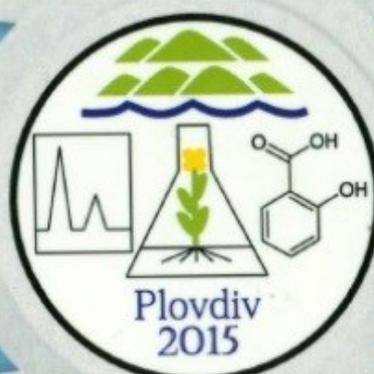


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SECONDARY METABOLITES FROM WILD AND *IN VITRO* GROWN *ARTEMISIA ALBA* AND *INULA BRITANNICA*

***Antoaneta Trendafilova*¹, *Milka Todorova*¹, *Samuel Peter*², *Victorya Genova*¹, *Evelyn Wolfram*²,
*Dimitar Dimitrov*³, *Ljuba Evstatieva*⁴, *Kalina Danova*¹**

¹*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*

²*Zurich University of Applied Sciences, Institute of Biotechnology, Wädenswil, Switzerland*

³*National Museum of Natural History, Bulgarian Academy of Sciences, 1000 Sofia, Bulgaria*

⁴*Institute for Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria*

Many species of Asteraceae family have been widely used for their curative properties in traditional medicine. *Artemisia alba* Turra is a widespread species in the southern parts of Europe and its aerial parts have been traditionally utilized as a stomach digestive and tonic in the form of a decoction. *Inula britannica* L. is widely distributed in Europe and Asia and is used as antibacterial, carminative, diuretic, laxative, and stomach remedies and for treating asthma, hepatitis and tumors.

The aim of this work was to study the capacity of *in vitro* cultures of these two species to accumulate biologically active secondary metabolites characteristic of the intact plants. To achieve the goal, plant material from both species was collected from natural habitats and worked up for isolation and identification of individual compounds. The structures of all compounds were elucidated by spectral methods (NMR, MS, IR, and UV). Sesquiterpenes and sesquiterpene lactones and triterpene palmitates, flavonoids, coumarins and dicaffeoylquinic acids were identified in *A. alba* and *I. britannica*. The isolated compounds were used as references for characterization of the metabolite profiles of *in vitro* culture derived plant materials. The obtained results showed a qualitative similarity in the investigated samples and the potential of *A. alba* and *I. britannica* as sources of the production of phytopharmaceuticals through plant biotechnology.

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ANTIBACTERIAL ACTIVITY OF TOTAL EXTRACTS AND FRACTIONS OF *IN VITRO* CULTIVATED MEDICINAL PLANTS

Azaiez Sana¹, Ben Slimene-Debez Imen¹, Limam Ferid¹, Tounsi Moufida¹, Hammami Majdi¹, Aneva Ina², Evstatieva Ljuba³, Jebara Moez³, Borji Manel³, Danova Kalina³

¹Center of Biotechnology Borj Cedria, Hammam Lif – 2050, Tunisia, e-mail address: limam_ferid@yahoo.fr

²Institute of Organic Chemistry with Centre of Phytochemistry, BAS, 1113 Sofia, Bulgaria, e-mail address: k_danova@abv.bg

