Chemical Constituents and Biological Activities of *Cirsium leucopsis*, *C. sipyleum*, and *C. eriophorum*

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Two endemic *Cirsium* species, *C. leucopsis* DC. and *C. sipyleum* O. Schwarz, and *C. eriophorum* (L.) Scop. growing in Turkey were investigated to establish their secondary metabolites, fatty acid compositions, and antioxidant and anticholinesterase potentials. Spectroscopic methods were used to elucidate the structures of thirteen known compounds (*p*-hydroxy-benzoic acid, vanillic acid, *cis*-epoxyconiferyl alcohol, syringin, balanophonin, 1′-*O*-methyl-balanophonin, apigenin, kaempferol-3-*O*-β-D-glucopyranoside, kaempferol-3-*O*-α-L-rhamnopyranoside, taraxasterol, taraxasterol acetate, β-sitosterol, β-sitosterol-3-*O*-β-D-glucopyranoside). *cis*-Epoxyconiferyl alcohol and 1′-*O*-methyl-balanophonin were isolated for the first time from *Cirsium* species. Palmitic acid (47.1 %) was found to be the main fatty acid of *C. leucopsis*, linoleic acid in both *C. sipyleum* (42.1 %) and *C. eriophorum* (37.8 %). Assays of β-carotene bleaching, scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals, 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium (ABTS) cation radicals, and superoxide anion radicals, as well as cupric reducing antioxidant capacity (CUPRAC) were used to determine the antioxidant activities of the extracts and isolated compounds. Vanillic acid, balanophonin, and kaempferol-3-*O*-α-L-rhamnopyranoside exhibited strong antioxidant activity. Taraxasterol was a potent inhibitor of acetyl- and butyrylcholinesterase activity, respectively.

Key words: Cirsium, Secondary Metabolites, Biological Activity

Introduction

The genus *Cirsium* Mill. (Asteraceae) consists of over 250 species distributed in Europe, North Africa, Asia, and North and Central America (Charadze, 1963; Davis and Parris, 1975; Petrak, 1979). In Turkey, there are 81 *Cirsium* taxa, and 28 of them are endemic (Davis and Parris, 1975; Davis *et al.*, 1988; Güner, 2000; Özhatay *et al.*, 2011). *Cirsium* species, known in Turkey as "köy göçerten, çarık kesen, eşek dikeni", are mainly distributed in East and Northeast Anatolia (Deliorman-Orhan *et al.*, 2007). In Turkish folk medicine, the decoction prepared from the seeds and roots of *Cirsium* species has been used for the treatment of haemorrhoids, and their flowers are a good remedy for peptic ulcer. In addition, their stems have been used for the treatment of cough and bronchitis in Anatolia (Deliorman-Orhan *et al.*, 2007). A literature survey revealed that flavonoids and their glycosides are the main secondary metabolites of *Cirsium* species, which also contain sterols, triterpenes, polyacetylenes, aliphatic aldehydes, and phenolic acids (Jordon-Thaden and Louda, 2003). They have various biological effects such as antimicrobial, antioxidant, antidiabetic, anti-inflammatory, vasorelaxant, astringent, hepatoprotective, antiphlogistic, and anticancer (Jeong *et al.*, 2008; Jung *et al.*, 2009; Ku *et al.*, 2008; Liu *et al.*, 2007; Nazaruk, 2008). The young flowers,

stems, and roots of some *Cirsium* species are consumed as a vegetable (Orhan *et al.*, 2009).

C. leucopsis DC. is an endemic and perennial species (60 – 100 cm tall). Its stem is continuously sinuate-winged, and is usually densely set with short to very long spines, often laxly paniculate at the tip. Its leaves are broadly oblong, herbaceous, bearing (like the decurrent wings) stiff yellowish spines of up to 5 – 10 mm, and are densely arachnoid-tomentose on both surfaces, or sometimes sparsely arachnoid (Davis and Parris, 1975). The stem of *C. sipyleum* O. Schwarz, which is an endemic and biennial species (60 – 90 cm tall), is stout at its base, and is sometimes sparingly branched, unwinged, and sparsely arachnoid. Its leaves have a spinose-strigose adaxial surface with setae (3 – 3.5 mm), otherwise glabrous, and a sparsely arachnoid to tomentose abaxial surface (Davis and Parris, 1975). *C. eriophorum* (L.) Scop. being a biennial species (40 – 250 cm tall) has a branched, unwinged, striate, and sparsely arachnoid stem. Its leaves have also a spinose-strigose adaxial surface with setae (1 – 2 mm), otherwise glabrous, and an arachnoid abaxial surface (Daşkın *et al.*, 2006).

