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Abstracts

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FG1

ANALYSIS OF COPPER-CHELATING ACTIVITY OF ISORHAMNETIN AND TAMARIXETIN

LOMOZOVÁ, Z.,[1] MACÁKOVÁ, K.,[1] KARLÍČKOVÁ, J.,[1] CATAPANO, M. C.,[2] MLADĚNKA, P.,[2]

[1] Department of Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: lomozovz@faf.cuni.cz

Isorhamnetin and tamarixetin are the main *O*-methylated metabolites of the flavonoid quercetin. Flavonoids represent a large group of polyphenolic compounds which belong to plant secondary metabolites and have been suggested to have a positive impact on human health. They are a common component of the human diet. Chelation of transient metal ions is one of their proposed mechanisms of action. Copper is an essential trace element necessary for many physiological processes. However, free copper ions can cause damage to various biomolecules and lead to tissue impairment.[1] Disorder of copper homeostasis can be treated with copper chelators. Due to the limited array of the currently used copper chelators, research of such compounds continues to be of clinical interest.[2] In this *in vitro* study, tamarixetin and isorhamnetin were tested for their interactions with copper at four (patho)physiologically relevant pH conditions ranging from 4.5 to 7.5 by competitive and non-competitive methods. Competitive studies showed that both compounds were active copper chelators and non-competitive studies showed that the preferred stoichiometries were mainly 3:2 and 2:1 (flavonoid:metal). Analysis of cupric ion reduction has also been performed. In conclusion, both compounds showed good ability to chelate and reduce copper ions.

The study was supported by Charles University (1080217 C).

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FG2

SCREENING OF ANTIPLATELET ACTIVITY OF ISOQUINOLINE ALKALOIDS

PARVIN, M.S.,[1] MACAKOVA, K.,[2]

[1]Department of Pharmaceutical Botany, Faculty of Pharmacy, Charles University, Czech Republic

[2]Department of Pharmacognosy, Faculty of Pharmacy, Charles University, Czech Republic

e-mail: parvins@faf.cuni.cz

Alkaloids are nitrogen containing secondary metabolites mostly found in plants and other organisms. They have been used for the treatment of many ailments such as cancer, malaria, diabetics, cardiovascular diseases (CVDs).[1] Platelet cells perform a significant role in haemostasis and uncontrolled regulation of platelets can lead to CVDs progression. However, antiplatelet therapy has its limits, and so current research seeks to find active substances with different mechanisms of action. In this study, we have measured antiplatelet aggregation activity of selected 14 isoquinoline alkaloids isolated from plants. Multiple Electrode Aggregometry has been used where multiple measurement is possible in one time. The screening was performed with the use of whole human blood. Arachidonic acid was used as aggregation inducer and acetylsalicylic acid (ASA) as a standard drug. Percentage of aggregation has been measured from correlation of base line and linear portion of the aggregation curve. The most active compounds were papaverine, scoulerine and bulbocapnine. The first two substances exhibited similar inhibitory activity as ASA at the concentration of 40μM while were significantly less active at lower concentrations. Initial screening revealed the most active substances that would need a series of experiments to determine the mechanism of their action. The compounds have already been tested for antagonism at thromboxane receptors using the stable thromboxane analogue U46619.

The study was supported by Charles University (SVV 260 412).

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FG3

PHYTOCHEMICAL ANALYSIS AND BIOLOGICAL ACTIVITY OF *AZORELLA COMPACTA*

ŠTŮSKOVÁ, M., TŮMOVÁ, L.

Department of Pharmacognosy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: stuskovm@faf.cuni.cz

Azorella compacta Phil. (syn. yareta, Apiaceae) is a compact evergreen cushion shrub growing at altitudes between 3000 and 5000 metres in the South American Andes. The plant is frequently used in traditional medicine in the form of infusions and decoctions to treat various diseases (cold, pain, asthma, diabetes etc.) and relieve altitude sickness.[1] The major secondary metabolites that have been described in azorella plant are diterpenoids. A few of the potential medical effects were observed, such as antihyperglycemic effect and *in vivo* inhibition of *Plasmodium berghei* growth in mice;[2] an antimicrobial activity and an anti-*Trypanosoma cruzi* activity were further confirmed.[3] Polyphenols are other metabolites found in the azorella plant. Previous experiments with aqueous extracts proved their antioxidant and immunomodulatory activity; however, it is not known which particular substances are responsible for the effects. The main aim of the study is to prove selected biological activities of aqueous and ethanolic extracts of the whole plant *Azorella compacta*. Isolation and finding the specific substances responsible for biological effect is another goal of the study. Due to the current problems of widespread diseases and the lack or insufficient clarification of some findings on the effects of azorella plant, anti-aggregation, antityrosinase, antiallergic and antiparasitic activities were selected for testing.

