UNIVERZITA KARLOVA
Farmaceutická fakulta
v Hradci Králové

EFSA-CDN

Workshop

Recent Progress in Pharmacognosy and Phytochemistry

23. - 24.6. 2022

Charles University, Faculty of Pharmacy
Garden of Medicinal plants
Hradec Králové, Czech Republic

BOOK OF ABSTRACTS

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MINISTRY OF EDUCATION,
YOUTH AND SPORTS

Workshop: Recent progress in Pharmacognosy and Phytochemistry

June 24 - 25, 2022, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic

Thursday, 23 June 2022

8:30-9:15	**Registration**
9:15-9:30	Opening of the Workshop by Dean of the Faculty and organizers prof. Lucie Cahlíková, prof. Karel Šmejkal
9:30-9:45	Introduction – Presentation research infrastructure efficiency and safety improvement of current drugs and nutraceuticals: advanced methods – new challenges (EFSA–CDN)
9:45-11:20	**Lectures (Chair prof. Karel Šmejkal)**
9:45-10:20	HPLC-Based approaches for the analysis of naturally occurring cannabinoids ***(prof. Satyajit Sarker)***
10:20-10:55	Phytochemical analysis of Cannabis sativa L. products on the market: what do we have on our hands today? ***(prof. Stefano Dall'Acqua)***
10:55-11:20	Chromatographic methods for the analysis of nutraceuticals based on plant extracts and their quality control in the Czech market ***(prof. Dalibor Šatínský)***
11:20-11:50	**Coffee break**
11:50-13:00	**Lectures and short lectures (Chair prof. Dalibor Šatínský)**
11:50 -12:25	Natural products as drug leads for neutrophilic inflammatory diseases – on-line lecture ***(prof. Tsong-Long Hwang)***
12:25-12:40	Isolation and identification of geranylated flavonoids from Paulownia tomentosa Steud. fruit and their anti-inflammatory activities ***(Dr. Lenka Molčanová)***
12:45-13:00	Selective inhibitory effects of tropical plant extracts and compounds on diarrheagenic bacteria and intestinal cancer cells ***(Dr. Tomáš Kudera)***
13:00-14:00	**Lunch break**
14:00-16:05	**Lectures (Chair Prof. Stefano Dall'Acqua)**
14:00-14:35	Challenges of phenolic compounds analysis in herbal drugs and preparations ***(prof. Franz Bucar)***
14:35-15:00	Recent advances in antimicrobial susceptibility testing of plant-derived volatile agents in vapour phase ***(prof. Ladislav Kokoška)***
15:00-15:20	Progress and trends in potential utilization of natural compounds as drugs - prenylated phenolics ***(prof. Karel Šmejkal)***
15:20-15:35	Citrus bergamia, isolation of compounds with LDLR and PCSK9 modulation properties, a food source of hypocholesterolemic agents ***(Dr. Stefania Sut)***
15:35-15:50	Study of cytostatic, cytotoxic and proapoptotic activity of Amaryllidaceae alkaloid montanine ***(Dr. Radim Havelek)***
15:50-16:05	Development and production of LC columns in the Chromservis Company ***(Ing. Jana Volková)***
16:05-19:00	**Coffee, small refreshment accompanied by practical demonstration of HPTLC analysis of secondary metabolites and 3D printing**
16:05-19:00	**Poster section**
17:00-19:00	Excursion to the Garden of Medicinal plants and Greenhouse ***(English and Czech group)***
19:00	Closing of the first day of Workshop

Workshop: Recent progress in Pharmacognosy and Phytochemistry

June 24 - 25, 2022, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic

Friday, 24 June 2022

9:00-10:35 **Lectures and short lectures (Chair Prof. Vincenza Andrisano)**

9:00-9:35 Large scale isolation and semi-synthesis of bioactive compounds from Olea europaea ***(prof. Leandros Scaltsounis)***

9:35-10:00 Isoquinoline alkaloids and their derivatives as a new class of antimycobacterial drugs ***(prof. Lucie Cahlíková)***

10:00-10:20 Ambelline derivatives as selective inhibitors of liver stage malaria in vitro ***(Dr. Kateřina Hradiská Breiterová)***

10:20-10:35 Ficus species: comparison of phytochemical profile and isolation of dominant compounds ***(Dr. Milan Malaník)***

10:35-11:00 **Coffee break**

11:00-12:50 **Lectures and short lectures (Chair prof. Lucie Cahlíková)**

11:00-11:35 Integrated analytical methodologies for Alzheimer's disease drug discovery ***(prof. Vincenza Andrisano)***

11:35-11:55 Alkaloids of norbelladine type from Narcissus pseudonarcissus cv. Carlton as inspiration for development of highly selective butyrylcholinesterase inhibitors ***(Dr. Abdullah al Mamun)***

11:55-12:15 Biological study of indole alkaloid from Vinca minor L. with anti-alzheimer's potential ***(Dr. Rudolf Vrabec)***

12:15-12:35 Isolation of alkaloids from Geissospermum vellosii and their biological activity ***(Dr. Marcela Šafratová)***

12:35-12:50 Bioguided analysis of Papaver rhoeas as a source of potential biologically active alkaloids ***(Dr. Jaroslav Jenčo)***

12:50-14:00 **Lunch Break**

14:00-15:35 **Lectures and short lectures (Chair prof. Franz Bucar)**

14:00-14:35 Gut microbiota need to be considered for explaining the activity of herbal medicine ***(prof. Rudolf Bauer)***

14:35-14:55 Santorini's main food crops agricultural side-products with bioactivity potential: “Fava” and the “Santorini cherry tomato” ***(Dr. Konstantina Vougogiannopoulou)***

14:55-15:15 Alkaloids isolated from Croton linearis Jacq. leaves: their antiprotozoal potentiality ***(Dr. Jesús García Díaz)***

15:15-15:35 Discovery of new structural features in natural products ***(Dr. Jana Křoustková)***

15:35-17:30 **Coffee, small refreshment accompanied by practical demonstration of HPTLC analysis of secondary metabolites and 3D printing**

17:30-22:00 **Workshop's discussion evening**

Program changes reserved.

Overview of poster presentations:

Determination of in vitro growth-inhibitory effect of essential oils from Indian medicinal plants against respiratory tract pathogens using new broth macrodilution volatilization method
Aishwarya Chaure

Evaluation of anti-bacterial activity of semi-synthetic alkaloid derivatives from medicinal plants of the Amaryllidaceae family
Adéla Diepoltová

Susceptibility of intestinal bacteria involved in colorectal cancer pathogenesis to phytochemicals and their synthetic analogs in vitro
Barbora Fišerová

Liquid matrix volatilization methods for susceptibility testing of respiratory bacteria to volatile agents in vapour phase
Markéta Houdková

Investigation of the phytochemical and cancer chemopreventive potential of Claoxylon longifolium leaves growing in southern Thailand
Chuanchom Khuniad

Discovery of anti-coronavirus bisbenzylisoquinoline alkaloids
Michal Kořínek

Discovery of a series, novel oleocanthal - based compounds as potent anticancer agents
Ioannis K. Kostakis

Investigation of selected biological activities of montanine-type alkaloids and their derivatives
Negar Maafi

Advanced chromatographic approaches in phenolic compounds profiling in archive Tokaj wines
Pavlína Moravcová

Celecoxib potentiates the in vitro anti-staphylococcal effect of oxacillin
Onyedika Emmanuel Okpala

Amaryllidaceae alkaloids as inspiration for the development of highly selective butyrylcholinesterase inhibitors: the relationship between structure, effect, and toxicity
Filip Pidaný

Nature mimicking drug design of spiro-2-oxindoles with 4H-pyran and chromen cores
Ruslan Redkin

Semi-synthetic derivatives of Amaryllidaceae alkaloid ambelline as potential lead structures for drug development
Aneta Ritomská

Exploitation of Greek medical and aromatic plants for the production of edible products
Konstantina Vougogiannopoulou

An innovative and high nutritional value cheese product, enriched with bioactive extracts of olive by-products
Konstantina Vougogiannopoulou

Production of meat products enriched with olive biophenols
Konstantina Vougogiannopoulou

Alkaloids of Dicranostigma franchetianum (Papaveraceae) and their antimycobacterial activity
Viriyanata Wijaya

HPLC-Based approaches for the analysis of naturally occurring cannabinoids

Satyajit D Sarker [1], Lutfun Nahar[1,2]

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Cannabis sativa L., a native herbaceous medicinal plant from Eastern and Central Asia, belongs to the family Cannabaceae. This plant is the main source of naturally occurring cannabinoids, which are often referred to as 'phytocannabinoids'. There are >100 phytocannabinoids reported to date. Among them, Δ^9-tetrahydrocannabinol (Δ^9-THC or THC) and cannabidiol (CBD) are two major cannabinoids. Δ^9-THC is the main contributor to the psychoactive property of *C. sativa*, but CBD offers antipsychoactive property. In addition to *C. sativa*, a few other plant species, e.g., *Acmella oleracea*, *Echinacea angustifolia*, *E. purpurea*, *Helichrysum umbraculigerum* and *Radula marginata*, also biosynthesize certain cannabinoids. Cannabinoids are one of the most-investigated groups of bioactive phytochemicals. These compounds bind to cannabinoid receptors (endocannabinoid system). Various analytical methods, e.g., GC- and HPLC-based techniques, are used for their analysis. However, HPLC (including UPLC or UHPLC) has emerged as the most popular analytical tool for the detection and quantification of naturally occurring cannabinoids in various matrices. Simple HPLC-UV or HPLC-PDA based methods are the most common in the analysis of cannabinoids, but HPLC-MS, HPLC-MS/MS, UPLC (or UHPLC)-PDA, UPLC (or UHPLC)-MS and UPLC (or UHPLC)-MS/MS methods are also now used routinely. In fact, MS detectors hyphenated with an HPLC or UPLC (or UHPLC) provide valuable data for precise identification of cannabinoids. This talk will present a critical overview of the relevant literature on the use of various HPLC-based analytical methods for the analysis of naturally occurring cannabinoids.

Phytochemical a**nalysis of *Cannabis sativa* L. products on the market: What do we have on our hands today?**

Stefania Sut[1], Stefano Dall'Acqua[1], Filippo Maggi[2], Riccardo Petrelli[2]

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Hemp (*Cannabis sativa* L.) is a versatile crop that has recently been considered a good opportunity for agriculture. Its cultivation is allowed for certified varieties producing δ-9-tetrahydrocannabinol (THC) below 0.2% of the dried weight of the plant material. The main industrial applications of hemp are production of fibre, and oil, furthermore, in some countries, hemp inflorescences are used for the production of food supplements and for extraction of non-psychotropic cannabinoids. A large part of consumers also use inflorescence to smoke or prepare herbal teas and foods. Large interest is present in cannabinoids, and for example, cannabidiol (CBD) has been registered as a drug in the EU (Epidiolex) for the treatment of some epilepsy forms. Many research groups are studying cannabidiol and other "minor" cannabinoids for possible applications in healthcare, medicine, as well as food supplements and cosmetics. In this regard, improving the knowledge of different hemp varieties or cultivars to obtain extracts enriched in specific phytoconstituents may be advantageous. Furthermore, new products with high concentrations of non-psychotropic cannabinoids are available on the market.

In this work, the specific phytochemical analysis of cannabis was discussed with an orthogonal approach of LC-DAD-MS, GC-MS, and NMR to evaluate phytoconstituents in different varieties. Quali-quantitative analysis of terpenes was obtained by GC-MS analysis and is an important trait of cannabis to obtain a chemical fingerprint. Flavonoids and cannabinoids were studied by LC-DAD-MS approach, observing differences in the various analyzed samples.