Cirsium species grown in Turkey have not been investigated phytochemically and biologically, except for *C. hypoleucum* (Deliorman-Orhan *et al.*, 2007; Orhan *et al.*, 2009; Özçelik *et al.*, 2005, 2008). The aim of the present study was to establish, for the first time, the secondary metabolites and some *in vitro* bioactivities of *C. leucopsis*, *C. sipyleum*, and *C. eriophorum*. Their *n*-hexane extracts were also analysed by capillary gas chromatography-mass spectrometry (GC/MS) to characterize their fatty acid composition.

Experimental

General

FT-IR spectra were recorded on a Perkin Elmer Spectrum 100 (Waltham, MA, USA) instrument, while UV spectra were recorded on a Thermo Scientific-Evoluation 300 UV-Visible (Waltham, MA, USA) and a Shimadzu UV 1601 (Kyoto, Japan) spectrophotometer, respectively. The ^{1}H NMR spectra were recorded on Varian Unity Inova 500 MHz (Sparta, NJ, USA) and Bruker Avance AV 300 MHz, 400 MHz, 500 MHz, and 600 MHz (Billerica, MA, USA) spectrometers. ^{13}C NMR spectra were recorded on Varian Unity Inova and Bruker Avance 500 spectrometers at 125 MHz using tetramethylsilane (TMS) as an internal standard. EI-mass spectra were recorded on a Jeol MS route (Tokyo, Japan) instrument. ESI-mass spectra (positive ion mode) were recorded on a QStar XL Hybrid LC/MS/MS spectrometer (Applied Biosystems, Framingham, MA, USA). For fatty acids analysis, a Thermo Scientific Polaris Q GC/MS/MS instrument equipped with a nonpolar Phenomenex DB5 fused (5 % phenyl-/95 % dimethylpolysiloxane) silica column (30 m × 0.32 mm, 0.25 μm film thickness) (Agilent, Santa Clara, CA, USA) was used. For recycling preparative high-performance liquid chromatography (HPLC) an LC 908 (Japan Analytical Instruments, Tokyo, Japan) instrument was equipped with a JAIGEL-ODS-M80 column (S-4 μm, 8 nm, 250 mm × 20 mm ID; YMC, Kyoto, Japan), a JAI RI-5 refractive index detector, and a JAI UV 310 detector (254 nm) (Japan Analytical Instruments). All activity assays were performed using a BioTek Power Wave XS microplate reader (Winooski, VT, USA).

Chemicals

β-Carotene, linoleic acid, quercetin, pyrocatechol, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,6-di-*t*-butyl-1-hydroxytoluene (BHT), butylated hydroxyanisole (BHA), 5,5-dithiobis-(2-nitro benzoic acid) (DTNB), nicotinamide adenine dinucleotide (NADH), phenazine metosulfate (PMS), nitroblue tetrazolium (NBT), acetylcholinesterase (AChE), butyrylcholinesterase (BChE), α-tocopherol, potassium peroxydisulfate ($K_2S_2O_8$), 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and copper(II) chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA), Tween-40, chloroform, dichloromethane, methanol, ethanol, aluminium nitrate, potassium acetate, silica gel 60 (0.063 – 0.200 mm, 70 – 230 mesh ASTM, Merck 1.07734), TLC plates (Alufolien Kieselgel 60F_{254}, Merck 1.05554, RP-18 F_{254}, 5 cm × 10 cm) were from Merck (Darmstadt, Germany). Polygoprep 100 – 20 (C18, 100 Å, 15 – 25 μm, Art. No. 711019.100) was from Macharey-Nagel (Düren, Germany). Galanthamine hydrobromide and neocuproine (2,9-dimethyl-1,10-phenanthroline) were from Sigma-Aldrich (Steinheim, Germany). Acetylthiocholine iodide and Folin-Ciocalteu reagent were from Applichem (Darmstadt, Germany). Butyrylthiocholine iodide was from Fluka (Steinheim, Germany). Sodium carbonate, ammonium acetate, sodium hydrogen phosphate, and sodium dihydrogen phosphate were from Riedel-de-Häen (Seelze, Germany). All reagents and chemicals used were of analytical grade.

Plant material

The aerial parts of *C. leucopsis*, *C. sipyleum*, and *C. eriophorum*, which were in the flowering stage, were collected from Uludağ, Bursa, Turkey, in September 2009, and identified by Prof. Bayram Yıldız (Department of Biology, Faculty of Science and Arts, Balikesir University, Balikesir, Turkey). Voucher specimens have been deposited in the herbarium of the Faculty of Pharmacy, Istanbul University, Istanbul, Turkey (ISTE: 95409, 95410, and 95411, respectively).

