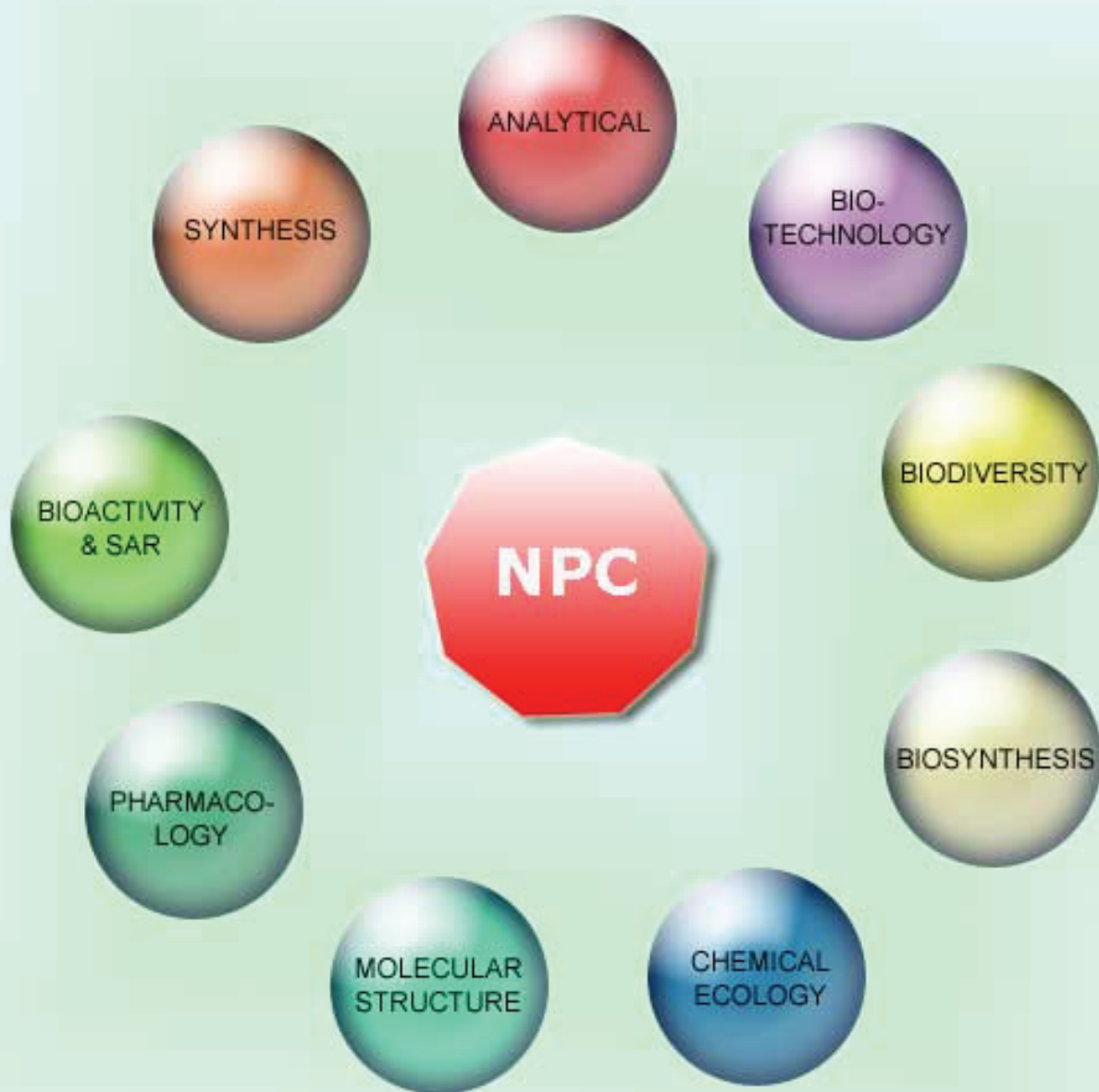


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## Phytochemical Profile of *Inula britannica* from Bulgaria

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The flower heads of *Inula britannica* L. of Bulgarian origin afforded sesquiterpene lactones (gaillardin, britannin, 11,13-dihydroinuchinenolide B, ivalin, pulchellin C), triterpenoids (3-*O*-palmitates of 16 $\beta$ -hydroxylupeol, 16 $\beta$ -hydroxy- $\beta$ -amyrin, and faradiol) and flavonoids (quercetin, luteolin, luteolin-7-*O*-glucoside). All compounds are known and they were identified by spectral methods. The observed differences in the chemical content of the chloroform and methanol extracts were also reflected in their free radical scavenging activity, evaluated by DPPH and ABTS assays. Intraspecific variability of *I. britannica* is discussed.

