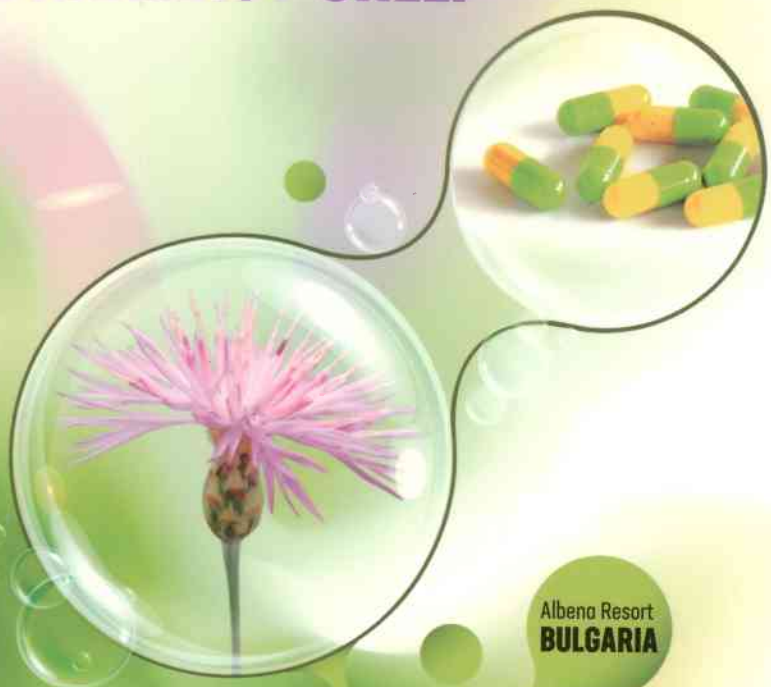
4th INTERNATIONAL CONFERENCE ON NATURAL PRODUCTS UTILIZATION

FROM PLANTS TO PHARMACY SHELF



Albena Resort
BULGARIA

4th International Conference on Natural Products Utilization

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PP 9

CURRENT STATE OF KNOWLEDGE ABOUT METABOLITE PROFILE AND BIOLOGICAL ACTIVITIES OF *THYMUS* SPP. IN EUROPE: A REVIEW

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Genus *Thymus* comprises more than 250 species of perennial herbaceous or fruticose plants, classified into 8 sections. Total 66 species with numerous subspecies and varieties are listed in Flora Europaea. Due to the taxonomic complexity and difficult identification of the species the studies focused on a relatively small part of the existing diversity. Usually samples of different species are bulked together for the purposes of the pharmaceutical industry, and they are designated as *Thymus* spp., which approach does not take into account the peculiarities in the phytochemical composition of the different species. The objective of the review is to make a survey on the phytochemical investigations and biological activities of the thymes occurring naturally in Europe. The major part of the studies focused on the determining of essential oil composition of the species. Very typical phenomenon for the genus is the chemical polymorphism, which is due both to the ecological factors, and to genetic variation. Phenolic compounds were studied to a lesser extent. A correlation was established between the phenolic content and antioxidant activity of extracts with different polarity. Besides antioxidant activity, antibacterial activity has received particular attention in many experimental designs.

The most frequently investigated species are relatively few, while the majority of thyme species in Europe still remain poorly studied, or not studied at all. This concern especially the endemic species distributed in remote and hardly accessible areas.

Acknowledgements: This work was supported by the NSF, Ministry of Education and Science, Bulgaria, Project DN 16/3.

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MONITORING OF THE ROSE OIL SAMPLES BY

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Bulgarian rose oil (*Rosa dama* after products for its fine arom indication since 2014. Geograp ditions and production techno chemical composition and qual

According to the internation are used for oil production. Ho some rose flowers undergo var lation. Both, the amount and th cantly. Although ethanol is a na content increases due to the fe used as a marker for this undesire rose oil is usually performed by C maintenance costs and time for methods, based on near infrared troscopy can provide a fast and n

The aim of the current study is simple, fast and non-destructive For this purpose, quantitative an samples from Bulgaria was perfor spectral measurements and suita NIR spectra with partial least squa

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SECONDARY METABOLITES PRODUCTIVITY IN *INULA BRITANICA* IS RELATED TO BIOMASS FORMATION AND PHYSIOLOGICAL ADAPTATION IN TISSUE CULTURE CONDITIONS

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Inula britannica is widely distributed throughout Western Europe and Turkey, reaching China through Iran and Pakistan. The species is of great importance for Traditional Chinese Medicine as well as Kampo medicine in Japan. Decoction of aerials or blossoms is used for treating asthma, and as an expectorant. Flowers are used as antibacterial, carminative, diuretic, laxative, stomach, tonic, rapid-healer, for hepatitis and tumors.