On account of the early stage of research, this study does not provide any valid data yet. The presentation will be focused on the introduction of the plant and planed methods.

The study is supported by Research Founding SVV 260 416 of Charles University.

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FG4

CYTOTOXIC EVALUATION OF ASTAXANTHIN MONOESTERS FROM MICROALGAE *HAEMATOCOCCUS PLUVIALIS*

FÁBRYOVÁ, T.,[1, 2] DA SILVA D. C., [3] ANDRADE P. B.,[3] VALENTÃO P.,[3] PEREIRA D. M.,[3] KOPECKÝ J., [2] CHEEL, J.,[2] TŮMOVÁ, L.,[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2]Laboratory of Algal Biotechnology-Centre ALGATECH, Institute of Microbiology of the Czech Academy of Sciences, Czech Republic

[3]REQUIMTE/LAQV, Laboratório de Farmacognosia, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, R. Jorge Viterbo Ferreira, nº 228, 4050-313 Porto, Portugal

e-mail: fabryovt@faf.cuni.cz

Reddish ketocarotenoid astaxanthin (AXT) has more powerful antioxidant capacity than *β*-carotene, vitamin E, zeaxanthin, lutein or canthaxanthin.[1] The ability of this compound to cope with various diseases, thus to protect human body, has been also examined. Nowadays, this pigment attracts more and more interest from various industries, such as nutraceutical and cosmetic due to its different bio-functional properties that can have a huge impact on human health or its nutrition. The major natural source of AXT is the freshwater microalgae *Haematococcus pluvialis*, in which this compound is being present mainly in the esterified form. AXT is esterified with different fatty acids that are well-known for having various biological properties. It is believed that they may bestow these properties to AXT esters. From *H. pluvialis* biomass, five AXT monoesters have been isolated in our laboratory by high performance countercurrent chromatography (HPCCC) and their identity was confirmed using the high-performance liquid chromatography–atmospheric pressure chemical ionization–high resolution tandem mass spectrometry (HPLC–APCI–HRMS/MS). The fraction of astaxanthin monoesters showed a cytotoxic activity against the human gastric cancer cells (AGS cell line) using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assay. Later, the cytotoxic effect of AXT esterified with linolenic acid (**1**), linoleic acid (**2**), palmitic acid (**3**), oleic acid (**4**) and stearic acid (**5**) has been examined over these cells, showing that only the compound **4** exhibits a cytotoxic activity against this cell line.

The study was supported by SVV 260294 and Charles University Grant's Agency (GAUK 1134217).

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FG5

ISOLATION OF ALKALOIDS FROM *VINCA MINOR* L. AND THEIR INHIBITORY ACTIVITY ON HUMAN CHOLINESTERASES

VRABEC, R.,[1] OPLETAL, L.,[2] LOČÁREK, M.,[2] MAŘÍKOVÁ, J.,[3] JENČO, J.,[2] HULCOVÁ, D.,[1] ŠAFRATOVÁ, M.,[1] JANOUŠEK, J.,[1]

[1] Department of Pharmacognosy, ADINACO Research Group, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Pharmaceutical Botany, ADINACO Research Group, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[3] Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: vrabecr@faf.cuni.cz

Vinca minor L. (*Apocynaceae*) is an evergreen trailing subshrub common in western and southern Europe, mostly as a groundcover in temperate gardens. It contains over 50 indole alkaloids. It has been discovered that the alkaloidal extract from overground parts exhibits selective inhibitory activity against human butyrylcholinesterase (BuChE) (IC_{50} 13,60 ± 0,83 μg/ml), contrary to acetylcholinesterase (AChE) (IC_{50} 191,58 ± 38,03 μg/ml). BuChE is responsible for cleavage of acetylcholine, especially at the advanced stage of Alzheimer‘s disease, and also for its aggregation of β-amyloid that increases its neurotoxicity. Thus, BuChE represents an interesting target for new drug development, and alkaloids from *V. minor* seems to be a promising source of the effective structures. Seven alkaloids have been isolated from the original fraction no. 147–214, which had been obtained from the alkaloidal extract using column chromatography with aluminium oxide. The alkaloids have been separated by TLC techniques on silica-gel, and identification of their structures was determined by HPLC/DAD/MS-ESI, GC/MS-EI, and NMR instruments. Four of these alkaloids have been isolated for the first time from *V. minor* ((+)-aspidofractinine, (+)-raucubaine, (-)-demethoxycarbonyltetrahydrosecodine, (-)-demethoxyalstonamide), whereas (-)-minovincine, minoriceine, and strictamine had already been isolated in previous research. All of the isolated alkaloids have been tested on the inhibitory activity of human AChE and BuChE. The most active alkaloids against BuChE were (-)-demethoxycarbonyltetrahydrosecodine (IC_{50} 0,65 ± 0,17 μM) and (-)-demethoxyalstonamide (IC_{50} 56,38 ± 2,58 μM). Other isolated alkaloids did not show a substantial effect on the inhibition of BuChE. None of the alkaloids exhibited significant activity against AChE.

