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Comparison of phenolic components and biological activities of two *Centaurea* sp. obtained by three extraction techniques

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ABSTRACT

Objective: The present study aimed to compare the polyphenol content, total phenolic, total flavonoid, antioxidant and antimicrobial activity of the extracts obtained from *Centaurea amaena* Boiss. & Balansa and *Centaurea aksoyi* Hamzaoğlu & Budak.
Methods: Both species were subjected to maceration, Soxhlet and ultrasonication extractions with methanol in order to macerated (ME), Soxhlet (SE) and ultrasonicated (UE) extracts. Their phenolic profiles were qualitatively examined by LC–MS. Their antioxidant activities were determined by phosphomolybdenum, β-carotene bleaching and DPPH assays. Agar diffusion and broth dilution methods were carried out to find the antimicrobial activity of these extracts against fifteen microorganisms.
Results: Quercetin, quercetin-3-β-D-glucoside and protocatechuic acid were the main components of the both extracts obtained by Soxhlet extraction. The highest phenolic and flavonoid contents were found in the UE for both species. All the extracts exhibited good total antioxidant and DPPH radical scavenging activity. UE obtained from *C. amaena* showed the highest antioxidant activity with the highest phenolic and flavonoid contents. The antibacterial activity of UE obtained from *C. amaena* was better than other extracts and antibacterial activity of *C. amaena* was also better than *C. aksoyi*.
Discussion: This study confirms that ultrasonic extraction may be an ideal, simple and rapid method to obtain polyphenol-rich extracts have antioxidant as well as antibacterial activity from both *Centaurea* species especially from *C. amaena*.

1. Introduction

Free radicals are generated by our body through various metabolic reactions [1]. Free radicals can conversely alter lipids, proteins and DNA and trigger a number of human diseases [2]. Our endogenous antioxidant defense systems (SOD, catalase, and peroxidase) controls their production exerting synergistic action with exogenous originating reducing compounds (vitamin E, vitamin C and β-carotene). The antioxidant defense system becomes inefficient when excess of free radical production. The oxidative stress is associated with pathogenesis of various disorders like neurodegenerative disorders, atherosclerosis, cancer, hypertension, aging and many more. Antioxidants are the substances which can scavenge free radicals and help to protect the body from oxidative stress induced damage [3]. Plants contain phytochemical antioxidants such as phenolics and flavonoids which can be used to scavenge the free radicals. The antioxidant potential of these substances is due to their redox properties [4]. Crude extracts of phenolic rich plants are raising interest in the food industry because they delay oxidative damage of lipids and improve the quality and their nutritional value. The importance of the antioxidants in plants in the maintenance of health and protection from disease is also increasingly of interest among scientists and consumers [5].

Centaurea L. (Asteraceae) includes about 500 species with wide distribution in the Mediterranean area and in Western Asia [6–9]. *Centaurea* is represented in Turkey with 194 species out of which 118 are endemic [10,11]. Common names for different species are star-thistle, cornflower, and knapweed. Some of the genus are known by various local names such as “peygamber

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çiceği", "zerdali dikeni", "çoban kaldiran", "Timur dikeni", "gökbaş", "sarıbaş", "acımık", "kötürüm", "kotonkıran"and "boğa dikeni" in Anatolia [7,12–16]. Some *Centaurea* are used in the popular medicine in some countries and in Turkish folk medicine to treat various ailments including stomach ache, abscesses, asthma, hemorrhoids, headache, inflammatory disorders, urogenital ailments, endocrine diseases, gastrointestinal symptoms, cardiovascular problems, parasitic and microbial infections, rheumatic pain, as cytostatic, astringent, diuretic, antineoplastic, tonic, allergenic, antinociceptive, antipyretic effects, antiulcerogenic activity, anti-atherosclerotic effect, wound healing, to reduce fever [6,7,16–21]. Many reports on *Centaurea* have indicated the presence of sesquiterpene lactones, flavonoids [6,20,22], lignans [23,24], alkaloids, simple phenolics [22], steroids, triterpenes, hydrocarbons, polyacetylenes, anthocyanins [16]. Some *Centaurea* have biological activities such as antimicrobial, antifungal, antiplasmodial [21,25], cytogenetic, antiulcerogenic activities, antioxidant properties, antiviral, antiprotozoal, anti-colon cancer and cytotoxic activity [12,14,16,26,27], anti-inflammatory [28], neuro-cytotoxic, cardiotonic [6]. Keeping in view the fact that there is no report concerning the phytochemical contents and bioactivities of *Centaurea aksoyi* and *Centaurea amaena* in the literature, it was thought worthwhile to evaluate total phenolics, antioxidant and antimicrobial activities of both plant. Also, different extraction methods for extraction of bioactive substances from both *Centaurea* were used in this report.