Shoot cultures of the plant were developed from material collected in Bulgaria. An experiment for elucidation of the combined effect of vitamins (Murashige and Skoog *vs.* Gamborg) and auxin and cytokinin treatments on secondary metabolite productivity, biomass formation and physiological status of shoot cultures was conducted.

Gamborg vitamins considerably stimulated plant growth as compared with Murashige and Skoog ones. Further on, the addition of benzyl adenine as cytokinin stimulated biomass formation in terms of axillary rosettes formation, however it suppressed aerial and root length and caused callusogenesis at explant base, this effect being slightly alleviated by 2-Naphthylacetic acid addition. Gamborg vitamins supplementation stimulated polyphenolics and total sesquiterpene lactones content *in* vitro. Interestingly, plant growth regulators affected polyphenolics and sesquiterpene lactones britannin and gaillardin ratio in a different way depending on the vitamins added. Biomass formation and secondary metabolite productivity were also found to be dependent on oxidative stress and photosynthetic pigments content of the cultures. The results are indicative of the relations between growth, development and secondary metabolite production in the plant organism.

Acknowledgements: This research was supported by the National Scientific Fund, Bulgaria (DN 09/11).

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PHOTOSTIMULATION
IN *ARTEMISIA ALBA* CE

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*[1] Institute of Organic Chemistr
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[2] The Czech Academy of Scienc
Czech Republic*

As a part of a broader progran
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Acknowledgements: We ac
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ted plant growth as compared the addition of benzyl adenine terms of axillary rosettes forma- length and caused callusogen- y alleviated by 2-Naphthylacetic tation stimulated polyphenolics vitro. Interestingly, plant growth iterpene lactones britannin and on the vitamins added. Biomass tivity were also found to be de- thetic pigments content of the ations between growth, develop- in the plant organism.

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PP 39

PHOTOSTIMULATION OF POLYPHENOLICS PRODUCTIVITY IN *ARTEMISIA ALBA* CELL AGGREGATES IN LIQUID MEDIA

Kalina Danova[1], Václav Motyka[2], Petre Dobrev[2], Antoaneta Trendafilova[1], Milka Todorova[1]

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As a part of a broader program for biotechnological development of *Artemisia alba*, non-differentiated cell aggregates were developed and grown in liquid media. Lines were initiated from sterile leaf explants and root cuttings, the first ones characterized by significantly higher lipid peroxidation and oxidative stress. Lines were maintained in liquid media supplemented with N^6-benzyladenine (BA) in combination with either indole-3-butyric (IBA) acid or 2-Naphthylacetic acid (NAA).

Light treatment stimulated polyphenolics production and increased stress hormones salicylic, abscisic and jasmonic acid. Interestingly, light affected differentially zeatin levels in relation to the initial explants from which they were initiated. Thus, while *trans*-zeatin content was generally enhanced by light treatment in the leaf-initiated lines, the *cis*-zeatin content was lowered in the same samples. This effect was media-dependent for root-initiated lines.

The initial physiological status also affected the morphology and biosynthetic capacity of the resulting lines. Thus, leaf initiated cell aggregates were heterogenous and to a higher degree non-differentiated, as compared with the ones initiated from root cuttings. This was accompanied by a higher polyphenolics productivity of the root-initiated lines. In addition NAA grown lines were more compact and with larger size as compared with IBA grown ones. This was also related to higher biosynthetic capacity of the NAA-grown lines. Qualitative characterization of the polyphenolics is in process in order to assess the potential of the obtained system as a prospective source of phytotherapeuticals.

Acknowledgements: We acknowledge the Czech Science Foundation (19-13103S) and Joint Scientific Research Project between CAS and BAS (Reg. No. 17-17).

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ROOTING AS A DECISIVE FACTOR AFFECTING ENDOGENOUS HORMONOME AND BIOSYNTHETIC CAPACITY OF *ARTEMISIA ALBA* TURRA *IN VITRO* MODEL

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In vitro culture model characterized by enhanced or suppressed rooting in essential oil bearing *Artemisia alba* Turra was developed with the aim to better understand interrelations between *in vitro* rooting and biochemical processes occurring in this plant.

Two main morphotypes were developed based on modification of cytokinin (N^6-benzyladenine) and auxin (indole-3-butyric acid) supplementation, either alone or in combination. Comparison was made based on the study of essential oil profile, polyphenolics levels, as well as endogenous hormonome *in vitro*.

It was revealed that normally rooting plants were characterized by elevated monoterpenoid/sesquiterpenoid ratio of their essential oil profile, higher salicylic and abscisic acid contents, bioactive cytokinin concentrations and *trans/cis*-zeatin ratio on one hand and by lowered polyphenolics levels and decreased jasmonic acid amounts on the other hand as compared with root suppressed plants.

The plant organism consists of different organs, which are constituted of different tissues, build up by cells. Production of secondary metabolites and phytohormones as well as their accumulation and translocation are dependent on the presence and communication between the highly specialized anatomical structures within the plant organism. Root morphogenesis was shown to play an important role in both biosynthetic features as well as physiology of *in vitro* cultured *A. alba* model, which could be applied in targeted cultivation of plant material with desired quality of this species.