An alternative extraction procedure for the development of new cannabis products was also discussed. Distillation was performed to explore the opportunity to obtain three different products, namely essential oil, residual water and "deterpenated plant material" (after distillation). The chemical composition of the three materials is described here. During the study, preparative HPLC was performed to isolate a compound that presented the same molecular weight of CBD and THC but eluted at different retention times. This compound was identified as "abnormal cannabidiol" a compound previously reported only by synthetic approaches, and the structure elucidation was performed by 1D and 2D NMR experiments, optical rotation power and HR-MS. The overall results allow to have a deep investigation on the chemical constituents of the most diffused cannabis products actually present on the market.

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Chromatographic methods for the analysis of nutraceuticals based on plant extracts and their quality control in the Czech market

Dalibor Šatínský[1], Jakub Fibigr[1], Pavlína Moravcová[1], Michaela Majorová[1]

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Nutraceuticals include a wide range of products that are designed to be taken because of their added nutrients and presumed health benefits. Global food supplement sales are experiencing rapid growth and supplements that based on plant extracts are among the most popular. The meteoric rise in sales coupled with the general lack of a commitment to pass effective regulation and quality control make this market more vulnerable to dishonest producers, increase the likelihood that supplements containing adulterants or low content of declared biologically active compounds are sold on the market, and a greater prevalence of safety and quality issues [1]. In this contribution, we will present an overview of various examples of chromatographic analyses of extracts with anthocyanins, chlorogenic acids, berberine, indole-3-carbinol, resveratrol, phytosterols, silymarin, and other nutraceuticals based on plant extracts and their quality control in the Czech market.

1. Fibigr J, Šatínský D, Solich P (2018) *Anal. Chim. Acta* 1036: 1-15

Natural products as drug leads for neutrophilic inflammatory diseases

Tsong-Long Hwang[1]

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Neutrophils are the most abundant leukocytes in humans and act as the first line of defence in innate immune response. However, an overwhelming activation of neutrophils also plays a critical pathogenic role in inflammatory diseases and autoimmune disorders, such as acute respiratory distress syndrome (ARDS) and psoriasis. The reactive oxygen species, proteases, and neutrophil extracellular traps released by activated neutrophils can damage cells and cause immune-inflammatory disorders. The abundant presence of neutrophils in the lungs and psoriatic skin lesions serves as a histopathological hallmark of ARDS and psoriasis. Neutrophil counts are significantly correlated with disease severity of ARDS and psoriasis. Hence, neutrophils can not only be used as pathogenic markers but also as candidate drug targets. A better understanding of the precise regulation of neutrophils in human health and disease is fundamental for designing novel therapies. The pharmacological approaches to discover drug lead compounds with specific targets for neutrophilic inflammation will be discussed

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Isolation and identification of geranylated flavonoids from *Paulownia tomentosa* Steud. fruit and their anti-inflammatory activities

Lenka Molčanová[1], Tereza Štaffová[1], Klára Sušovská[1], Miriama Dupľáková[1], Jakub Treml[2], Veronika Leláková[2,3], Petr Maršík[4], Karel Šmejkal[1]

[1]Department of Natural Drugs; [2]Department of Molecular Pharmacy, Faculty of Pharmacy, Masaryk University, Palackého tř. 1946/1, CZ-61200 Brno, Czech *Republic;* [3]Veterinary Research Institute, Hudcova 296/70, CZ-62100 Brno, Czech *Republic;* [4]*Department of Food Quality and Safety, Czech University of Life Sciences Prague, Kamýcká 129, CZ-16500 Prague 6–Suchdol, Czech Republic.*

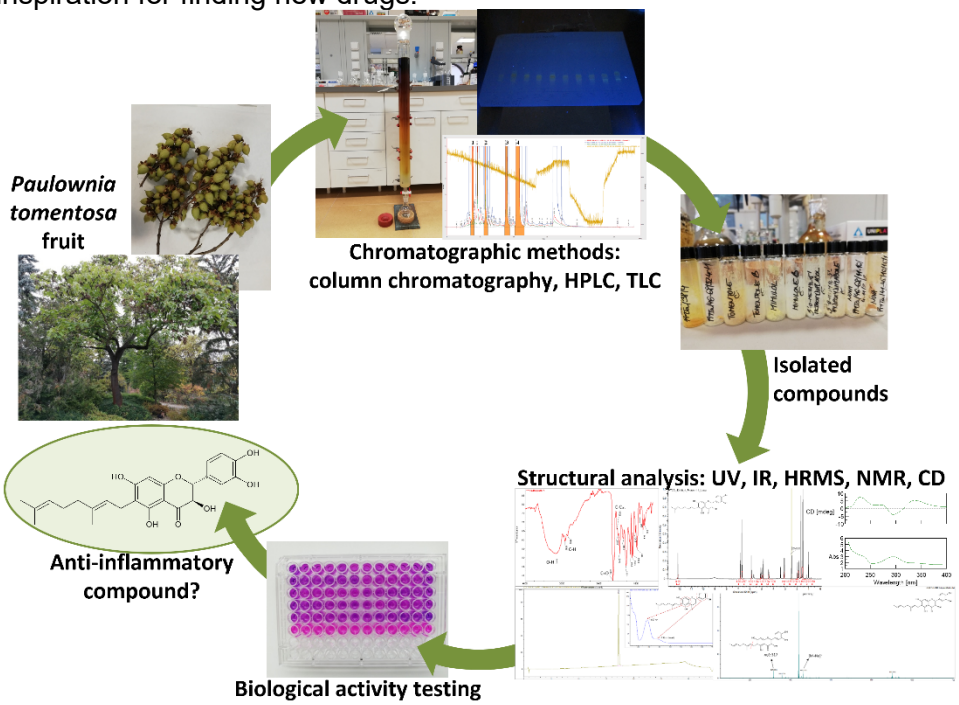
Introduction: *Paulownia tomentosa* Steud. (Paulowniaceae), a traditional Chinese medicinal plant, had been used for many centuries as component of remedies for many illnesses. It is a rich source of secondary metabolites, mainly geranylated flavonoids, which are currently studied for their promising biological activities, such as anti-inflammatory, antioxidant, antimicrobial, or cytotoxic.

The aim of work: Our work was focused on the isolation of compounds from chloroform portion of the ethanolic extract of *P. tomentosa* fruit.

The methods used: Compounds were isolated using different chromatographic methods, such as column chromatography, high-performance liquid chromatography, and thin layer chromatography. The structures were elucidated using ultraviolet and infrared spectroscopy, high-resolution mass spectrometry, and 1D and 2D nuclear magnetic resonance spectroscopy. The absolute configurations were determined using circular dichroism spectroscopy.

The major results: A series of geranylated flavanones and flavones, and other compounds (phenolics, a triterpene, and acylglycerols) were isolated and eighteen of these compounds were obtained from a natural source for the first time. Selected compounds were evaluated for cytotoxicity, anti-inflammatory, and antioxidant activities. Eight compounds were more active than the standard anti-inflammatory drug prednisone in the assay for inhibition of the NF-κB signaling pathway, therefore they may have the potential for treating the inflammation.

Conclusion: We believe that natural compounds, such as geranylated flavonoid from *P. tomentosa*, can still influence the modern healthcare and can be interesting source of inspiration for finding new drugs.



Selective Inhibitory Effects of Tropical Plant Extracts and Compounds on Diarrheagenic Bacteria and Intestinal Cancer Cells

Tomas Kudera[1], Ivo Doskocil[2], Barbora Fiserova[1], Marie Korytakova[1], Hana Salmonova[2], Ladislav Kokoska[1].

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Bacterial diarrhoea remains a global health problem, especially in developing tropical countries [1]. Moreover, dysbiosis caused by diarrheagenic bacteria and inappropriate antimicrobial treatment has been associated with the increased risk of intestinal carcinogenesis [2]. In many tropical countries, there is still a rich tradition of the use of local plants for the treatment of gastrointestinal disorders, whereas several phytochemicals have already been employed in the development of internationally available pharmaceuticals, dietary supplements, and herbal medicines used for intestinal ailments [3]. However, many of these plant-derived products have not been systematically studied for their selective biological activities against intestinal bacteria and cells. Therefore, *in vitro* inhibitory activities of 35 ethanolic extracts derived from 32 Cambodian and Philippine antidiarrheal medicinal plants together with 10 phytochemicals and their synthetic analogues were determined by broth microdilution method against 12 diarrheagenic bacteria [4]. Furthermore, their toxicity to intestinal cancer cells (Caco-2 and HT-29) using thiazolyl blue tetrazolium bromide cytotoxicity assay and safety to six beneficial intestinal bacteria (bifidobacteria and lactobacilli) and intestinal normal cells (FHs 74 Int) were determined [5]. Six antibiotics and one anticancer drug commonly employed in the treatment of the respective intestinal infections and cancers were used as positive controls. The extracts of *Ancistrocladus tectorius*, *Artocarpus blancoi*, and *Pentacme siamensis* produced significant growth-inhibitory effects against diarrheagenic bacteria at the concentrations nontoxic to intestinal normal cells. Moreover, the extract of *P. siamensis* was relatively safe to beneficial bacteria. One phytochemical and two phytochemical synthetic analogues, namely chloroxine, nitroxoline, and zinc pyrithione, exhibited selective antibacterial actions with lesser effects on beneficial bacteria. However, their antimicrobially active concentrations were toxic to intestinal normal cells. Plant extracts of *A. blancoi*, *Ehretia microphylla*, *Lagerstroemia cochinchinensis*, *Melastoma saigonense*, and *P. siamensis* as well as phytochemicals 8-hydroxyquinoline and sanguinarine produced selective antiproliferative activities against intestinal cancer cells. The results suggest that certain Cambodian and Philippine plants are promising materials for further research regarding the isolation and identification of their active constituents that might be utilized in the development of new selective antibacterial and antiproliferative agents for the treatment of infectious diarrhoea and associated intestinal cancer diseases. From that perspective, the findings also indicate that 8-hydroxyquinoline alkaloids and metal-pyridine derivative complexes are chemical structures derived from plants with such a promising bioactive properties.

Acknowledgements: This research was financially supported by the Czech University of Life Sciences Prague [projects IGA.20223102] and METROFOOD-CZ research infrastructure project [MEYS Grant No: LM2018100], including access to its facilities.

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Challenges of phenolic compounds analysis in herbal drugs and preparations

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Phenolic compounds are one of the most widely occurring secondary plant constituents, and are rich in herbal drugs, vegetable, and fruit diets as well as beverages of plant origin.

Quality control of these products largely involves analytics of phenolics, including flavonoids, coumarins, cinnamic acids, tannins, among others. Due to plethora of related structures as well as the complex matrix in which these compounds are embedded, one has to face a number of challenges when analysing these compounds, like selective extraction and isolation, artefact formation or isomerization.

This lecture will present a survey of our work on plant phenolic compounds. Aside from different solubilities, glycoside hydrolysis during extraction has to be considered as could be seen for stilbenes in *Reynoutria japonica* rhizome preparations [1]. In case of analysis of ethanolic extracts from Lavandulae flos pre-and post-distillation material, salvianolic acid A could be confirmed as artefact arising from hydro-distillation [2]. A combination of HPLC, GC-MS and NMR analyses was necessary for elucidation of isomeric hydroxyketones in seeds of *Aframomum melegueta* [3]. LC-ESI-MS analysis provides valuable information in case of C-glycosylflavones which is exemplified by analysis of extracts from heather and spelt. In addition, in vivo metabolism has to be taken into account, such as flavonol-O-glycosides from roseroot which were rapidly catabolised by colonic microbiota [4].

