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Chemical Composition and Antimicrobial Activity of Essential Oils from *Centaurea pannonica* and *C. jacea*

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The chemical composition and antimicrobial activity of the essential oils obtained by hydrodistillation from *Centaurea pannonica* (Heufel) Simonkai and *C. jacea* L. (Asteraceae), were investigated. The essential oils were analyzed by GC and GC-MS. Forty five and twenty nine compounds were identified in the two oils, respectively. *C. pannonica* oil was rich in fatty acids (43.7%), with 9-octadecanoic acid (34.0%) and (*Z,Z*)-9,12-octadecadienoic acid (8.6%) as the major compounds. In contrast, the essential oil of *C. jacea* was dominated by oxygenated sesquiterpenes (43.2%), among which caryophyllene oxide (23.5%) and spathulenol (8.9%) were the major constituents. However, the oil was also characterized by an important fatty acid fraction (15.5%), with 9-octadecanoic acid (8.9%) and hexadecanoic acid (6.6%) being the main components. The antimicrobial activities of the essential oils were evaluated by the microdilution method against three Gram-positive and three Gram-negative bacteria, and one yeast. Both oils exhibited significant antimicrobial activity, especially against Gram-positive bacteria.

Keywords: *Centaurea pannonica* (Heufel) Simonkai, *Centaurea jacea* L., essential oil, fatty acids, sesquiterpenes, antimicrobial activity.

The genus *Centaurea* L. is represented by approximately 40 species in Serbia, distributed across all parts of the country [1]. Some members of this genus, such as *C. cyanus* L., *C. benedictus* L., *C. calcitrapa* L. and *C. scabiosa* L. are used in folk medicine as diuretic, emmenagogue, cholagogue, astringent and antiseptic agents, and in the treatment of fever and tumors [2,3]. The taxonomy of the genus is still complex. The taxonomic scheme currently preferred is the segregation of the genus into several sections, mostly based on morphological characters. Both taxa investigated here belong to the morphologically complicated section *Jacea* (Cass.) DC [4].

C. pannonica (Heufel) Simonkai is an erect perennial plant with pink flower heads on branched stems, 30-80 cm in height, widely distributed on dry land. It is traditionally used for stomach diseases [3,5]. *C. jacea*, known under the common name brown knapweed, is distributed across Europe. It is a perennial herb, very variable in height, with large terminal pink flowers, flowering from June to September. It is used in Serbian folk medicine as a diuretic, antidiabetic and febrifuge [5,6]. Concerning the secondary metabolites of *Centaurea* sp., sesquiterpene lactones are predominant and are of taxonomic significance [7-12]. In addition, flavonoids, lignans, steroids, hydrocarbons, polyacetylenes and alkaloids have also been isolated from this genus [13-17]. Previous phytochemical investigations on the volatile compounds have focused on the relationship between chemical composition and taxonomy [18-21]. Some essential oils obtained from *Centaurea* sp. were found to possess antifungal and antibacterial activities [22,23]. To the best of our knowledge, there is no previous report on the volatile constituents of *Centaurea* taxa belonging to the section *Jacea* (Cass.) DC. Therefore, to fulfill this gap,

Table 1: Chemical composition (relative % peak area) of the essential oils of *C. pannonica and C. jacea*.
[a]RI, retention indices calculated against C9-C24 *n*-alkanes on HP-5MS (1) and HP-Innowax (2) capillary columns, respectively.

