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Terpenoids in four *Inula* species from Bulgaria

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Abstract: Phytochemical study of the chloroform extract of the aerial parts of *Inula germanica* L., *I. ensifolia* L., *I. conyza* (Griess.) DC. and *I. salicina* L. led to the identification of 33 terpenoids. *β*- and *α*-amyrin, lupeol, taraxasterol, *ψ*-taraxasterol and their 3-*O*-acetates and 3-*O*-palmitates were identified by GC/MS. In addition, the structures of 3-*O*-palmitates of mainaladiol, arnidiol, faradiol and 16-hydroxylupeol were confirmed by NMR. *ent*-Kaur-16-en-19--oic acid and its 15*α*-(3-methylpentanoyloxy) and 15*α*-(3-methylbutanoyloxy) derivatives were isolated from *I. conyza*. Ten closely related sesquiterpene lactones (germacranolides and melampolides) were found in *I. germanica* and their structural identification was performed by spectral analyses. *I. ensifolia* and *I. salicina* were free of sesquiterpene lactones and diterpenoids. All triterpenoids and diterpenoids, grazielia acid, desacetylovatifolin and 8-(2-methylbutanoyloxy)-1(10),4,11(13)-germacrutrien-6,12-olide-14-oic acid are described for the first time in the studied species. The principal component analysis was used to find a relationship between the investigated up to now *Inula* species, growing in Bulgaria.

Keywords: *Inula*; triterpenoids; diterpenoids; sesquiterpene lactones; PCA.

INTRODUCTION

Genus *Inula* (Asteraceae) includes more than 100 species distributed mainly in Africa, Asia and Europe. The genus is paraphyletic and heterogeneous concerning several diverse characters, which makes determining taxa difficult.[1,2] *Inula* species are an inexhaustible source of new chemical diversity and approximately 500 secondary metabolites have been identified to date, some of which with relevant pharmacological activity, such as antiproliferative, antiviral, antibacterial, antifungal, anti-inflammatory, antitumour, cytotoxic, antiprotozoal,

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etc.[3–6] Undoubtedly, the most important and representative compound classes of secondary metabolites in this genus are sesquiterpene lactones. However, further important metabolites were also found, *e.g.*, thymol and chlorogenic acid derivatives, sterols and flavonoids. Sesquiterpene lactones possess a common biosynthetic origin, and are useful chemical characters for differentiating subtribes within several major tribes, or to study infra- and intraspecific variability in certain genera of Asteraceae family.[7]

So far, the phytochemical studies of *Inula* species, growing in Bulgaria, namely *I. oculus-christi*,[8,9] *I. britannica*,[10] *I. aschersoniana*[11] and *I. bifrons*[12] revealed the presence of various terpenoids, among which the most distinctive was the group of sesquiterpene lactones. Continuing our research on *Inula* species, herein we describe the terpene constituents of four *Inula* species – *Inula germanica* L., *I. ensifolia* L., *I. conyza* (Griess.) DC. and *I. salicina* L. The species selected for the study are the representatives of two groups according to the classification of Anderberg.[2] *Inula salicina*, *I. germanica* and *I. ensifolia* belong to the *Inula salicina* group, diagnosed by xeromorphic leaves and with ligulate peripheral flowers, 1–1.5 as long as the involucre. *Inula conyza* is a part of *Inula decurrens* group. While *I. ensifolia* and *I. conyza* are clearly distinct morphologically, the identification of *I. germanica* and *I. salicina* possesses more difficulties. *Inula ensifolia* is characterized by lanceolate and acute leaves with the parallel venation, and *I. conyza* – by much wider leaves and very short (up to 1 mm) ligulae of the ray flowers. *I. germanica* can be distinguished from *I. salicina* by the shorter ligulae, equal to or slightly exeding the involucrum, and by forming many flower heads (capitulae) usually, while *I. salicina* is characterized by ligulae clearly longer than the involucrum, and by the smaller number of the flower head (one to few). Therefore, the comparison of their chemical content could bring additional insights into their relationships.