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2. Dostal A (2021) *Master thesis,* University of Graz.
3. Gröblacher B, Maier V, Kunert O, Bucar F (2012) *J Nat Prod* 75: 1393-1399.
4. Turek I (2018) PhD Thesis, University of Graz

Recent advances in antimicrobial susceptibility testing of plant-derived volatile agents in vapor phase

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Volatile plant compounds are important as pharmaceuticals, food flavors, and agrochemicals. However, antimicrobial applications based on their volatility have not been fully developed yet. The lack of appropriate methods for evaluation their activity in vapor phase is one of main limiting factors in this area. Due to the high volatility and hydrophobicity of plant volatiles, conventional laboratory methods of antimicrobial susceptibility testing face specific problems in the research of volatiles, including affection of the results of standard biological assays [1,2]. In the past decades, several methods have been developed with aim to study the potential of vapors of volatile agents to inhibit growth of pathogenic microorganisms. Methods based on the solid matrix volatilization principle (e.g., disc volatilization assay) are simple to carry out but they also have many disadvantages, such as high consumption of material and labor [3]. Recently, both micro- and macro-dilution volatilization assays based on the liquid matrix volatilization principle have been developed for the evaluation of the antimicrobial potential of volatile agents in vapor phase in our laboratory [4,5]. These assays are suitable for simple and rapid susceptibility testing of microbial pathogens to volatiles in the liquid and the vapor phase and allow a cost- and labour-effective high-throughput screening of volatile agents using commercially available microtubes or microplates. Both methods have been validated for research and development of applications in the areas of agricultural (e.g., controlled-atmosphere agents and fumigants), food (e.g., active, or smart packaging agents and materials) and pharmaceutical (e.g., inhalation drugs) products.

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5. Houdkova M, Chaure A, Doskocil I, Havlik J, Kokoska L (2021) *Molecules* 26: 4179.

Progress and trends in potential utilization of natural compounds as drugs - prenylated phenolics

Karel Šmejkal[1], Martina Fojtíková[2], Josef Mašek[2], Milan Malaník[1], Lenka Molčanová[1], Jakub Treml[3]

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Natural substances often have a pleiotropic effect and can affect several cellular processes in parallel. They can have parallel anti-inflammatory and antibacterial effects, together with the current antiviral effect. Their mechanism of action is complex. However, the problem of natural substances is often their limited solubility and consequently also problematic bioavailability [1]. Series of prenylated phenols were isolated from Paulowniaceae, Moraceae, and Euphorbiaceae plants [2-5]. As part of the lecture, we will introduce the isolation and identification of prenylated phenols with potential antiviral and anti-inflammatory effects, we will describe their bioactivity, their formulations to increase solubility, and will describe the possibilities of their further development. We described the effects of phenolics in vitro in cellular or biochemical systems on the production and release of inflammation-related cytokines; their effects on the inhibition of cyclooxygenases and lipoxygenases, and also some in vivo experiments confirming activity. At the end, an improvement of solubility by incorporating of tested substances into liposomes was presented.

Acknowledgements: The work was supported by Czech Science foundation, project no. 21-38204L Complexes of selected transition metals with plant-derived compounds with anti-NF-kappa B and pro-PPAR dual activities.

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Citrus bergamia, isolation of compounds with LDLR and PCSK9 modulation properties, a food source of hypocholesterolemic agents.

Stefania Sut[1], Irene Ferrarese[1], Maria Giovanna Lupo[1], Ilaria Rossi[1], Giovanni Panighel[1], Nicola Ferri[1], Stefano Dall'Acqua[1]

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Bergamot (*Citrus bergamia*) is a fruit native to southern Italy with traditional uses for fever, sore throat, mouth, skin, respiratory and urinary system infections [1-2]. Fruit extracts can improve immune response, cardiovascular function [1] and inflammatory bowel disease [3]. As other citrus, bergamot contains an essential oil and many non-volatile constituents. These latter are mostly flavonoids, coumarins, and limonoids. Some derivatives have been considered as HMG-CoA reductase inhibitors due to their peculiar chemical structure and are claimed as active compounds for the cholesterol lowering properties of the extracts [4]. A recent review on clinical trials suggests that bergamot polyphenol fraction can lower low-density lipoprotein-cholesterol (LDL-C) and total cholesterol levels, indicating a potential interest in this fruit as a source of hypocholesterolemic agents [1]. This opportunity is of great interest because bergamot is a fruit and can be introduced in the diet as a hypocholesterolemic functional food. Up to now the importance of bergamot constituents as hypocholesterolemic agents is still to be fully elucidated, and more research is needed to lighten possible molecular targets and mode of actions useful to assess doses and to establish its safety. The aim of this work was to study the effects of isolated constituents from *C. bergamia* on key players of cholesterol homeostasis. For this reason, extract, and isolated compounds were tested in cultured human hepatoma cell line Huh7 for their potential modulating properties of both LDL receptor (LDLR) and proprotein convertase subtilisin/kexin type 9 (PCSK9) expression.

The phytochemical composition of *C.bergamia* extract was assessed by LC-DAD-MS, and the main constituents were isolated by semipreparative HPLC and their structure were elucidated using MS, 1D and 2D NMR experiments. The dried extract contains mostly neohesperidin (5.25%), and the total flavonoid content was 25%. Thirteen different compounds were tested, and a significant effect was observed for flavonoids, especially melitidin, narirutin and neohesperidin that were able to induce the expression of both LDLR and PCSK9 in a similar manner of simvastatin. These results allowed us to ascribe at least in part the claimed bioactivity of *C.bergamia* to some of its flavonoids. Thus, the identification of the active compound of bergamot represents one linkage of the molecular targets, LDLR and PCSK9, and the hypocholesterolemic effect of the plant.

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Study of Cytostatic, Cytotoxic and Proapoptotic Activity of Amaryllidaceae Alkaloid Montanine

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Introduction: Isoquinoline alkaloid constituents of Amaryllidaceae plants gained importance in the last decades due to the growing interest in their potential for medicinal use. Montanine-type subclass constitute a less abundant and limited group of alkaloids within the Amaryllidaceae. The representative structure of this group is montanine, which has demonstrated notable degree of *in vitro* cytotoxicity on cancer cells in previous studies [1, 2]. However, many questions remain unanswered, and the need to deepen mechanistic understanding of montanine-type alkaloids activity becomes evident. Furthermore, montanine may be considered as a valuable starting compound for the semisynthesis and structure-activity relationship studies.

Aim of work: Thus, in this work, we have studied *in vitro* antiproliferative, cytotoxic and proapoptotic activity of montanine and montanine analogues. We reisolated montanine alkaloid from *Hippeastrum taxa* [3] in an amount sufficient for semisynthetic transformations. Then, we assayed the antiproliferative activities of montanine and a series of fifteen semisynthetic montanine derivatives against a panel of 8 cell lines from different tumor types. In the next step, the anticancer potential, and mechanisms of montanine were studied.

Methods: Montanine or its semisynthetic derivates were initially investigated by tetrazolium salt proliferation assay. To reveal further insights into the causes of montanine bioactivity, we conducted Trypan blue exclusion assay, xCELLigence system measurements, Annexin V-apoptosis assay, mitochondrial membrane potential assay, flow cytometry cell cycle analysis, caspase -3/-7, -8 and -9-activation assay, Western blotting and immunofluorescence staining for DNA double-strand breaks marker γH2AX.

Results: Among montanine and its analogues tested, montanine, derivative 12 and 14 showed the highest cytostatic activity in the initial single-dose screening. However, since the native montanine has exhibited evidence of the greatest antiproliferative activity among all alkaloidal compounds tested, we evaluated the cytotoxicity and cell death mechanisms related to montanine. Montanine exhibited considerable cytostatic effect by causing G1-phase accumulation with a concomitant decrease in the percentage of S-phase cells, as shown by the downregulation of cdc25A and the upregulation of p27 and phosphorylated Chk1 Ser345.

Moreover, our results revealed that montanine triggered MOLT-4 cells apoptosis with marked decrease in mitochondrial membrane potential. This apoptosis-mediated cytotoxicity, however, was not accompanied by DNA double-strand breaks induction.

Discussion: Our findings provide new insights about the mechanisms of cytostatic, cytotoxic or proapoptotic effects of montanine alkaloid in lung adenocarcinoma A549 and leukemic MOLT-4 cancer cells models.

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Large scale isolation and semi-synthesis of bioactive compounds from *Olea europaea*

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Extra virgin olive oil, the main product of *Olea europaea* is a well-known source of polyphenols which has attracted scientific attention in recent years because of its biological activities and its attribution in many aspects of human health. Although olive oil primarily consists of oleic acid (up to 80 %) and other fatty acids, some minor phenolic compounds, comprising the 1-2 % of the total content, are generally considered to be responsible for the various health benefits of olive oil. The most characteristic compounds in this group are hydroxytyrosol, tyrosol, the two glucosylated seco-iridoids Oleuropein and Ligstroside and the two corresponding decarboxymethylated aglycons Oleacein and Oleocanthal.

Oleocanthal and oleacein are characterized by a dialdehyde core, connected by an ester moiety with tyrosol and hydroxytyrosol respectively. These two compounds, and especially oleocanthal, have been identified as the agents responsible for the pungency of extra virgin oil. Oleocanthal's recent discovery as COX inhibitor, with similar effect to that of ibuprofen, has dramatically increased its interest both for the study of biological properties but also for the development of new non-steroidal anti-inflammatory drugs (NSAIDS) based on its structure. Additionally, according to many data, oleocanthal demonstrated promising anticancer and neuroprotective activities with no toxic effects. Regarding oleacein, several studies suggests that this compound possesses antimicrobial, anti-proliferative, anti-inflammatory, cardio protective and antioxidant activity by modulating the Nrf2 pathway. Thus, there is a high demand for these two dialdehydes, in order to initiate more in-depth biological studies, however, their low content in olive oil prevents large scale isolation due to apparent high-cost efficiency of the process.

The great interest of these two high-added value natural compounds triggered the development of various synthetic approaches, all involving multi-step total synthesis, with low total yields.

Thus, there is still a need for an improved process for the production of compounds such as oleocanthal or oleacein and their analogues, which does not have the drawbacks of the processes of the prior art.

Our work is focused on finding alternative strategies to manage the residues of olive oil industry, following two axes. Firstly, the development of liquid/ liquid or solid/liquid extraction followed by partition chromatography techniques for the isolation of these compounds in multi gram scale. Secondly the use of some of these compounds such as oleoside, EDA as starting material for the hemi-synthesis of oleacein and oleocanthal as well as new analogues and their evaluation as potential antitumor agents.

Isoquinoline alkaloids and their derivatives as a new class of antimycobacterial drugs

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Tuberculosis (TB) is a widespread infectious disease caused by *Mycobacterium tuberculosis* (Mtb). According to the Global Tuberculosis Report 2021, issued by the World Health Organization (WHO), the latent form of Mtb has infected about a quarter of the world's population, but only a small part (5–10%) will develop this bacterial disease [1]. The increasing incidence of multidrug-resistant (MDR), and extensively drug-resistant (XDR) strains has created a need for new antiTB agents with new chemical scaffolds to combat the disease. Thus, the key question is: how to search for new antiTB and where to look for them? One of the possibilities is to search among natural products.

In order to search for new antiTB drug, we screened isolated alkaloids in our lab within previous phytochemical studies against Mtb H37Ra and four other mycobacterial strains (*M. aurum, M. avium, M. kansasii,* and *M. smegmatis*). In order to expand portfolio of tested compounds several series of semisynthetic derivatives of selected alkaloids (e.g., berberine, galanthamine, haemanthamine and others) were developed and tested. Derivatization of berberine in position C-9 was connected with a significant increase in antimycobacterial activity against all tested strains (MICs 0.39–7.81 μg/mL). Similarly, derivatization of galanthamine in position C-6 was connected with increase of activity against Mtb H37Ra (MICs 1.56–15.625 μg/mL).

The most active compounds were also evaluated for their *in vitro* hepatotoxicity on a hepatocellular carcinoma cell line (HepG2), exerting lower IC50 values than their MIC values, further corroborating their potential as potent and safe antimycobacterial agents.

