

## Abstracts

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# Role in terpenoid biogenesis of plant growth regulators in *Artemisia alba* Turra shoot cultures

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## Further Information

Congress Abstract

Full Text

*Artemisia alba* Turra is characterized by a variability of the terpenoid components of its essential oil, due to environmental, climatic and genetic factors [1]. Previously we established that auxin and cytokinin treatments affected its essential oil profile *in vitro* [2]. Here, relations between its terpenoid profile, thylakoid and chloroplast morphology and photosynthetic apparatus structure with endogenous cytokinin levels were studied, in response to exogenous plant growth regulators (PGRs) treatments. It was established that irrespectively of the different indole-3-butyric acid (IBA) and benzyladenine (BA) modifications applied, essential oils of the aerials of *A. alba* demonstrated two general terpenoid profile types based on their monoterpenoid/sesquiterpenoid ratios. Thus, plants with well-expressed rooting and normally represented chloroplast morphology (PGR-free control and IBA treated) expressed elevated levels of primary metabolites – 81.4% (0.5 mg/l IBA) and 144% (1.0 mg/l IBA) of bioactive cytokinins sum, while in plants with suppressed rooting (0.5 mg/l IBA + 0.2 mg/l BA and 1.0 mg/l IBA + 0.2 mg/l BA) this parameter dropped to 51.8% and 63.3%, respectively (sum of bioactive CKs in controls was accepted as 100%). Similar dependencies were observed for secondary metabolites. Monoterpenoid/sesquiterpenoid ratio dropped more than twice in the second group of plants, where also alterations of PSII and chloroplast were recorded. Knowing that monoterpenoid biogenesis is spatially bound to chloroplasts in the plant cell [3], the observed relations might be an indication of the possible mediatory role of endogenous CKs in terpenoid biogenesis by means of affecting thylakoid morphology and alteration of the efficacy of biogenetic pathways, spatially situated in the chloroplast.

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**Keywords:** *Artemisia alba* shoot cultures, plant growth regulators, endogenous cytokinins, terpenoid profile, chloroplast.

## References:

- [1] Radulović N, Blagojević P. Volatile profiles of *Artemisia alba* Turra from contrasting serpentine and calcareous habitats. *Nat Prod Commun* 2010; 5: 1117 – 1122
- [2] Danova K, Todorova M, Trendafilova A, Evstatieva L. Cytokinin and auxin effect on the terpenoid profile of the essential oil and morphological characteristics of shoot cultures of *Artemisia alba*. *Nat Prod Commun* 2012; 7: 1 – 2
- [3] Bouvier F, Suire C, d'Harlingue A, Backhaus RA, Camara B. Molecular cloning of geranyl diphosphate synthase and compartmentation of monoterpene synthesis in plant cells. *Plant J* 2000; 24: 241 – 252

## Abstracts

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# HPTLC and HPTLC-coupled Bioassays for Bioprocess Control of Medicinal Plant In Vitro Cultures from the Balkan region

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## Further Information

Congress Abstract

Full Text

An *in vitro* germplasm collection has been established for the delivery of biologically active compounds of medicinal and aromatic plants of the Balkans. Representatives of the species *Pulsatilla montana*, *Pulsatilla slaviankae*, *Pulsatilla halleri* and *Clinopodium vulgare* have been subjected to a media optimization study by means of alteration of plant growth regulators treatments. The aim of this study was to demonstrate the suitability of HPTLC phytochemical fingerprints combined with the assessment of antioxidant (DPPH and  $\beta$ -Carotene/Linolenic Acid Bleaching HPTLC assay) as well as optimized Xanthine Oxidase [1] and Lipase [2] inhibiting HPTLC assays (enzymatic bioautography). For *Pulsatilla* species, a mixture of ethyl acetate, formic acid, acetic acid and water (68/8/6/18) and for *Clinopodium vulgare* a slight modification (68/6/8/18) has been identified for optimal separation. Chlorogenic acid and rutin were applied as references. Total polyphenolic content [3] of the cultures was assessed spectrophotometrically as a sum parameter for one class of target secondary metabolites. Phytochemical fingerprints with adequate derivatisation (Flavonoids and Anisaldehyde-Sulfuric acid) demonstrate visual differences in number and nature of *in vitro* formed substances as well as differences in colour fluorescence at 366nm or visible zones in white light. Compared to control vouchers from *ex situ* samples exhibiting also yellow zones, *in vitro* cultures of *Pulsatilla* sp. show only a large number of blue phenolcarboxylic acids zones. *In vitro* cultures of *C. vulgare* exhibit also two faint yellow zones in *in vitro* samples, one at the R<sub>f</sub> value of rutin. Inhibition zones of oxidant and enzyme reactions corresponding to phytochemical zones at the comparable R<sub>f</sub> heights render valuable rapid and cost effective data and facilitates the control and optimization of the plant *in vitro* culture processes. *C. vulgare* culture showed compounds with Xanthine Oxidase and Lipase inhibition. In conclusion, the adapted HPTLC separation for *Pulsatilla* sp and *Clinopodium vulgare* combined with rapid activity screening demonstrate the potential of HPTLC and its hyphenated bioassay methods as a relatively easy and feasible *in-process* control tool. The obtainable data is the basis for further optimization.