EXPERIMENTAL

General

Column chromatography was carried out on Silica gel 60 (230–400 mesh, Merck). Thin-layer chromatography (TLC) on silica gel 60 F_{254} plates (Merck) was used for monitoring the separation of the extracts and for preparative TLC. The spots were visualised by spraying with concentrated H_2SO_4 followed by heating at 120 °C. IR spectra were obtained on a Shimadzu FTIR IR Spirit spectrometer with ATR. The 1D and 2D NMR (^{1}H- and ^{13}C-NMR, COSY, HSQC and HMBC, NOESY) spectra were recorded on a Bruker Avance II+ 600 NMR spectrometer, with the operating frequency 600 MHz (^{1}H) and 150 MHz (^{13}C), using the residual solvent signal (δ 7.26 in ^{1}H- and 77.00 ppm in ^{13}C-NMR for $CDCl_3$) as a reference.

Plant material

Plant material was collected in full flowering stage from native populations in Bulgaria during 2017. The aerial parts were air-dried and kept in a dark and cool place until extraction. *Inula germanica* L. (GPS 42°0'27.94"N 23°37'47.72"E, SOM176698) aerial parts were collected from Rila Mts, *I. ensifolia* L. (41°29'30.34"N, 23°26'59.71"E, SOM176700) – from

Chereshnitza village, Struma River Valley, *I. salicina* (41°53'18.76"N 23°22'13.18"E, SOM 176701) – from Rila Mts., and *I. conyza* (Griess.) DC. (41°45'16.25"N 24°23'57.13"E, SOM1387) – from Western Rhodopes Mts. Plant species were identified by Dr. Ina Aneva (Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences). Voucher specimens (SOM) have been deposited with the Herbarium of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences.

Extraction

Air-dried and powdered aerial parts (50 g) from *I. ensifolia* (IE), *I. salicina* (IS), *I. conyza* (IC) and *I. germanica* (IG) were extracted with chloroform (3×500 mL) at room temperature to give the corresponding chloroform extracts (1.4, 1.5, 1.6 and 2.1 g in total, respectively).

Fractionation of the chloroform extracts and isolation of individual compounds

A portion of the chloroform extract (0.5–1.0 g) was dissolved in $CHCl_3$ and subjected to CC on silica gel, using *n*-hexane/ethyl acetate (EtOAc) mixtures with increasing polarity (from 10:1 to 0:1). Fractions were monitored by TLC (silica gel, *n*-hexane/Et_2O, 2:1) and compared with standards *β*-amyrin palmitate, *β*-amyrin acetate, *β*-amyrin, faradiol-3-*O*-palmitate and *β*-sitosterol. Fractions, containing triterpene acetates (R_f 0.8–0.9) and free triterpene alcohols (R_f 0.5–0.6) were directly analysed using GC/MS, whereas fractions containing triterpene fatty acid esters (R_f 0.9–1.0) and 16-hydroxy triterpene fatty acid esters (R_f 0.6–0.8) were subjected to alkaline hydrolysis.

CC of *I. ensifolia* (IE) chloroform extract (1.0 g) afforded 8 fractions: IE-1 (triterpene fatty acid esters, 335 mg), IE-2 (triterpene acetates, 50 mg), IE-3 (free triterpene alcohols, 230 mg), IE-4 (16 mg), IE-5 (16-hydorxy triterpene fatty acid esters, 110 mg), IE-6 (25 mg), IE-7 (50 mg) and IE-8 (98 mg). Prep. TLC ($CHCl_3$/Et_2O, 50:1) of IE-4 (16 mg) afforded manil-adiol-*O*-palmitate (**16**, 1.7 mg). Prep. TLC ($CHCl_3$/Et_2O, 50:1) of IE-6 (25 mg) afforded 16*β*-hydroxylupeol 3-*O*-palmitate (**17**, 1.1 mg) and faradiol-3-*O*-palmitate (**19**, 1.5 mg). Prep. TLC (*n*-hexane/Et_2O, 1:1) of a portion of IE-7 (10 mg) afforded β-sitosterol (**20**, 3 mg).

