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EFFECT OF TISSUE CULTURE ON THE FLAVONOID PROFILE AND ENDOGENOUS AUXIN AND CYTOKININ LEVELS IN *ARTEMISIA ALBA TURRA IN VITRO*

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UPLC-PDA-QDa was used for analysis of flavonoids in field collected *A. alba* aerals and blossoms. Comparison was done with the *in vitro* cultured plant. Endogenous cytokinins and auxins were analyzed by LC/MS. Tissue culture generally lowered flavonoid amounts *in vitro* as compared with the field collected material. However, flavones/flavonoles ratio seemed to be strongly affected by *in vitro* developmental patterns. Thus, plants with suppressed rooting and intensive callusogenesis (in cytokinin (CK) and combined auxin and cytokinin treatments) exhibited a significant drop in the latter parameter, this profile being similar to the one of the field collected samples. On the other hand, rooting in control and auxin treated plants was related to considerably higher flavone/flavonole ratio. Then, callusogenesis *in vitro* was related to a drop of the sums of the CKs *trans*-Zeatin, dihydrozeatin and isopentenyl adenine free forms and conjugates and a rise in *cis*-Zeatin, as well auxin conjugates indole-3-acetyl acid-aspartate and oxo-indole-3-acetic acid. The variability in secondary metabolites production is strongly dependent on the physiological response of plants to stimuli of their surrounding environment. Therefore, understanding these processes could be a key tool for the targeted production of desired plant secondary metabolites by plant cell and tissue culture.

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**POLYPHENOLIC CONTENT AND BIOLOGICAL ACTIVITY OF
IN VITRO CULTIVATED *SIDERITIS SCARDICA* L.**

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Sideritis scardica L. is a Balkan endemic species, widely utilized in indigenous traditional medicine for treatment of pulmonary ailments, infections and as a wound healing remedy. The low germination capacity and high collection pressure *in situ* are a challenge in finding complementary approaches for the delivery of raw plant material for fundamental studies and secondary metabolite delivery of this plant. Shoot cultures of the plant were established of surface sterilized seeds, collected at Shabran peak, Slavianka Mountain (germination within two weeks was 1%) and maintained on Murashige and Skoog medium with Gamborg vitamins supplementation. Further on, auxin (benzyl adenine) and cytokinin (naphthalene acetic acid) were applied in order to obtain *in vitro* multiplication. Total phenolics and flavonoids contents were assayed spectrophotometrically. Optimized HPTLC procedures for Lipase and acetylcholinesterase (AChE) assays were applied. Plant growth regulators (PGR) significantly stimulated *in vitro* multiplication and survival as compared with PGR-free media. Plant growth regulators free, as well as media modifications with auxin exceeding cytokinin concentrations were shown to be superior in terms of phenolic and flavonoid production *in vitro*. The extract showed to exhibit profound inhibitory activity in the bioautographic assays (with one and 2-4 components with probable Lipase and AChE inhibition activity, respectively). Further research is in process for the elucidation of the chemical nature of components exhibiting enzymatic activity inhibition.

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PHYTOCHEMICAL STUDY ON *INULA BRITANNICA* L. OF BULGARIAN ORIGIN

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Inula britannica is used in Chinese folk medicine to treat digestive disorders, bronchitis and inflammation, bacterial and viral infections, some types of cancer. More than 40 sesquiterpene lactones some which biologically active have been isolated so far. With exception of Serbian ones, other European populations of the species have not been studied in details.

From the studied Bulgarian taxa of this species, five sesquiterpene lactones - pulchellin C, gaillardin, britannin, 11,13-dihydroinuchinenolide B, and ivalin were isolated. In addition, three triterpenoids - 3-O-palmitates of 16 α -hydroxylupeol, 16 α -hydroxy- β -amyrin, and faradiol, known esters of the corresponding triterpene diols as well as luteolin, quercetin, luteolin-7-O-Glu, 1,5-dicafeloylquinic acid were found. For isolation, classical CC and PTLC techniques were used, and structures proved by spectral methods. The absence of secoguaianolides in Bulgarian *I. britannica* populations is a similar feature with other European populations of the species, where these compounds have not been established at all. Noteworthy, the lack of secoeudesmanolides distinguished Bulgarian *I. britannica* from the Serbian one. The literature survey revealed that gaillardin, britannin and pulchellin C among the described in this work lactones possess significant antitumor activity. Britannin was shown to be the main lactone in the studied samples, followed by gaillardin.

Additionally, total phenolic and flavonoid contents in flowers and leaves were determined by spectrophotometric methods. Antioxidant capacity was assessed by measurement of DPPH and ABTS radical scavenging activity. The most active methanol extract from flowers correlated with the highest amount of phenolic compounds.

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