemic areas. In the chronic stage of the disease, certain health issues (e.g., cardiac and gastrointestinal problems) might develop that may become life-threatening. Due to the limited therapeutic options (benznidazole, nifurtimox), there is a need for new drug candidates [1]. In this work, we screened fifty-eight natural and semi-synthetically modified ecdysteroids against *T. cruzi* epimastigotes. Antitrypanosomal activity was found for *E*- and *Z tert*-butyl oxime ether-containing ecdysteroids and ecdysteroid 2,22- and 3,22-dicinnamic esters [2,3]. Based on this, new derivatives were semi-synthesised, in which the newly identified pharmacophores were combined into new derivatives of 20-hydroxyecdysone. This led to more active compounds and provided the two best hits until now, both containing a cinnamic ester group at C-2 and an *E*- or *Z* tert-butyl oxime ether function at C-6. The compounds did not possess cytotoxic activity [4]. Our further goal is to prepare new ecdysteroid derivatives with enhanced antitrypanosomal activity, and this work is currently in progress.

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11 – SHORT LECTURE

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Bioactive components from *Epicoccum sorghinum*

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Natural products are important sources for drug discovery and development throughout the last two centuries. They demonstrate tremendous chemical and structural diversity which are hard to be matched by synthetic libraries of small molecules, and continuously inspire novel findings in biology, chemistry, industry and medicine. Fungi produce numerous prominent bioactive secondary metabolites, which have been utilized in agriculture, food, or pharmaceutical industries such as cyclosporine, lovastatin, and penicillin. Therefore, the chemical and biological exploration of fungal natural products continued to be helpful for the discovery of bioactive components [1].

Sorghum (Kaoliang) species are important crops with high economic value and several applications [2]. In Taiwan, sorghum has been used in the wine industry, and "Kinmen Kaoliang Liquor" is a well-known Asian brand. Fungal contamination is one of the major threats affecting the production of sorghum grain resulting in economic losses, and causing human and animal health problems. Several fungal species can infect sorghum grain and generate some toxic secondary metabolites. *Epicoccum sorghinum* is one of the major fungal contaminants of sorghum grains and a potent producer of mycotoxins such as tenuazonic acid (TeA) [3]. However, except for TeA, few studies focused on chemical compounds produced by this fungus. To explore the potential biological and toxic effects of *E. sorghinum*, a chemical investigation was carried out on the ethyl acetate extract of the fungus because it showed cytotoxic activity against a triple-negative breast cancer cell line, MDA-MB-231 (54.82% inhibition at 20 μg/mL). The present lecture deals with the compounds isolated by our group from *E. sorghinum*.

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12 – SHORT LECTURE

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Designing novel spiropyrazoline oxindoles to dual target p53-MDM2/X PPIs

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Natural products (NPs) and their analogues have been historically a source of inspiration to pharmacotherapy, in particular drug discovery, as they cover unexplored chemical space that is not occupied by commercially available molecule libraries. Despite their limited application in drug discovery, the identification of biologically relevant fragments is a powerful strategy in the discovery and development of new drugs. NPs-derived spirooxindoles are privileged scaffolds and several analogues are undergoing clinical trials, as anticancer candidates [1]. In our research group, we have been focusing on the development of five-membered spirooxindoles to activate p53 tumor suppressor function [2].

The p53 protein is one of the most promising targets in cancer research since it is inactivated in cancer cells. Hence, the dual inhibition of p53 interactions with MDM2 and MDMX is an efficient approach to fully activate wild type p53 function. Despite several clinical candidates have entered clinical trials as MDM2 inhibitors, they all show toxicity and develop chemoresistance, and don't inhibit p53-MDMX protein-protein interaction (PPI). Currently, no small molecule is undergoing clinical trials as dual inhibitor of p53-MDM2/X PPIs. Therefore, it is imperative to develop new chemical families to inhibit both MDM2 and MDMX to consequently fully reactivate the p53 function [3].