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Ambelline derivatives as selective inhibitors of liver stage malaria *in vitro*

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Malaria is a severe parasitic protozoal infection of global importance caused by unicellular protozoa from *Plasmodium* genus. According to the WHO, around 40% of the global population live in threatened areas. The most important factors related to malaria treatment are prevention, early diagnosis, appropriate and effective medication. That brings us to a serious problem – development of drug resistance in *Plasmodium* spp. which is the crucial incentive for new potential drugs research [1]. 49% of all drugs and 67% of small-molecule drugs approved between 1981-2019 are in certain connection with natural compounds [2]. This proves, that natural compounds and their derivatives are still an important source where new potential drugs can be sought. In the treatment of malaria, it was quinine at first and most recently it is sesquiterpene lactone artemisinine and its semisynthetic derivatives.

One of the interesting groups of bioactive compounds are alkaloids of Amaryllidaceae family which belongs to the most important alkaloid families with almost 600 of various Amaryllidaceae alkaloids (AmA) isolated and structurally described, so far. In our study, over 70 AmA and their semisynthetic derivatives were screened *in vitro* due to their activity against the liver stage malaria caused by *Plasmodium berghei* sporozoites. The most promising activities against the *P. berghei* liver stage were shown by aromatic derivatives of ambelline. Compound LC-104 with IC_{50} = 0.048 ± 0.014 μM was deemed to be the most active one (primaquine IC_{50} = 5.74 ± 0.86 μM). Considering the inactivity against the blood stage of *P. falciparum*, this compound seems to be an interesting selective inhibitor of malaria liver stage.

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Ficus species: comparison of phytochemical profile and isolation of dominant compounds

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The genus *Ficus* is composed of around 900 species, including trees, shrubs, and lianas. Belonging to the family Moraceae suggests that *Ficus* species should be a rich source of prenylated flavonoids that are known for their anti-inflammatory, antimicrobial, and many more activities. Therefore, it is of great significance to explore the phytochemical profile of those neglected *Ficus* species to find further natural compounds with promising bioactivities and plausible pharmacokinetic properties and enable us to conduct more detailed research on structure-activity relationship.

For this purpose, thirty-eight ethanolic extracts from different parts (leaves, twigs, bark, roots) of ten *Ficus* species have been prepared. Extracts have been subjected to HPLC-DAD analysis and obtained UV spectra have been compared with the library of UV spectra of compounds isolated previously at the Department of Natural Drugs. Surprisingly, only the roots of *F. cyathistipula* contained flavonoids, whereas coumarins were dominant compounds in the roots of *F. pumila* and all parts of *F. carica*. Other extracts did not contain constituents absorbing UV radiation; therefore, these extracts have been subsequently subjected to HPLC-ELSD analysis that proved the presence of compounds lacking a chromophore, probably including triterpenes, phytosterols, and fatty acids. Based on these findings, a taxonomic revision of *Ficus* species based on the phytochemical profile is surely warranted as it would simplify the orientation in this genus and its subgenera.

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Integrated analytical methodologies for Alzheimer's disease drug discovery

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In neurodegeneration, the selection of new lead compounds of natural and synthetic origin is a challenging task and involves various essential steps, the first being the identification/validation of new targets, then the selection of molecules able to bind to the target(s), and finally the study of the effects of hitting the target at molecular, cellular, and whole animal level.

In the case of Alzheimer's disease (AD), the most common form of dementia in adults, the enzyme acetylcholinesterase (AChE) has been the first target for the development of new drug inhibitors since the discovery of the cholinergic deficit in the central nervous system. However, basic research showed that cognitive impairment could also be due to a cascade of toxic biochemical events leading to the accumulation in the brain of proteins such as ß-amyloid (Aβ) and hyper-phosphorylated tau protein. Consequently, beta-secretase (BACE1), one of the enzymes that cleave APP (amyloid precursor protein) generating abnormal levels of toxic amyloid peptides, GSK3β, a tau protein phosphorylating kinase, and amyloid aggregation have become important targets in AD drug discovery.

Once the disease targets are selected, the determination of the activity of the new compounds must be carried out quickly and in a way that allows the verification of the design hypothesis. Indeed, different types of interactions with specific biological targets can mediate drug activity, and the estimation of these interactions may elucidate the mechanism of action.

To this aim, in the first instance, a screening of a large number of compounds is required for the selection of a few lead compounds. Secondly, specific methods able to elucidate the mechanism of action of selected compounds are employed, before the ultimate and most advanced tools, transgenic animal models of the disease can be used to study the effects of single compounds on the disease phenotype.

Here we report the development of purposely designed integrated methodologies to define the multifunctional activity profile of small molecules of natural and synthetic origin for the discovery of new AD drugs. With the regards to the screening of the activity of chemical collection, affinity chromatography on HPLC immobilized-enzyme columns (or immobilized enzyme reactors, IMER) is shown as a promising methodology for fast applications. Human recombinant AChE and BACE1 monolithic micro-IMERs (immobilized enzyme reactor) have been developed for on-line automated HPLC inhibition studies (IC_{50} and mechanism of inhibition). Secondly, fluorescence, circular dichroism (CD), mass spectrometry (MS) and atomic-force microscopy (AFM) methods are optimized for the inhibition of spontaneous Aβ aggregation, elucidating at which intermediate level of the Aβ aggregation cascade the inhibitors stop the process (monomer, soluble oligomers, protofibrils, fibrils). Moreover, MS, CD and fluorescence are applied to investigate amyloid chemical reactivity with specific ligands able to produce covalent modifications that affect the peptides aggregation pattern. MS is also combined with UHPLC to investigate the mechanism of action of GSK3β inhibitors. By the application of these integrated approaches, new leads as the prototype of new classes of multifunctional compounds for AD treatment were discovered, active in the AD transgenic animal.

Alkaloids of norbelladine type from *Narcissus pseudonarcissus* cv. Carlton as inspiration for development of highly selective butyrylcholinesterase inhibitors

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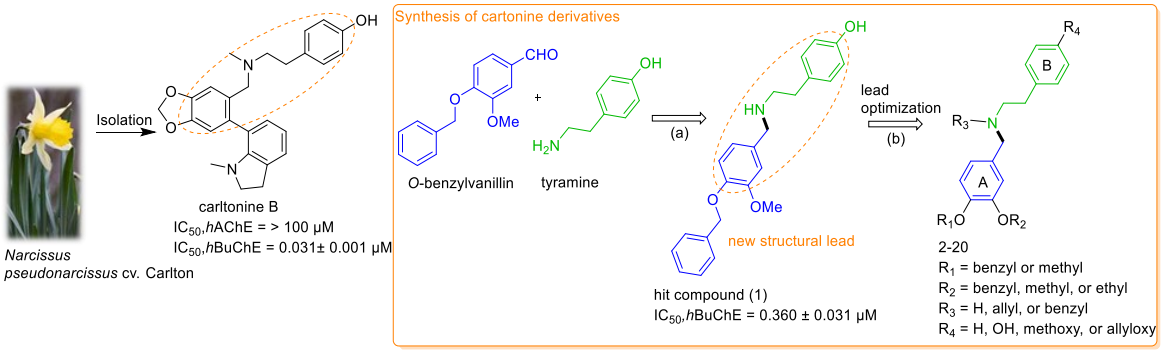
This Alzheimer´s disease (AD) is the most common age-related neurodegenerative disease, consisting of many cognitive and neuropsychiatric manifestations, that result in progressive disability and eventual incapacitation. Current therapy of AD's mild-to-moderate stages relies on the administration of acetylcholinesterase (AChE) inhibitors, represented by galantamine, donepezil, and rivastigmine [1]. In the later stages of AD, ACh hydrolysis is preferentially controlled by another cholinesterase named butyrylcholinesterase (BuChE). Its activity may be increased by 40% to 90%, moreover BuChE may contribute to the pathogenesis of type 2 diabetes mellitus (T2DM) by causing insulin resistance and Parkinson's diseases.

The plants of Amaryllidaceae family are a potential source of biologically active natural compounds. This study has been focused on the isolation of minor alkaloids with potential biological activity connected to AD and subsequently used as a template for the development of new lead compounds. Novel alkaloids carltonine B have been isolated from the alkaloidal extract of fresh bulbs of *Narcissus pseudonarcissus* cv. Carlton, exhibited selective *in vitro* *h*BuChE inhibition potency with the IC_{50} value of 31nM [2]. Unfortunately, these alkaloids are present in plant material only in trace amounts. Therefore, we have decided to use a crucial structural fragment e.g. (4-[2-(benzylamino) ethyl] phenol moiety) from carltonine B, which is responsible for high *h*BuChE inhibition activity, for the preparation of a pilot series of compounds (**1** – **20**) structurally inspired by these alkaloids (Scheme 1) [3]. Seven compounds were found to possess *h*BuChE inhibition profile, with IC_{50} values below 1 μM. The most significant inhibition activity was demonstrated by compound **6** with the IC_{50} value of 72 nM, and an excellent selectivity pattern over *h*AChE, reaching a selectivity index of almost 1400. Further, enzyme kinetic analysis reveals that compound **6** binds into active side of *h*BuChE enzyme with a reversible mode. The *in vitro* study was further established by *in silico* evaluation. Therefore, it can be concluded that optimization of further norbelladine analogues, potentially applicable in the treatment of neurodegenerative diseases is an interesting direction in the development of highly selective BuChE inhibitors.



*Scheme **1**: Design of novel cholinesterase inhibitors derived from hit compound 1 and norbelladine-type Amaryllidaceae alkaloids isolated from Narcissus pseudonarcissus cv. Carlton, namely carltonine B*

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Biological study of indole alkaloid from Vinca minor L. with anti-Alzheimer's potential

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During our research was discovered that alkaloids from Vinca minor L. possess a selective inhibition activity against human butyrylcholinesterase (hBuChE), a less known but crucial enzyme in the pathology of Alzheimer's disease (AD). One of the compounds, namely 2-ethyl-3[2-(3-ethylpiperidinyl)-ethyl]]-1H-indole, isolated from this species for the first time, exerted unusual inhibitory hBuChE activity (IC_{50} 0.65 μM). The alkaloid also inhibited prolyloligo-peptidase (IC_{50} 58 μM); another enzyme involved in AD's pathogenesis. These results led us to further examination. The enzyme pharmacokinetic study revealed the binding mode to the active site of the hBuChE to be as reversible and competitive, while in silico simulations, such as molecular docking and dynamics, clarified the binding pose. Parallel artificial membrane permeability assessment in vitro predicted this compound's ability to penetrate the blood-brain barrier by passive diffusion. This alkaloid also tentatively seemed non-cytotoxic, as showed by a cytotoxicity test on the panel of ten different cell lines at the concentration of 10 μM. Since this structure can also be prepared synthetically, our compelling results support future biological studies of this compound and the exploration of potentially better analogs.

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Isolation of alkaloids from *Geissospermum vellosii* and their biological activity

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The genus *Geissospermum* (Apocynaceae) are Amazonian trees native to Brazil, commonly found in the eastern region. Native tribes use aqueous and ethanolic extracts of bark for various diseases, e.g., malaria, cancer, and bacterial infections. The species of *Geissospermum vellosii* is a rich source of indole and β-carboline type of alkaloids. There are few phytochemical studies of this genus. However, almost none of the study is dealing with the isolation of alkaloids. The biological activity of extracts from *G. vellosii* is broad and copies the native use of decoction. The preliminary screening study for determining cholinesterase inhibition of extract from the bark of *G. vellosii* showed interesting inhibition activity against huBuChE IC_{50} = 0.37 ± 0.05 µg/ml, and 15 alkaloids were identified by GC/MS and TLC. Primary ethanolic extract was prepared from 40 kg of dried crushed bark. The alkaloidal extract was prepared with different solvents (diethyl-ether and chloroform) depending on the polarity. The purified diethyl-ether extract (53 g) was separated using column chromatography to give 16 fractions. After purification and crystallization were isolated five compounds so far. The inhibitory activity against recombinant human AChE and BuChE, GSK-3β of isolated alkaloids and their blood-brain barrier penetration was determined.