Extraction and isolation

The dried and powdered aerial parts of *C. leucopsis* (408 g), *C. sipyleum* (690 g), and *C. eriophorum* (969 g) were successively macerated with *n*-hexane (3 × 24 h; 2 L, 3.5 L, 5 L, respectively), acetone (3 × 24 h; 2 L, 3.5 L, 5 L, respectively), and methanol (3 × 24 h; 2 L, 3.5 L, 5 L, respectively) at 25 °C. After filtration through a filter paper, the solvents were evaporated under reduced pressure until dryness. The *n*-hexane extracts of *C. leucopsis* (CLH; 6.46 g), *C. sipyleum* (CSH; 21.73 g), *C. eriophorum* (CEH; 31.50 g), the acetone extracts of *C. leucopsis* (CLA; 9.46 g), *C. sipyleum* (CSA; 16.35 g), *C. eriophorum* (CEA; 24.33 g), and the methanol extracts of *C. leucopsis* (CLM; 33.22 g), *C. sipyleum* (CSM; 31.37 g), *C. eriophorum* (CEM; 48.76 g) were subjected to column chromatography over silica gel. Their elution was started with *n*-hexane and followed, increasing the polarity of the element, by acetone and methanol, respectively. Similar fractions were combined according to the thin-layer chromatography (TLC) analysis. TLC plates were visualized by spraying with cerium(IV) sulfate dissolved in 10% sulfuric acid and observation under UV light. Final purification was carried out on silica gel and Sephadex columns, preparative TLC plates, and by preparative HPLC. Thirteen known compounds were obtained: *p*-hydroxy-benzoic acid (**1**; 45.7 mg) [preparative TLC, dichloromethane/acetone (2:1, v/v)], vanillic acid (**2**; 21.4 mg) [preparative TLC, dichloromethane/acetone (2:1)], *cis*-epoxyconiferyl alcohol (**3**; 10.5 mg) [preparative TLC, toluene/diethyl ether (1:1)], syringin (**4**; 2.6 mg) [white solid; purified by washing with methanol (6×), purity confirmed by TLC analysis], balanophonin (**5**; 7.8 mg) [preparative TLC, dichloromethane/acetone (5:1)], 1′-*O*-methyl-balanophonin (**6**; 5.4 mg) [preparative TLC, dichloromethane/acetone (2:1)], apigenin (**7**; 81.2 mg) [preparative TLC, dichloromethane/acetone (1:1)], kaempferol-3-*O*-β-D-glucopyranoside (**8**; 1 mg) [preparative HPLC, water/methanol (1:1)], kaempferol-3-*O*-α-L-rhamnopyranoside (**9**; 58.6 mg) [preparative HPLC, water/methanol (1:1)], taraxasterol (**10**; 85.7 mg) [preparative TLC, toluene/diethyl ether (5:1)], taraxasterol acetate (**11**; 60.6 mg) [preparative TLC, *n*-hexane/dichloromethane (2:1)], β-sitosterol (**12**; 37.8 mg) [preparative TLC, *n*-hexane/dichloromethane (1:1)], and β-sitosterol-3-*O*-β-D-glucopyranoside (**13**; 9.4 mg) [white solid; purified by washing with methanol (5×), purity confirmed by TLC analysis].

Derivatization of the n-hexane extracts for GC/MS analysis and GC/MS conditions

Conditions for the esterification of the *n*-hexane extracts and subsequent GC/MS analyses were according to Şabudak *et al.* (2009). Chromatographic analysis was carried out on a Thermo Scientific Polaris Q GC/MS/MS instrument.

Identification of fatty acids

Fatty acids were identified by comparison of their retention times and mass spectra with those of authentic samples, and/or the NIST and Wiley spectra, as well as literature data.

HPLC conditions

The following conditions were applied for chromatographic separations: column, C18 (250 mm × 20 mm ID, S-4 μm); solvent, methanol/water (1:1); flow rate, 3 mL/min; UV detector.

Determination of total phenolics and flavonoids

The contents of phenolics (Slinkard and Singleton, 1977) and flavonoids (Moreno *et al*., 2000) in the crude extracts of the three *Cirsium* species were expressed as pyrocatechol and quercetin equivalents, respectively, and were calculated from their absorbance according to the following equations:

absorbance (760 nm) = 0.0316 pyrocatechol (μg/mL) + 0.0422 ($R^2 = 0.9952$),

absorbance (415 nm) = 0.0269 quercetin (μg/mL) + 0.0393 ($R^2 = 0.9989$).