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FG6

NMR ELUCIDATION OF ACETYLCARANINE

MAŘÍKOVÁ, J.,[1] HOŠŤÁLKOVÁ, A.,[2] KUNEŠ, J.,[1]

[1] Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: marikoj2@faf.cuni.cz

The presented alkaloid was isolated from *Nerine bowdenii* (Amarylidaceae) at the Department of Pharmaceutical Botany. The Amarylidaceae family is very rich in alkaloids containing isoquinoline scaffold.

The isolated substance was characterized by ^{1}H and ^{13}C NMR experiments as well as Heteronuclear Single Quantum Correlation (HSQC), Heteronuclear Multiple Bond Correlation (HMBC) and Correlation Spectroscopy (COSY) experiments. Other spectroscopic methods such as HRMS and optical rotation were also used.

The determination of this structure will be discussed.

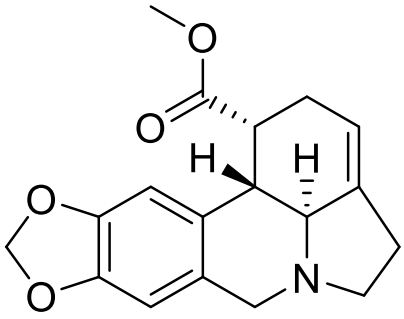

Figure 1 The identified structure of acetylcaranine [1]

The study was supported by Czech Science Foundation (project GA ČR 18-17868S) and Charles University (project SVV 260 401).

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FG7

BROUSSONETIA PAPYRIFERA AS A RICH SOURCE OF VARIOUS SECONDARY METABOLITES

MALANÍK, M.,[1] PAULÍKOVÁ, M.,[1] POMYKALOVÁ, K.,[1] ŠAFÁŘOVÁ, H.,[1] POSPÍŠILOVÁ, T.,[1] ŠMEJKAL, K.,[1]

[1] Department of Natural Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1-3, CZ-612 42 Brno, Czech Republic

e-mail: malanikm@vfu.cz

Broussonetia papyrifera (L.) L'Hér. ex Vent. (Moraceae), known as paper mulberry, is a deciduous tree naturally occurring in Southeast Asia. This plant is used in traditional Chinese medicine for various medicinal purposes. Extracts of *B. papyrifera* exhibit antioxidant, anti-inflammatory, antidiabetic and antimicrobial properties[1]. Several isolated compounds have huge potential to be used in medicine as they exert significant biological activities.

In the present work, chromatographic separation of chloroform part of ethanolic extract of the branches and twigs of *B. papyrifera* led to the isolation of 29 compounds belonging to the group of flavonoids, coumarins, lignans, stilbenoids, and fatty acid derivatives. The structures of the substances were determined by HRMS, and by 1D and 2D NMR. Broussofluorenone C (**23**) and (*R*)-8-methoxymarmesin (**11**) have been introduced as new compounds and further compounds (**1, 3, 6, 9, 10, 12, 19, 20, 28, 29)** have been isolated from *B. papyrifera* for the first time. The effect on insulin signaling cascade and NF-κB inhibitory activity of selected phenolic substances have been evaluated in *in vitro* assays.

The study was supported by grant IGA VFU Brno 303/2019/FaF.

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FG8

ISOLATION AND IDENTIFICATION OF ACTIVE CONSTITUENTS FROM *PAULOWNIA TOMENTOSA* STEUD. FRUIT

MOLČANOVÁ, L., [1] DALL'ACQUA, S., [2] ŠMEJKAL, K., [1]

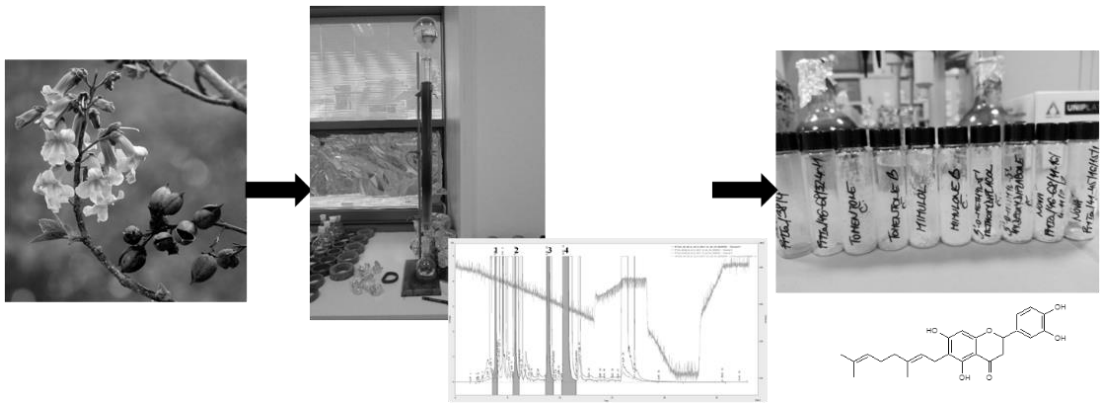
[1] Department of Natural Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

