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BOOK OF ABSTRACTS

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BIOACTIVE COMPOUNDS IN NATIVE, IN VITRO OBTAINED, AND *EX VITRO* ADAPTED PLANTS OF *CENTAUREA DAVIDOVII* URUM.

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C. davidovii Urum. (*Asteraceae*) is a Bulgarian endemic plant, occurring only in Stara planina Mt and is represented by few and very fragmented populations. The development of a biotechnological method for *in vitro* propagation of the species and subsequently *ex vitro* acclimatation is an approach for the conservation of this endangered species. The purpose of this study was to evaluate the polyphenol, flavonoid and sesquiterpene lactone content in plant extracts derived from native, *in vitro* obtained and *ex vitro* adapted plants of *C. davidovii*.

In vitro cultures were initiated from seeds of *C. davidovii.* Whole plantlets were regenerated and cultivated for several months on agar-solidified nutrient media differing by their composition: basal MS medium, MS medium with half-strength macrosalts, MS media supplemented with plant growth regulators (0.5 mg/l BAP, or 1.4 mg/l BAP and 3.5 mg/l TDZ), and liquid MS medium. Plantlets were *ex vitro* adapted and successfully acclimated to open-air conditions, where flowering was observed in some individuals in the first spring. The contents of the total flavonoids and the total phenols were determined spectrophotometrically, separately in the leaves and the roots of the in vitro plantlets, and then compared to those in the leaves of the native plants. The lactone 8α-(5'-hydroxyangeloyl)-salonitenolide was determined by HPLC in leaf samples of *C. davidovii* native plants, *in vitro* obtained plantlets and *ex vitro* acclimated plants. The composition of the nutrient medium influenced the contents of all studied bioactive substances; however, the differences were more important, according to the plant organs. The highest lactone concentration was detected in the leaves of the flowering acclimated plants: 44.1 mg/g DW, which was several times more than in the other leaf samples.

The obtained results revealed that the *ex vitro* acclimated *C. davidovii* could be a source of sesquiterpene lactones with potential biological activity as well as the application of biotechnological methods for propagation and conservation of rare and endangered species.

Keywords: *Centaurea*, *in vitro* cultures, sesquiterpene lactones, flavonoids, polyphenols

POTENTIAL OF *I. BRITANNICA* FOR BIOTECHNOLOGICAL PRODUCTION OF BIOLOGICALLY ACTIVE SESQUITERPENE LACTONES

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Inula britannica is a biregional element (Euro-Siberian and Irano-Turanian regions) widely distributed in Western Europe and Turkey, extending eastward to China through Iran and Pakistan. It is an important plant species used in Traditional Chinese Medicine (TCM) and Kampo Medicines. It is known as 'Xuan Fu Hua' in TCM. It is also known as British yellowhead or meadow fleabane. Its decoction is used as antibacterial, carminative, diuretic, laxative, stomach, tonic remedies, and for treating asthma, hepatitis and tumors. Major bioactive secondary metabolites in *I. britannica* are sesquiterpene lactones. In the present study the capacity of in vitro cultivated *I. britannica* to accumulate sesquiterpene lactones characteristic of the intact plant was investigated. Plant material was collected from the region of Western Balkan mnt. in Bulgaria. *In vitro* culture was initiated by surface sterilization of stem segments of the plant in 0.5 benzyl adenine supplemented medium. After axillary shoots formation stock cultures were further maintained in pant growth regulators free medium. The aerial parts of the wild collected plant afforded pulchellin C, gailiardin, britannin, ivalin and 11,13-dihydro-inuchinanolide. Their structures were determined by spectral methods. The isolated compounds were used as references for characterization of the sesquiterpenoid lactones profile of in vitro culture derived plant material. Qualitative similarity of the sesquiterpene lactone profiles of the intact plant and in vitro derived material was shown by TLC using sulfuric acid spray reagent for visualization. These results are an indication of the potential of I. britannica as a biotechnological source of controlled delivery of valuable phytopharmaceuticals.

Keywords: *I. britannica*, sesquiterpene lactones, in vitro culture, bitechnologiacl delivery of phytopharmaceuticals

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BIOTECHNOLOGICAL APPROACHES FOR THE TARGETED DELIVERY OF ESSENTIAL OILS WITH DEFINED COMPOSITION FROM ARTEMISIA ALBA TURRA SHOOT CULTURES

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The essential oil of the fragrant shrub *A. alba*, is characterized by high variability which has been attributed by different authors to ecological, climatic or genetic factors. The established spasmolytic and antimicrobial activities of the oil have motivated our research to develop biotechnological approaches for the controlled delivery of raw plant material with targeted terpenoid profile of its essential oil. Previously it was established that plant growth regulators (PGR) treatment strongly influenced the terpenoid profile of the oils *in vitro*. In continuation of this work, we developed a broader experimental model for affecting essential oil content in PGR free and benzyl adenine (BA) and indole-3-butyric acid (IBA) treatments individually and in combination. Essential oils were obtained by micro-steam distillation-extraction. Identification and quantification was performed by GC and GC/MS techniques. Essential oils were compared based on the ratios between the total monoterpenoid and sesquiterpenoid contents in them (M/S ratio). As a result three distinctive in vitro culture systems for essential oil production were obtained. While different combinations between IBA and BA led to an M/S ratio of 1.8 – 2.7, the treatment with each of the two regulators separately, led to a drop of sesquiterpenoids in the oils. Thus individual BA treatment increased 1.5 times the levels of sesquiterpenoids in the oils and IBA treatment increased these compounds even further to a level of M/S 5.2. Interestingly, PGR free control displayed similar terpenoid profile to IBA treated plants and all described effects were weakly dependent on the concentrations of the applied PGR. Further research is in progress to evaluate the potential of the developed *in vitro* systems for non-volatile extractable biologically active compounds.

Keywords: *Artemisia alba*, *in vitro* culture, plant growth regulators, essential oil variability, terpenoid profile

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