2. Material and methods

2.1. Plants material

C. aksoyi Hamzaoğlu & Budak was collected from Yozgat: between Şefaatli and Yerköy, Karanlıkdere valley, south slope of Adatepe, Turkey, ca 905 m, 11 Jun 2011 (39°34′02″ N, 034°40′15″ E). *C. amaena* Boiss. & Balansa was collected from the Yılanlı Mountain ascent, above quarry, rocky slopes, Turkey, 1275 m, July 2011–2012 (38°42′82″N, 35°25′20″E). Plants were collected during their flowering season and identified. The voucher specimens have been deposited at the Herbarium of the Department of Biology, Erciyes University, Kayseri, Turkey (Voucher no.: *C. aksoyi*-AAksoy 2411, *C. amaena*-AAksoy 2406).

2.2. Extraction

2.2.1. Maceration

Ground plant material (10 g) and methanol (100 mL) were put in a series of the Erlenmeyer flasks (100 mL). No additional stirring was applied. The extraction was carried out at room temperature in the dark for 3 d. After 3 d of maceration, the liquid extract was separated from the solid residue by filtration. The solid residue was washed twice with 20 mL of methanol. The filtrates were collected and the solvent was evaporated in a rotary vacuum evaporator at 40 °C to obtain macerated extract (ME) [29].

2.2.2. Soxhlet extraction

Powdered plant samples (10 g) were extracted using a Soxhlet type extractor with 100 mL methanol for 7 h. Then, the Soxhlet extract (SE) was filtered and evaporated to dryness in vacuum at 40 °C with a rotary evaporator [30].

2.2.3. Ultrasonic extraction

The sonication was performed for 1 h with methanol, at a ratio of plant material (g) to solvent (mL) 1:10 and (25 ± 0.1) °C using an ultrasonic cleaning bath (Bandolen Sonorex). The power and frequency were set at 140 W and 5 kHz, respectively. The temperature was controlled and maintained at the desired level with water circulating from a thermostat bath by means of a pump. The extract was filtered and evaporated as described in previous section [31]. This yield of the ultrasonicated (UE) extract (% percent) was determined and prepared extracts were stored at 4 °C until further analyzed.

2.3. LC–MS analysis of phenolic compounds in the extracts

A liquid chromatography was equipped with electrospray ion sources mass spectrometer working with Mass Hunter software package. Zorbax SB-C18 (150 × 2.1 mm, 1.8 μm) column was used. The flow rate was 0.25 mL/min and the injection volume 10 μL. Positive and negative ion mode was performed. The nitrogen temperature was 350 °C at a flow rate of 8 L/min. The mobile phase composition was: (5/95:h/h) methanol:water (eluent A) and methanol (eluent B) both containing 0.01% formic acid and 5 mmol/L ammonium formate. The gradient program was as follows: 5% B (0–1 min), 30% B (1–3 min), 60% B (3–4 min), 60% B (4–5 min), 70% B (5–6 min), 80% B (6–8 min), 5% B (8.01 min), 5% B (8.01–10 min).

Gallic acid, cinnamic acid, caffeic acid, ferulic acid, chlorogenic acid, protocatechuic acid, ellagic acid, catechin hydrate, epicatechin, epigallocatechin gallate, rosmarinic acid, syringic acid, quercetin, quercetin-3-β-D-glucoside, myricetin, phloridzin hydrate, rutin, p-coumaric acid, o-coumaric acid, kaempferol, bergapten and psoralen were used as standard.

2.4. Determination of total phenolics

Folin-Ciocalteu assay was used to determine the total phenolic contents of the extracts [32]. Forty microliter aliquot of the extract was mixed with 200 μL of Folin-Ciocalteu reagent and 600 μL (20% Na_2CO_3) of sodium carbonate. The mixture was vortexed and allowed to stand for 2 h at room temperature. Absorbance was then measured at 765 nm using the spectrophotometer. Gallic acid was used in the construction of the standard curve. The values were expressed in terms of milligrams of gallic acid equivalents (mg GAE) per gram extract.

2.5. Determination of total flavonoids

Total flavonoids were assessed by Aluminum chloride colorimetric method as described by Pourmorad *et al* [33]. The extract was mixed with methanol (1.5 mL), aluminum chloride (0.1 mL), 1 mol/L potassium acetate (0.1 mL) and distilled water (2.8 mL). The absorbance of the mixture was measured at 415 nm. The results are expressed as mg of quercetin equivalents (QE)/g extract.

2.6. Determination of antioxidant activity

2.6.1. Total antioxidant activity

Phosphomolybdenum assay was used Prieto *et al* [34]. The extract was mixed with 4 mL of reagent solution (0.6 mol/L

sulfuric acid, 28 mmol/L sodium phosphate and 4 mmol/L ammonium molybdate). The tubes were incubated at 95 °C for 90 min. The absorbance was measured at 695 nm. The antioxidant activity was expressed as mg of ascorbic acid equivalents (AAE)/g extract.