CC of *I. salicina* (IS) chloroform extract (0.5 g) afforded 7 fractions: IS-1 (triterpene fatty acid esters, 202 mg), IS-2 (triterpene acetates, 21 mg), IS-3 (free triterpene alcohols, 45 mg), IS-4 (16-hydorxy triterpene fatty acid esters, 12 mg), IS-5 (5.6 mg), IS-6 (54 mg) and IS-7 (149 mg). Prep. TLC ($CHCl_3$/Et_2O, 50:1) of IS-5 (5.6 mg) afforded 16-hydroxylupeol-*O*-palmitate (**17**, 1.1 mg), arnidiol-*O*-palmitate (**18**, 1.3 mg) and faradiol-*O*-palmitate (**19**, 1.2 mg). Prep. TLC (*n*-hexane/Et_2O, 1:1) of a portion of IS-6 (10 mg) afforded β-sitosterol (**20**, 2.5 mg).

CC of *I. conyza* (IC) chloroform extract (0.5 g) afforded 10 fractions: IC-1 (138 mg), IC-2 (8.5 mg) and IC-3 (21 mg) contained triterpene fatty acid esters, triterpene acetates and free triterpene alcohols, respectively. Prep. TLC (*n*-hexane/Et_2O, 5:1, x2) of IC-4 (21.3 mg) gave 10.2 mg of diterpene acid **21**. Prep. TLC (*n*-hexane/Et_2O, 5:1, x3) of IC-6 (11.8 mg) afforded mixture of diterpene acids **22** and **23** (3.3 mg). Prep. TLC (*n*-hexane/Et_2O, 5:1, x2) of IC-7 (20 mg) gave additional amount **22** and **23** (5.9 mg). Prep. TLC (*n*-hexane/Et_2O, 1:1) of a portion of IC-9 (15 mg) yielded *β*-sitosterol (**20**, 6.5 mg).

CC of *I. germanica* (IG) chloroform extract (1.0 g) afforded 11 fractions of which IG-1 (265 mg) and IG-2 (118 mg) contained triterpene fatty acid esters and triterpene acetates, respectively. CC ($CHCl_3$/Et_2O, 50:1) of IG-4 (58 mg) gave **16** (6.2 mg) and a mixture of triterpenes **17**–**19** (16.8 mg). CC ($CHCl_3$/Et_2O, 50:1) of IG-5 (93 mg) afforded **18** (28 mg), 4.5 mg of **20** and a mixture of free triterpene alcohols (12.8 mg), which were further analyzed by GC/MS. Prep. TLC (*n*-hexane/EtOAc, 3:1, x2) of IG-8 (30 mg) afforded grazielia acid (**24**, 1.1 mg), 8-(2-methylbutanoyloxy)-1(10),4,11(13)-germacratrien-6,12-olide-14-oic acid (**25**,

1.0 mg), 14-hydroxy-8*β*-angeloyloxymelampolide (**32**, 0.8 mg), 2*α*-acetoxy-desacetyl-laurenobiolide (**33**, 1.8 mg), ovatifolin (**27**, 5.6 mg) and a mixture of germanin A and B (**28** and **29**, 4.4 mg). Prep. TLC ($CHCl_3$/acetone, 3:1, x2) of IG-9 (35 mg) afforded desacetylovatifolin (**26**, 1.3 mg) and mixture of 2α,14-dihydroxy-8*β*-angeloyloxymelampolide and 2*α*,14-dihydroxy-8*β*-[2-methylbutyryloxy]-melampolide (**30** and **31**, 12.2 mg).

Hydrolysis of fatty acid esters and methylation of free fatty acids

Hydrolysis of the fatty acid esters of the mixtures of triterpenes (each 10 mg) was performed with 1 M KOH in MeOH (2 mL) at 50 °C for 12 h. After cooling, the reaction mixture was diluted with water (5 mL) and extracted with Et_2O (3 times, 5 mL each). The combined Et_2O extracts were washed with water (5 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The free triterpene alcohols mixture was analysed with GC/MS.

The aqueous layer was further acidified with 1 M HCl and re-extracted with Et_2O (3 times, 5 mL each). The combined Et_2O extracts were washed with water (5 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum to give free fatty acid fraction. The latter was further methylated with methanolic 1 % H_2SO_4 (2 mL) at 50 °C for 2 h. After cooling, 5 % aq. NaCl (5 mL) were added to the reaction mixture and extracted with *n*-hexane (3 times, 5 mL each). The combined *n*-hexane extracts were washed with water, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The resulting methyl esters of fatty acids were analysed by GC/MS.

