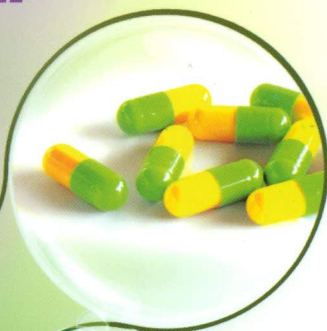


# 4<sup>th</sup> INTERNATIONAL CONFERENCE ON NATURAL PRODUCTS UTILIZATION

## FROM PLANTS TO PHARMACY SHELF



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## TARGETED BIOTECHNOLOGICAL PRODUCTION OF CYTOTOXIC FLAVONOIDS IN BALCAN ENDEMIC *SIDERITIS* *SCARDICA* GRIESB

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Shoot cultures of Balkan endemic *Sideritis scardica* were initiated and treatments with plant growth regulators (PGR) and activated charcoal (AC) were performed. Cytotoxicity of the ethyl acetate and butanol fractions of the methanolic extract of commercial samples of *S. scardica* was tested on MCF7 (human breast adenocarcinoma cell line) and compared with the same preparations of the *in vitro* cultured plant in PGR-free medium. Chemical characterization of the samples was performed by LC/MS analysis.

While the ethyl acetate fraction exhibited marked cytotoxic activity, no negative effect on cell growth, as compared with the control non-treated cells, was recorded for the butanol preparation of the commercial sample. All *in vitro* samples, cultivated in the control medium, showed weak cytotoxicity against the tested adenocarcinoma cell line.

Chemical analysis demonstrated that cytotoxicity of the most active ethyl acetate fraction of the commercial sample was related to its enrichment in flavone derivatives and especially with the predominance of apigenin, hypolaetin and isoscutellarein glycosides, combined with significant drop of phenylethanoids. On the contrary, the butanol fraction was highly enriched in phenylethanoids, present together with the flavones.

Interestingly, AC treatments stimulated significantly biomass and flavonoids (luteolin, apigenin and hypolaetin derivatives) as compared with PGR application.

The results demonstrate the high cytotoxicity of flavones, and are indicative of the possible cytoprotective effect of phenylethanoids in *Sideritis scardica* extract. The conducted biotechnological experiment led to the achievement of differential stimulation of phenolics productivity *in vitro*, which could be a promising approach in obtaining plant biomass with desired properties.



## CURRENT STATE OF KNOWLEDGE ABOUT METABOLITE PROFILE AND BIOLOGICAL ACTIVITIES OF *THYMUS* SPP. IN EUROPE: A REVIEW

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Genus *Thymus* comprises more than 250 species of perennial herbaceous or fruticose plants, classified into 8 sections. Total 66 species with numerous subspecies and varieties are listed in Flora Europaea. Due to the taxonomic complexity and difficult identification of the species the studies focused on a relatively small part of the existing diversity. Usually samples of different species are bulked together for the purposes of the pharmaceutical industry, and they are designated as *Thymus* spp., which approach does not take into account the peculiarities in the phytochemical composition of the different species. The objective of the review is to make a survey on the phytochemical investigations and biological activities of the thymes occurring naturally in Europe. The major part of the studies focused on the determining of essential oil composition of the species. Very typical phenomenon for the genus is the chemical polymorphism, which is due both to the ecological factors, and to genetic variation. Phenolic compounds were studied to a lesser extent. A correlation was established between the phenolic content and antioxidant activity of extracts with different polarity. Besides antioxidant activity, antibacterial activity has received particular attention in many experimental designs.

The most frequently investigated species are relatively few, while the majority of thyme species in Europe still remain poorly studied, or not studied at all. This concern especially the endemic species distributed in remote and hardly accessible areas.

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## SECONDARY METABOLITES PRODUCTIVITY IN *INULA BRITANICA* IS RELATED TO BIOMASS FORMATION AND PHYSIOLOGICAL ADAPTATION IN TISSUE CULTURE CONDITIONS

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*Inula britannica* is widely distributed throughout Western Europe and Turkey, reaching China through Iran and Pakistan. The species is of great importance for Traditional Chinese Medicine as well as Kampo medicine in Japan. Decoction of aerals or blossoms is used for treating asthma, and as an expectorant. Flowers are used as antibacterial, carminative, diuretic, laxative, stomach, tonic, rapid-healer, for hepatitis and tumors.