Bioguided analysis of *Papaver rhoeas* as a source of potential biologically active alkaloids

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Papaver rhoeas is a common plant in many regions around the world. It is regarded as a source of many bioactive compounds with beneficial health effects. In folk medicine *P. rhoeas* is known for centuries for their pharmacological properties in treatment of various diseases [1]. Extracts of P. rhoeas are studied for their soothing abilities in anxiety-related digestive problems, and are studied as a potent antitussive, antispasmodic, antigenotoxic, antimutagenic, antineoplastic as well as bactericide agents [2]. Recent studies showed interesting activities of its extract, that prevents against neurodegenerative diseases such as Alzheimer disease. Previous phytochemical investigation has revealed the presence of various alkaloids [1, 3].

As a part of our on-going screening of plant extracts for analytical bio-guided extraction methodologies, the alkaloidal extract from aerial parts of *P. rhoeas* was studied. The summary extract was separated into individual fractions by Flash chromatography. Obtained fractions were examined for their biological activities. Fractions with highest biological activities underwent further separation to individual subfractions using preparative chromatography, and by preparative thin layer chromatography. Based on instrumental analysis by HPTLC, HPLC-MS and GC-MS the main biologically active constituents were analyzed by to elucidate their chemical composition, identified, and compared with routinely used phytopharmaceuticals for the treatment of Alzheimer disease.

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Gut microbiota need to be considered for explaining the activity of herbal medicine

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Despite intensive research the active principles and mechanisms of action of many herbal medicines and medicinal plants are still not known. Moreover, the bioavailability of many plant constituents is rather low, why they are not likely to act systemically. Therefore, alternative approaches to explain their activity have to be considered.

Gut microbiota and the human body form a symbiosis which is essential for our health and well-being. Dysbiosis can lead to serious diseases, like inflammation, obesity, asthma, diabetes, and even cancer. Therefore, gut microbiota may be a relevant target for herbal medicinal products and may help to understand their effects [1].

For example, *Faecalibacterium. prausnitzii* has been identified as a major actor of human intestinal health [2], the mucin-degrading bacterium *Akkermansia muciniphila* has been linked to obesity and type 2 diabetes (T2D) [3], and members of the genus *Fusobacterium* have been identified as potential causative agents in colorectal carcinomas [4].

In order to study the interaction of medicinal plant extracts with gut microbiota, we have established a research platform, which allows the analysis of metabolization of plant constituents by LC-HRMS, and microbiome shifts by 16S RNA sequencing [5]. We are now going to study also the interaction of plants used for mental health via microbiome-gut-brain axis [6].

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Santorini's main food crops agricultural side-products with antioxidant activity: "Fava" and the "Santorini cherry tomato"

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Introduction: Santorini is a Greek island, with breath-taking volcanic landscapes and picturesque villages. The inhabitants of Santorini have accomplished to preserve traditional crops of the island, [1] such as the "fava" (*Lathyrus clymenum* L., Fabaceae) and the drought-tolerant "Santorini cherry tomato" (*Solanum lycopersicum,* Solanaceae), while both are PDO products. Except their nutritional value, there is an untapped potential of the respective agricultural side-products as rich sources of antioxidants and phytonutrients, that can be readily used in nutraceutical/cosmeceutical applications [2,3].

Aim: The aim of this work is to investigate the phytochemistry and antioxidant activity of the agri-food waste originating from the processing of the Santorini tomato (skin/seeds), and Santorini fava (perisperm). Our study was focused on the glycoalkaloids of the tomato, and the polyphenols of the fava.

Methods: The initial material (residual seeds/skin from the production of tomato puree, and fava perisperm from sperms peeling) was dried, extracted with water, or water/ethanol and filtered. In a continuous line, the water extracts were enriched with the aid of Amberlite XAD7HP resin, while for the purification of the tomato glycoalkaloid fraction Sephadex LH-20 was used. Samples were analyzed by means of HRMS/MS ESI (±), using a UHPLC system hyphenated to a hybrid LTQ-Orbitrap Discovery Mass Spectrometer. Total Phenolic Content (TPC) of extracts was evaluated with the Folin-Ciocalteu colorimetric assay and expressed in mg gallic acid equivalent/g of extract. In vitro antioxidant activity of extracts was assessed with the DPPH radical scavenging assay, and IC_{50} was expressed in mg/mL.

Conclusion: The extract profiling of both fava and tomato agri-food waste, revealed the efficiency of adsorption resin treatment for the preparation of enriched extracts. Indeed, UHPLC-HRMS ESI (±) profiling resulted in the identification of a variety of Solanaceae dietary glycoalkaloids in Santorini tomato agri-food waste, such as *α*-tomatine, esculeoside A, hydroxytomatine, and β1-hydroxytomatine. Santorini fava perisperm was found rich in dietary antioxidants such as catechins (catechin/epicatechin, dimers of epicatechin/epicatechin gallate), benzoic acid analogues and flavonoids. The hydroalcoholic and the resin enriched extract of fava perisperm showed high values of TPC (134.37 and 261.20 mg GA/g extract, respectively) while DPPH assay showed that they are both very effective scavengers, with an IC_{50} of 0.025 and 0.069 mg/mL. This work highlights the importance of two traditional PDO products of Santorini, as sources of nutritional glycoalkaloids (tomato), and antioxidant phytochemicals (fava), that can be used in the formulation of nutraceuticals.

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Alkaloids isolated from *Croton linearis* Jacq. leaves: their antiprotozoal potentiality.

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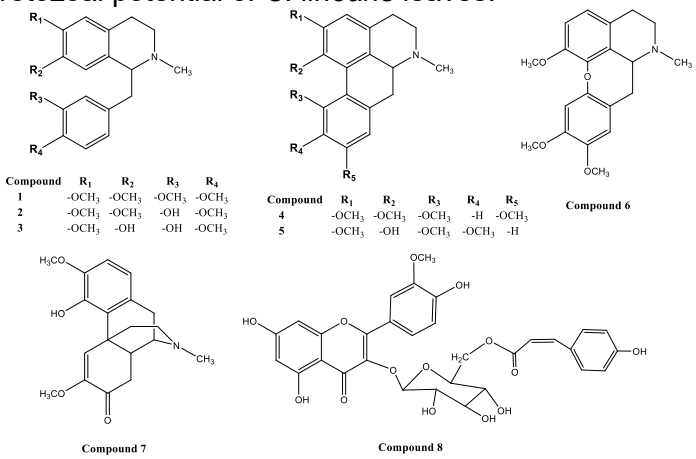
Leaves of *Croton linearis*, known as "rosemary", are widely used in folk medicine in Caribbean countries to treat fever and colds (associated to infections)[1], that's why in the present work we accomplish to evaluate the *in vitro* antiprotozoal activity of compounds isolated from *C. linearis* leaves. Compounds were isolated from an ethanolic extract of *C. linearis*, using flash chromatography and semi-preparative HPLC-DAD-MS (High Performance Liquid Chromatography – Diode Array Detection – Mass Spectrometry). Isolated compounds were characterized by MS and 1D and 2D NMR (Nuclear Magnetic Resonance) spectroscopy[2]. Antiprotozoal activity against *Leishmania infantum* MHOM/MA (BE)/67, *Trypanosoma cruzi* (Tulahuen CL2, β-galactosidase strain (nifurtimox-sensitive)), *Trypanosoma brucei brucei* Squib 427 strain (suramin-sensitive) or *Trypanosoma brucei rhodesiense* (strain STIB-900) and chloroquine-resistant strain *Plasmodium falciparum* K1 were assessed *in vitro* by microdilution method, as well as direct counting in optical microscopes[3]. In addition, cytotoxicity against MRC-5 cells (human fetal lung fibroblast cells) was determined [3]. Eight compounds were isolated and characterized (**Figure 1**): laudanosine (**1**), laudanidine (**2**), , reticuline (**3**), corydine (**4**), glaucine (**5**), cularine (**6**) and 8,14-dihydrosalutaridine (**7**) and the flavonoid glycoside isorhamnetin-3-*O*-(6''-*O*-*p*-*trans*-coumaroyl)-*β*-glucopyranoside (**8**). Reticuline showed a weak activity against *L. infantum* (IC_{50}=148.0 ± 1.2 µM), while the flavonoid was active against *T. cruzi* (IC_{50}=35.6 ± 2.3 µM). Moreover, reticuline, laudanidine and 8,14-dihydrosalutaridine showed moderate antiplasmodial activity with IC_{50} values of 46.8 ± 0.6, 17.7 ± 0.6 and 16.0 ± 0.5 µM, respectively, but no cytotoxicity was observed in a concentration up to 64.0 µM. This is the first report on the antiplasmodial activity of laudanidine and 8,14-dihydrosalutaridine. The results show the antiprotozoal potential of *C. linearis* leaves.



Compound	R_1	R_2	R_3	R_4
1	$-OCH_3$	$-OCH_3$	$-OCH_3$	$-OCH_3$
2	$-OCH_3$	$-OCH_3$	$-OH$	$-OCH_3$
3	$-OCH_3$	$-OH$	$-OH$	$-OCH_3$

Compound	R_1	R_2	R_3	R_4	R_5
4	$-OCH_3$	$-OCH_3$	$-OCH_3$	$-H$	$-OCH_3$
5	$-OCH_3$	$-OH$	$-OCH_3$	$-OCH_3$	$-H$

Fig. 1 Structures of compounds isolated from leaves of C. linearis

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Discovery of New Structural Features in Natural Products

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Although it may seem that bulbs of the Amaryllidaceae family have been largely explored, our work on their alkaloids shows they still have something to offer. A new pharmacophore in Alzheimer's disease research, where galanthindole is condensed with tyramine, has been identified in the carltonine type of alkaloid [1]. Interestingly regarding structural elucidation, an axial chirality has been described for this structural group. Even though galanthindole alkaloids have long been known, only Řezanka et al. [2] described atropisomerism for them.

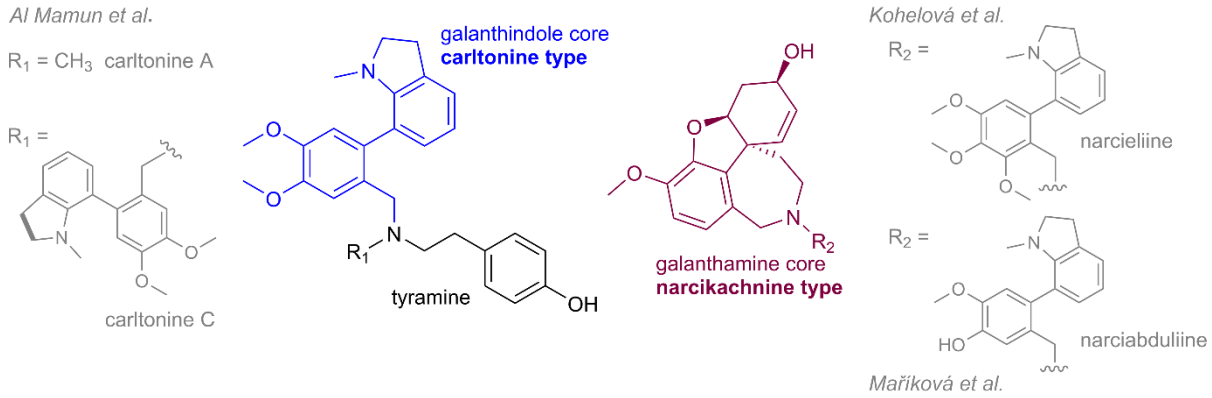

Figure 1 Recently discovered new types of Amaryllidaceae alkaloids having an atropisomerism

As the determining technique, dynamic NMR analysis was performed with an increasing temperature. Furthermore, atropisomerism was identified later in a narcikachnine type of Amaryllidaceae alkaloids [3,4].

Unfortunately, no samples were obtained for X-ray analysis. NMR spectroscopy was the key analysis in all identifications; nevertheless, MS and optical methods took place in the identification.

Recently, more alkaloids with galanthindole core were discovered in our ongoing study, and this short talk focuses on their structure analysis.