Antioxidant activity of extracts and isolated compounds

The β-carotene-linoleic acid test system (Miller, 1971), DPPH free radical scavenging activity (Blois, 1958), ABTS cation radical decolorization (Re *et al.*, 1999), superoxide anion radical scavenging activity (Nishikimi *et al.*, 1972), and cupric reducing antioxidant capacity (CUPRAC) (Apak *et al.*, 2004) methods were used to determine the antioxidant activity of the extracts and isolated compounds.

Anticholinesterase activity of extracts and isolated compounds

The spectrophotometric method developed by Ellman *et al.* (1961) was employed to determine the acetyl- (AChE) and butyrylcholinesterase (BChE) inhibitory activities of the extracts and isolated compounds.

Statistical analysis

The results of the antioxidant and anticholinesterase activity assays are given as the mean $\pm$SD of three parallel measurements. The statistical significance was estimated using a Student's t-test, p values < 0.05 were regarded as significant.

Results and Discussion

The phytochemical investigation on *C. leucopsis*, *C. sipyleum*, and *C. eriophorum* resulted in the isolation of thirteen known compounds: *p*-hydroxybenzoic acid (**1**) (Zhang *et al.*, 2009), vanillic acid (**2**) (Zhang *et al.*, 2009), *cis*-epoxyconiferyl alcohol (**3**)

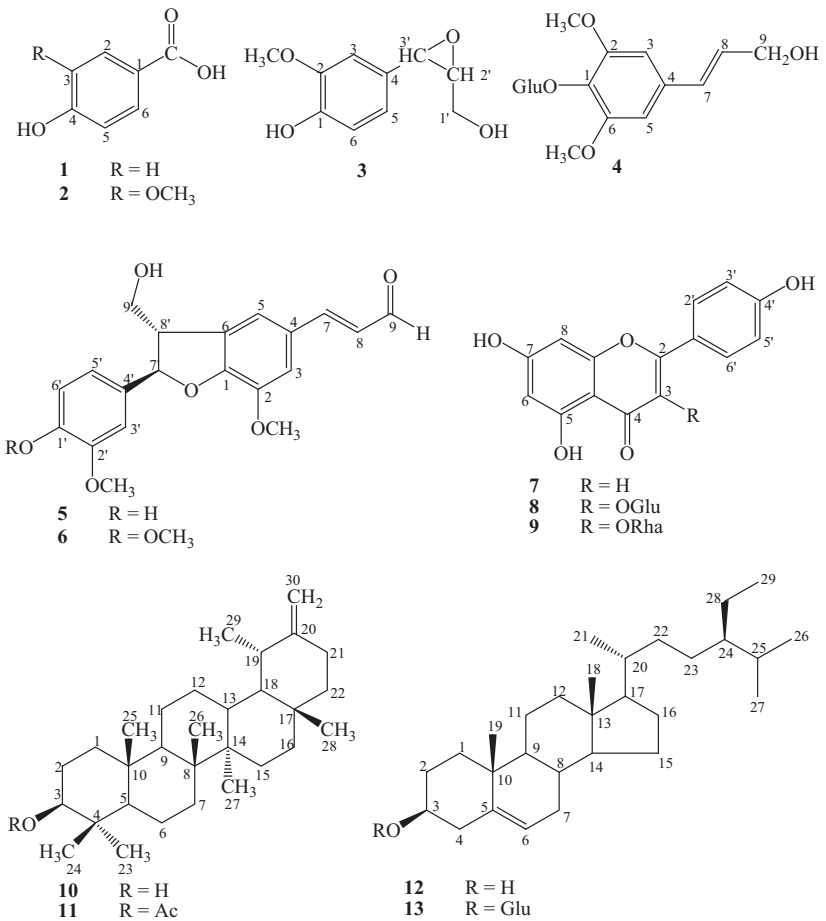

Fig. 1. Chemical structures of compounds **1** – **13**.

(Guz and Stermitz, 2000; Hacıbekiroğlu and Kolak, 2011), syringin (**4**) (Kim *et al.*, 2007), balanophonin (**5**) (Sy and Brown, 1999), 1′-*O*-methyl-balanophonin (**6**) (Ho *et al.*, 2004), apigenin (**7**) (Goulas *et al.*, 2012), kaempferol-3-*O*-*β*-D-glucopyranoside (**8**) (Wu *et al.*, 2009), kaempferol-3-*O*-*α*-L-rhamnopyranoside (**9**) (Correia *et al.*, 2008), taraxasterol (**10**) (Khalilov *et al.*, 2003), taraxasterol acetate (**11**) (Khalilov *et al.*, 2003), *β*-sitosterol (**12**) (Kolak *et al.*, 2005), and *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**13**) (Lee *et al.*, 2002) (Fig. 1). The structures of the isolated compounds were determined on the basis of spectroscopic evidence [UV, IR, ^{1}H and ^{13}C NMR (APT, DEPT), HMQC, HMBC, MS]; their spectroscopic data were in full agreement with those of standard samples. In the present study, *cis*-epoxyconiferyl alcohol (**3**) and 1′-*O*-methyl-balanophonin (**6**) were isolated for the first time from *Cirsium* species.