[2] Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Italy

e-mail: lenka.molcanova1993@gmail.com

Paulownia tomentosa Steud. (Paulowniaceae), a traditional Chinese medicine plant, is a rich source of multifarious secondary metabolites, mainly of phenolic character. Flavonoids, lignans, phenolic glycosides, phenolic acids, terpenoids, quinones, glycerides, and miscellaneous other compounds have been isolated from different parts of this plant. Recent interest in *P. tomentosa* has focused on isolation and identification of prenylated or geranylated flavonoids, which exhibit interesting biological activities, such as antioxidant, antimicrobial, anti-inflammatory, cytotoxic, and others[1]. More than sixty compounds with a prenyl or a geranyl side chain attached to the flavonoid skeleton at position C-6 have been isolated from leaves, flowers, and fruit of *P. tomentosa* till today.

Chromatographic separation of fractions obtained from *P. tomentosa* fruit led to the isolation of 14 flavonoid derivatives. The structures were determined by evaluation of the UV, MS, and NMR data. Eight of these compounds were isolated from a natural source for the first time.



The study was supported by IGA VFU Brno 311/2019/FaF and IMA VFU Brno 2019-FaF-04.

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FG9

IDENTIFICATION OF PLANT PHENOLICS AS NOVEL PPARγ AGONISTS

TREML, J.,[1] DIRSCH, V.,[2] VÁCLAVÍK, J.,[3] ŠMEJKAL, K.,[3]

[1] Department of Molecular Biology and Pharmaceutical Biotechnology, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Czech Republic

[2] Department of Pharmacognosy, Faculty of Life Sciences, University of Vienna, Austria

[3] Department of Natural Products, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Czech Republic

e-mail: tremlj@vfu.cz

Peroxisome proliferator-activated receptor-γ (PPARγ) is a nuclear receptor protein playing an essential role in lipid and glucose homeostasis. It is recognized as the receptor of the thiazolidinediones—a class of drugs used to manage hyperglycaemia.[1]

Aim of this project was to determine PPARγ agonist activity of plant phenolics isolated from *Paulownia tomentosa* (Thunb.) Steud., Paulowniaceae and *Morus alba* L., Moraceae. Potential agonists among the compounds isolated at Dpt. of Natural Drugs were preselected using virtual screening based on binding to PPARγ (AutoDock Vina; PDB ID: 1FM6). Only the compounds binding to binding site with satisfactory binding affinity were chosen.

The selected compounds were then tested for agonist activity using PPARγ luciferase reporter gene transactivation assay. HEK293 cell culture was transiently transfected with PPARγ expression plasmid, reporter plasmid (tk-PPREx3-luc), and pEGFP-N1 as internal control.

After 18h of incubation luminescence and fluorescence was measured and expressed as ratio. The two most active compounds were geranylated flavanones from *P. tomentosa*: diplacone and mimulone. Both compounds exhibited dose-dependent activation of PPARγ with its peak at 3 μM: 2.6-fold and 3.6-fold, resp. ($p \leq 0.0001$). Whereas rosiglitazone (positive control) showed 7.4-fold activation. Both compounds therefore have an antidiabetic potential.

The research stay in Vienna was supported by AKTION semester scholarship. Special thanks to prof. V. Dirsch and her research group for hosting me.

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FG10

ISOLATION AND IDENTIFICATION OF *SCELETIUM TORTUOSUM* CONSTITUENTS

RJAŠKOVÁ, V.,[1] ÇIÇEK, S.,[2] ZIDORN, C.,[2] ŠMEJKAL, K.,[1]

[1] Department of Natural Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

[2] Department of Pharmaceutical Biology, Institute of Pharmacy, Faculty of Mathematics and Natural Sciences, Christian-Albrecht University of Kiel, Germany

e-mail: v.rjaskova@gmail.com

Sceletium tortuosum (L.) N.E.Br. (Aizoaceae), also known as kanna, is a succulent plant from South Africa. It is known to contain several types of alkaloids, from these the mesembrine alkaloids are the most abundant. Structure of the mesembrine alkaloids is based on 3a-aryl-*cis*-octahydroindole ring. Main alkaloids from this group are mesembrine, mesembrenol, mesembrenon, mesembranole. Studies of biological activities have shown that mesembrine alkaloids act primarily as monoamine releasing agents and secondarily as serotonine reuptake inhibitors. These compounds have also displayed inhibitory activity on phosphodiesterase 4[1,2].