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"Determination of *in vitro* growth-inhibitory effect of essential oils from Indian medicinal plants against respiratory tract pathogens using new broth macrodilution volatilization method"

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Recently nebulised antibiotics have been preferred over systemic therapies for treating respiratory infections (e.g., cystic fibrosis) [1]. However, the delivery of aerosolised antimicrobial particles in the lower respiratory tract can be problematic due to the particle size of drugs. In this context, plant-derived products, especially essential oils (EOs), maybe interesting alternatives. The unique physicochemical feature of high volatility allows easy inhalation and uniform distribution of EOs' active substances [2]. Therefore, the current study aims to evaluate the antibacterial potential of 3 essential oils [*Cymbopogon citratus* (DC.) Stapf, *Cyperus scariosus* R.Br., and *Trachyspermum ammi* (L.) Sprague] obtained from Indian medicinal plants against bacteria associated with respiratory infections (*Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus pneumoniae, and Streptococcus pyogenes*) using recently developed broth macrodilution volatilization method. This assay combines the principles of broth microdilution volatilization and standard broth macrodilution methods and enables rapid, simple, cost- and labour-effective screening of volatile agents [3]. The highest antibacterial activity was observed for *T. ammi* seeds EO against *H. influenzae* with respective minimum inhibitory concentration (MIC) values of 128 and 256 µg/mL in liquid and vapour phase, followed by S. *aureus*, *S. pneumoniae*, and *S. pyogenes* with MIC values 512 µg/mL for both phases. EOs *of C. citratus* (leaves) *and C. scariosus* (rhizomes) exhibited the same level of antibacterial activity in liquid and vapour phases against all bacterial strains tested except *H. influenzae* which was more susceptible with MIC values of 256 and 512 µg/mL. The results demonstrate the practical applicability of this assay for the screening of volatile agents. Moreover, this study also suggests the potential of Indian EOs (e.g., *T. ammi* seeds EO) for application in the inhalation therapy; however, further research on the cytotoxicity and *in vivo* evaluation is necessary to verify its practical use.

Acknowledgement: This research was financially supported by the Czech University of Life Sciences Prague [projects IGA.20223102] and METROFOOD-CZ research infrastructure project [MEYS Grant No: LM2018100], including access to its facilities.

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Evaluation of anti-bacterial activity of semi-synthetic alkaloid derivatives from medicinal plants of the Amaryllidaceae family

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A loss of control over antibiotic-resistant pathogens has become a global issue due to severe and often untreatable bacterial infections. This state is reflected in complicated treatment, health costs, and higher mortality [1]. Facing this reality, the crucial need for action adverting a global crisis in health care is imperative. It may be time to look back at herbal remedies that have been proven by different human cultures for centuries for their promising antimicrobial effects. Antimicrobial and chemo-preventive properties have been demonstrated in many plant-based natural products [2]. These molecules could be used alone or combined with currently used antibiotics as promising antimicrobial agents. In our project, a collection of semi-synthetically achieved montanine-type derivatives of alkaloids from *Amaryllidaceae* family were at first subjected to the basic antibacterial screening of anti-infective potential. Eight reference bacterial strains were employed to determine the minimal inhibitory concentrations (MIC). Promising antibacterial activities, predominantly against gram-positive reference strains (MIC=3.9–62.2 μmol/L), were recognized in four candidates. Then, two derivatives were chosen for advanced studies considering their antibacterial aspects. In derivative with internal laboratory designation NMA-5 the activity corresponding to MIC=15.6–62.5 μmol/L against five gram-positive bacterial clinical isolates was revealed. The second derivative, NMA-12, showed similarly promising activity, MIC=7.8–125 μmol/L, against the identical clinical isolates. NMA-12 was further subjected to synergy testing, so-called “Checkerboard studies”, to reveal the relations between semi-synthetized candidate compounds and commercially used antibiotics in the action against reference bacterial strain (methicillin-resistant *Staphylococcus aureus*). For this purpose, six antibiotics with a different mechanism of action were included in these assays. Checkerboard studies allow recognizing four different types of relationship between two tested compounds: synergistic, additive, indifferent, and antagonistic. In the candidate compound, NMA12, a synergistic effect within different combinations of concentrations of the candidate compound with ciprofloxacin, linezolid, and tigecycline was revealed. Furthermore, for the more relevant consideration of the potential of this compound to become a promising anti-infective drug the *in vivo* toxicity testing using an invertebrate animal model, *Galleria mellonella* will be included.

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Susceptibility of intestinal bacteria involved in colorectal cancer pathogenesis to phytochemicals and their synthetic analogs *in vitro*

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Colorectal cancer (CRC), defined as an adenocarcinoma of large intestine, is the second most deadly cancer that caused 0.9 million deaths in 2020 worldwide [1]. China and the United States have the highest estimated number of new cases [2]. Gut dysbiosis is one of the factors associated with an increased risk of developing intestinal cancer. Various phytochemicals and their synthetic analogs (e.g., quinoline derivates) have been found to inhibit gut pathogenic microorganisms [3]; however, their effect on CRC associated microorganisms has not been determined yet. Therefore, the aim of this study was to test *in vitro* growth-inhibitory effects of ten substances (berberine, bismuth subsalicylate, ferron, 8-hydroxyquinoline, chloroxine, nitroxoline, salicylic acid, sanguinarine, tannic acid, and zinc pyrithione), together with six conventional antibiotics (ceftriaxone, ciprofloxacin, chloramphenicol, metronidazole, tetracycline, and vancomycin) against CRC-causing pathogens (*Clostridium septicum, Esherichia coli, Fusobacterium necrophorum, Fusobacterium nucleatum, Peptostretococcus anaerobius* and *Streptococcus bovis*) using broth-microdilution method assessing minimum inhibitory concentrations (MIC) [4,5]. Nitroxoline (MICs = 8-16 µg/ml), zinc pyrithione (MICs = 4-32 µg/ml) and chloroxine (MICs = 4-64 µg/ml) have been found to be the most active substances. *E. coli* and *S. bovis* were the most susceptible bacteria with MICs ≥ 4 µg/ml. These findings indicate that 8-hydroxyquinoline alkaloids and coordination complexes of zinc are chemical structures with potential to inhibit growth of pathogenic gut microorganisms associated with CRC development.

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Liquid matrix volatilization methods for susceptibility testing of respiratory bacteria to volatile agents in vapour phase

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Respiratory infections belong to the leading causes of morbidity and mortality throughout the world [1]. Inhalation therapy is a possible way of treatment when the active agents are delivered directly to the site of infection in the respiratory system [2]. Plant volatile products such as essential oils (EOs) are of great potential for inhalation due to their volatility. However, a lack of knowledge on antimicrobial activity of these vapours is a main obstacle for their practical use. Due to the high volatility and hydrophobicity of plant volatiles, conventional laboratory methods of antimicrobial susceptibility testing face specific problems. In recent decades, several methods have been developed with the aim of studying the potential of vapours of volatile agents to inhibit the growth of pathogenic microorganisms. Methods based on the solid matrix volatilization principle (e.g., disc volatilization assay) are simple to carry out but they also have many disadvantages, such as high consumption of material and labour [3]. Recently, broth micro- and macro-dilution volatilization assays based on the liquid matrix volatilization principle have been developed for the evaluation of the antimicrobial potential of volatile agents in vapour phase in our laboratory [4,5]. These assays are suitable for simple and rapid susceptibility testing of respiratory bacterial pathogens to volatiles in the liquid and the vapour phase and allow a cost- and labour-effective high-throughput screening of volatile agents using commercially available microtubes or microplates. Both methods have been validated for research and development of applications and technologies for the treatment of bacterial respiratory infections based on volatile antimicrobials [6,7].

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Investigation of the phytochemical and cancer chemopreventive potential of *Claoxylon longifolium* leaves growing in southern Thailand

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In consideration of the high levels of mortality and morbidity caused by cancer, there is a “real need” to focus on cancer prevention [1]. Medicinal plants represent a rich source of chemopreventive agents [2]. *Claoxylon longifolium* (Euphorbiaceae) is a flowering plant, which has been used in Thai traditional medicine and cuisine. Previous phytochemical screenings of this species have reported that the leaves possess alkaloids, terpenes, and flavonoids [3, 4], with promising chemopreventive potential. This study aimed to determine the phytochemical constituents and cancer chemopreventive potential of *C. longifolium* leaves. Powdered leaves of *C. longifolium* were subjected to sequential Soxhlet extraction, with *n*-hexane, dichloromethane, and methanol. The three resultant crude extracts were then screened for cytotoxicity using the MTT assay with AREc32 cells. Non-cytotoxic concentrations were then assessed for their ability to induce Nrf2 activity using a luciferase based cellular reporter assay system. The results revealed that the methanolic extract (0.4 mg/mL) was the most active with a 24.9-fold induction of luciferase activity (relative to control). This extract was the partitioned using C18 Solid Phase Extraction (SPE) into 4 fractions (F1-F4) which were then subjected to the MTT assay and Luciferase assay as previously described. The highest increase in Nrf2 induction was found with fraction F1 (3 mg/mL) followed by fraction F2 (1 mg/mL) and then fraction F3 (0.1 mg/mL) reaching induction folds of 11.5, 5.3 and 2.1, respectively. Phytochemical analysis of these fractions by 1D and 2D nuclear magnetic resonance revealed three known compounds: L-tyrosine, vitexin and rosmarinic acid. The evaluation of the bioactivities associated with the isolated compounds are ongoing. The present study highlights that further study of *C. longifolium* is justified as a potential source of chemopreventive agents for prevention of human cancer.

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Discovery of anti-coronavirus bisbenzylisoquinoline alkaloids.

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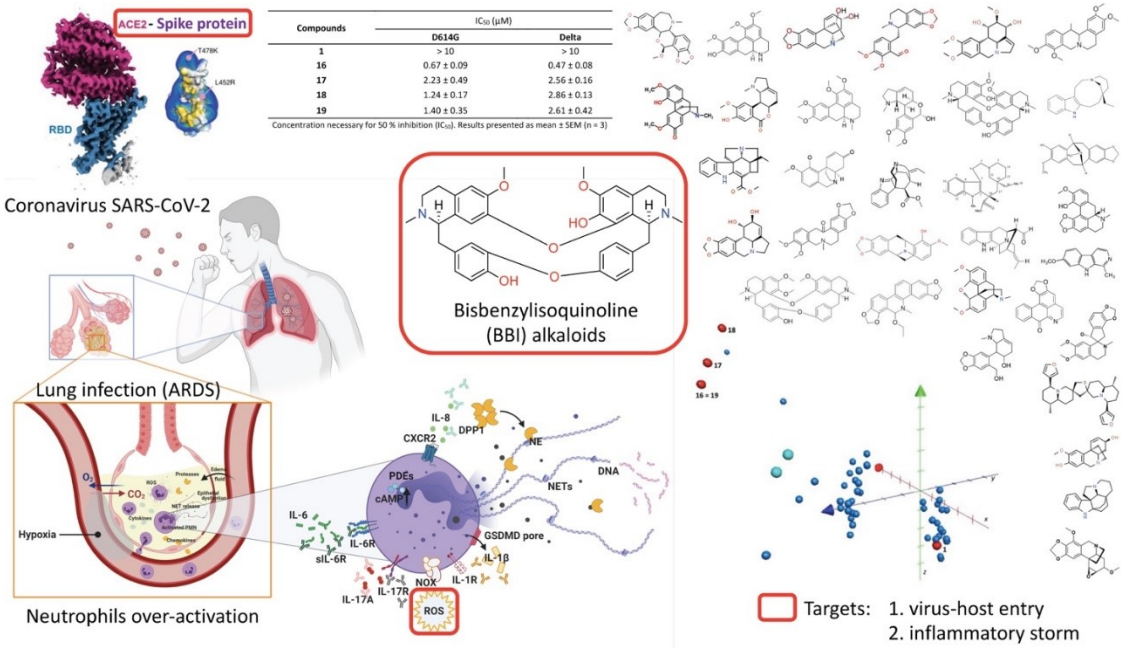
Coronaviruses belong to a group of dangerous viruses seriously affecting human health and society. The first coronaviruses were isolated in the 1960s, however until now there is a lack of effective anti-viral treatments. Natural alkaloids represent a promising class of bioactive agents. In this study, a wide range of alkaloids was screened in vitro for anti-inflammatory and anti-coronavirus 229E activities, and the active alkaloids were further explored by pseudovirus assay and ChemGPS-NP. We identified a group of bisbenzylisoquinoline alkaloids isolated from *Berberis vulgaris* with promising effects against coronaviruses *in vitro.* Moreover, recent evidence has revealed that excessive reaction of the immune system contributes to coronavirus-related severe complications. Therefore, we further studied the anti-inflammatory effects in human neutrophils. All the 99 alkaloids were isolated at Pharmaceutical Faculty of Charles University and tested for biological activity at Chang Gung University in Taiwan.