**Keywords:** *Inula britannica*, Asteraceae, Sesquiterpene lactones, Triterpenoids, Flavonoids, DPPH and ABTS assays.

The genus *Inula* (Asteraceae) consists of approximately 100 species, distributed mainly in Asia and Europe. Many studies have been carried within the genus due to the significant structural diversity and biological activity of sesquiterpene lactones, diterpenes, triterpenes, and flavonoids, isolated from a number of *Inula* species [1,2]. *I. britannica* is used in Chinese folk medicine to treat digestive disorders, bronchitis and inflammation, bacterial and viral infections, as well as some tumors [2b]. That is why most of the studies are on *I. britannica* of Chinese origin. The application in traditional medicine requires extensive phytochemical study and pharmacological evaluations of this species. It should be noted that sesquiterpene lactones were the largest group. More than 40 lactones of different skeletal types – germacranolides, eudesmanolides, guaianolides, secoeudesmanolides, secoguaianolides, and lactone dimers, have been described so far. To the best of our knowledge, from the European taxa only Russian [3] and Serbian [4] have been investigated up to now.

In continuation of our studies on species of the Asteraceae family this work is a part of a phytochemical investigation of Bulgarian taxa from the genus *Inula*. The CHCl<sub>3</sub> extract of the flowers of *I. britannica* was fractionated by column chromatography on silica gel. IR control allowed selection of the fractions (characteristic absorption band at 1770-1740 cm<sup>-1</sup>) for further determination of the lactone profile. Thus, the sesquiterpene lactones ivalin [5], britannin [3a,4], gaillardin [4,5a], 11,13-dihydroinuchinenolide B [4], and pulchellin C [3b,4] have been isolated and identified. All these compounds have been previously found in *I. britannica* of different origins. With exception of ivalin, the other compounds were reported for the Serbian taxon [4]. From the latter one, 1,10-secoeudesmanolides – 14-(3-methylpentanoyl)-6-deoxybritannilactone, 14-(3-methylbuta-noyl)-6-deoxybritannilactone, and 14-(2-methylpropanoyl)-6-deoxybritannin, as well as 4H-tomentosin have been isolated. Varieties of lactones with secoeudesmane and secoguaiane skeletons have been reported before in the Asian populations of *I. britannica* [1,2]. It should be noted that no one of these substances was detected in the studied Bulgarian sample.

It was found (IR control) that the less polar fractions did not contain sesquiterpene lactones, but the absorption band at 1720 cm<sup>-1</sup> showed the presence of compounds bearing ester groups. Further on, prep. TLC yielded the known triterpenoids 3-*O*-palmitates of 16 $\beta$ -hydroxylupeol, 16 $\beta$ -hydroxy- $\beta$ -amyrin, and faradiol. Only the first one has been found in a Turkish population of *I. britannica* so far [6a], while the other two compounds are isolated now for the first time from this species, but they have been detected earlier in *Achillea alexandri-regis* [6b] and *Calendula officinalis* [6c], respectively.  $\beta$ -Sitosterol and  $\beta$ -amyrin were also detected. More or less, different triterpenoids have been isolated from the genus *Inula*, but esters of long-chain fatty acids have been detected only in one Turkish population of *I. britannica* [6a]. It should be noted that the literature data for triterpenoids in *Inula* species are insufficient for discussion of their chemotaxonomic significance.

Besides the described above compounds, 3 flavonoids and 1,5-dicafeloylquinic acid were isolated from the MeOH extract. Luteolin, luteolin-7-*O*-glucoside and quercetin are known for *I. britannica*, while 1,5-dicafeloylquinic acid was detected in *I. viscosa* [2]. Being very common plant components and usually in high concentrations, at this point of the investigation, these compounds were not regarded as compounds of chemotaxonomic interest.

Further, TLC comparison of extracts from flowers and leaves showed that both plant organs accumulated the same sesquiterpene lactones. Regarding phenolic components, it was found that luteolin, luteolin-7-*O*-glucoside, and 1,5-dicafeloylquinic acid were present in both leaves and flowers, while quercetin was detected only in flowers. Finally, the observed differences in the chemical composition of the chloroform and methanol extracts were also reflected in their free radical scavenging activity, evaluated by DPPH and ABTS assays (Table 1). As can be seen, both methanol extracts (from flowers and leaves) possessed the highest antioxidant activity and maximal total phenolic and flavonoid contents. A good correlation between antiradical activity in the studied extracts and their total phenolic content was also observed ( $R^2 = 0.9954$  and  $R^2 = 0.9910$  for ABTS and DPPH, respectively).

**Table 1:** Total phenolic (TPC) and flavonoid (FC) contents in different *I. britannica* extracts and their radical scavenging activity.