In the flora of Turkey, the genus *Cirsium* is classified into three sections: *Epitrachys* DC., *Cirsium* Mill., and *Cephalonoplos* (Neck.) DC. (Davis and Parris, 1975; Davis *et al.*, 1988; Güner, 2000). Our findings indicated that the *Cirsium* and *Epitrachys* sections contain different groups of secondary metabolites. As shown in Table I, *C. leucopsis*, which belongs to the *Cirsium* section, was found to contain the lignans **3** and **4**, while *C. sipyleum* and *C. eriophorum*, which are grouped into the *Epitrachys* section, possess the phenolic acids, neolignans, and kaempferol glycosides **1**, **2**, **5**, **6**, **8**, and **9**. While apigenin (**7**), taraxasterol (**10**), and *β*-sitosterol (**12**) are encountered in both sections, taraxasterol acetate (**11**) was obtained from *C. sipyleum* and *C. eriophorum*, and *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**13**) was isolated from *C. leucopsis*.

The fatty acid composition in the *n*-hexane extracts of *C. leucopsis*, *C. sipyleum*, and *C. eriophorum* was determined by GC and GC/MS analysis. The major fatty acids of *C. sipyleum* and *C. eriophorum* were identified as linoleic acid (42.1 % and 37.8 %, respectively) and palmitic acid (28.3 % and 23.1 %) (Table II), while reversely, palmitic acid (47.1 %) was the dominant, and linoleic acid (15.8 %) a lesser constituent of *C. leucopsis*. Orhan *et al.* (2009) reported that the main fatty acid of *C. hypoleucum*, which is grouped into the same section with *C. leucopsis,* is also palmitic acid.

Total phenolic and flavonoid contents of the *n*-hexane, acetone, and methanol extracts, respectively, of *C. leucopsis*, *C. sipyleum*, and *C. eriophorum* were determined as pyrocatechol equivalents (PEs) and quercetin equivalents (QEs), respectively. The acetone extracts were found to be rich in flavonoids (Table III).

The antioxidant activity tests of the *n*-hexane, acetone, and methanol extracts of *C. leucopsis*, *C.*

Table I. Compounds isolated from *C. leucopsis*, *C. sipyleum*, and *C. eriophorum*.

Isolated compound	*Cirsium* section	*Epitrachys* section	
	C. leucopsis	*C. sipyleum*	*C. eriophorum*
Phenolic acids			
p-Hydroxy-benzoic acid (**1**)		×	×
Vanillic acid (**2**)		×	×
Lignans			
cis-Epoxyconiferyl alcohol (**3**)	×		
Syringin (**4**)	×		
Neolignans			
Balanophonin (**5**)		×	×
1′-*O*-Methyl-balanophonin (**6**)		×	×
Flavonoids			
Apigenin (**7**)	×	×	×
Kaempferol-3-*O*-*β*-D-glucopyranoside (**8**)		×	×
Kaempferol-3-*O*-*α*-L-rhamnopyranoside (**9**)		×	×
Triterpenoids			
Taraxasterol (**10**)	×	×	×
Taraxasterol acetate (**11**)		×	×
Steroids			
β-Sitosterol (**12**)	×	×	×
β-Sitosterol-3-*O*-*β*-D-glucopyranoside (**13**)	×		

×, Compound isolated.

Table II. Fatty acid composition (in %) of *C. leucopsis*, *C. sipyleum*, and *C. eriophorum*.