³Institute of Biodiversity and Ecosystem Research, BAS, 1113 Sofia, Bulgaria

Methicillin-resistant *Staphylococcus aureus* (MRSA) causes skin and soft tissue infections, as well as toxin-associated diseases such as toxic shock syndrome. It is characterized by occurrence of strains highly resistant to antibiotics¹. *Listeria monocytogenes* causes infections of the central nervous system, and bacteremia in immunocompromised and elderly people. *Bacillus cereus* is a food-borne bacterium². The activity against these three pathogens was tested by the disc diffusion method of the total hexane (Hex), methanolic (Met) and chloroform (Chl) extracts, as well as ethyl acetate (EtOAc), butanol (BuOH) and water (W) fractions of the methanolic extracts of *in vitro* cultured *Hypericum perforatum*, *H. richeri*, *H. rumeliacum*, *Sideritis scardica* and *Inula britannica*, collected in Bulgaria, as well as *Lavandula dentata*, collected in Tunisia. The highest activities against MRSA had *H. richeri* (EtOAc), *Inula britannica* aeriols (Hex) and *S. scardica* (Chl). Most active against *B. cereus* were the (Chl) extracts of aeriols of *I. britannica* and *H. perforatum* (as well as the EtOAc of the latter), and against *L. monocytogenes* highest activity had the (Chl) extracts of *H. perforatum*, *H. richeri* and *L. dentata*. Of all (BuOH) fractions, activity was exhibited only by the aeriols of *I. britannica* against MRSA. With the detection limit of method applied, (MeOH) extracts and (W) fractions did not exhibit notable activity. Further study is in process to elaborate on different test systems to probe more polar extracts and fractions of the studied species.

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BIOTECHNOLOGICAL DELIVERY OF PHYTOPHARMACEUTICALS OF MEDICINAL PLANTS GERmplasm OF THE BALKAN REGION

Kalina Danova¹, Evelyn Wolfram², Milka Todorova³, Antoaneta Trendafilova¹, Sashka Krumova¹, Václav Motyka⁴, Petre Dobrev⁵, Yuliana Markovska⁵, Krassimira Idakieva¹, Yuliana Raynova¹, Tonya Andreeva³, Beat Meier²

¹Institute of Organic Chemistry with Centre of Phytochemistry, BAS, 1113 Sofia, Bulgaria

²Institut für Biotechnologie, Zurich University of Applied Sciences, 8820 Wädenswil, Switzerland

³Institute of Biophysics and Biomedical Engineering, BAS, 1113 Sofia, Bulgaria

⁴Institute of Experimental Botany, CAS, 165 02 Prague 6, Czech Republic

⁵Faculty of Biology, Sofia University St. Kl. Ohridski, 1164 Sofia, Bulgaria

The aim of the work was the complex biotechnological development of medicinal and aromatic plants characteristic for the Balkans. *Hypericum*, *Pulsatilla*, *Sideritis*, *Inula britannica* and *Artemisia* species were collected from Bulgaria. Tissue culture initiation, maintenance and development were based on modification of vitamin content, plant growth regulators and agar supplementations and light regime treatments. Essential oils were prepared by micro-steam distillation of fresh material, and characterized by GC-MS chromatography. Extracts were purified and compounds identified by chromatographic and spectroscopic techniques, respectively. Endogenous plant hormones were studied by LC/MS. HPTLC fingerprinting coupled with DPPH assays was applied in order to assess secondary metabolite and active compound content simultaneously. Polyphenolic contents, enzymatic activities, molecular markers of oxidative stress were measured spectrophotometrically. Structural and functional alterations of photosynthetic membranes were characterized by 77 K fluorescence spectroscopy, electrophoretic profile and enzymes were characterized by 10 % SDS-PAGE and zymography. Overall 67 shoot accessions in solid medium, 8 genetically non-transformed roots and 8 suspension lines in liquid culture were developed. Marker compounds with phytopharmaceutical potential identified and confirmed *in vitro* and optimizations performed for the stimulation of antioxidant compounds content. Approaches were suggested for the delivery of essential oils with defined terpenoid profile, based on morphogenesis, physiological status and endogenous hormonal production. Obtained results will be used for scientifically based targeted delivery of plant material with defined secondary metabolite profile in bioreactor system.