The results of this research would provide new insights into the development of novel anti-coronavirus natural products. Further, this study provides basis for academic collaboration among the researchers in Czech Republic and Taiwan and explore the traditional medicinal systems in Europe and Asia. In the future we would like to explore modern metabolomic techniques to evaluate target drug-containing extracts.



Compounds	IC50 (μM) D614G	Delta
1	> 10	> 10
16	0.67 ± 0.09	0.47 ± 0.08
17	2.23 ± 0.49	2.56 ± 0.16
18	1.24 ± 0.17	2.86 ± 0.13
19	1.40 ± 0.35	2.61 ± 0.42

Concentration necessary for 50 % inhibition (IC_{50}). Results presented as mean ± SEM (n = 3)

Discovery of a Series, Novel Oleocanthal - Based compounds as Potent Anticancer Agents.

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Olive Oil (OO), an important component of the Mediterranean diet, is a well-known source of polyphenols. This group of compounds, consisting of 2% of the total component, is considered to be responsible for the biological activities and the health benefits of olive oil. Among them, two phenyl alcohols, i.e., tyrosol and hydroxytyrosol and their two decarboxylated analogs, i.e., oleacein and oleocanthal, respectively, are acknowledged as the key ingredients, responsible for the health benefits of EVOO / are the subject of intense scientific study due to their important biological activities. The outstanding interest in these high-value natural compounds, and the inability to isolate them in significant and pure amounts, has triggered the development of various synthetic approaches, all involving multistep total synthesis, with low overall total yields [1]. In this regard, herein, we describe the development of a concise and scalable procedure for the synthesis of various oleocanthal analogues. The synthesis is performed by a convenient biomimetic and stereo-controlled approach, starting from oleuropein, an abundant raw material in olive leaves. We have already prepared various oleocanthal analogs with potent cytotoxic activity against several cancer cell lines, to in vitro and in vivo experiments.

Acknowledgment: This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH –CREATE –INNOVATE (project code: T2EDK-02423).

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Investigation of selected biological activities of montanine-type alkaloids and their derivatives

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The Amaryllidaceae plant family is one of the most important alkaloid-containing plant families with potent biological properties such as antitumor, antimicrobial, antimalarial, and significant anti-neurodegenerative activities [1]. Among all Amaryllidaceae alkaloids, montanine-type alkaloids are characterized by 5,11-methanomorphanthridine ring system and are known for their antiproliferative, antimalarial, antirheumatic and most recently for their antimicrobial activity. Montanine, pancracine and manthine have demonstrated single digit IC_{50} against various cancerous cell lines in discrete studies. Montanine itself, in addition to moderate and dose-dependent acetylcholinesterase inhibitory activity, has shown activity against pathogenic *E. coli*, *P. aeruginosa*, *S. aureus* and *S. epidermis* [2].

In the current study, a library of approximately 100 new semi-synthetic derivatives of montanine-type alkaloids were prepared, using montanine and 3-*O*-methylpancarine as starting material in order to compare the impact of C-3 or C-2 substitution on activity. 3-*O*-Methylpancarine was achieved through intramolecular rearrangement of haemantamine [2]. The derivatives were selectively screened for various biological tests such as cytotoxicity, acetylcholinesterase and butyrylcholinesterase inhibitory, antibacterial and antimycobacterial activity evaluation. Complementary hepatotoxicity test was performed on selected active compounds.

Results present 4 active compounds with potential antimycobacterial properties, 2 candidates as antibacterial agents, and 4 compounds with single digit IC_{50} against acetylcholinesterase and butyrylcholinesterase.

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Advanced chromatographic approaches in phenolic compounds profiling in archive tokaj wines

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The presented study is focused on the characteristics of archive Tokaj wines, especially in terms of their benefits to the human body in the form of antioxidant activity of phenolic compounds [1]. The samples of archive Tokaj wine come from vineyards of the Slovak part of the Tokaj wine region. The Tokaj wine region is one of the few areas producing special wine made from grapes affected by noble rot Botrytis cinerea under particular environmental conditions, which leads to the production of naturally sweet wines with a unique aroma [2].

More than 60 archive samples were evaluated in terms of phenolic substances profile, including hydroxybenzoic and hydroxycinnamic acids, stilbenes, and flavan-3-ols; the 18 phenolic compounds were identified in these samples. Individual phenolic substances, which differ significantly in their polarities, structural types, and amounts, were detected by ultra-high performance liquid chromatography method with diode array detection.

Although the UHPLC-DAD method was considered acceptable for application in the characterization of these types of complicated matrices, the two-dimensional liquid chromatography (2D-LC) has been tested as an effective technique for the separation of chemical compounds in complex sample matrices, such as wine. The 2D-LC achieves high peak capacity, reflected by decreased compound overlap, by combining two columns with various separation approaches, such as hydrophilic interactions or PEG stationary phases combined with reversed phases (pentafluorophenyl, RP-Amide, biphenyl, and phenyl-hexyl phase) [3].

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Celecoxib potentiates the *in vitro* anti-staphylococcal effect of oxacillin.

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Staphylococcus aureus is a human pathogen that causes musculoskeletal infections (MI) such as osteomyelitis and septic arthritis [1]. They are inflammation-related infections that pose a severe health challenge globally [2,3]. Antibiotics have been used successfully in treating bacterial infections [4]. However, this is threatened by the emergence of antibiotic-resistant bacteria and the reduction in the development of new antibiotics [5]. Therefore, there is a current search for new antibiotics to combat *S. aureus* associated infections. The synergistic action of drug combination is one of the strategies used for overcoming bacterial resistance. Synergy shows that when two or more drugs are combined, their activity becomes more potent than when used alone, producing a more significant effect [6]. Celecoxib is a non-steroidal anti-inflammatory drug (NSAIDs) used to treat inflammation caused by MI [7]. Previous findings demonstrated the antimicrobial activities of celecoxib and their ability to enhance the efficacy of some antibiotics (e.g., linezolid and vancomycin) through synergistic interaction [8]; therefore, they are seen as a potential drug candidate to potentiate the efficacy of antibiotics. This study investigated the in vitro synergistic interactions of celecoxib with oxacillin against *S. aureus* strains. The minimum inhibitory concentrations (MICs) were determined by the broth microdilution method using 96-well microtiter plates [9]. The fractional inhibitory concentrations (FICs) were assessed by the checkerboard assay [10]. The combinatory effects against ten strains of *S. aureus* were subsequently determined, where ΣFIC≤0.5 = synergy., ΣFIC>0.5-4 = no interaction., and ΣFIC>4 = antagonism [10]. In this study, celecoxib showed a robust synergistic interaction (∑FICs 0.087 to 0.471) with oxacillin against most tested S. aureus strains at concentrations ranging from 16 to 0.5 µg/mL. Although, previous reports showed that celecoxib enhances the activities of certain antibiotics against *S. aureus* [8]; however, to our best knowledge, this is the first study of the synergistic interaction of celecoxib with oxacillin against S. aureus.

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Amaryllidaceae alkaloids as inspiration for the development of highly selective butyrylcholinesterase inhibitors: the relationship between structure, effect, and toxicity

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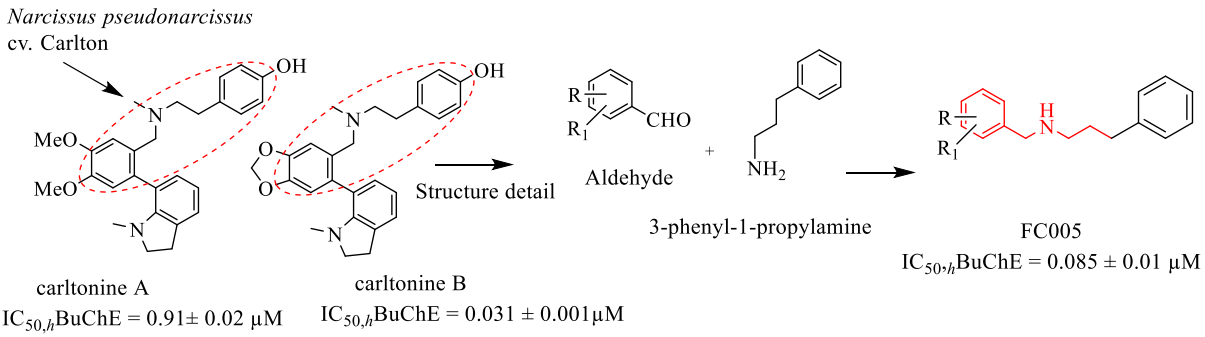
Alzheimer's disease (AD) is a serious irreversible progressive neurodegenerative age-dependent disorder characterized by memory loss and progressive cognitive impairment.[1] With increasing life expectancy, it is estimated that by the end of 2050, the number of affected people will be close to 150 million. Although the exact etiology of AD has not been fully elucidated, among the common features of the disease is dysfunction of the cholinergic system, which is one of the current targets for the treatment of the disease. As reported by the cholinergic hypothesis, damaged nerve cells in the brain with AD lead to an abnormal decrease in acetylcholine (ACh), and, at the same time, low enzyme levels are a symbolic pathological characteristic strongly associated with cognitive function. As AD progresses, levels of acetylcholinesterase in the brain decrease sharply by 90%, while butyrylcholinesterase (BuChE) increases to 165% of normal levels, taking over the hydrolysis of ACh. Based on this, BuChE is a potential target for the treatment of advanced AD.[2]

Carltonine A and B alkaloids demonstrated an exceptional selective inhibition potential of *h*BuChE in tens of nanomoles (IC_{50} = 0.031 ± 0.001μM). Unfortunately, these alkaloids are present in plant material only in trace amounts. The aim of this work is the preparation of synthetic compounds inspired by alkaloids of the belladin-type with a subsequent study of the structure and biological activity relationship. Cytotoxicity against human SH-SY5Y neuroblastoma and the HepG2 hepatocellular tumor line was measured for selected derivatives. For drugs that act in the central nervous system, it is crucial that they enter the CNS. PAMPA assay was established for selected compounds to detect blood–brain barrier permeability.



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Nature mimicking drug design of spiro-2-oxindoles with 4*h*-pyran and chromen cores

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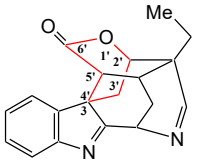
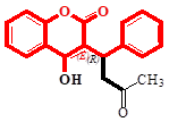
Introduction. Coumarin (2*H*-chromen-2-on) and its derivatives belongs to natural phenylpropanoids and have been found in many plant species (*Apiaceae, Rutaceae, Fabaceae etc.*). It is fascinating class natural compounds widely applicated in medicine and modern technology as far-famed useful fluorescent labelling reagents for trace determination of different proteins probes and photochromic materials with excellent luminescence in the blue-green part of the spectrum [1]. Coumarin derivatives are equally important for pharmacology. These compounds commonly applicated at World medicine as choleretics (e.g., 4-methyl-umbelliferon), anticoagulants (4-OH-coumarin derivatives – warfarin, dicoumarol). Other biological activities such as anticancer, antimicrobial, antiviral, etc., have also been discovered [2]. From the other side natural spiro-oxindoles containing spiro-fused pyran or chromene motifs are also distributed at plants, e.g., the akuammiline alkaloid scholarisine A, a dense, polycyclic compound isolated from *Blackboard tree* (*Alstonia scholaris)*, a plant used in traditional Chinese medicine to treat various respiratory diseases [3]. Accordingly, developing of new compounds containing of spiro-oxindole platform spiro-fused 4*H*-pyran- and chromen- cores in "*hybrid*" molecules are highly attractive approach for potential pharmacologically active substances research [4].