2.6.2. β-Carotene bleaching assay

Antioxidant activity of the extract was assessed by β-carotene bleaching system as described by Cao *et al* [35]. β-carotene was dissolved in chloroform. An aliquot of this solution was added to linoleic acid and Tween 40. The chloroform was removed. Then, distilled water saturated with oxygen was added. The mixture was added to extract solution. The test emulsion was incubated at 50 °C for 2 h, when the absorbance was measured at 470 nm. The same procedure for BHT (Butylated hydroxytoluene) was carried out.

2.6.3. DPPH method

The scavenging ability to 2,2-diphenyl-1-picrylhydrazyl (DPPH) was assessed according to Lee *et al* [36]. 0.1–4.0 mg/mL concentration of the extract was added to DPPH solution (0.1 mmol/L). The absorbance was read at 517 nm after 30 min incubation period. IC_{50} (concentration required to scavenge 50% DPPH free radicals) value was determined graphically. BHT was used as control.

Percent inhibition (%) was calculated in the following way:

Inhibition % = ($A_{\text{absorbance of the control}} - A_{\text{absorbance of the sample}}$/$A_{\text{absorbance of the control}}$) × 100.

2.7. Determination of antimicrobial activity

The following microorganisms were tested: *Aeromonas hydrophila* ATCC 7965, *Bacillus cereus* RSKK 863, *Escherichia coli* ATCC 25922, *E. coli* O157:H7 932, *Klebsiella pneumoniae* FMC 5, *Listeria monocytogenes* 1/2B, *Morganella morganii*, *Mycobacterium smegmatis* RUT, *Proteus mirabilis* BC 3624, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* NRRLE 4463, *Staphylococcus aureus* ATCC 29213, *Yersinia enterocolitica* ATCC 1501, *Candida albicans* ATCC 1223 and *Aspergillus parasiticus* DSM 5771.

The agar-well diffusion assay was used [29]. The bacteria were suspended in sterile nutrient broth. *C. albicans* and *A. parasiticus* were suspended in malt extract and potato dextrose broth, respectively. Suspensions of microorganisms were 1.5 × 10^6–10^7 colony-forming units (cfu/mL). The medium was poured into Petri plates (9 cm). Then the agars were allowed to solidify. Subsequently, wells (6 mm) were made in the inoculated media. 50 µL of each extract (30 mg/mL) was aseptically filled into the well. *Y. enterocolitica*, *C. albicans* and *A. parasiticus* was incubated at 25 °C for 24–48 h in the inverted position. The other bacteria were incubated at 37 °C for 18–24 h. Inhibition zone was measured. The methanol was used as a control. Tetracycline (10 mg/mL), Oxacillin (10 mg/mL) for bacteria and Natamycin (30 mg/mL) for *C. albicans* and *A. parasiticus*, standard antibiotics were also used. All the tests were performed in duplicate and the results were presented as averages.

2.8. Determination of minimum inhibitory concentration (MIC)

A micro-well dilution assay was used to determination of minimum inhibitory concentrations of the extracts [37]. Dilutions of the extracts were prepared in a 96 well microtitre plates to get final concentration ranging from 0.234 to 30 mg/mL. A total of 10 µL of the bacterial strains which were adjusted to 0.5 McFarland were inoculated on to the microplates. Total volume in each well was 200 µL. Contents of each well were mixed and incubated at 37 °C (25 °C for yeasts) for 24 h. The lowest concentration, which did not show any visual growth, was determined as MIC.

2.9. Statistical analysis

The measurements were performed in triplicate and the results were averaged. Analysis of variance was performed by ANOVA procedures (SPSS 10.0 for Windows). Percentage data were transformed using arcsine $\sqrt{x}$ before ANOVA. Means were separated at the 5% significance level by the least significant difference (*LSD*) test.

3. Results

3.1. Amounts of total phenolic and flavonoids

The percent yields, total phenolics of *C. aksoyi* and *C. amaena* extracts were different from each other (Table 1). The extract yields of *C. aksoyi* ranged from 4.59% to 11.42% (w/w) while the extract yields of *C. amaena* ranged from 7.19% to 14.99% (w/w) (Table 1). The yields for both species obtained by Soxhlet extraction technique were the highest. There were

Table 1

Total phenolic, flavonoid contents, antioxidant and antiradical activities of two *Centaurea* species.