Compound	RI_1[a]	RI_2[a]	*Centaurea pannonica* (%)	*Centaurea jacea* (%)
(*E*)-2-Hexenal	851	1210	0.2	0.5
2-Pentyl-furan	990	1231	0.5	1.1
n-Octanal	996	1289	0.1	-
(*E,E*)-2,4-Heptadienal	1010	1443	0.3	-
Benzyl alcohol	1033		1.1	4.2
n-Nonanal	1102	1392	3.2	1.6
(*E*)-2-Nonenal	1163	1528	0.2	-
2-Hydroxy-benzoic acid	1179	1390	-	0.8
n-Decanal	1199	1500	1.3	0.9
(*E*)-2-Decenal	1265	1639	0.4	-
Dihydroedulan II	1288	1485	0.4	1.2
Dihydroedulan I	1291	1512	0.2	-
Theaspirane A	1296		0.3	-
Theaspirane B	1312		0.3	-
(*E,E*)-2,4-Decadienal	1310	1812	0.5	-
E-*β*-Damascenone	1378	1765	0.4	7.3
β-Elemene	1391	1572	0.4	-
β-Caryophyllene	1417	1580	1.2	-
Widdrene	1448	1598	0.3	-
α-Humulene	1455	1660	0.3	-
Geranyl acetone	1458	1863	0.7	1.3
Germacrene D	1482	1701	1.0	-
β-Ionone	1486	1950	0.6	1.3
β-Selinene	1492	1717	0.3	-
Tridecanal	1507	1817	0.3	-
2,4-Bis-(1,1-dimethylethyl)-phenol	1512		-	1.1
2,3,3-Trimethyl-2-cyclohexanone	1560		-	3.1
1,5-Epoxysalvial-4(14)-ene	1562	1924	-	2.5
Spathulenol	1580	2120	6.0	8.9
Caryophyllene oxide	1585	1987	8.0	23.5
Salvial-4-(14)-en-1-one	1595	2010	0.7	2.3
Nor-copaanone	1610	2156	0.5	1.7
Vulgarol B	1615	2345	0.6	-
1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	1626		1.3	2.5
Fonenol	1635		0.8	-
β-Eudesmol	1652	2238	1.6	1.8
Heptadecane	1700	1700	0.2	-
Valerenal	1715		-	0.7
Octadecane	1800	1800	0.2	-
1,15-Pentadecanediol	1812		0.2	-
Hexadecanal	1815	2108	0.3	0.7
6,10,14-Trimethyl-2-pantadecanone	1842	2131	0.9	1.9
Hexadecanol	1872	2384	0.9	-
Ethyl linoleate	1894		0.2	-
(*E,E*)-Farnesyl acetone	1920	2386	-	1.3
Hexadecanoic acid	1965	2911	0.7	6.6
Manoyl oxide	1987		-	0.7
n-Eicosane	2000	2000	-	0.7
11,14,17-Eicosatrienoic acid methylester	2054		0.2	-
(*Z,Z*)-9,12-Octadecadienoic acid	2130	3150	8.6	-
9-Octadecanoic acid	2140	3154	34.0	8.9
Tricosane	2300	2300	-	0.6
Pentacosane	2500	2500	1.0	-
Heptacosane	2700	2700	0.8	0.1
Total			82.2	89.8
$[\alpha]_D^{20}$	-0.98 (hep, *c* 2.56)		-0.41 (hep, *c* 1.70)	

we report here the chemical composition and antimicrobial activity of the essential oils obtained from the aerial parts of *C. pannonica* and *C. jacea* L., both growing wild in Serbia.

Table 2: The chemical classes of compounds isolated from *C. pannonica* and *C. jacea* essential oils.

Compound class	*C. pannonica*		*C. jacea*	
	%	No. of compounds	%	No. of compounds
Aliphatics				
Alkanes, alkenes	2.2	4	1.4	3
Alcohols	2.2	3	4.2	1
Aldehydes	6.8	10	3.7	4
Ketones	0.9	1	6.3	3
Fatty acids and aliphatic esters	43.7	5	15.5	2
Terpenoids				
Sesquiterpene hydrocarbons	3.5	6	-	-
Oxygenated sesquiterpenes	18.7	7	43.2	7
Diterpenes	-	-	0.7	1
Phenylpropanoids	-	-	1.1	1
Miscellaneous	1.3	2	2.6	3
Compounds with 13 carbons	2.9	7	11.1	4
Total	82.2	45	89.8	29

Hydrodistillation of the aerial parts of *C. pannonica* and *C. jacea* yielded ca. 0.043% and 0.025% (v/w), respectively, of colorless essential oils with pleasant odors. The identified compounds are listed in Table 1, according to their retention index on the HP-5MS column. Forty-five compounds were identified representing 82.2% of the total essential oil from *C. pannonica*. Unsaturated and aliphatic acids (43.7%) were the major group (Table 2). Among them, 9-octadecanoic acid (34.0%) and (*Z,Z*)-9,12-octadecadienoic acid (8.6%) were the main components. Oxygenated sesquiterpenes constituted 18.7% of the total oil, with caryophyllene oxide (8.0%) and spathulenol (6.0%) as the major compounds. Aldehydes represented also a large portion of this oil (6.8%) with *n*-nonanal being the major constituent (3.2%). Twenty-nine constituents were identified representing 89.8% of the total oil of *C. jacea*. The oil was characterized by a high content of oxygenated sesquiterpenes (43.2%), and fatty acids (15.5%). Among the oxygenated sesquiterpenes, caryophyllene oxide (23.5%) and spathulenol (8.9%) were the main constituents. Furthermore, 9-octadecanoic acid (8.9%) and hexadecanoic acid (6.6%) were determined as the major compounds of the fatty acid portion. These compounds are rather more typical constituents of epicuticular waxes than of essential oils [24]. Also, *E*-*β*-damascenone formed 7.3%, of the oil of *C. jacea*. Both essential oils were characterized by the absence of monoterpenes and were also deficient in odoriferous phenylpropanoids.

