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BIOGENETIC RELATIONSHIP BETWEEN SOME *INULA* SPECIES GROWING IN BULGARIA BASED ON THEIR PHYTOCHEMICAL PROFILE

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Inula (Asteraceae) is a large genus with more than 100 species distributed in Africa, Asia and Europe. *Inula helenium* L., *Inula racemosa* Hook.f. and *Inula britannica* L. are frequently used in ethnomedicine. *Inula* species are continuously the target of scientific investigations to find their active principles and new pharmacological applications, or from chemotaxonomical point of view. Recently we have started phytochemical investigation of *Inula* species growing in Bulgaria. Extracts from *I. helenium*, *I. aschersoniana* Janka, *I. britannica* L., *I. oculus-christi* L., *I. germanica* L., *I. conyza* (Griess.) Meikle, *I. ensifolia* L. and *I. bifrons* (L.) L. were studied. It was found that the methanol extracts of all of them contained chlorogenic, 1,5-, 3,5- 3,4 and 4,5-dicaffeloylquinic acids. Thus, these compounds could not be used as chemotaxonomic markers in this case. Further, the lack of sesquiterpene lactones in *I. conyza*, *I. ensifolia*, and *I. bifrons* distinguished these species from the other four. With exception of *I. germanica* sesquiterpene lactones were studied in *I. helenium* (alantolactone, isoalantolactone), *I. aschersoniana* (parthenolide, diepoxycostunolide, inusoniolide), *I. britannica* (ivalin, britannin, gaillardin, 11,13-dihydroinuchinenolide B, pulchellin C), *I. oculus-christi* (gaillardin, pulchellin E, pulchellin C, 9β,10β-epoxygaillardin, 9α,10α-epoxy-2-epi-gaillardin, 2α-acetoxy-4α,9β-dihydroxy-1β-guaia-11(13),10(14)-dien-12,8α-olide, 4α,15α-epoxypulchellin E). It has been found that species produced different type of lactones: 12,6-olides in *I. aschersoniana* and 12,8-olides in the other 3 species. Another significant feature for *I. aschersoniana* was the production of germacranolides and a 4,5-secoguaianolide. Eudesmanolides (ivalin and its C-3 oxygenated derivatives- pulchellin B and C) and guaianolides (gaillardin and biogenetically related to it) isolated from *I. britannica* and *I. oculus-christi* showed their similarity. The eudesmanolides found in *I. helenium* indicated that it is closer to the latter two species. The obtained results could help in determining the place of these species within the general scheme of the genus.

Key words: *Inula*, Asteraceae, sesquiterpene lactones, biogenetic relationship

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GENUS *INULA* L. IN BULGARIA – DISTRIBUTION AND NATURAL HABITATS

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Genus *Inula* is represented by 12 species in Bulgaria. Most of them are widely distributed across the country and three species are considered rare. One species – *I. spiraeifolia* L. is protected by the Biodiversity Act and is included in the Red Data Book of Bulgaria. According to their ecological characteristics, the species can be subdivided into four groups. The first group includes species occurring on limestone in open places (for example, *I. oculus-christi* L. and *I. aschersoniana* Janka). Frequently they are part of the habitats 62A0 Eastern sub-mediterranean dry grasslands, 8210 Calcareous rocky slopes with chasmophytic vegetation and 5210 Arborescent matorral with *Juniperus* spp. The second group consists of species which are found mostly on calcareous soils but occur also under wide range of ecological conditions (for example, *I. bifrons* /L./ L., *I. conyza* L.). The third group includes only one species – *I. ensifolia* L. which is found exclusively in forest habitats (mostly 9530 (Sub-) Mediterranean pine forests with endemic black pines and 95A0 High oro-Mediterranean pine forests). However, other species can also be found in the forests, especially in the peripheral parts (for example, *I. helenium* L.). The fourth group includes the most tolerant species, occurring in variety of environmental conditions, like *I. britannica* L., *I. germanica* L. and *I. hirta* L. The results of the study are discussed in the light of the species conservation and sustainable use.