Here, we report our recent results in the development of spiropyrazoline oxindoles as dual inhibitors of p53-MDM2/X PPIs. Structure-based optimization of the hit compound to mimic p53 pivotal amino acids was performed while introducing chemical diversity of substituents. The most promising compounds were synthesized and evaluated in cancer cell lines harboring wild type p53 and overexpressing MDM2 and/or MDMX. The most active compounds were also evaluated in an enzyme immunoassay for heterocomplexes p53-MDM2 and p53-MDMX. Four new

spiropyrazoline oxindole derivatives inhibited both p53-MDM2/X PPIs in the nanomolar range while one was highly selective disrupting p53-MDM2 PPI. These compounds are invaluable leads for developing new dual MDM2/X inhibitors [4].

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13 – SHORT LECTURE

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Investigation of antimicrobial and antitumor properties of selected Euphorbiaceae species

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Plants of Euphorbiaceae has been traditionally used for medicinal purposes in many regions. The present study deals with thirty-two fractions prepared from 8 selected medicinal plants belonging to different genera of Euphorbiaceae based on their traditional medicinal information. The fractions were subjected to biological screening, including antimicrobial and anticancer assay. The antimicrobial activities were evaluated using standard disc diffusion method against thirteen bacteria (Gram-positive, Gram-negative) and four fungal strains, while the anticancer activity was tested on human colon adenocarcinoma cells by MTT assay. *C*hloroform and ethyl acetate fractions of *Shirakiopsis indica* demonstrated the highest antimicrobial activity against *Candida glabrata* ATCC 2001. The most sensitive strains were *Candida parapsilosis* and *C. glabrata*; at least one fraction of all species showed any activity against these fungi. Among the fractions, *n*-hexane and chloroform fractions of *Euphorbia atoto* exhibited strong antiproliferative activity against Colo 205 cell line with IC_{50} 0.24±0.06 µg/mL, and 0.23±0.04 µg/mL, respectively. Meanwhile, *Mallotus rufidulus* chloroform fraction showed the best activity against Colo 302 cell line (IC_{50} 7.10±0.60 µg/mL).

Euphorbia atoto was investigated for bioactive metabolites, and five compounds were isolated using various chromatographic techniques. Structure elucidation was performed by NMR and mass spectroscopic analysis.

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14 – SHORT LECTURE

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Antioxidant and pharmacokinetic studies on new semi-synthetic nitrogen-containing diarylheptanoids derived from curcumin

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Hydrocurcumins are the main Phase I metabolites of curcumin, the main bioactive compound of the widely known spice and traditional Asian medicine turmeric. Hydrocurcumins just like the parent compound possess a diverse pharmacological profile but, compared to curcumin, they have a much better bioavailability. Based on its pharmacokinetic properties and antioxidant activity, hexahydrocurcumin (HHC) is the most promising lead among them [1]. A big portion of approved drugs contain nitrogen, in most cases as heterocycle. Nitrones are less apparent, but due to their radical scavenger properties are also being investigated. It has been found that the introduction of nitrone moity to Trolox increases its antioxidant potential [2].

In this study, we aimed to synthesise new derivatives of hexahydrocurcumin and investigate their antioxidant capacity as well as their pharmacokinetic properties. Along the naturally occurring gingerenone A and dihydrogingerenone A, nitrogen-containing diarylheptanoids were prepared, such as nitrones, oximes and heterocyclic derivatives. The antioxidant properties of the synthesized compound were tested on DPPH and oxygen radical absorbing capacity (ORAC) assay. Solubility and membrane permeability (on PAMPA model) were also measured. Some pharmacokinetic properties were calculated *in silico.*

Diverse results were obtained. Concerning most investigated properties, there were some new compounds more potent than HHC. While on the DPPH assay none of the new derivatives showed significantly better activity compared to HHC, on the ORAC assay several had lower IC_{50} values. The best activity was found for a methyl-nitrone that had 0.18 µM IC_{50} value, about half of that of the reference compound. Generally, the nitrone and oxime derivatives showed better water solubility, while the heterocyclic compounds had better membrane permeability compared to HHC. Our results show that some of the new nitrogen-containing diarylheptanoids have comparable potential to that of hexahydrocurcumin, and are worth of further investigation.