Rt [min][a]	Compound[b]	Composition (%)		
		C. leucopsis	*C. sipyleum*	*C. eriophorum*
6.56	Capric acid	–	–	0.2
12.00	Lauric acid	–	–	1.5
14.39	10-Undecanoic acid	–	–	1.7
28.00	Myristic acid	1.0	1.5	2.2
31.57	Palmitoleic acid	2.5	1.1	0.3
31.98	Palmitic acid	47.1	28.3	23.1
32.30	Phytol	5.1	t[c]	3.5
35.61	Stearic acid	3.5	4.3	4.7
35.92	Oleic acid	11.0	16.5	15.9
36.70	Linoleic acid	15.8	42.1	37.8
37.77	Linolenic acid	9.5	2.6	1.2
38.99	Arachidic acid	2.4	3.1	1.6
39.36	Docosane	–	–	5.6
40.16	Heptacosane	2.1	0.5	–
43.82	Behenic acid	–	–	0.7
	Total	100	100	100

[a] Retention time.
[b] Compounds listed in order of elution from a nonpolar Phenomenex DB-5 fused silica column. They were analysed as their methyl esters.
[c] Trace.

sipyleum, *C. eriophorum*, and of the isolated compounds **1**–**7** and **9**–**13** were carried out using β-carotene bleaching, DPPH free radical scavenging, ABTS cation radical scavenging and superoxide anion radical scavenging activities, and CUPRAC assays. After elucidation of the structure of compound **8**, the remaining amount was too low to conduct the biological assays. None of the extracts exhibited antioxidant activity in these tests, except for those of *C. eriophorum*. The acetone and methanol extracts of *C. eriophorum* exhibited almost the same ABTS cation radical scavenging activity as the standard compound, (+)-catechin at 100 μg/mL (Fig. 2). All of the tested compounds **1**–**7** and **9**–**13**, which did not have CUPRAC, inhibited lipid peroxidation very weakly (< 40% inhibition) at all concentrations tested (10, 25, 50, and 100 μg/mL). The DPPH free radical scavenging assay of these compounds indicated that vanillic acid (**2**) and balanophonin (**5**) possessed the best antioxidant capacity with 72% and 64% inhibition, respectively, at 100 μg/mL whereas the others were inactive (Fig. 3). The antioxidant activities of the compounds determined in the ABTS assay are shown in Fig. 2. For each sample, four concentrations were tested. At the highest tested dose (100 μg/mL), vanillic acid (**2**) and balanophonin (**5**) exhibited almost the same ABTS cation radical scavenging activity (85% inhibition) as (+)-catechin, and kaempferol-3-*O*-α-L-rhamnopyranoside (**9**) produced the strongest inhibition (over 85%) among the tested extracts and isolated compounds. The inhibition of ABTS cation radical formation ranged from 40% to 90% at the highest tested dose (100 μg/mL) and from 5% to 52% at the lowest tested dose (10 μg/mL). Fig. 4 illustrates the ability of compounds **1**–**4**, **6**, and **9** to scavenge superoxide anion radicals. Vanillic acid (**2**) and kaempferol-3-*O*-α-L-rhamnopyranoside (**9**) exhibited higher inhi-

Table III. Total phenolic and flavonoid contents of the extracts[a].

Extract	Phenolic content (μg PEs/mg extract)[b]	Flavonoid content (μg QEs/mg extract)[c]
CLH	33.97 ± 0.37	24.67 ± 3.11
CLA	30.06 ± 1.57	68.87 ± 3.19
CLM	16.38 ± 1.68	14.04 ± 0.65
CSH	32.67 ± 0.34	21.96 ± 3.15
CSA	19.86 ± 1.68	67.37 ± 1.78
CSM	17.17 ± 1.01	11.82 ± 0.65
CEH	10.54 ± 1.82	3.90 ± 1.02
CEA	15.09 ± 1.29	32.11 ± 0.57
CEM	6.00 ± 1.29	16.32 ± 1.72

[a] Values are means ±SD of three parallel measurements.
[b] PEs, pyrocatechol equivalents.
[c] QEs, quercetin equivalents.
CLH, *n*-hexane extract of *C. leucopsis*; CLA, acetone extract of *C. leucopsis*; CLM, methanol extract of *C. leucopsis*; CSH, *n*-hexane extract of *C. sipyleum*; CSA, acetone extract of *C. sipyleum*; CSM, methanol extract of *C. sipyleum*; CEH, *n*-hexane extract of *C. eriophorum*; CEA, acetone extract of *C. eriophorum*; CEM, methanol extract of *C. eriophorum*.

