AMAPSEEC

Association for Medicinal and Aromatic Plants of Southeast European Countries

9th CMAPSEEC

Conference on Medicinal and Aromatic Plants of Southeast European Countries



26-29 May 2016
Plovdiv, Bulgaria

PP 158

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 16â-hydroxylupeol, 16â-hydroxy-â-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 pulchelin 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.

Acknowledgments:
Swiss National Science Foundation (BSRP, grant No. IZEBZ0_142989; DO2-1153)

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PHYTOCHEMICAL, ANTIOXIDANT, CYTOTOXIC AND ANTITUMOR SCREENING OF DIFFERENT *INULA OCULUS-CHRISTI* EXTRACTS FROM BULGARIA

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Species from genus *Inula* (Asteraceae) are known as herbal medicines. Continuing our research on *Inula* species, growing in Bulgaria we have studied chloroform and methanol extracts of *I. oculus-christi* leaves and flowers and their antioxidant, cytotoxic and antitumor properties.

Preliminary phytochemical screening revealed that aerial parts of this species contained triterpenyl palmitates, â-sitosterol, a sesquitepene lactone (gaillardin), flavonoids (luteolin, 6-methoxyluteolin, 5,6,7-trihydroxy-4'-methoxyflavone, apigenin and desmethoxycentaureidin) and mono- and dicaffeoylqunic acids. The highest amount of phenolics was found in methanol extracts of both flowers and leaves (68.34±0.66 and 66.95±1.13 mgGA/g dry extract, resp.), while flowers were richest in flavonoids (42.63±0.75 mg/g). The antioxidant potential was evaluated by DPPH and ABTS assays (mMol Trolox /g dry extract). A good correlation was observed between DPPH and ABTS radical scavenging activity and phenolic/flavonoid content in the studied extracts. The most active was the methanol extract from flowers, for which was found to contain flavonoids and phenolic acids.

Cytotoxicity on normal (MDCK II) and cancer (A549) cells of the extracts was also tested and cell viability was assessed by crystal violet staining after 24 and 48 hrs. Studied extracts showed potential anti-cancer effects. Most prominent results were obtained by chloroform extracts (rich in gaillardin) causing more severe cell damages. In both cell types different alterations were observed, indicating interaction of some components of the extracts with different signal pathways into normal and cancer cells. Revealing the exact mechanisms of action into cells require identification of different components into extracts and cell components they interact with.

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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* aerials 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-acetyc acid-aspartate and oxo-indole-3-acetic acid. The variability in secondary metabolites production is strongly dependent on the 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.

Acknowledgements: Bulgarian-Swiss Research Program (BSRP, grant No. IZEBZO_142989; DO2-1153); Joint Scientific Research Project between the CAS and BAS (reg. No. 29); the Czech Science Foundation (16-14649S). World Federation of Sciences – Bulgarian National Scholarship Programme for young researchers.