Gas chromatography/mass spectrometry

Analyses were carried out with an Agilent 7890B (Agilent, USA) gas chromatograph equipped with a flame ionization detector (FID) and mass selective detector (MSD) Agilent 5977A. A HP-5MS capillary column (5 %-phenyl)-methylpolysiloxane, 30 m×0.25 mm; 0.25 μm film thickness, Agilent) was used with Helium as carrier gas at a flow rate of 1.7 mL/min. The GC oven temperature gradient started at 60 °C, followed by a ramp of 5 °C/min to 300 °C, and then held for 20 min. The samples were analysed with a split ratio of 5:1 and injection volume of 1 μL. The injector temperature was 280 °C. The temperatures of the MSD and the source were 150 and 230 °C, respectively. Mass spectra were taken at 70 eV and the mass range was from *m*/*z* 45 to 800. The identification of triterpene alcohols, triterpene acetates and fatty acid methyl esters was based on comparison of their EI-mass spectra with the NIST14 and home-made databases.

Statistical analysis

Principal component analysis (PCA) was performed using the PAST 4.0 software to determine the chemical variation and relationship between the species.

RESULTS AND DISCUSSION

The chloroform extracts obtained by extraction of the aerial parts of *I. germanica* L. (IG), *I. ensifolia* L. (IE), *I. conyza* (Griess.) DC. (IC) and *I. salicina* L. (IS) were submitted to a column chromatography using *n*-hexane/EtOAc mixtures with increasing polarity. A preliminary study of the non-polar fractions by TLC revealed the presence of triterpene compounds – alcohols and their esters (acetates and long-chain aliphatic esters). The fractions containing fatty acid esters were hydrolysed in an alkaline medium. GC/MS analysis of the obtained triterpene alcohols and fatty acid methyl esters, as well as of the fractions containing free triterpene alcohols and their acetates, led to the identification of 19 triterpenoids of

β- and *α*-amyrin, lupeol, taraxasterol and *ψ*-taraxasterol type (Table S-I of the Supplementary material to this paper and Fig. 1). Thus, *β*- and *α*-amyrin (**1** and **2**) were characterized by a base peak at *m*/*z* 218, but differed in the relative intensities of the peaks at *m*/*z* 189 and 203: *β*-amyrin (**1**) had *m*/*z* 203 peak around twice the intensity of the *m*/*z* 189 peak, while *α*-amyrin (**2**) spectrum showed both peaks with similar intensities.[13] The mass spectrum of lupeol (**3**) exhibited a base peak at *m*/*z* 189, which is characteristic for the fragmentation of triterpenoids with a lupane skeleton bearing a hydroxyl group in position 3. Other abundant fragment ions were at *m*/*z* 203 and 207. The first can be related to the retention of an additional methylene group from the C ring with respect to the fragment ion at *m*/*z* 189, and the second represents the fragment obtained from the fragmentation of the C ring before the dehydration reaction led to the formation of the ion at *m*/*z* 189.