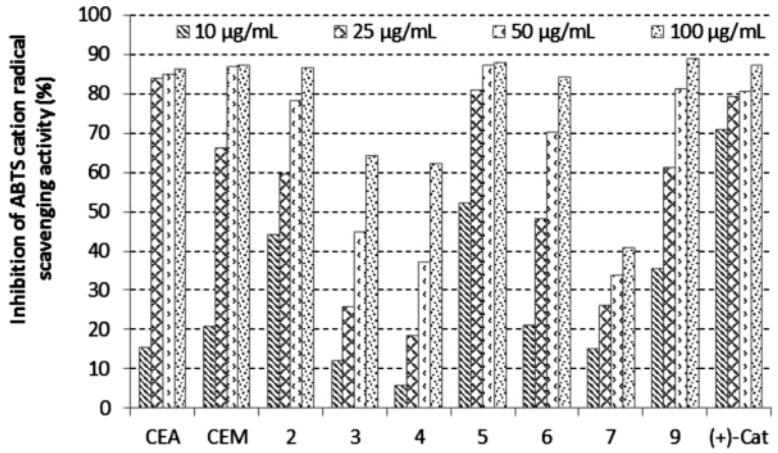

Fig. 2. Inhibition (in %) of the ABTS cation radical scavenging activity of the acetone (CEA) and methanol (CEM) extracts of *C. eriophorum*, isolated compounds **2**–**7**, **9**, and (+)-catechin [(+)-Cat]. Values are means ± SD, $n = 3$, $p < 0.05$, significantly different from each other with Student's *t*-test.

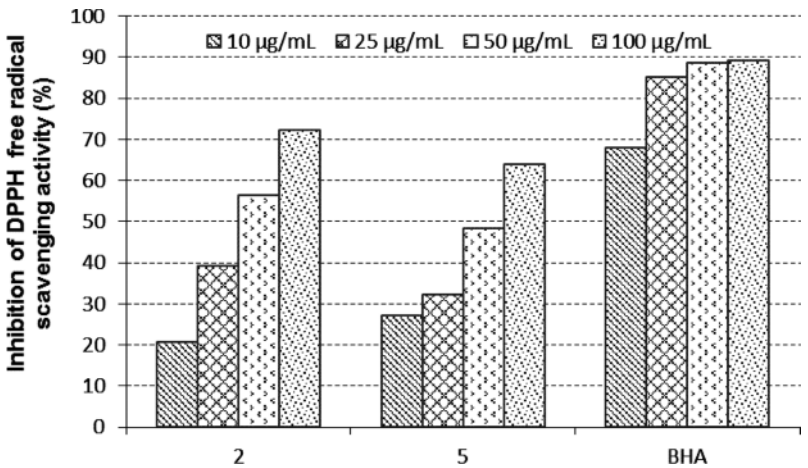

Fig. 3. Inhibition (in %) of the DPPH free radical scavenging activity of compounds **2** and **5**, and butylated hydroxyanisole (BHA). Values are means ± SD, $n = 3$, $p < 0.05$, significantly different from each other with Student's *t*-test.

bition than gallic acid, a standard compound, in the superoxide anion radical scavenging activity assay at 50 μg/mL. Pro-oxidants are chemicals that induce oxidative stress, either by generating reactive oxygen species or by inhibiting antioxidant systems. As shown in Fig. 4, the antioxidant activity of compound **6** did not increase as a function of its concentration; it may have a pro-oxidant effect.

The neurodegenerative Alzheimer's disease (AD) is an important health problem for elderly people. AD is associated with a characteristic loss of memory which is accompanied by an increase of acetylcholinesterase activity. Some clinical effects of medicinal plants have been closely related to their antioxidant activity (Gu and Weng, 2001), *i. e.* the antioxidants may be relevant in slowing AD progression and minimizing neuronal degeneration (Howes *et al.*, 2003). Since some synthetic acetylcholinesterase inhibitors (tacrine, donepezil) used for the treatment of AD have several adverse effects, herbal extracts and their secondary metabolites have been tested *in vitro* and *in vivo* as anticholinesterase inhibitors, and some of them exhibited a strong effect (Hacıbekiroğlu and Kolak, 2011). In the present study, the anticholinesterase activity of the *C. leucopsis*, *C. sipyleum*, and *C. eriophorum* extracts, and of the isolated com-

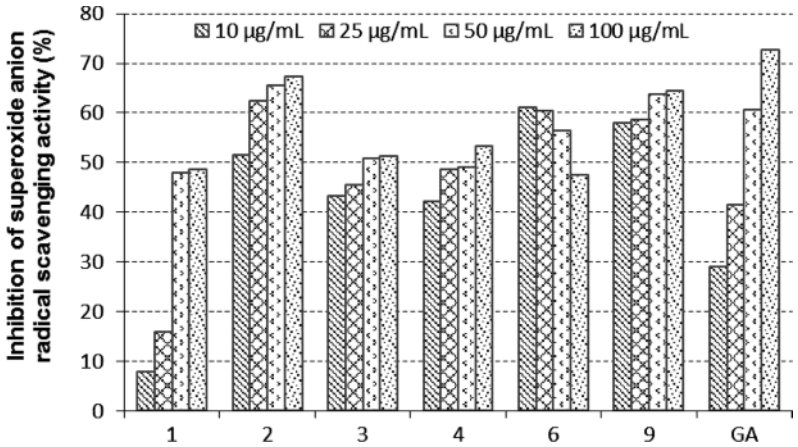

Fig. 4. Inhibition (in %) of the superoxide anion radical scavenging activity of compounds **1**–**4**, **6**, **9**, and gallic acid (GA). Values are means ± SD, $n = 3$, $p < 0.05$, significantly different from each other with Student's *t*-test.

