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COMPONENTS AND ANTIOXIDANT ACTIVITY OF FRUITS OF *Cirsium palustre* AND *C. rivulare*

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In the hexane extract of C. palustre *and* C. rivulare *fruits, fatty acids, sterols, triterpenes, and volatile compounds were analyzed by the GC-MS-FID method. In the methanolic extracts, total phenol content was estimated. The antioxidant activity of both extracts was measured with DPPH assay and expressed in % scavenged DPPH.*

Keywords: *Cirsium palustre*, *Cirsium rivulare* fruits, fatty acids, sterols, triterpenes, volatile compounds, GC-MS-FID, total phenol content, DPPH.

Cirsium palustre (L.) Scop. is a biennial plant, and *C. rivulare* (Jacq.) All. is a perennial plant, both from the Asteraceae family occurring in Europe. Their fruit is called cypsela, which is surrounded by the pappus, 2.5–3.5 mm long in *C. palustre* and 3.5–5.5 mm long in *C. rivulare*. One plant may produce up to 2000 fruits [1]. Seeds of the Asteraceae family are rich in lipids that possess high biological activity. Many publications concern investigations of this class of natural compounds [2–5]. Among *Cirsium* species, their fruits have been little examined. In *C. vulgare*, unsaturated 18:1, 18:2, and 18:3 fatty acids and phytosterols were recognized [6]. The chemical compounds of *C. palustre* and *C. rivulare* fruits have not been examined so far. The fruits were exhaustedly extracted with hexane in a Soxhlet apparatus. The yield of extraction was 12.08% and 12.52% for *C. palustre* and *C. rivulare*, respectively. Extracts were obtained as dark-yellow liquids with characteristic odor. Qualitative and quantitative analysis of fatty acids, phytosterols, and triterpenes in the extracts was established by GC-MS-FID, and the results are presented in Tables 1 and 2. The composition of fatty acids in both extracts is similar: unsaturated fatty acids linoleic (18:2) and oleic (18:1) acids are dominant. The difference is in the mutual proportion of these acids; in *C. palustre* extract it is 4:1, and in *C. rivulare*, 2:1. The main phytosterol in both extracts is β-sitosterol. Some difference is observed in the volatile fraction. In *C. palustre* fruits the content of the main compound, limonene, is more than 80%, and in sum 13 components were identified. In *C. rivulare* limonene is also dominant, but its content is only 57.2% and more compounds (26) were identified (Table 3).

Defatted *C. palustre* and *C. rivulare* fruits were extracted with methanol under reflux, and the methanolic extracts were obtained as orange-brown, viscous solid substance with 9.2% and 7.9% yield, respectively. In extracts the total phenol content was estimated by the colorimetric method with Folin–Ciocalteu reagent. The content of phenolics is relatively low; in the extract of *C. palustre* it amounts to 8.7 mg/g, and in *C. rivulare* it amounts to 11.8 mg/g.

For the hexane and methanol extracts (concentration of sample 1 mg/mL) of *C. palustre* and *C. rivulare* fruits, the antioxidant activity was also examined. The method with spectroscopic measurement of the percent scavenged $DPPH^{\cdot}$ free radicals was used. The activity of the hexane extracts was 35.56% and 14.22%, respectively. The methanolic extracts were more active; the percent scavenged free radicals was 91.36% and 88.55%, respectively, but there was no correlation between the activity and the total phenolic content.

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TABLE 1. The Composition and Content of Fatty Acids in the Hexane Extract of *C. palustre* and *C. rivulare* Fruits (GC, mass %)

Fatty acid	*C. palustre* extract	*C. rivulare* extract	Fatty acid	*C. palustre* extract	*C. rivulare* extract
14:0	0.1	0.1	18:1(9)	16.0	25.8
15;0	<0.05	<0.05	18:0	4.1	2.8
16:1(9)	0.1	0.2	20:1(11)	0.2	0.2
16:0	8.9	10.4	20:0	0.5	0.5
17:0	0.1	0.1	22:0	0.2	0.2
18:2(9,12)	69.6	59.6	24:0	0.1	0.2

TABLE 2. The Composition and Content of Phytosterols and Triterpenes in the Hexane Extract of *C. palustre* and *C. rivulare* Fruits

Compound	*C. palustre*		*C. rivulare*	
	mass %, GC	mg/g extract	mass %, GC	mg/g extract
Cholesterol (internal standard)	4.41	0.4	5.03	0.4
Campesterol	7.63	0.69	4.46	0.36
Campestanol	Tr.	–	Tr.	–
Stigmasterol	4.74	0.43	2.86	0.23
Ergost-8(14)-en-3-ol	0.84	0.08	0.83	0.07
β-Sitosterol	46.88	4.25	33.39	2.66
Sitostanol	4.30	0.39	6.6	0.53
β-Amyrin	0.42	0.04	5.0	0.4
α-Amyrin	2.45	0.22	4.84	0.39
Stigmasta-5,24-dien-3-ol	2.87	0.26	1.89	0.15
24-Methylenecycloartanol	0.63	0.06	3.47	0.28
Total phytosterols	67.26	6.1	50.03	4.0
Total triterpenes	3.5	0.32	13.31	1.07

Tr.: < 0.05%.