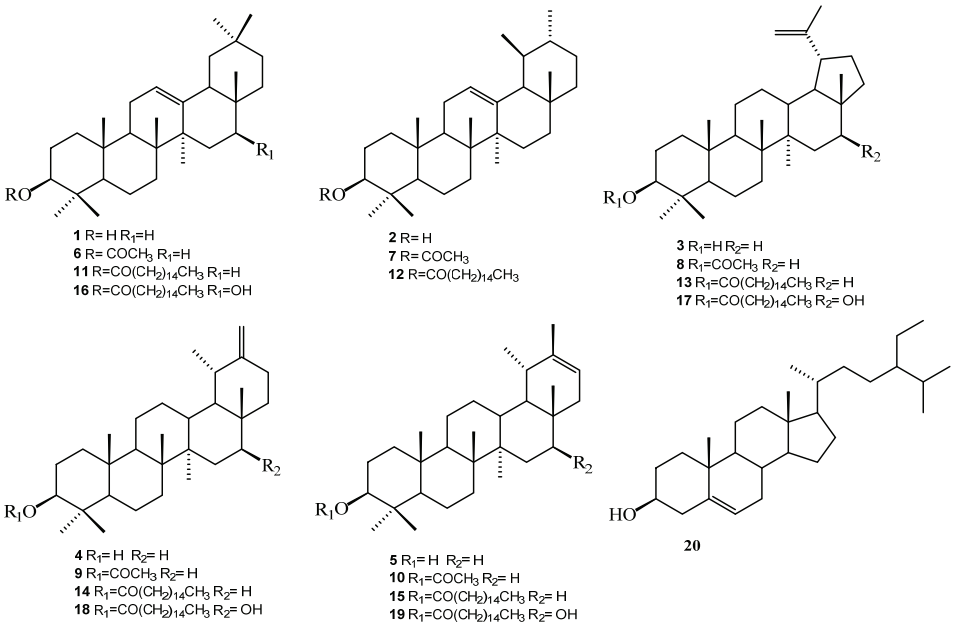


Fig. 1. Triterpenoids and sterols in *Inula* species.

The mass spectra of taraxasterol (**4**) and *ψ*-taraxasterol (**5**) revealed very intense ions at *m*/*z* 207 (80 and 85 %, respectively) and 189 (100 %), whose formation can be assumed by the cleavage of ring C, with a transfer of a hydrogen atom, followed by the loss of water.[13] The intensity of ions at *m*/*z* 218 and 203 was significantly lower than in *β*- and *α*-amyrin. The acetates **6**–**10** exhibited a molecular ion at *m*/*z* 468, an ion at *m*/*z* 408 corresponding to the loss of AcOH $[M-60]^+$ and the characteristic ions for the corresponding triterpene skeleton types. The structures of 16-hydroxy derivatives **16**–**19** were additionally confirmed by

NMR[14,15] after their isolation and purification by prep. TLC. *β*-Sitosterol (**20**) was also detected in all studied extracts by TLC comparison with authentic standard and confirmed by GC/MS of the fractions containing this compound.

The obtained results showed both similarity and difference in the studied samples (Table II).

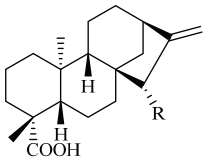
TABLE II. Distribution of triterpenoids in *Inula* species

Triterpenoid	IS	IG	IC	IE
Alcohols				
β-Amyrin (**1**)	+	+	+	+
α-Amyrin (**2**)	+	+	+	+
Lupeol (**3**)				+
Taraxasterol (**4**)	+	+	+	+
ψ-Taraxasterol (**5**)	+	+	+	+
Acetates				
β-Amyrin acetate (**6**)	+	+	+	+
α-Amyrin acetate (**7**)		+	+	
Lupeol acetate (**8**)	+		+	+
Taraxasterol acetate (**9**)	+	+	+	+
ψ-Taraxasteol acetate (**10**)	+	+	+	+
Palmitates				
β-Amyrin palmitate(**11**)	+	+	+	+
α-Amyrin palmitate (**12**)	+	+	+	+
Lupeol palmitate (**13**)				+
Taraxasterol palmitate (**14**)	+	+	+	+
ψ-Taraxasterol palmitate (**15**)	+	+	+	+
Maniladiol palmitate (**16**)		+		+
16*β*-Hydroxylupeol-3-*O*-palmitate (**17**)	+	+		+
Arnidiol palmitate (**18**)	+	+		+
Faradiol palmitate (**19**)	+	+		+

As can be seen, *I. conyza* was the only sample, which did not contain 16-hydroxy derivatives. All identified compounds are described for the first time in the here presented study of species. Fatty acid esters of triterpene alcohols have been previously found in *I. britannica*,[10,16] *I. oculus-christi*[8] and *I. bifrons*.[12] The literature data on triterpene compounds in *Inula* species are scarce and so far, there are few reports of triterpene alcohols and their esters in *I. japonica, I. helenium* and *I. cappa* only.[4] Triterpenoids of *β*- and *α*-amyrin, lupeol, taraxasterol and *ψ*-taraxasterol type are frequently found in many genera of Asteraceae family such as *Achillea*, *Chrysanthemum*, *Jurinea*, *Calendula*, *Taraxacum*, *etc.*[14,15,17–19] and therefore, could not be used as chemotaxonomic markers.