Key words: *Inula*, Asteraceae, medicinal plants, habitats

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ANTIOXIDANT, CYTOTOXIC, ACETYLCHOLINESTERASE AND TYROSINASE ENZYME INHIBITORY ACTIVITIES OF SIX *INULA* SPECIES FROM BULGARIA

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Continuing our research on *Inula* species, growing in Bulgaria we have focused our attention on phenolic constituents and antioxidant, cytotoxic and enzyme inhibitory activity of methanol extracts of six *Inula* species - *I. aschersoniana* Janka., *I. conyza* DC., *I. ensifolia* L., *I. oculus-christi* L., *I. bifrons* (L.) L. and *I. germanica* L. HPTLC was used for qualitative analysis of phenolic acids. Total phenolic content was determined by Folin–Ciocalteu method. The free radical scavenging potential was assessed against $DPPH^{\cdot}$ and $ABTS^{+\cdot}$ radicals. Acetylcholinesterase inhibition was evaluated using Ellman's method, while tyrosinase inhibition - by the microtiter plate assay reported by Masuda (2005). The cytotoxic activity (10 to 300 μg mL^{-1}) and analysis of cell morphology were determined by crystal violet assay. Only the *I. oculus-christi* and *I. aschersoniana* exstracts reached IC50 for the malignant cells (A549) on 48 h, while viability was around 75% for the non-cancer ones (MDCK II). HPTLC comparison of the methanol extracts with commercial standards showed presence of chlorogenic, 1,5-, 3,5-, 3,4- and 4,5-dicaffeoylquinic acids. *I. ensifolia*, *I. conyza* and *I. germanica* (119.92 ± 0.95, 96.87 ± 0.85, 85.81 ± 1.01 mg GAE gE^{-1}, resp.) contained the highest amounts of phenolics and demonstrated the highest percentage of inhibition of DPPH radicals at concentration of 200 μg mL^{-1} (69.41 ± 0.55, 68.58 ± 0.52 and 60.17 ± 0.3%, respectively). *I. bifrons* and *I. germanica* extracts were found to be the most active *Inula* samples with TEAC values 0.257 ± 0.012 and 0.242 ± 0.011 mg mL^{-1}, respectively. These two samples also showed the best inhibitory potential against tyrosinase enzyme (IC50 0.123 ± 0.0 and 0.159 ± 0.032 mg mL^{-1}, resp.). All tested extracts demonstrated weak anti-AChE activity (Inh % 4 - 17). A good correlation between total phenolic content and antioxidant, acetylcholinesterase and tyrosinase enzyme inhibitory activities was observed by calculating Pearson's correlation coefficients.

Key words: *Inula*, Asteraceae, phenolic acids, biological activity

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EVALUATION OF INHIBITORY POTENTIAL OF *LIGUSTICUM ALATUM* (Bieb.) Sprengel ESSENTIAL OILS AND EXTRACTS AGAINST ENZYMES INVOLVED IN ALZHEIMER DISEASE AND TYPE II DIABETES

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In the present work the essential oils, hexane and methanol extracts obtained from the leaf and fruits of *Ligusticum alatum* (Bieb.) Sprengel were investigated for chemical composition, free radical (DPPH$^{\cdot}$ and ABTS$^{+\cdot}$) scavenging potential and inhibitory effects against acetylcholinesterase (from *Electrophorus electricus*) and porcine pancreatic α-amylase. Acetylcholinesterase inhibition of the samples was evaluated using Ellman's method. Antidiabetic potentials of the extracts and the oils were evaluated via measurement of inhibition of α-amylase enzyme using KI/I2 method. Epoxy-trans-pseudoisoeugenyl angelate (22.4%), (E)-anethole (13.8%), caryophyllene oxide (8.7%), β-caryophyllene (6.3%) and γ-himachalene (6.6%) were detected in the leaf. In the fruits, (Z)-β-farnesene (50.3%), (E)-anethole (13.2%), epoxy-trans-pseudoisoeugenyl angelate (12.8%), zizanene (5.8%), γ-himachalene (4.9%) and α-zingiberene (4.9%) were found as major constituents. The essential oils and the extracts of *L. alatum* demonstrated anti-acetylcholinesterase activity ranged between 43% and 58%. The highest TEAC values were obtained for the extract obtained with methanol (2.47 ± 0.18 mM) and the essential oil (1.78 ± 0.11 mM) from the leaf. The extracts demonstrated weak inhibitory effect (Inh. % up to 20.63 ± 0.37) towards to α-amylase. The present work is the first contribution about biological potential of *Ligusticum alatum* fruit and leaf extracts.