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A REVIEW OF THE ANTIINFLAMMATORY AND ANTITUMOR POTENTIAL OF ASTERACEAE SPECIES

Camelia P. Stefanache¹, Evelyn Wolfram², Kalina Danova³, Adriana Trifan⁴, Doina Danila¹

¹ National Institute of R&D for Biological Sciences/ "Stejarul" Biological Research Centre, Piatra Neamt, Romania

² Zurich University of Applied Sciences, Institute of Biotechnology, Waedenswil, Switzerland

³ Bulgarian Academy of Sciences, Institute of General and Inorganic Chemistry

⁴ Faculty of Pharmacy, "Gr. T. Popa" University of Medicine and Pharmacy, Iasi, Romania

The Asteraceae family comprises a large number of medicinal species, traditionally used for their hepatoprotective, antioxidant, antibacterial and anti-inflammatory properties. Phytochemical investigations of the Asteraceae family have revealed a vast variety of bioactive compounds (triterpenoids, flavonoids, phenols, coumarines, volatile oil, sesquiterpen-lactones). In the recent years, several Asteraceae species were tested for the antiinflammatory and antitumor activities.

Studies on *Artemisia* species showed good antitumor activity of the extracts, extractive fractions and isolated compounds when tested on different human cancer cell lines¹. The crude extract of *Calendula* showed promising antitumor activity on tumor cell lines derived from various types of human cancer. *In vitro* antiproliferative activities of *Inula* sesquiterpen-bearing fraction against human gastric and cervix cancer cell lines are reported². Studies on the sesquiterpen-lactones fractions of *Arnica* showed apoptotic and antimetastatic activity in several types of cancer, including human melanoma³.

Inflammation is involved in the etiology of a multitude of disorders such as rheumatoid arthritis, cardiovascular diseases etc. Studies on Asteraceae species led to the identification of several potent antiinflammatory agents that might be useful in the management of inflammatory and related diseases.

In the context of major medical breakthroughs, the treatment of cancer is still limited. Studies on plant bioactive compounds might lead to the development of new management strategies for cancer and inflammatory diseases. Previous screenings highlighted that Asteraceae species might be potential candidates for the isolation of new therapeutic agents.

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POLYPHENOLICS PRODUCTION IN *ARTEMISIA ALBA* IS RELATED TO DEVELOPMENTAL PATTERNS AND ENDOGENOUS STRESS HORMONES *IN VITRO*

*Evelyn Wolfram*¹, *Samuel Peter*¹, *Vaclav Motyka*², *Petre Dobrev*², *Milka Todorova*³, *Ljuba Evstatieva*⁴, *Kalina Danova*³, *Antoaneta Trendafilova*³

¹ Zurich University of Applied Sciences, Institute of Biotechnology, Wädenswil, Switzerland

² Institute of Experimental Botany, Czech Academy of Sciences, Prague, Czech Republic

³ Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

⁴ Institute for Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria

Though many studies have been dedicated to characterization of essential oil content of the fragrant shrub *Artemisia alba* Turra, scarce information exists on the content of non-volatiles in this plant. In the present work *in vitro* cultures of the plant were treated with plant growth regulators in order to affect its developmental patterns¹. UPLC-PDA-QDa analysis was used for quantitative and qualitative determination of flavonoids and phenolic acids, as twelve flavonoids and 5 phenolic acids were identified based on their UV and MS data as well as by comparison with authentic standards. Amount of flavonoids was expressed as mg quercetin equivalents per gram dry plant material, and of phenolic acids – as mg 3,5-DCQA per gram dry plant material. Luteolin was found to be the main flavonoid, 3,5-DCQA is the major phenolic acid. Total phenolics and flavonoids, malondialdehyde and hydrogen peroxide were assayed spectrophotometrically, endogenous stress hormones (salicylic acid, as well as jasmonic acid and its conjugates) were quantified by LC/MS². It was established that inhibition of rooting and stimulation of callusogenesis *in vitro* were related to inhibition of the production of some main flavonoids, but led to elevation of phenolic acid content, drop of both stress hormones and preservation of oxidative stress and lipid peroxidation levels, as compared with non-treated control. The results imply the role of lower molecular polyphenolics in maintaining physiological status of the plant *in vitro*.