Shoot cultures of the plant were developed from material collected in Bulgaria. An experiment for elucidation of the combined effect of vitamins (Murashige and Skoog vs. Gamborg) and auxin and cytokinin treatments on secondary metabolite productivity, biomass formation and physiological status of shoot cultures was conducted.

Gamborg vitamins considerably stimulated plant growth as compared with Murashige and Skoog ones. Further on, the addition of benzyl adenine as cytokinin stimulated biomass formation in terms of axillary rosettes formation, however it suppressed aerial and root length and caused callusogenesis at explant base, this effect being slightly alleviated by 2-Naphthylacetic acid addition. Gamborg vitamins supplementation stimulated polyphenolics and total sesquiterpene lactones content *in vitro*. Interestingly, plant growth regulators affected polyphenolics and sesquiterpene lactones britannin and gaillardin ratio in a different way depending on the vitamins added. Biomass formation and secondary metabolite productivity were also found to be dependent on oxidative stress and photosynthetic pigments content of the cultures. The results are indicative of the relations between growth, development and secondary metabolite production in the plant organism.

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## PHOTOSTIMULATION OF POLYPHENOLICS PRODUCTIVITY IN *ARTEMISIA ALBA* CELL AGGREGATES IN LIQUID MEDIA

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As a part of a broader program for biotechnological development of *Artemisia alba*, non-differentiated cell aggregates were developed and grown in liquid media. Lines were initiated from sterile leaf explants and root cuttings, the first ones characterized by significantly higher lipid peroxidation and oxidative stress. Lines were maintained in liquid media supplemented with N<sup>6</sup>-benzyladenine (BA) in combination with either indole-3-butyric (IBA) acid or 2-Naphthylacetic acid (NAA).

Light treatment stimulated polyphenolics production and increased stress hormones salicylic, abscisic and jasmonic acid. Interestingly, light affected differentially zeatin levels in relation to the initial explants from which they were initiated. Thus, while *trans*-zeatin content was generally enhanced by light treatment in the leaf-initiated lines, the *cis*-zeatin content was lowered in the same samples. This effect was media-dependent for root-initiated lines.

The initial physiological status also affected the morphology and biosynthetic capacity of the resulting lines. Thus, leaf initiated cell aggregates were heterogenous and to a higher degree non-differentiated, as compared with the ones initiated from root cuttings. This was accompanied by a higher polyphenolics productivity of the root-initiated lines. In addition NAA grown lines were more compact and with larger size as compared with IBA grown ones. This was also related to higher biosynthetic capacity of the NAA-grown lines. Qualitative characterization of the polyphenolics is in process in order to assess the potential of the obtained system as a prospective source of phytotherapeutics.

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## ROOTING AS A DECISIVE FACTOR AFFECTING ENDOGENOUS HORMONOME AND BIOSYNTHETIC CAPACITY OF *ARTEMISIA ALBA* TURRA *IN VITRO* MODEL

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*In vitro* culture model characterized by enhanced or suppressed rooting in essential oil bearing *Artemisia alba* Turra was developed with the aim to better understand interrelations between *in vitro* rooting and biochemical processes occurring in this plant.

Two main morphotypes were developed based on modification of cytokinin (*N*<sup>6</sup>-benzyladenine) and auxin (indole-3-butyric acid) supplementation, either alone or in combination. Comparison was made based on the study of essential oil profile, polyphenolics levels, as well as endogenous hormone *in vitro*.

It was revealed that normally rooting plants were characterized by elevated monoterpenoid/sesquiterpenoid ratio of their essential oil profile, higher salicylic and abscisic acid contents, bioactive cytokinin concentrations and *trans/cis*-zeatin ratio on one hand and by lowered polyphenolics levels and decreased jasmonic acid amounts on the other hand as compared with root suppressed plants.

The plant organism consists of different organs, which are constituted of different tissues, build up by cells. Production of secondary metabolites and phytohormones as well as their accumulation and translocation are dependent on the presence and communication between the highly specialized anatomical structures within the plant organism. Root morphogenesis was shown to play an important role in both biosynthetic features as well as physiology of *in vitro* cultured *A. alba* model, which could be applied in targeted cultivation of plant material with desired quality of this species.

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