Table IV. Inhibiton of cholinesterase activity by extracts and compounds **1**–**7** and **9**–**13** at 200 μg/mL[a].

Sample	Inhibition of AChE (%)	Inhibition of BChE (%)
CLH	16.50 ± 2.84	NA[b]
CLA	19.42 ± 4.13	36.94 ± 1.54
CLM	5.25 ± 2.01	NA
CSH	22.70 ± 2.36	6.75 ± 0.15
CSA	21.39 ± 2.23	24.87 ± 3.20
CSM	9.90 ± 1.89	5.33 ± 0.81
CEH	48.74 ± 2.89	42.51 ± 1.96
CEA	56.98 ± 2.16	38.79 ± 0.47
CEM	46.64 ± 2.93	29.87 ± 0.86
p-Hydroxy-benzoic acid (**1**)	16.99 ± 0.97	NA
Vanillic acid (**2**)	NA	28.02 ± 1.22
cis-Epoxyconiferyl alcohol (**3**)	16.67 ± 0.28	41.71 ± 0.78
Syringin (**4**)	NA	46.08 ± 1.13
Balanophonin (**5**)	NA	6.19 ± 1.61
1′-*O*-Methyl-balanophonin (**6**)	20.00 ± 1.11	59.29 ± 1.06
Apigenin (**7**)	39.02 ± 1.05	56.11 ± 1.19
Kampferol-3-*O*-*α*-L-rhamnopyranoside (**9**)	NA	NA
Taraxasterol (**10**)	55.49 ± 1.27	72.55 ± 1.55
Taraxasterol acetate (**11**)	30.20 ± 2.22	NA
β-Sitosterol (**12**)	NA	28.61 ± 1.65
β-Sitosterol-3-*O*-*β*-D-glucopyranoside (**13**)	NA	NA
Galanthamine[c]	76.08 ± 0.39	76.52 ± 0.41

[a] Values are means ± SD of three parallel measurements ($p < 0.05$).
[b] NA, not active.
[c] Standard drug.

pounds **1**–**7** and **9**–**13** was determined at 200 μg/mL, and galanthamine was used as a standard compound. The acetone extract of *C. eriophorum* was moderately inhibitory, whereas the other extracts were inactive (Table IV). Taraxasterol (**10**) exhibited the highest acetyl- and butyrylcholinesterase inhibitory activities among the tested compounds in this study (Table IV), while Gurovic *et al.* (2010) claimed that it has no acetylcholinesterase inhibitory effect. 1′-*O*-Methyl-balanophonin (**6**) and apigenin (**7**) were found to possess significant inhibitory activity against butyrylcholinesterase.

Conclusions

To our knowledge, this is the first phytochemical and biological report on *C. leucopsis*, *C. sipyleum*, and *C. eriophorum*. This study indicates that the presence of lignans in the *Cirsium* section and of phenolic acids, neolignans, and kaempferol glycosides in the *Epitrachys* section could be of chemotaxonomic importance, along with their fatty acid profiles. Further phytochemical investigations on other *Cirsium* species grown in Turkey are needed to confirm this assumption. In the current work, *cis*-epoxyconiferyl alcohol (**3**) and 1′-*O*-methyl-balanophonin (**6**) were obtained for the first time from the genus *Cirsium*. This report demonstrated also the relationship between the chemical structures and biological activities of some isolated compounds. *p*-Hydroxy-benzoic acid (**1**) and vanillic acid (**2**) have the same skeleton. The strong antioxidant capacity of vanillic acid (**2**) in scavenging the DPPH free radical, ABTS cation radical, and superoxide anion radical, respectively, may be explained by a methoxy group at the C-3 position, while *p*-hydroxy-benzoic acid (**1**) was found to be inactive in these assays. Taraxasterol (**10**) possessed strong acetyl- and butyrylcholinesterase inhibitory activity, while its acetate **11** had a weak inhibitory effect against acetylcholinesterase. On the other hand, the relationship mentioned above was not observed for balanophonin (**5**) and its methyl derivative **6**.

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