In this work we present isolation of eight compounds from *S. tortuosum* plant. Dried plant collected in South Africa was extracted with methanol. The methanolic extract was subjected to liquid-liquid extraction with hexane and chloroform. Hexane and chloroform extracts were separated by several column chromatographies. We used semi-preparative HPLC with UV and ELSD detection for isolation of pure compounds. Five compounds were isolated from the hexane part of methanolic extract and three from the chloroform part.

The study was supported by IGA VFU Brno 320/2016/FaF, IGA VFU Brno 307/2017/FaF and IMA VFU Brno 2019-FaF-02.

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FG11

ANTIMICROBIAL PROPERTIES OF CANNABINOIDS FROM CANNABIS INDICA

HELCMAN, M., TREML, J., ŠMEJKAL, K., BURCLOVÁ, Z.

Department of natural drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

e-mail: martin.helcman@gmail.com

Tested compounds were isolated from C. indica with the use of chromatographic methods (column chromatography, analytical TLC, analytical and semi-preparative HPLC). Six compounds obtained in the pure form were identified as major cannabinoids (CBG, CBGA, THCA, δ9-THC,CBDA, CBN). These compounds, plus the standard of CBD, were then used for the research of their antimicrobial properties against gram-positive and gram-negative bacteria. Firstly, minimum inhibition concentrations were measured. As an example of gram-positive bacteria we chose Staphylococcus epidermidis, because of its ability to form biofilm. Activity of the subinhibition concentrations of cannabinoids against biofilm formation was also the subject of this research. Mutant strain of Chromobacterium violaceum was selected as an example of gram-negative bacteria. We also used this strain to study the effect of the subinhibition concentrations of the cannabinoids against the quorum sensing, the type of cell-cell communication, which plays the key role in the regulation of bacterial behavior. As a result of this research, we observed strong bacteriostatic activity of the cannabinoids (excluding CBGA) against gram-positive bacteria, but almost no effect on gram-negative bacteria, which is in line with the previous experiments.1 Specific anti-biofilm activity was observed for the CBGA. Quorum-quenching activity will be the subject of further research.

The study was supported by the grant 310/2019/FaF from IGA VFU Brno

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FG12

ISOLATION OF AMARYLLIDACEA ALKALOIDS FROM *HIPPEASTRUM* CULTIVAR FERRARI AND EVALUATION FOR THEIR BIOLOGICAL ACTIVITIES

ALSHAMMARI, L.,[1] KUNEŠ, J.,[2] HAVELEK, R.,[3] CAHLÍKOVÁ, L.,[1]

[1] ADINACO Research Group, Department of Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[3] Department of Medical Biochemistry, Faculty of Medicine in Hradec Králové, Charles University, Czech Republic

e-mail: alshamml@faf.cuni.cz

Hippeastrum is a well-known ornamental Amaryllidaceae genus native to South America and comprises approximately 60 species, about 30 of which are found in Brazil; the majority are endemic and poorly studied.[1] This genus has been traditionally used to cure piles, tumors and various inflammatory disorders such as asthma. Physiological activities reported for plants of this genus include psychopharmacological, against *Trichomonas vaginalis* and cytotoxicity.[2]

The summary ethanolic extract was prepared from fresh bulbs of *Hippeastrum cv.* ferrari and separated on column chromatography. More than three hundred fractions were collected and pooled together based on TLC into fifteen subfractions. So far, fourteen alkaloids in pure form have been isolated belonging to different structural types. The isolated compounds were identified by comparison of obtained analytical data (MS, NMR, optical rotatory) with the literature data. All isolated alkalois were assayed for their biological activities- e.g. inhibition of HuAChE and HuBuChE, POP (prolyloligopeptides), GSK-3β (glycogen synthase kinase-3β), anticancer potential (cytotoxicity against panel of cancerous and noncancerous cell lines) and others. Within isolated alkaloids montanine displayed strong cytotoxicity against all tested cancer cell with IC_{50} values between 1.04 – 1.99 μM.

The study was supported by SVV 260 412 project.