The high content of oxygenated sesquiterpenes and fatty acids are characteristic of *Centaurea* species. Hexadecanoic and dodecanoid acids were the main compounds of *Centaurea* species from Turkey and Bulgaria [25-27], whereas the main components from Greek *Centaurea* essential oils were caryophyllene oxide and spathulenol, as well as hexadecanoic acid [20,28]. However, this is the first time that unsaturated

Table 3: Antimicrobial activity of the essential oils of *C. pannonica and C. jacea* aerial parts.

Microorganism	Essential oil of *C. pannonica*		Essential oil of *C. jacea*		Standard	
	MIC[a]	MBC[a]	MIC[a]	MBC[a]	MIC[b,c]	MBC[b,c]
Bacteria						
Gram-positive						
E. faecalis	0.63	0.63	2.50	5.00	0.31	0.31
M. lysodeikticus	2.50	5.00	2.50	5.00	0.62	1.25
S. aureus	0.31	0.63	0.08	0.16	0.62	1.25
Gram-negative						
E. coli	>5.00	>5.00	>5.00	>5.00	0.31	0.62
K. pneumoniae	1.25	1.25	1.25	2.50	0.31	0.31
P. aeruginosa	>5.00	>5.00	>5.00	>5.00	>2.50	>2.50
Yeast						
C. albicans	2.50	5.00	2.50	2.50	0.31	0.31

[a]Values given as μL/mL; [b]The standard drugs used were tetracycline for bacteria and nystatin for *C. albicans*; [c]in μg/mL

fatty acids have been identified as main components in *Centaurea* sp. essential oils. In previous reports, they were found only in significantly lower amounts [20,27]. Another significant characteristic of both oils was the presence of C-13 compounds (2.9% and 11.1% respectively) in appreciable amounts. It is the first time that *E*-*β*-damascenone has been identified in such a high percentage in the genus *Centaurea*. In previous reports this compound ranged up to 2.1% [26-28].

The results of the antimicrobial assay showed that both oils exhibited significant activity against the tested strains (Table 3). Gram-positive bacteria were more sensitive than Gram-negative bacteria and yeast. This finding agrees with previous reports [22]. The oils of *C. pannonica* and *C. jacea* were found to have strong bactericidal effects against methicillin-resistant *S. aureus* (MBC of 0.63 and 0.16 μL/mL respectively), moderate activity against *E. faecalis, M. lysodeikticus*, *K. pneumoniae* and *C. albicans* (MBC 0.63-5.00 μL/mL respectively) and no inhibition against *E. coli* and *P. aeruginosa* MIC, MBC >5.00 μL/mL. The inhibitory effect may be attributed to their main compounds, such as 9-octadecanoic acid and (*Z,Z*)-9,12-octadecadienoic acid, caryophyllene oxide, and spathulenol, since, according to the literature, these components were found to be effective against several bacteria and fungal pathogens [29,30]. The presence of long chain aldehydes, alcohols, and ketones should also be taken into consideration. It is also possible that the minor components may be involved in some type of synergism with the other active compounds [31,32].

As a conclusion, section *Jacea* differs from the previously studied sections, especially with regard to its high content of unsaturated fatty acids. Also, the level of C-13 compounds is of interest. More species belonging to section *Jacea* should be studied, since their chemical profile could be used as a chemotaxonomic marker. Moreover, the obtained results support the idea that *Centaurea* essential oils could be promising sources of antimicrobial agents.

Experimental

Plant material: Aerial parts of *C. pannonica* (Heufel) Simonkai and *C. jacea* L. were collected during the flowering period (September, 2008) in Divostin (Šumarice, Kragujevac district, Central Serbia), at ca. 200-250 m altitude. Voucher specimens of the plants were deposited to the Herbarium of the Department of Botany, Faculty of Biology, University of Belgrade: no. 16387 (*C. pannonica*) and no. 16388 (*C. jacea*).