TABLE 3. Volatile Compounds Identified in SPME Extracts, %

Compound	RI lit.	*C. palustre*	*C. rivulare*	Compound	RI lit.	*C. palustre*	*C. rivulare*
Tricyclene	927	1.0	–	*trans*-*p*-Mentha-2,8-dienol	1100	–	0.8
α-Pinene	936	0.3	–	*cis*-*p*-Mentha-2,8-dienol	1116	–	0.5
Camphene	950	–	0.1	Cymen-9-ol	1157	–	1.5
Hexanoic acid	962	–	2.7	Terpinen-4-ol	1164	–	6.4
6-Methylhept-5-en-2-one	968	–	1.4	α-Terpineol	1176	–	0.7
5-Methylheptan-3-one	970	–	1.3	8,9-Dehydrothymol	1190	–	0.6
Sabinene	973	–	0.5	*trans*-Carveol	1200	–	1.0
β-Pinene	978	0.7	0.2	Carvotanacetone	1220	–	1.5
2-Pentylfurane	981	0.5	0.8	Linalyl acetate	1239	–	0.9
Myrcene	987	1.6	1.4	Bornyl acetate	1270	0.2	0.8
p-Cymene	1015	8.4	9.1	γ-Nonanolide	1318	–	0.4
Limonene	1025	80.6	57.2	Tetradecane	1400	0.9	–
γ-Terpinene	1051	1.1	0.9	*ar*-Curcumene	1473	–	0.3
Terpinolene	1082	0.2	0.2	γ-Curcumene	1475	–	0.5
Linalool	1086	–	1.7	δ-Selinene	1490	1.9	–
Dodecane	1100	1.3	–	Total identified		98.7	93.3

EXPERIMENTAL

GC-MS-FID Analysis. Volatile compounds from SP-ME (solid phase microextraction) extracts, fatty acid methyl esters and derivatized phytosterols, were analyzed by GC-MS-FID. The analyses were performed on a Trace GC Ultra coupled with a DSQII mass spectrometer (Thermo Electron). A simultaneous GC-FID and MS analysis was performed using a MS-FID splitter (SGE Analytical Science). Operating conditions for volatile compounds and FAME are: capillary column Rtx-1MS (60 m × 0.25 mm i.d., film thickness 0.25 μm); temperature program 60°C (0.5 min) – 300°C (30 min) at 4°C/min; injector and detector temperatures 280°C and 300°C, respectively; carrier gas helium with flow rate 1.5 mL/min.

Operating conditions for TMS-phytosterols are: capillary column HP-5 (30 m × 0.25 mm i.d., film thickness 0.25 μm); temperature program 100°C (1 min – 10°C/min) – 250°C (15 min – 4°C/min) – 300°C (30 min); injector and detector temperatures 310°C and 320°C, respectively; carrier gas helium with flow rate 1.0 mL/min.

Plant Material. *C. palustre* and *C. rivulare* fruits were collected at the end of June and in July 2009 and 2010 in the vicinity of Bialystok (Poland). Voucher specimens were deposited in the Herbarium of the Department of Pharmacognosy, Medical University of Bialystok, Poland (No. CP 06014 and CR 00006).

Extract Preparation. The ground fruits (25 g) were extracted, first exhaustedly with *n*-hexane in a Soxhlet apparatus and then once with methanol under reflux. The solvents were evaporated *in vacuo*.

SPME Sampling. The SPME fiber (DVB/CAR/PDMS) and the holder were obtained from Supelco Ltd. (Bellefonte, USA). The fiber was first conditioned according to the manufacturer′s instructions. A sample of 25 g fresh-crushed fruits in a 100 mL glass vial with a silicone septum was kept for 10 min in a water bath at 60°C to achieve partition equilibrium between the sample and the air in the vial, and after that the SPME fiber was exposed to absorb the analytes for 60 min.

Fatty Acid Methyl Ester (FAME) Preparation. The FAMEs were obtained by heating under reflux 100 mg hexane fruit extract with 5 mL 2% NaOH (methanolic solution). After alkaline hydrolysis, 5 mL of anhydrous 14% boron trifluoride (Sigma-Aldrich, St. Louis, USA) in methanol was added. Samples were heated for 30 min in a water bath under reflux, and then 4 mL of heptane was added, after which the solution was cooled down and 20 mL of NaCl added. The heptane solution (2 mL) was placed in a screw-capped tube and dried with anhydrous $MgSO_4$. The solution was analyzed by GC-MS-FID.

Phytosterol Sample Preparation. Phytosterols were transformed to trimethylsilyl ethers. The samples were prepared according to the literature [7].

Identification of Lipophilic Compounds. Identification of volatile compounds from fruits was based on a comparison of their MS spectra with computer library NIST 98.1, Wiley Registry of Mass Spectral Data, 8th Ed., and MassFinder 4.1 along with the relative retention indices (RI, nonpolar column). Fatty acids were determined as FAMEs, and the phytosterols were analyzed as TMS derivatives along with their mass spectrum and compared with MS spectra of computer library NIST 98.1 and Wiley Registry of Mass Spectral Data, 8th Ed. The retention times were compared with standard data.

Total Phenolic Content. The content of total phenolics in the methanolic extracts was determined by the Folin-Ciocalteu method described previously [8].

Antioxidant Activity of Extracts. Antioxidant activity was determined by the method described by Cuended et al. [9], which consisted of spectroscopic measurement of the intensity of the color change in solution, depending on the amount of DPPH free radical. The reaction mixture contained $DPPH^{\cdot}$ (Sigma-Aldrich, Steinheim, Germany), 0.004% methanolic solution (400 μL), and extract dissolved in 1600 μL of methanol. The final concentration of extract was 1 mg/mL. The control solution was prepared using only $DPPH^{\cdot}$ and methanol. After 30 min absorbance at 517 nm was determined. All tests were performed in triplicate.

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