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FG13

ALIPHATIC AND AROMATIC DERIVATIVES OF MONTANINE-TYPE ALKALOIDS AND THEIR CYTOTOXIC ACTIVITY

MAAFI, N.,[1] CAHLÍKOVÁ, L.,[1] KUNEŠ, J.,[2]

[1] ADINACO Research Group, Department of Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: negarm@faf.cuni.cz

The Amaryllidaceae plant family are known as a fruitful source of particular alkaloids which possess different range of bioactivities, pointedly antitumor, antimalarial, antibacterial and cytotoxic activity. Among these compounds, montanine-type alkaloids are characterized by 5,11-methanomorphanthridine ring system and known for their potential cytotoxic activity. Montaine and coccinine isolated from *Haemanthus humilis* Jacq., are showed to have in vitro IC_{50} value between 1.9 and 23.3 μM against 6 different cancerous cell lines.[1] In another study manthine and 3-*O*-methylpancracine were synthesized through rearrangement of haemantamine and showed GI_{50} values between 3 to 31 μM on 6 cancerous cell lines.[2] These evidences introduce montanine-type structure as a potential cytotoxic framework to be investigated.

This project is focused on different chemical group replacement on montanine type alkaloids framework in order to improve the cytotoxic activity and also to figure out the possible SAR of these compounds. Since these alkaloids are rare in natural sources, based on previous publications, they were synthesized using haemantamine intermolecular nucleophilic rearrangement, [2] and various chemical group were tried in position 3. In this presentation we will report the procedure of preparation of about 20 aliphatic and aromatic esters and ethers and results from their cytotoxic activity.

The study was supported by SVV 260412 project.

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FG14

DERIVATIVES OF AMARYLLIDACEAE ALKALOID AMBELLINE AS POTENTIAL DRUGS

RITOMSKÁ, A.,[2] HULCOVÁ, D.,[2] MAŘÍKOVÁ, J.,[1] CAHLÍKOVÁ, L.,[2]

[1]Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2]ADINACO Research Group, Department of Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

email: ritomska@faf.cuni.cz

Twenty-one derivatives of crinane-type alkaloid ambelline were developed. All of them were inspected for their potential inhibitory activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Esters of ambelline seem to be promising selective inhibitors of BuChE, which plays significant role in later stages of AD when levels of BuChE rapidly increase. The benefits of BuChE inhibition are predicted based on its symptomatic-relief mode of action, but also on suggestion an involvement of this enzyme in regulating disease progression. Seven aromatic derivatives with different substitutions on the attached aromatic ring were endowed with remarkable inhibitory potency against *h*BuChE (IC_{50} < 5 μM), highlighting three top-ranked compounds as follows: 11-*O*-(1-naphtoyl)ambelline, 11-*O*-(2-methybenzoyl)ambelline, and 11-*O*-(2-methoxybenzoyl)ambelline with IC_{50} values of 0.10 ± 0.01 μM, 0.28 ± 0.02 μM, and 0.43 ± 0.04 μM. Notably, four derivatives displayed selective *h*BuChE inhibition profile with selectivity index higher 100. *In vitro* investigation was supported by computational studies predicting compound's plausible binding modes in the active sites of *h*BuChE. To predict CNS availability logBB was calculated and the data correlated well with those obtained from PAMPA assay. Based on the obtained data all compounds should be able to permeate blood-brain barrier.

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FG15

ALKALOIDS AS POTENTIAL DRUGS IN THE TREATMENT OF ALZHEIMER'S DISEASE

HULCOVÁ, D.,[1] ŠAFRATOVÁ, M.,[1] HOŠŤÁLKOVÁ, A.,[2] CHLEBEK, J.,[2] OPLETAL, L.,[2] CAHLÍKOVÁ, L.,[2]

[1] Department of Pharmacognosy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: hulcovd@faf.cuni.cz

Alkaloids are a very important group of secondary metabolites with a number of interesting biological effects (anticancer, analgesic, anticholinesterase, antimalarial, …). Among the best known and most important substances are for example Ergot alkaloids used in therapy diseases of the circulatory system, Vinca alkaloids such as vincristine and vinblastine with anticancer activity or Amaryllidaceae alkaloid galanthamine which is used in therapy of Alzheimer´s diseases (AD).[1]

AD is one of the most frequent causes of dementia in the world. AD consisting of many cognitive and neuropsychiatric manifestations as is damage of memory, speech, orientation and others. During AD in the brain occurs to pathological changes of some enzyme systems that result in loss of neurotransmitter acetylcholine (Ach) and formation of amyloids plaques and neurofibrillary tangles (NFTs). NFTs consisting of paired helical filaments, with the main component being hyperphosphorylated τ-protein. Phosphorylation of τ-proteins is primarily dependent on glycogen synthase kinase-3β (GSK-3β) and cyclin-dependent kinase 5.[2] Alkaloids of different structural types have been screened for their potency to inhibit GSK-3β at a concentration 50 μM. Promising results have been demonstrated by two alkaloids 5/205 from *Vinca minor* (IC_{50} = 4.08 ± 0.14 μM) and GV 8-3b from *Geissospermum Vellosii* Alemao (IC_{50} = 7.18 ± 1.12 μM).