From the chloroform extract of *I. conyza* were isolated 3 compounds with very similar NMR spectral characteristics, indicating that they were diterpenoids with *ent*-kaurane skeleton and identified as *ent*-kaur-16-en-19-oic acid **(21)**,[20] *ent*-15*α*-(3-methylpentanoyloxy)-kaur-16-en-19-oic acid (**22**)[12] and *ent*-15*α*-(3-

-methylbutanoyloxy)-kaur-16-en-19-oic acid (**23**),[21] Fig. 2. The diterpene acids **22** and **23** have been recently found in *I. bifrons*[12] of Bulgarian origin, while *ent*--kaur-16-en-19-oic acid **(21**) is reporting for the first time in *Inula* species.

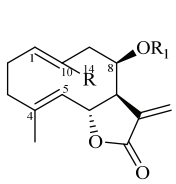


21 R = H
22 R = $OCOCH_2CH(CH_3)CH_2CH_3$
23 R = $OCOCH(CH_3)CH_2CH_3$

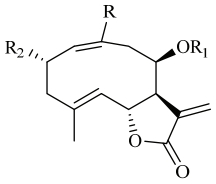
Fig. 2. Diterpene acids in *I. conyza.*

The literature survey showed only four reports on the content of diterpenoids in five species of genus *Inula – I. nervosa*, *I. cappa*, *I. japonica*, *I. britannica* and *I. bifrons.*[12,22–24] With the exception of *I. nervosa* and *I. cappa*, all other species contained diterpenoids with *ent*-kaurene skeleton, but differing in the nature and position of the substituents. According to the taxonomic classification of Anderberg,[2] *I. conyza*, *I. bifrons*, *I. britannica* and *I. japonica* belong to the section *Enula*, while *I. nervosa* and *I. cappa* are representatives of the section Duhaldea. Therefore, the presence of *ent*-kaurene diterpenoids in the section *Enula* could be of chemotaxonomic importance. Further investigations are needed to confirm this suggestion.

The preliminary study of the chloroform extracts of *I. germanica*, *I. ensifolia*, *I. conyza* and *I. salicina* by IR spectroscopy have shown the presence of sesquiterpene lactones in *I. germanica* only (characteristic absorption band at 1750–1765 cm^{-1}). Further separation of this extract by CC led to isolation of 10 closely related compounds with cyclodecadiene carbon skeleton and *trans*-*α*-methylene-*γ*-lactone ring at C-6 (**24**–**32**) or C-8 (**33**), Fig. 3. The lactones were isomers differing in the configuration of the double bonds: germacranolides with *trans*-$\Delta^{1,10}$ and *trans*-$\Delta^{4,5}$ (**24**–**27** and **33**) and melampolides with *cis*-$\Delta^{1,10}$ and *trans*-$\Delta^{4,5}$ (**28**–**32**) as well as in the nature of the substituents at C-2, C-8 and C-14.



24 R=COOH R_1=Ang
25 R=COOH R_1=2MeBu
26 R=CH_2OH R_1= H
27 R=CH_2OAc R_1= H



28 R=COOH R_1=Ang R_2= H
29 R=COOH R_1=2MeBu R_2= H
30 R=CH_2OH R_1=Ang R_2= OH
31 R=CH_2OH R_1=2MeBu R_2= OH
32 R=CH_2OH R_1=Ang R_2= H

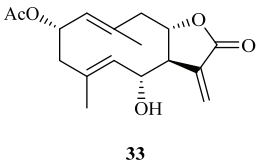


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Fig. 3**.** Sesquiterpene lactones from *I. germanica.*

