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P65. Effect of *in vitro* culture on the content of biologically active sesquiterpene lactones in *Inula britannica* shoots

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

Inula britannica is widely distributed in Western Europe and Turkey, extending eastward to China through Iran and Pakistan [1,2]. The plant known as 'Xuan Fu Hua', British yellow head, or meadow fleabane is an important plant species used in Traditional Chinese Medicine (TCM) and Kampo Medicines. Its decoction is utilized as antibacterial, carminative, diuretic, laxative, stomach, tonic remedies, and for treating asthma, hepatitis and tumours [3]. Major bioactive secondary metabolites in *I. britannica* are sesquiterpene lactones [3].

Recently, it has been found that *in vitro* cultivated *I. britannica* produced sesquiterpene lactones characteristic of the intact plant [4]. These results prompted us to study the effect of culture medium composition on the biosynthetic capacity of the plant *in vitro*.

For optimization of sesquiterpene lactone production vitamin supplementation (Murashige and Skoog and Gamborg), as well as plant growth regulators (benzyl adenine and 1-Naphthalene acetic acid) were modified in overall 10 treatments. Plant extracts were obtained from air-dried and ground plant material by extraction with chloroform. The quantity of the main sesquiterpene lactones in the studied extracts was determined by GC/MS. Based on the obtained results up-scale of sesquiterpene production by cultivation of the plant in liquid culture bioreactor system is under development.

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P195. Biotechnological delivery of medicinal plant material indigenous to the Balkan region with antioxidant and acetylcholinesterase inhibitory activity

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Keywords: PhytoBalk project, HPTLC, acetylcholinesterase assay

Protocols for the *in vitro* cultivation of selected medicinal and aromatic plants of the Balkan region have been established within the frames of the Bulgarian-Swiss joint research PhytoBalk project. Cultures of various genera such as *Pulsatilla*, *Hypericum*, *Sideritis*, *Inula*, *Clinopodium* and *Artemisia* have been successfully cultivated and their secondary metabolite content had to be assessed with suitable bioprocess control assays.

HPTLC is an analytical tool of long term tradition in quality control of medicinal plants extracts. Separated compounds are fixed on the solid silica phase like a compound library. By direct coupling of visualizable antioxidant and enzyme reactions on the HPTLC plate, this compound library can also be used for activity screening. The enzymatic bioautographic Acetylcholinesterase (AChE) assay was optimized and applied for screening of conventional and *in vitro* cultured medicinal plants from the Balkan region [1]. Antioxidant properties were evaluated using HPTLC fingerprints combined with DPPH assay. Some inhibitory spots in the bioautographic AChE assay were identified as false positive results depended on the stationary phase and the detection method used. Some compounds have the potency to imitate potential active constituents that could be differentiated from real enzyme inhibitors by using a control assay [2]. A correct assignment of active compounds was possible with an adapted bioautographic AChE procedure and a control assay.

Nevertheless, extracts of *in vitro* cultured *Sideritis scardica* and different *Hypericum* species showed to contain active compounds that are able to inhibit AChE. Antioxidant properties were identified in *Clinopodium vulgare*, different *Pulsatilla* species and *Sideritis scardica*. It can be concluded that the bioautographic AChE assay offer a rapid and simple tool for screening of secondary metabolite profiles for potential AChE inhibitory activities, indeed active spots need to be examined critically.

Acknowledgements: BSRP, grant No. IZEBZ0, 142989; D02-1153

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LMP6. Sustainable utilization of medicinal and aromatic plants by means of conventional and tissue culture breeding techniques

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Keywords: *Anthriscus cerefolium*, *Levisticum officinale*, *Satureja hortensis*, essential oils, *ex situ* conservation

Anthriscus cerefolium, Apiaceae, is traditionally utilized as an expectorant and bitter tonic. *Levisticum officinale* Koth, Apiaceae is a perennial plant native of Europe, known as carminative and spasmolytic folk medicine. *Satureja hortensis*, Lamiaceae has culinary, decorative, and medicinal applications; its tea being traditionally applied for treatment of cramps, muscle pains, nausea, indigestion, diarrhea and infections.

The crops were established at the farm of SEKEM at Bilbase Sharkia, as well as in Minya Governorate Gharib farm (situated at 500 km to the South) in Egypt. Dominant components of the essential oils, determined by GC/MS were methyl-eugenol, estragole and 2,4-dimethoxyallylbenzene in *A. cerefolium*, β -phellandrene and α -terpinylacetate in *L. officinale* and carvacrol, γ -terpinene and α -cimene in *S. hortensis*. The oil of *S. hortensis* showed higher capacities in scavenging free radicals (243.55 \pm 0.17 and 55.60 \pm 0.60 μ M Trolox equivalents/g oil, by ABTS and DPPH methods, respectively) than those of *L. officinale* essential oil (25.79 \pm 0.40 and 3.80 \pm 0.54, respectively), most probably determined by the presence of phenolic functional group in the molecule of carvacrol. Climatic conditions affected the qualitative characteristics of the oils, as Minya Governorate cultivated *S. hortensis* displayed elevated carvacrol/ γ -terpinene ratio and *A. cerefolium* – higher estragole and 2,4-dimethoxyallylbenzene levels, as compared with the Bilbase Sharkia cultivated plants. Sterile germination was performed in the dark in half-strength Murashige and Skoog medium, supplemented with Gamborg vitamins, 2 g/l glycine and 20 g/l sucrose in the dark. Germination percentage within a three weeks period was 52.54, 39.62 and 51.16% for *A. cerefolium*, *L. officinale* and *S. hortensis*, respectively. Adaptation and cultivation of medicinal and aromatic plants in less fertile and high salinity areas is of great importance for the sustainable utilization of the climatic conditions and the promotion of novel crops with therapeutic potential for the climatic conditions of Egypt.

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LMP7. Bioautographic enzyme assays and antioxidant polyphenolics screening of biotechnologically derived medicinal and aromatic plants of the Balkan region

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Keywords: medicinal and aromatic plants of the Balkans, *in vitro* production of phytopharmaceuticals, Xanthine Oxidase, Glucosidase, RITA temporary immersion system

An *in vitro* collection of medicinal and aromatic plants of the Balkan region has been established within the frames of the Bulgarian-Swiss joint research PhytoBalk project. Species included representatives of the *Hypericum*, *Pulsatilla*, *Sideritis*, *Inula*, *Artemisia* and *Clinopodium* genera. Optimizations were carried out, based on different approaches such as modification of plant growth regulators and vitamin treatments, regeneration through different explant types, application of activated charcoal, as well as light regime, solid and liquid medium cultivation. Spectrophotometric determination of phenolics and flavonoids was utilized for the rapid and cost effective feed-back to applied optimizations. HPTLC fingerprinting coupled with DPPH assays was applied in order to assess secondary metabolite and active compound content simultaneously. Enzymatic bioautographic assays using Xanthine Oxidase (XOD) and Glucosidase were optimized and applied. Within the *Hypericum* representatives studied, Balkan endemic *H. rumeliacum*, as well as the high mountainous *H. richeri* were selected as superior producers of hypericins and the hypericin non-producing *H. calycinum* demonstrated the highest polyphenolics levels, as well as highest radical scavenging activity *in vitro*. *Pulsatilla* sp., as well as *Clinopodium vulgare* were shown to possess the highest activity in XOD assays and extracts of *in vitro* cultured *Sideritis scardica*, *Pulsatilla slevianikae* and *Inula britannica* showed to contain active compounds that are able to inhibit effectively glucosidase. Based on the results demonstrated, a differentiated culture in liquid medium based on RITA temporary immersion system is being developed.

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