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FG16

ALKALOIDS OF THE AMARYLLIDACEAE FAMILY AS POTENTIAL DRUGS IN THERAPY OF DISEASES OF AFFLUENCE

BREITEROVÁ, K.,[1] MAŘÍKOVÁ, J.,[2] KOUTOVÁ, D.,[3] CAHLÍKOVÁ, L.,[1]

[1] Department of Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[3] Department of Medical Biochemistry, Faculty of Medicine in Hradec Králové, Charles University, Hradec Králové, Czech Republic

e-mail: breiterk@faf.cuni.cz

51 % of all drugs and 65 % of small-molecule drugs approved between 1981 – 2014 are in certain connection with natural compounds[1]. This proves that natural compounds and their derivatives are still an important source where new potential drugs can be sought. One of interesting groups of bioactive compounds are alkaloids. Amaryllidaceae family belongs among the twenty most important alkaloidal families with almost 600 of various Amaryllidaceae alkaloids isolated and structurally described so far.

34.3 kg of fresh bulbs of *Narcissus* cv. Professor Einstein were processed to obtain 31.7 g of summary alkaloidal extract. This extract was subjected to separation by different chromatographic methods. At the end, twenty-five alkaloids were isolated and identified by GC-MS, ESI-MS, NMR, X-ray, optical rotation and literature. One compound was identified as a new unpublished alkaloid of lycorine structure type - 7-oxonorpluviine. All compounds isolated in sufficient amount have undergone series of bioassays associated with Alzheimer‘s disease, cytotoxicity and activity against the liver stage malaria *in vitro*. The most promising is cytotoxic activity of pancracine – hence it went through study of the cell cycle and apoptosis induction interference.

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FG17

SEMISYNTHETIC DERIVATIVES OF AMARYLLIDACEAE ALKALOID HAEMANTHAMINE AS POTENTIAL DRUGS IN THE TREATMENT OF ALZHEIMER'S DISEASE

PERINOVA, R.,[1] KOHELOVA. E.,[1] SPULAK, M.,[2] MARIKOVA, J.,[2] HULCOVA, D.,[1] CAHLIKOVA, L.[1]

[1] ADINACO Research Group, Department of Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Bioorganic and Organic Chemistry, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic

e-mail: perinovr@faf.cuni.cz

Plants of the Amaryllidaceae family contain approximately 85 genera and 1100 species, have a wide distribution through both tropical and sub-tropical regions worldwide. Alzheimer's disease (AD) is the most prevalent neurodegenerative disease worldwide with complex etiology and multifaceted pathophysiology and data indicate an exponential rise in the number of cases of this disease. The well-known Amaryllidaceae alkaloid (AA) galanthamine is marketed drug for AD therapy under the commercial name Reminyl© (galanthamine hydrobromide).[1]

Studies also pointed out various pharmacological properties of semisynthetic derivatives of some Amaryllidaceae alkaloids. One of the most interesting AA alkaloids is alkaloid haemanthamine (HMT), which is widely distributed through Amaryllidaceae plants. Based on our previous results, where we reported promising anti-cholinesterase activity of pilot series of HMT derivatives, we decided to continue in preparation of further semisynthetic derivatives. [1,2]

Several new aromatic and aliphatic esters and pilot ethers have been synthesized for structure-activity relationship (SAR). New compounds were identified by 1D and 2D NMR, GC/MS and ESI-MS methods. All newly developed compounds were screened for different biological activities connected with potential treatment of AD. Active compounds are studied in more detail (e.g. type of inhibition, docking studies, logBB etc.).

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FG18

SEPARATION OF STEREOISOMERS FROM *ZEPHYRANTHES CITRINA*

KOHELOVÁ E.,[1] JENČO J.,[1] CAHLÍKOVÁ, L.,[1] MAŘÍKOVÁ, J.[2]

[1] Department of Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: kohelove@faf.cuni.cz

Zephyranthes is a genus of bulbous perennial plants belonging to Amaryllidaceae family that consists of about 90 various species. Phytochemical screening of their biologically active constituents revealed diverse group of compounds especially Amaryllidaceae alkaloids having various pharmacological activities including anticancer, anticholinesterase, antiviral, antifungal and anti-inflammatory activity. To date, ten alkaloids of various structural types have been reported in *Zephyranthes citrina*[1].