The comparison of their spectral data with the literature data allowed the identification of grazielia acid (**24**),[25] 8-(2-methylbutanoyloxy)-1(10),4,11(13)--germacrutrien-6,12-olide-14-oic acid (**25**),[26] desacetylovatifolin (**26**),[27] ovatifolin (**27**),[28] germanin A (**28**),[29] germanin B (**29**),[30] 2*α*,14-dihydroxy-8*β*-angeloyloxymelampolide (**30**),[31] 2*α*,14-dihydroxy-8*β*-[2-methylbutyryloxy]-melampolide (**31**),[31] 14-hydroxy-8*β*-angeloyloxymelampolide (**32**)[31] and 2*α*-acetoxy--desacetyllaurenobiolide (**33**).[31] To the best of our knowledge, there are two reports on the content of sesquiterpenoids in *I. germanica* only. Germanin A and B (**28** and **29**) were isolated from *I. germanica* of Russian origin,[29] while lactones **27**, **30**–**33** were found in the sample cultivated in the Botanical garden of Berlin.[31] It is worth to mention that the lactones **24**–**26** are described now for the first time in *Inula* species.

In this study, no sesquiterpene lactones were detected in *I. conyza*, *I. salicina* and *I. ensifolia.* These results did not correlate with a previous investigation of *I. salicina*,[32] which reported the presence of alantolactone and isoalantolactone in this plant. Probably, the studied taxon is a new chemotype.

The obtained up to now data for the content of terpenoids in 8 *Inula* species growing in Bulgaria (*I. oculus-christi*,[8,9] *I. britannica*,[10] *I. aschersoniana*,[11] *I. bifrons*,[12] *I. germanica*, *I. conyza*, *I. salicina* and *I. ensifolia*) were analysed by the principal component aanalysis (PCA) to demonstrate their relationship. PCA performed on the different skeletal types of sesquiterpene lactones, di- and triterpenoids showed that the first two principal components accounted 80.74 % of the total variations (Fig. 4). Considering the contributions to the variances, PC 1 (61.02 % of the total variations) accounted for the positive contributions of germacranolides (GeSL), melampolides (MeSL) and triterpenoids with oleane (OleT), ursane (UrsT), taraxane (TarT) and *ψ*-taraxane (psi-TarT) carbon skeleton and negative contributions of sesquiterpene lactones with guaiane (GuSL) and eudesmane (EuSL) framework. The second component PC 2 (19.72 % of the total variations) was positively related to all types of sesquiterpene lactones (GeSL, MeSL, EuSL, GuSL, PsGuSL and SeGuSL), and negatively related to diterpenoids (DiT) and also all skeletal types of triterpenoids (OleT, UrsT, LupT, TarT and psi-TarT).

As depicted from the biplot (Fig. 4), the samples could be grouped as follows: *I. germanica* and *I. ascherosniana* constituted the first group (**A**), which was characterized by the presence of germacranolides. The other skeletal types of sesquiterpene lactones, namely melampolides in *I. germanica* and seco-guaianolides in *I. ascherosniana* are probably responsible for their placement on different sides of PC2. The second group (**B**) combined *I. oculus-christi*, *I. britannica* and *I. bifrons*, which contained eudesmanolides, guaianolides and pseudoguaianolides. The presence of diterpenoids places *I. bifrons* on the negative side of PC2. The species included in the third group (**C**) *I. conyza*, *I. ensifolia* and *I. salicina* were

characterized with the presence of triterpenoids and the lack of sesquiterpene lactones and diterpenoids. It is worth to mention that the lactone ring was 12,8-fused in all lactones found in *I. britannica*, *I. oculus-christi* and *I. bifrons* (group B), while *I. germanica* and *I. aschersoniana* (group A) produced predominantly 12,6--olides. The obtained results did not correlate well with the classification of Anderberg[2] based mainly on the morphological data. Thus, *I. germanica* differed from the species in *Inula salicina* group by the presence of sesquiterpene lactones. These compounds were absent from the plant material of *I. salicina* and *I. ensifolia*. *I. conyza* produced diterpenoids similarly to *I. bifrons*[12] from the same *Inula decurens* group. The lack of sesquiterpene lactones places *I. conyza* closer to the representatives of the *Inula salicina* group.