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**Keywords:** HPTLC, bioprocess control, bioassay-coupled-HPTLC, bioautography, *in vitro* plant culture.

## References:

- [1] Ramallo IA, Zacchino SA, Furlan RLE. A Rapid TLC Autographic Method for the Detection of Xanthine Oxidase Inhibitors and Superoxide Scavengers. *Phytochem Anal* 2006; 17: 15 – 19
- [2] Hassan AMS. TLC Bioautographic Method for Detecting Lipase Inhibitors. *Phytochem Anal* 2012; 23: 405 – 407
- [3] Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L *BMC Complement Altern Med* 2012; 12: 221 – 233

## Abstracts

Georg Thieme Verlag KG Stuttgart · New York

# Biotechnological yield of phytopharmaceuticals of valuable Balkan medicinal plants

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## Further Information

Congress Abstract

Full Text

*In vitro* germplasm collection has been developed for the controlled delivery of biologically active compounds of medicinal and aromatic plants of the Balkans. Representatives of the *Hypericum* and *Pulsatilla* genera, *Sideritis scardica*, *Inula britannica*, *Artemisia alba* were collected from Bulgaria [1]. Overall 71 shoot accessions in solid medium, 8 genetically non-transformed roots and 8 suspension lines in liquid culture were developed. *In vitro* culture modifications were performed based on vitamin content, plant growth regulators, agar supplementations, active charcoal and light regime treatments. Total polyphenolic contents were assayed spectrophotometrically. HPTLC fingerprinting coupled with DPPH assays was applied in order to assess secondary metabolite and active compound content. Plant extracts were purified and compounds with phytopharmaceutical potential identified by chromatographic and spectroscopic techniques, respectively. Essential oils were prepared by micro-steam distillation of fresh material, and characterized by GC-MS chromatography. Based on the conducted optimizations, three different *in vitro* systems were established for production of essential oils with modified terpenoid profile for *A. alba* (with high, medium or low values of the monoterpenoid/sesquiterpenoid ratio in the oils); high mountainous *H. richeri* was selected as a superior producer of hypericins and the hypericin non-producing *H. calycinum* – as a producer of polyphenolics with radical scavenging activity *in vitro*; biotechnological delivery of sesquiterpene lactones gailardin and britannin of *I. britannica* was obtained. The obtained processes were suggested for up-scale in RITA temporary immersion bioreactor system.

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**Keywords:** Balkan medicinal and aromatic plants, *in vitro* germplasm collection, secondary metabolites, RITA temporary immersion system.

## References:

- [1] Todorova M, Trendafilova A, Krumova S, Idakieva K, Genova V, Markovska Y, Raynova Y, Evstatieva L, Wolfram E, Danova K. Interdisciplinary interaction for the biotechnological development of Balkan medicinal plant species. Proceedings book of the Seminar of Ecology 2014, 24 – 25 April, Union of Scientists, Bulgaria 2015; 95 – 98

## Abstracts

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# Effect of activated charcoal on the developmental patterns, polyphenolics productivity and photosynthetic activity of *Sideritis scardica* in vitro

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## ► Further Information

Congress Abstract

Full Text

*Sideritis scardica* is a Balkan endemic species traditionally used for the treatment of pulmonary emphysema and angina pectoris [1]. It is threatened in half of the countries of its occurrence (less than 2,500 mature individuals in Bulgaria and over 250 in Serbia) [2]. The intensive collection pressure has imposed the necessity of its conservation by means of *in situ* and *in vitro* approaches. Tissue culture of the plant has been initiated by surface sterilization of its seeds, collected in the Slavjankae Mountain in Bulgaria. As a part of a broader program for the biotechnological delivery of phytopharmaceuticals with antioxidant activity of this plant, modifications of organic and inorganic factors of the culture medium have been experimented. Here we present the results of the effect of activated charcoal (AC) on *S. scardica* shoot cultures. Low (0.02, 0.05) as well as high (0.2 and 0.5 g/l) concentrations has been applied. It was established that the higher AC concentrations (0.2 and 0.5 g/l) led to a profound stimulation of axillary shoot formation, enlargement of leaf area and stimulation of polyphenolic production. The values obtained for photosystem II quantum yield revealed that the control (untreated) and all carbon-treated variants were physiologically fit. However, a slower apparent electron transport rate for 0.2 and 0.5 g/l treated plants was established, as compared with the control and 0.02 and 0.05 g/l treated ones. It appears that the production of higher leaf mass and the increase in leaf area in 0.2 and 0.5 g/l treated *S. scardica* is a compensatory reaction/mechanism that allows for more efficient light utilization. The remarkable effect of activated charcoal on biosynthetic capacity and physiological status of the plant, without the addition of organic supplements such as plant growth regulators, seems to be a prospective approach in tissue culture optimization of the plant *in vitro*.

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**Keywords:** Balkan endemic *Sideritis scardica*, *in vitro*, activated charcoal, photosynthetic activity, polyphenolics production, developmental patterns.

## References:

- [1] Ivancheva S, Stantcheva B. Ethnobotanical inventory of medicinal plants in Bulgaria. J Ethnopharmacol 2000; 69: 165 – 172
- [2] The IUCN Red List of Threatened Species, <http://www.iucnredlist.org/details/203271/0>