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BIOPROCESS CONTROL OF IN VITRO CULTURES OF SELECTED MEDICINAL PLANTS FROM THE BALKAN REGION USING PHYTOCHEMICAL HPTLC FINGERPRINTS COMBINED WITH ENZYMATIC BIOAUTOGRAPHY

*Evelyn Wolfram*¹, *Sarah Bräm*¹, *Beat Meier*¹, *Kalina Danova*²

¹ Zürich University of Applied Sciences, Phytopharmacy Research Group, 8820 Wädenswil, Switzerland

² Institute of Organic Chemistry Centre of Phytochemistry, Bulgarian Academy of Science, Sofia, Bulgaria

HPTLC is an analytical tool of long term tradition in quality control of medicinal plant extracts. Separated compounds are fixed on the solid silica phase like a compound library. By direct performance of visualizable enzyme reactions on the HPTLC plate, this compound library can also be used for activity screening.

Enzymatic bioautographic assays using Xanthine Oxidase (XOD)¹, Acetylcholinesterase (AChE)² and β -Glucosidase³ were optimized and applied for screening of less studied medicinal plants extracts and fractions from conventional and *in vitro* cultivation. HPTLC standardized fingerprint analysis was performed with automated equipment from (CAMAG, Muttenz) and HPTLC plates (Merck, Darmstadt).

Some inhibitory spots were identified as false positive results due to artefacts occurring when HPTLC plates were detected in remission mode after bioautographic analysis. Some compounds have the potency to imitate potential active constituents that need to be differentiated from real enzyme inhibitors. Furthermore phenolic compounds and tannins can react with Fast Blue B (FBB) salt to form brown coloured bands that are difficult to assess.

Nevertheless extracts of *in vitro* cultured *Sideritis scardica* and *Pulsatilla slaviankae* showed to contain active compounds that are able to inhibit AchE and β -glucosidase. AchE inhibitors were moreover identified in *Clinopodium vulgare* whereas *Pulsatilla montana* showed potential XOD inhibiting compounds.

It can be concluded that bioautographic enzyme assays offer a rapid and simple tool for screening of secondary metabolite profiles for potential health beneficial activities, indeed active spots need to be examined critically.

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**ANTIOXIDANT DEFENSE AND POLYPHENOLIC PRODUCTION IN
ARTEMISIA ALBA SHOOT CULTURES**

*Yuliana Raynova*¹, *Krassimira Idakieva*¹, *Yuliana Markovska*², *Evelyn Wolfram-Schilling*³,
*Kalina Danova*¹

¹*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*

²*Faculty of Biology, Sofia University "St Kliment Ohridski", Sofia, Bulgaria*

³*Zürcher Hochschule für angewandte Wissenschaften (ZHAW), Life Sciences und Facility Management -
Institut für Biotechnologie, Wädenswil, Switzerland*

Artemisia alba Turra is a fragrant shrub used in traditional medicine¹. The aim of the present work is to assess its *in vitro* capacity as a potential phytopharmaceuticals producer and to study the impact of exogenously applied plant growth regulators (PGR) on the protein profile and the enzyme activities in *A. alba* shoot cultures.

In vitro shoot cultures were initiated as previously described¹. Soluble proteins were extracted from fresh plant material and the protein content of aerial, root and callus samples of *A. alba* was measured according to Bradford. Relative abundance of major proteins and their molecular masses was analyzed by SDS-PAGE. Enzyme activities were estimated spectrophotometrically and by zymography^{2,3}. Polyphenolics were assayed spectrophotometrically and by CAMAG HPTLC equipment and software.

Aerials were rich in protein content (~0.01 g/g fresh leaf) compared with underground parts (~0.005 g/g fresh tissue). Marked differences in their electrophoretic profile were observed. Application of PGR affected the activities of antioxidant enzymes: superoxide dismutase, catalase, ascorbat peroxidase, guaiacol peroxidase and glutathione reductase. Changes in the polyphenol content and molecular markers of lipid peroxidation and oxidative stress also were observed.

Results imply, that the interplay between enzymatic and non-enzymatic defense in *A. alba in vitro* is related to the auxin: cytokinin ratio, as well as to the concentrations of the regulators applied.

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