Plant parts	Extract	TPC [mgGA/gDM]	FC [mgC/gDM]	ABTS [μMT/gDM]	DPPH [μMT/gDM]
Leaves	CHCl <sub>3</sub>	0.4±0.02	0.3±0.02	1.6±0.1	0.7±0.1 <sup>a</sup>
Flowers	CHCl <sub>3</sub>	0.1±0.02	0.1±0.02	1.1±0.1	0.7±0.02 <sup>a</sup>
Leaves	MeOH	2.3±0.1	0.6±0.01	15.5±0.2	13.7±0.7
Flowers	MeOH	7.9±0.4	0.9±0.02	44.4±0.4	37.6±0.6

<sup>a</sup>Values with the same letter are not significantly different,  $p \leq 0.05$ . Standard deviations were calculated on the base of three samples.

Diversity of lactones found in the so far studied taxa of *I. britannica* of Asian and European origin revealed significant intraspecific variability. Nevertheless, on the basis of the skeleton type of lactones it could be suggested that secoeudesmanolides and secoguaianolides characterized the Asian populations. On the other hand, Bulgarian and Russian (European) populations are free of these types of lactones. Further, secoeudesmanolides and one secoguaianolide have been isolated from a Serbian taxon, but bicyclic lactones are principal components. So, the existence of secoeudesmanolides and secoguaianolides in the European *I. britannica* cannot be ruled out. Thus, the domination of guaianolides and eudesmanolides or their seco-derivatives characterize European and Asian taxa, respectively. Further phytochemical investigation on *I. britannica* will clarify the lactone profile of European populations.

## Experimental

**Plant material:** *I. britannica* was collected from a natural locality in the Southern Balkan Region in Bulgaria. A voucher specimen (SOM 172474) was deposited in the Herbarium of the Institute of Biodiversity and Ecosystem Research, Bulg. Acad. Sci.

**Extraction and isolation:** A portion (1 g) of air-dried flower heads and leaves of *I. britannica* were extracted with CHCl<sub>3</sub> and MeOH. The corresponding crude extracts were compared by TLC (Silica gel 60 and RP-18, F<sub>254</sub> (Merck), CHCl<sub>3</sub>-diethyl ether, 50:1; CHCl<sub>3</sub>-acetone, 10:1 and 1:1; MeOH-H<sub>2</sub>O, 1:1) and used for determination of the total phenolics, flavonoids and antioxidant capacity. Further, the CHCl<sub>3</sub> and MeOH extracts from flower heads (30 g) of the plant were worked up for isolation of the individual compounds. The CHCl<sub>3</sub> extract (1.5 g) was fractionated by column chromatography (CC) on silica gel using CHCl<sub>3</sub>-acetone mixtures with increasing

polarity to give 11 fractions (F<sub>1</sub>-F<sub>11</sub>). Further separation by CC and prep. TLC (silica gel, *n*-hexane-diethyl ether, 5:1) of Fr. F<sub>2</sub> (50 mg) afforded 3-*O*-palmitates of 16β-hydroxyulpeol (5.7 mg), 16β-hydroxy-β-amyirin (5.3 mg), and faradiol (5.0 mg). The presence of β-amyirin and β-sitosterol was proved by TLC of F<sub>3</sub> (silica gel, *n*-hexane-diethyl ether, 1:1) using these compounds as standards. Prep. TLC (silica gel, CHCl<sub>3</sub>-acetone, 5:1) of F<sub>7</sub> (47 mg) afforded ivalin (7 mg) and britannin (14 mg). Gaillardin (33 mg) and pulchellin C (18 mg) were obtained from F<sub>9</sub> (65 mg) and F<sub>11</sub> (92 mg), resp. after recrystallization (CHCl<sub>3</sub>). Prep. TLC (silica gel, CHCl<sub>3</sub>-acetone, 5:1) of F<sub>10</sub> (18 mg) yielded 11,13-dihydroinuchinenolide B (7 mg). The MeOH extract (0.5 g) was separated into 2 fractions F-1 and F-2 by CC (Sephadex LH-20, MeOH). Prep. TLC (silica gel RP-18, MeOH-H<sub>2</sub>O, 1:1) of F-2 (50 mg) yielded luteolin (9 mg), quercetin (1.8 mg), luteolin-7-*O*-glucoside (2.0 mg), and 1,5-dicafeloylquinic acid (6.4 mg). All isolated compounds were proved by comparison of their <sup>1</sup>H NMR spectral data with those in the literature.

**Determination of the content of total phenolic compounds (TPC) and total flavonoids (FC):** TPC and FC were determined by procedures described in ref. [7a,b] and expressed as mg gallic acid equivalents per g dry plant material [mgGA/gDM] and mg (+)-catechin equivalents per g dry plant material [mgC/gDM], respectively.

**DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity and ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)] assays:** The DPPH and ABTS assays were performed according to the procedures described by Thaipong *et al.* [7c] and the antioxidant activity was expressed as μM Trolox equivalents per g dry plant material [μMT/gDM].

**Statistical analysis:** Correlation coefficients ( $R^2$ ) for determination of the relationship between the radical scavenging activity and the TPC and FC were calculated using MS Excel software.

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