Essential oil extraction: Air-dried plant material (70 g) from each taxon was cut into small pieces, and the essential oils were obtained by hydrodistillation in 500 mL H_2O for 2 h in a modified Clevenger apparatus [33]. The oils, taken up in 2 mL of capillary GC grade *n*-heptane and dried over anhydrous sodium sulfate, were stored at -4°C and subsequently analyzed by GC and GC–MS. The yield was defined as the volume of essential oil obtained relative to the weight of dry aerial parts.

Gas chromatography: Analysis was carried out on a Perkin Elmer 8500 gas chromatograph with FID, fitted with a Supelcowax-10 fused silica capillary column (30 m x 0.32 mm I.D.; film thickness: 0.25 μm). The column temperature was programmed from 75° to 250°C at a rate of 2.5°C/min. The injector and detector temperatures were programmed at 230° and 300°C, respectively. Injection volume for all the samples was 2 μL of pure oil.

Gas chromatography-mass spectrometry: The composition of the volatile constituents was established by GC–MS analyses, which were performed on a Hewlett-Packard 5973–6890 system operating in EI mode (70 eV) equipped with a split/splitless injector (220°C), a split ratio of 1/10, using a non polar fused silica HP-5 MS capillary column (30 m x 0.25 mm (i.d.), film thickness: 0.25 μm) and a polar HP-Innowax capillary column (30 m x 0.25 mm (i.d), film thickness: 0.50 μm). The temperature program for the HP-5 MS column was from 60°C (5 min) to 280°C at a rate of

4°C/min and for the HP-Innowax column from 60°C to 260°C at a rate of 3°C/min. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. Injection volumes of each sample were 2 μL.

Identification of compounds: Retention indices for all compounds were determined according to the Van den Dool approach [34], using *n*-alkanes (C9-C24) as standards. Identification of the components was based on comparison of their MS with those of the Wiley Library [35] and those described in the literature [36,37].

Optical rotation: The $[\alpha]_D^{20}$ values were determined at 20ºC at 589 nm in *n*-heptane on a Perkin-Elmer 341 Polarimeter.

Antimicrobial assay: The antimicrobial activity of the essential oils was evaluated against the following Gram-negative bacteria: *Escherichia coli* (ATCC 25922), two clinical strains of *Klebsiella pneumoniae* (FSB 26), and *Pseudomonas aeruginosa* (FSB 37), as well as against the following Gram-positive bacteria: *Micrococcus lysodeikticus* (ATCC 4698), *Enterococcus faecalis* (ATCC 29212), and *Staphylococcus aureus* (ATCC 25923), and the yeast *Candida albicans* (ATCC 10259). All microbial strains were obtained from the Faculty of Biochemistry and Chemistry, University of Belgrade and Institute for Health Protection of Kragujevac, Serbia.

The bacterial strains were cultured on nutrient agar for 24 h at 37ºC, while *Candida albicans* (ATCC 10259) was cultured on Sabouraud dextrose agar at 28ºC for 48 h. The minimal inhibitory concentrations (MIC) were determined by the microdilution method [38] with slight modification [39]. The essential oils were diluted in Müeller-Hinton broth supplemented with Tween-80 (1:10). A series of two-fold dilutions of the oils, ranging from 5.00-0.08 μL/mL, was tested in a microtiter plate (96 wells) with the addition of 0.01 mL bacterial spore suspension (6.5×10^6 CFU/mL) and yeast spore suspension (3×10^4 CFU/mL). Tetracyclin and nystatin (10.00–0.08 μg/mL), were used as positive controls. Resazurin sodium salt (0.5%, w/v) (Alfa Aesar) was used as indicator, in order to estimate visually any change in color from violet to pink indicating reduction of the dye due to bacterial growth. The MIC values were determined as the lowest concentration of the oils inhibiting the visible growth of each micro-organism. To determine MBC, broth was taken from each well and inoculated in Müeller-Hinton broth for 24 h at 37°C for bacteria or in Sabouraud dextrose agar for 48 h at 28°C for the yeast. The MBC was defined as the lowest concentration (where violet color was visible) of the essential oil at which the inoculated microorganism was completely killed. The experiments were performed in duplicate.

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