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15 – SHORT LECTURE **doi: 10.14232/syrmpnpr.2023.15**

Phytochemical investigations of Euphorbia desmondii

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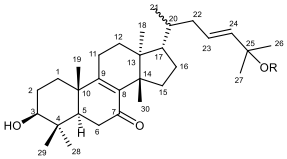
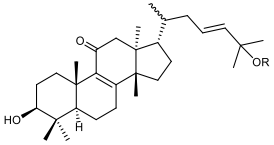
E. desmondii Keay & Milne-Redh. belongs to Euphorbiaceae family [1, 2]. It is native to West and Central African regions and has a height of up to 5.5 m [1, 3]. Together with its related species, *E. kamerunica*, they are used as village palisade to protect the communities from foreign invasion [2].

The aim of the present work was to isolate biologically active compounds from aerial parts of *E. desmondii*. Powdered plant material (1950 g) was extracted with methanol by percolation. Solvent-solvent partition of water-chloroform mixture gave the organic phase, which was subjected to open column chromatography on polyamide using step gradient elution with MeOH-water mixtures (20 – 100%), to yield five fractions. 60% MeOH fraction was subjected to a series of chromatographic techniques such as normal and reverse phase vacuum liquid chromatography, normal and reversed phase HPLC, PLC, and crystallization methods. Structures of isolated compounds were established using NMR and HRMS data.

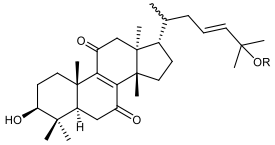
Phytochemical investigation of the 60% MeOH fraction afforded the isolation of 42 triterpenoid compounds. The triterpenoids were subdivided into three types (A, B and C) based on the presence or absence of keto group at C–7 and C–11 as shown on the figure below. 33 of the isolated compounds are new natural products.



TYPE A



TYPE B



TYPE C

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16 – SHORT LECTURE

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New adulteration pattern of *Boswellia* extracts

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Frankincense is a drug obtained from species of the genus *Boswellia*. The religious and medical application of *Boswellia* dried exudates is deeply rooted in human culture. The medical application of frankincense extract is supported by current pharmacological investigations, especially in the treatment of inflammatory related conditions, such as osteoarthritis [1]. Quality criteria of extracts applied for medical and food purposes are defined in Pharmacopoeias (USP-NF and Ph. Eur.) by setting up a minimal level of 1% for marker compounds 3-*O*-acetyl-11-keto-β-boswellic acid (AKBA), 11-keto-β-boswellic acid (KBA), respectively. Frankincense extract in food supplements is mostly characterised by its total boswellic acid content determined by acid-base titration. The availability of *Boswellia* extract is limited due to specific ecological niche of species and increasing demand for agricultural land. These conditions might result in adulteration of *Boswellia* containing products. The American Botanical Council has already published a Laboratory Guidance Document in 2022. The guidance provides botanical, genetic and chemical methods for characterisation of *B. serrata* resins and extracts.

The aim of our work was to analyse *Boswellia* extracts produced for industrial purposes and used as ingredients in food supplements. Fourteen extracts were purchased from China and Europe. USP *Boswellia* extract was used as standard. The AKBA and KBA content was determined by HPLC (Ph. Eur. 10). The total boswellic acid content was determined by acid-base titration. The carboxylic acid content of the extracts was screened and quantified using new targeted UHPLC-HRMS method.

The total boswellic acid content of the extracts was determined by acid-base titration. Although, the 67–95% total boswellic acid content was in accordance with amounts declared on the label; the HPLC measurement confirmed minimum 1% AKBA and ABA contents in two products. Targeted UHPLC-HRMS method was used to screen and quantify possible adulterants (malic acid, benzoic acid, oxalic acid, tartaric acid, citric acid) responsible for the acidic content of the extracts. Citric acid was detected in 10 out of 14 extracts in which the citric acid content was between 6% and 11%.

Based on our measurements we found that high ratio of frankincense extracts did not meet the criteria set up in Pharmacopoeias. Our results might suggest a new adulteration pattern for *Boswellia* extract: which is the addition of citric acid to extracts to set up the acidic content of the final product.

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