Blackboard tree – Alstonia scholaris

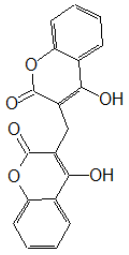


Alstonisine A

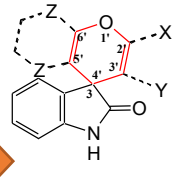


Anticoahulant **Warfarine**, 58 mg/Kg LD_{50} (rats) ΔG -8.1 kcal/mol (*H. sapiens* Oxidoreductase)

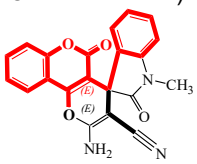
Melilotus officinalis (L.)



Dicoumarol



Designed and synthesised of new compounds library



Disigned "Hybrid" spiro-spiro-indolo-chromen **4.12n** 100 mg/kg LD_{50} (rats) ΔG -11.0 kcal/mol (*H. sapiens* Oxidoreductase)

The aim of work Design and synthesis of new biologically active compounds with coumarin, 4*H*-pyran- and spiro-2-oxindole moieties

Methods. AutoDockVina virtual molecular docking on *Homo sapiens* oxidoreductase (PDB ID 1yb5), parallel synthesis, multi-component reactions; proofing of the structure (*UV*-, NMR spectroscopy, MS etc.), *in vivo* pharmacology (*per os* toxicity, anticoagulant activity).

Major results and conclusion. The obtained spirooxindoles are bioisosteric to natural alkaloids of the scholarizine group and include 4-hydroxy-coumarin core. Most of the binding-scores (lowest binding-score ΔG energy, kcal/mol) obtained by docking on *H. sapiens* oxidoreductase molecular target a subset of anticoagulant-like library compounds to finding of "hit structure" **4.12n** (-11.0 kcal/mol ≈ predicted $logK_i$ 8.09 nM) *vs* Warfarine (-8.1 kcal/mol ≈ predicted $LogK_i$ 5.95 nM). Meanwhile, this "*nature mimicking*" drug design approach has also demonstrated improving of target compounds *in vivo* toxicity *vs* reference Warfarin.

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Semi-synthetic derivatives of Amaryllidaceae alkaloid ambelline as potential lead structures for drug development

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Amaryllidaceae alkaloid ambelline, belonging to the crinane-type subgroup, lacks any significant biological activity. However, its analogs prepared by the C-11 hydroxyl group's derivatization possess various pharmacological properties.

Within the current study, thirty-two derivatives were developed and tested for inhibitory activity of cholinesterases and in vitro cytotoxicity to screen their biological activity.

Seven aromatic derivatives (**6-8**, **11**, **12 19**, **26**) with different substitutions on the attached aromatic ring showed inhibitory potency against hBuChE (IC_{50} < 5 μM), of which 11-*O*-(1-naphtoyl) ambelline (**26**) was the most promising, with an IC_{50} value of 0.10 ± 0.01 μM. *In vitro* investigation was supported by computational studies predicting compounds' plausible binding modes in the active sites of *h*BuChE [1].

The cytotoxic potential of all derivatives was determined on a panel of nine human cancer cell lines and one noncancerous cell line. Molecules with the most pronounced cytotoxic activity contain a methyl group (**10**), methoxy group (**14**-**17**), or ethoxy group (**18**) on C11's aromatic ring. 11-*O*-(4-chloro-3-nitrobenzoyl) ambelline (**32**) had the most satisfactory cytotoxic potency among the ambelline derivatives, with IC_{50} ranging from 0.6 ± 0.1 μM (MCF-7) to 9.9 ± 0.2 μM (PANC-1). Derivative **32** was active even against resistant tumor cell lines, such as HT-29 and PANC-1.

The most active selective inhibitors of hBuChE are not cytotoxic and could be used as lead structures for a new series of ambelline derivatives, hence the need for further research.

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Exploitation of Greek medical and aromatic plants for the production of edible products

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Traditional aromatic plants, widely consumed in European countries as well as the Mediterranean area have gained consumers' preference. For this reason, herbs, and spices, are nowadays in the center of scientific attention. The aim of this study is the exploitation of selected Greek plants with final target the development of innovative commercial edible products. [1] Plants from the area of Taygetos mountain were collected, dried, and extracted. The studied plants were *Cerastium candidissimum*, *Cistus creticus*, *Achillea ligustica*, *Helichrysum stoechas*, *Lathyrus grandifloras*, *Sideritis clandestina*, *Thymbra capitata*, *Origanum scabrum*, *Salvia fruticosa*, *Mentha spicata,* and *Hypericum perforatum* [2,3]. The extraction of the plants was based on green methodologies like maceration, Microwave-Assisted-Extraction (MAE) and Supercritical Fluid Extraction (SFE). Water and mixtures of water/isopropanol (8:2 v/v) were used as extraction solvents. The chemical profiles of the extracts were analyzed via RP-HPLC-DAD and LC-MS. Moreover, all the extracts were evaluated for their antioxidant properties and their Total-Phenolic-Content (TPC) with *in vitro* assays. The chemical and antioxidant profiles, in combination with the consumers' preferences, were combined and the alcoholic macerated extract of *O. scabrum* (with DPPH IC50 at 0.056 mg and TPC of 170.39 mg GA/g extract) was chosen for further fractionation. Moreover, the extract was treated with Centrifugal-Partition-Chromatography (CPC) and a chosen fraction, was purified with prep-RP-HPLC. Rosmarinic acid was identified as the major metabolite. Altogether, the results suggest that *O. scabrum* can be a source of products and/or molecules for the food industry with antioxidant properties.

The authors declare no conflict of interest; Funding: KALIMERA, T2EDK-03534

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An innovative and high nutritional value cheese product, enriched with bioactive extracts of olive by-products

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During edible olive processing, huge amounts of by-products are produced with a very high polluting potential and create huge environmental problems in many country areas. Nevertheless, they are a source of valuable bioactive ingredients. Simple phenols such as hydroxytyrosol and tyrosol, secoiridoids (such as oleacein, oleocanthal and oleuropein), Verbascoside, as well as structural analogues thereof (such as lactones) are some of the bioactive compounds of olive by-products, with established beneficial effects on human health, proven by a plethora of scientific data. In 2012, the European Food Safety Authority (EFSA), recognizing the beneficial effect of these ingredients on human health, issued an opinion in favor of a specific health claim for olive oil biophenols, described in EU Regulation 432/2012. Moreover, recently, the European Union, by decision (Regulation 2017/2373 of 14 December 2017), approved the use of hydroxytyrosol on the market as a novel food ingredient, in accordance with Regulation of the European Parliament (EC) No 258/97. So, in recent years many food products, enriched with olive ingredients, have been developed and marketed by the food industries, with unexpected positive results. Among them, cheese products are an integral part of a balanced and healthy diet, due to its valuable nutrients. They are rich in protein, calcium, vitamins, and minerals. However, one of the major problems facing the cheese sector is the limited durability of cheese products, resulting in restricted exports in the food market.

In this work, using modern extraction methodologies, such as the adsorption resins technology (ART), hydroxytyrosol -enriched extracts from by-products of the debittering process of edible olives were produced. Then, three different types of cheese products were studied (Sheep Milk Cheese, Goat Milk Cheese, and Cream Cheese) and the olive extracts addition to the different steps of the cheese-making process (initial raw milk and curd) were investigated. Moreover, by applying HPLC-DAD analytical methodologies, the concentration levels of hydroxytyrosol and its derivatives, in the enriched cheese products, were determined.

Overall, in the context of the present study, significant amounts of hydroxytyrosol and specific biomarkers were detected in the final cheese products, while their long-term self-life extension is under investigation.

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Production of meat products enriched with olive biophenols

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Olive by-products is at the center of commercial interest due to their high content in bioactives. Among them, the agricultural side-products produced during the debittering process of edible olives have a very high polluting potential and create huge environmental problems in many country areas. Nevertheless, they are a source of valuable bioactive ingredients. Simple phenols such as hydroxytyrosol (HT) and tyrosol (T), secoiridoids (such as oleacein, oleocanthal and oleuropein), Verbascoside as well as structural analogues thereof (such as lactones) are some of the bioactive compounds of olive by-products, with established beneficial effects on human health, proven by a plethora of scientific data. In 2012, the European Food Safety Authority (EFSA), recognizing the beneficial effect of these ingredients on human health, issued an opinion in favor of a specific health claim for olive oil biophenols, described in EU Regulation 432/2012. Moreover, recently, the European Union, by decision (Regulation 2017/2373 of 14 December 2017), approved the use of hydroxytyrosol on the market as a novel food ingredient, in accordance with Regulation of the European Parliament (EC) No 258/97. So, in recent years, many food products, enriched with olive ingredients, have been developed and marketed by the food industries, with unexpected positive results. Among them, meat and meat products are important and suitable vehicles for human beings to convey the essential nutrients that may improve their health. They are ideal sources of soluble minerals (Fe- heme, Mg, K, Zn and Se), high-quality proteins (20–25%), vitamins (A, thiamine, riboflavin, niacin, retinol, B6, folic acid, B12, D and K), essential fats, amino acids and many other nutrients having a specific function to the body.

The production of innovative consumer meat products such as burgers, enriched with natural antioxidants that will be stable in cooking conditions is an ongoing challenge for the meat industry and the main goal of this study. In this work, using modern extraction methodologies, such as the adsorption resins technology (ART), hydroxytyrosol-enriched extracts from by-products of the debittering process of edible olives were produced. Then, bioactive extracts were added in the marinade mixture of burger products, in two different concentrations and after the cooking process, their organoleptic characteristics were evaluated. Moreover, by applying HPLC-DAD analytical methodologies, the concentration levels of hydroxytyrosol and its derivatives in the cooked and uncooked burger products were determined.

Overall, in the context of the present study, significant amounts of hydroxytyrosol and specific biomarkers were detected in the burger meat product after the cooking process, while the antioxidant profile of the Hydroxytyrosol-enriched meat product is under investigation.

Acknowledgements: The authors declare no conflict of interest. The authors are grateful to Operational Programme “Central Greece 2014-2020 “PYTHOLEO” (project code: STER1-0018066

Alkaloids of *Dicranostigma franchetianum* (Papaveraceae) and their antimycobacterial activity

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Tuberculosis (TB) is a widespread infectious disease caused by Mycobacterium tuberculosis. The increasing incidence of multidrug-resistant (MDR), and extensively drug-resistant (XDR) strains has created a need for new antiTB agents with new chemical scaffolds to combat the disease. Thus, the key question is: how to search for new antiTB and where to look for them? One of the possibilities is to search among natural products (NPs).

In order to search for new antiTB drug, we proceed detailed phytochemical study of whole plant of *Dicranostigma franchetianum* to isolate wide spectrum of isoquinoline alkaloids (IAs) and screen them against *Mycobacterium tuberculosis* H37Ra and four other mycobacterial strains (*M. aurum, M. avium, M. kansasii, and M. smegmatis*). The chemical structures of the isolated alkaloids were determined by a combination of MS, HRMS, 1D, and 2D NMR techniques, and by comparison with literature data. Alkaloids 3 and 5 showed moderate antimycobacterial activity against all tested strains (MICs 15.625–31.25 μg/mL).