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

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

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

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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 patterns[1]. 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 (salycilic acid, as well as jasmonic acid and its conjugates) were quantified by LC/MS[2]. 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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References:

[1] Danova K, Todorova M, Trendafilova A, Evstatieva L (2012) Natural Product Communications 7: 1-2.
[2] Dobrev P, Vaňková R (2012) In: Shabala S, Cuin TA (Eds) Plant Salt Tolerance: Methods and Protocols, Methods in Molecular Biology, vol. 913. Humana Press, Springer Science + Business Media, New York Heidelberg Dordrecht London, pp. 251-261.