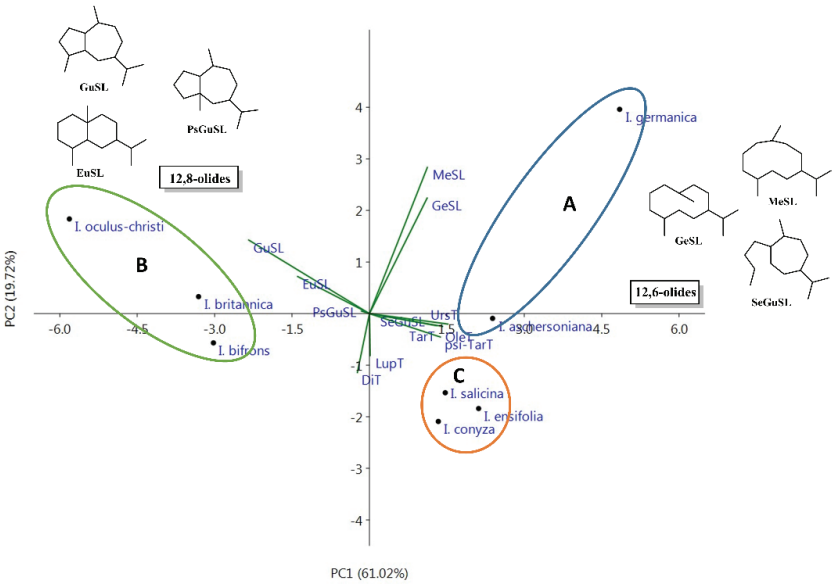

Fig. 4. Biplot (PCA) performed on the skeletal types of sesquiterpene lactones, di- and triterpenoids in *Inula* species growing in Bulgaria; germacranolides (GeSL), eudesmanolides (EuSL), guaianoilides (GuSL), pseudoguaianolides (PsGuSL), secoguaianolides (SeGuSL), diterpenoids (DiT), triterpenoids with oleane (OleT), ursane (UrsT), lupane (LupT), taraxane (TarT) and psi-taraxane (psi-TarT) skeleton.

CONCLUSION

The phytochemical studies of *I. germanica*, *I. ensifolia*, *I. conyza* and *I. salicinai,* growing in Bulgaria led to the identification of various classes of terpenoids: sesquiterpene lactones, diterpenoids, and triterpenoids. The most significant differences were observed with respect to sesquiterpene lactones and diterpenoids. They could serve as chemotaxonomic markers to clarify taxonomic problems in the genus *Inula*, which has been based mainly on morphological features so far.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/11011, or from the corresponding author on request.

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ИЗВОД

ТЕРПЕНОИДИ У ЧЕТИРИ *Inula* ВРСТЕ ИЗ БУГАРСКЕ

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Фитохемијска студија хлороформског екстракта надземних делова *Inula germanica* L., *I. ensifolia* L., *I. conyza* (Griess.) DC. и *I. salicina* L. довела је до идентификације 33 терпеноида. *β*- и *α*-амирин, лупеол, тараксастерол, *ψ*-тараксастерол и њихови 3-*O*-ацетати и 3-*O*-палмитати су идентификовани помоћу GC/MS. Поред тога, NMR потврђује структуре 3-*O*-палмитата маиналадиола, арнидиола, фарадиола и 16-хидроксилупеола. *ент*-Каур-16-ен-19-оична киселина и њени 15*α*-(3-метилпентаноилокси) и 15*α*-(3-метилбутаноилокси) деривати изоловани су из *I. conyza*. У *I. germanica* је пронађено десет блиско повезаних сесквитерпенских лактона (гермакранолида и меламполида) и њихова структурна идентификација је извршена спектралним анализама. *I. ensifolia* и *I. salicina* су биле без сесквитерпенских лактона и дитерпеноида. Сви тритерпеноиди и дитерпеноиди, гразиелиа киселина, десацетиловатифолин и 8-(2-метилбутаноилокси)-1(10),4,11(13)-гермакрутриен-6,12-олид-14-оична киселина су први пут описани у проучаваној врсте. Анализа главних компоненти (PCA) је коришћена за проналажење везе између до сада истраживаних врста *Inula* које расту у Бугарској.

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