Key words: *Ligusticum alatum*, composition, α-amylase, acetylcholinesterase, antioxidant

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PHENOLIC PROFILE, ANTIOXIDANT, ACETYLCHOLINESTERASE AND TYROSINASE ENZYME INHIBITORY ACTIVITIES OF ENDEMIC *JURINEA TZAR-FERDINANDII* Davidov

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Jurinea tzar-ferdinandii Davidov (genus *Jurinea*, Asteraceae family) is a Balkan endemic plant with limited distribution in Bulgaria and Romania. Recently, we have started phytochemical investigation of this species. From the chloroform extract obtained from the aerial parts of *J. tzar-ferdinandii* were isolated and identified 26 compounds, belonging to three main groups of secondary metabolites: triterpenoids, sesquiterpene lactones and nonpolar flavonoids. Continuing our research on this species, we have focused our attention on the phenolic constituents, antioxidant and enzyme inhibitory activities of the methanol extract. CC and PTLC of the methanol extract obtained from aerial parts led to isolation and identification of apigenin, luteolin, apigenin-7-O-glucoside, apigenin-4'-O-glucoside, apigenin-7-O-gentiobioside, luteolin-4'-O-glucoside, rutin and narcissin, chlorogenic acid and 1,5-dicaffeoylquinic acid. Structures of all compounds were elucidated using spectral methods (NMR, MS and UV). Antioxidant activity of the extract was tested against ABTS+• radical cations using TEAC (Trolox equivalent antioxidant capacity) assay. Acetylcholinesterase and tyrosinase inhibitions of the sample were evaluated using Ellman's method and the microtiter plate assay reported by Masuda, respectively. The measured TEAC value of the extract was 0.259 ± 0.008 mg mL^{-1}. The methanol extract demonstrated weak anti-AChE activity (8.3 Inh %) and low inhibitory potential against tyrosinase enzyme (IC50 0.208 ± 0.008 mg mL^{-1}). The present work is the first report on polar phenolic constituents and biological activity of the J. tzar-ferdinandii methanol extract.

Key words: *Jurinea tzar-ferdinandii*, flavonoids, phenolic acids, biological activity

GENUS *THYMUS* IN BULGARIA – A NEW PROJECT AIMED AT REVEALING OF SPECIES' METABOLITE PROFILE AND GENETIC DIVERSITY

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Genus *Thymus* comprises more than 250 species of perennial herbaceous or fruticose plants, classified into eight sections. Total of 66 species with numerous subspecies and varieties are listed in Flora Europaea. Twenty species occur naturally in Bulgaria. One of them is endemic to the country (*Thymus perinicus* /Velen./ Jalas), four are endemics to the Balkan Peninsula (*T. albanus* Heinr. Braun ex Wettst., *T. comptus* Griseb., *T. longedentatus* /Degen & Urum./ Ronniger, *T. stojanovii* Degen) and other three are sub-endemics (*T. atticus* Čelak., *T. moesiacus* Velen., *T. sibthorpii* Benth.). The studies on the genetic variation at species and within-species level will help the elucidation of some unresolved taxonomic issues and at the same time will serve as characteristics of the genetic resources of the species. The species of genus *Thymus* provoke substantial interest worldwide from phytochemical point of view, due to their diverse biological activities with potential for application in pharmaceutical, cosmetic and food industries. They have been used since the ancient times to treat diseases of the respiratory and digestive system, as well as of colds. They possess expectorant, antiseptic, fungicide, spasmolytic, carminative, sedative, diaphoretic and diuretic activity. The main objective of the present project is to perform a complete genetic and phytochemical study on the species of genus *Thymus* in Bulgaria. Distinguishing the taxa according to their morphology, chorology, ecological and genetic characteristics, metabolite content, essential oil composition, expression of antioxidant activity will allow composition of a general picture of the species of genus *Thymus* in Bulgaria. Results of the studies will be a basis for determining the place of each species within the general scheme of the genus. The information gathered will serve as a scientific basis for the initial stages of cultivation of the prospective (with best phytochemical characteristics) species, as well as the endangered ones.

Key words: Thyme, diversity, taxonomy, active compounds

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