So far, 26 alkaloids from 30 kg fresh bulbs of *Zephyranthes citrina* have been isolated by liquid-liquid extraction and commonly used chromatographic methods. All compounds were identified by MS and NMR techniques. Several alkaloids were obtained in the mixture of isomers. Haemanthidine, an alkaloid of *β*-crinane structure type, has been isolated in our lab in form of mixture of 4 isomers. Within previous studies this alkaloid showed promising cytotoxic activity against different cancer cell lines. The aim of this study was separation of individual isomers and study of their biological activity, since the biological activity of individual isomers is unknown. Direct resolution of haemanthidine into its enantiomers was achieved by normal-phase TLC on silica gel plates impregnated with optically pure *L*-tartaric acid and *L*-histidine as chiral selectors. The mobile phase that enabled the best resolution was combination of cyclohexane-ethyl acetate-isopropanol-diethylamine (45:45:5:5), the spots were detected with Dragendorff's reagent and under UV light. The studies of biological activities of each isomer are underway.

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FG19

BIOLOGICAL EVALUATION OF ALKALOIDS ISOLATED FROM *NARCISSUS CV.* CARLTON AND ANTIPROLIFERATIVE POTENTIAL OF THEIR SEMISYNTHETIC DERIVATIVES

AL MAMUN, A.,[1] CAHLÍKOVÁ, L.,[1] MAŘÍKOVÁ, J.,[2]

[1]Department of Pharmaceutical Botany, Faculty of Pharmacy, Charles University, Czech Republic

[2]Department of Bioorganic and Organic Chemistry, Faculty of Pharmacy, Charles University, Czech Republic

e-mail: almamuna@faf.cuni.cz

Plants of genus *Narcissus* L. the most common genus of the Amaryllidaceae family have been used in traditional medicine worldwide. Most of the species can hybridize and some hybrids cultivars have been reported as potential sources of galanthamine and further Amaryllidaceae alkaloids (AA).[1] *Narcissus pseudonarcissus cv.* Carlton is an abundant species of *Narcissus* L. genus, often used for commercial extraction of galanthamine which is the most active AA used in the clinical management of mild to moderate stages of Alzheimer's disease (AD). So far, thirteen known AA have been isolated from *Narcissus pseudonarcissus cv.* Carlton. One undescribed isomer of hippeastrine and three new compounds belonging to belladine structure types have been isolated. Compounds isolated in sufficient amounts were screened for acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), prolyloligopeptidase (POP), glycogen synthase kinase-3 beta (GSK3β) and beta-secretase1 (BACE1) inhibition activity. Hippeastrine isomer and new compounds named carltonine A, carltonine B, and carltonine C demonstrate BuChE inhibition activity in μM and nM concentrations (10.07μM, 91nM, 3nM and 14.84μM respectively).[2] The next part of current study was the preparation of semisynthetic derivatives of galanthamine and screening of their biological activities connected with AD and oncological diseases.

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FG20

NEUROPROTECTIVE ACTIVITY OF *NUPHAR LUTEA* L. ALKALOID

WIJAYA, V.,[1] OPLETAL, L.,[1] HULCOVÁ, D.,[1] KUNEŠ, J.,[2] MAŘÍKOVÁ, J.,[2] CHLEBEK, J.,[1]

[1] Department of Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: wijayav@faf.cuni.cz

During screening of potential plant inhibitors against human acetylcholinesterase (*h*AChE) and butyrylcholinesterase (*h*BChE) at our department, a *Nuphar lutea* alkaloidal extract demonstrated potent and selective *h*BChE inhibitory activity (IC_{50} value of 11.73 ± 1.05 μg/mL), against *h*AchE was inactive (IC_{50} value > 100 μg/mL). From a dried plant material of *N. lutea* (12.25 kg; leaves and rhizomes) was prepared a summary ethanol extract (37.7 g) which was subsequently fractionated with diethyl ether (Et_2O-A; 1.7 g and Et_2O-B; 34.4 g), chloroform ($CHCl_3$-B; 0.34 g), and ethyl acetate (EtOAc-B; 0.95 g) by liquid-liquid extraction. The Et_2O-B was separated by columm chromatography on neutral alumina with step elution using petroleum ether, chloroform, and ethanol to collect 266 fractions which were monitored by TLC analysis and 22 joined fractions were obtained. Subsequently, 5 fractions were separated by using different chromatographic techniques (flash chromatography and preparative TLC) to isolate five pure alkaloids (thiobinupharidine, neothiobinupharidine, two unpublished diastereomers of thiobinupharidine, and one unpublished diastereomer of deoxynupharidine). Their structures were elucidated with mass spectrometry (EI, ESI), NMR, and optical rotation. *h*AChE and *h*BChE inhibitory activity of pure alkaloids was determined using a modified Ellman's method[1]. The diastereomer of deoxynupharidine showed moderate activity against *h*BChE with an IC_{50} value 69.30 ± 5.00 μM, other compounds were considered inactive (IC_{50} values > 100 μM).

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