Acknowledgements: This research was supported by the Czech Science Foundation (19-13103S) and the Joint Scientific Research Project between the CAS and BAS (Reg. No. 17-17).

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PHENYLPROPANOIDS FROM … WITH ANTIOXIDANT ACTIV…

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[1] *Department of Pharmacology, Phar… Pharmacy, Medical University of Sofia*
[2] *Department of Pharmacognosy, Fac… Sofia, 2 Dunav Str., 1000 Sofia, Bulgar…*

Wild basil (*Clinopodium vulgare* L.) plant widespread in Bulgaria. Aerial … for treatment of diabetes, gastric … ates symptoms associated with ma… ing. The aim of the study was to iso… *C. vulgare* aerial parts. Ultrasound … % methanol aqueous solution at … tion of all compounds of interest. … several fractions by low-bar liquid … umn. The subsequent ultra high-pe… with high resolution mass spectron… HRMS system consisting of an Q Ex… with a heated HESI-II source. DPPH… antioxidant activity determination. … was used as positive control. All inv… nificant radical scavenging and ferr… HRMS results, comparison with ref… major compounds in CVE fractions… Moreover rosmarinic acid, the mai… rity) and identified.

The obtained results revealed th… phenylpropanoids with significant … induced oxidative stress.

Acknowledgements: The study … tist-8232/20.11.2018 from the M… University of Sofia.

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IDENTIFICATION AND QUANTIFICATION OF BIOLOGICALLY ACTIVE COMPOUNDS IN *MELISSA OFFICINALIS* L. EXTRACT

Mariana Kamenova-Nacheva, Maya Tavlinova, Yana Nikolova, Krasimira Dikova, Irena Zagranyarska, Martin Ravutsov, Zhanina Petkova, Svilen Simeonov, Kalina Alipieva, Svetlana Momchilova, Antoaneta Trendafilova, Angel Konakchiev, Svetlana Simova, Desislava Gerginova, Kalina Kostova, Vladimir Dimitrov

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Lemon balm (*Melissa officinalis*) is a perennial herbaceous plant in the Mint family (Lamiaceae). The leaves of *M. officinalis* are used as herb, in teas, and also as flavouring. The tea of lemon balm, the essential oil, and the extract are used in traditional and alternative medicine, including aromatherapy. In traditional medicine, it is used in the treatment of headache, migraine, toothache, earache, flatulence, indigestion, cramping, nausea, anxiety anemia, vertigo, syncope, asthma, bronchitis, amenorrhea, heart failure, arrhythmias, insomnia, epilepsy, depression, psychosis, hysteria, stomach disorders, ulcers, wounds, rheumatisms and torticollis. Pharmacological reports indicate that *M. officinalis* exhibits a variety of actions including antioxidant and anticholinesterase activities [1]. Several studies have demonstrated the lemon balm's antioxidant activity, obtained through high amounts of flavonoids, rosmarinic acid and phenolic contents [2].

Herein we present a complete characterization of dry extract of *M. officinalis*, obtained after extraction with alcohol, evaporation and subsequent drying with Mini Spray Dryer. We performed a chemical profiling of biologically active compounds in dry extract of *M. officinalis* using high performance thin layer chromatography (HPTLC), UHPLC-ESI-MS (with single quadrupole MS), SFC/HPLC-ESI-MS/MS (with triple quadrupole MS) and 1D and 2D NMR spectroscopy. For quantitative determination of the main components – rosmarinic acid, caffeic acid and rutin, HPTLC and UHPLC-PDA analyses have been done.

Acknowledgements: Support of this work by the Research and Development and Innovation Consortium, Sofia Tech Park (project D-062-2018/20.06.2018) is gratefully acknowledged.

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NEW AURONOLIGNANS FR

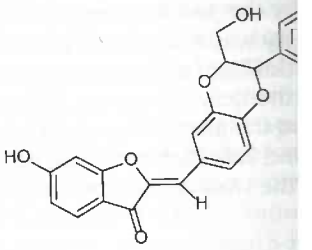
Miroslav Novaković[1], Iris Đorđevi Todorović[1], Slobodan Milosavljev

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Cotinus coggygria Scop. is a bush Anacardiaceae widely distributed flavonoid compounds – flavones, rones, chalcones, biflavones, phen logical activities.

Aurones are subclass of flavono Well known aurone isolated from figuration of the double bond. F that consist of flavone and lignin m ignans are silybins A and B, isosily *anum* with remarkable hepatopro

In this study, we report isolatio ignans **1** and **2** from *C. coggygria* 2017. Air dried heartwood was ex and methanol. Extract was fractio Final separation and purification phased HPLC. The structure elucid and 2D NMR (COSY, NOESY, HSQC



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