Plants	Extract	Extraction yields (%)	Total phenolics (mg GAE/g extract)	Total flavonoid (mg QE/g extract)	Total antioxidant activity (mg AAE/g extract)	IC_{50} (µg/mL)
C. aksoyi	ME	8.48 ± 0.11	3.28 ± 0.77^{b}	6.96 ± 0.01^{b}	142.95 ± 0.06^{a}	107.92^{b}
	SE	11.42 ± 0.02	4.97 ± 0.51^{a}	5.98 ± 0.02^{c}	135.38 ± 0.11^{b}	107.42^{d}
	UE	4.59 ± 0.57	5.65 ± 0.29^{a}	7.37 ± 0.02^{a}	136.38 ± 0.07^{b}	107.85^{c}
C. amaena	ME	12.28 ± 0.16	3.62 ± 0.77^{c}	5.83 ± 0.02^{c}	144.47 ± 0.09^{b}	77.85^{b}
	SE	14.99 ± 0.65	7.67 ± 0.29^{b}	7.17 ± 0.02^{b}	147.37 ± 0.14^{a}	63.18^{d}
	UE	7.19 ± 0.50	12.92 ± 0.77^{a}	7.69 ± 0.05^{a}	147.52 ± 0.06^{a}	71.95^{c}
BHT						5.52^{a}

ME: Classical extract; SE: Soxhlet extract; UE: Ultrasound extract. Total phenolic content expressed as gallic acid equivalent (GAE), total flavonoid content expressed as quercetin equivalent (QE), total antioxidant activity expressed as ascorbic acid equivalent (AAE). In each column, means of three independent experiments (± SD) with different superscript letters are significantly different ($P < 0.05$).

statistical differences among the total phenolic and flavonoid contents with respect to the plant species and the extraction method ($P < 0.05$). In the case of both *C. aksoyi* and *C. amaena*, the highest amounts of total phenolic and flavonoid were determined in the extracts obtained by ultrasonic extraction and the lowest amount was found by the maceration. The highest phenolic content was found in the UE for both *Centaurea* species followed by SE and ME. The total phenolic content in *C. aksoyi* extracts obtained from different extraction techniques varies from 3.28 to 5.65 mg GAE/g while in case of *C. amaena*, its ranges from 3.62 to 12.92 mg GAE/g. The flavonoid contents of *C. aksoyi* extracts were found to be 6.96, 5.98 and 7.37 mg QE/g extract for ME, SE and UE, respectively. Also, the flavonoid contents of *C. amaena* extracts were found to be 5.83, 7.17 and 7.69 mg QE/g extract for ME, SE and UE, respectively (Table 1).

3.2. Total antioxidant activity

As shown in Table 1, the extracts of both *Centaurea* species had strong total antioxidant activities independent on extraction techniques. There were statistical differences in the total antioxidant activity of the extracts ($P < 0.05$). As can be seen in Table 1, the UE of *C. amaena* extract exhibited the highest total antioxidant effect. The UE of *C. amaena* also included the largest amount of total phenolic and flavonoid compounds. While, the ME obtained from *C. amaena* showed the least total antioxidant and antiradical activity (IC_{50} = 77.85 μg/mL) with the least total phenolic and flavonoid contents. The *C. amaena* extract obtained by maceration showed the least total antioxidant effect (144.47 ± 0.1 mg AAE/g) and antiradical activity (IC_{50} = 77.85 μg/mL). In the contrast, the ME for *C. aksoyi* had the highest antioxidant effect (142.95 mg AAE/g). The lowest antioxidant activity was found in the SE of *C. aksoyi* (135.38 mg AAE/g) (Table 1).

Table 2
The quantity of some phenolic compounds determined in SE of *C. aksoyi* and *C. amaena* by LC–MS (μg/mL).

Compounds	*C. aksoyi*	*C. amaena*
Rosmarinic acid	–	191.40
Rutin	–	155.99
Quercetin-3-β-D-glucoside	1 009.74	349.60
Quercetin	280.85	261.90
Campherol	–	291.20
Protocatechuic acid	474.40	420.10
Chlorogenic acid	492.40	–
Caffeic acid	–	–
Syringic acid	–	221.50
p-coumaric acid	–	212.30
Ferulic acid	–	273.90
Total	2 257.39	2377.89

–: Not detected.

3.3. Phenolic composition

The phenolic acids and flavonoids of SE of both species were detected by the LC–MS method and are showed in Table 2. The amount of each compound is demonstrated as μg/mL. The major component present in the SE of *C. aksoyi* was identified as quercetin-3-β-D-glucoside followed by chlorogenic acid, protocatechuic acid and quercetin. The major component present in the SE of *C. amaena* was identified as protocatechuic acid followed by quercetin-3-β-D-glucoside, champherol, and ferulic acid.

3.4. DPPH scavenging activity

The radical scavenging activities of the extracts were determined by the DPPH method. The findings are indicated in Table 3. The percent inhibitions on DPPH radical of the extracts (6.66–133.3 μg/mL) were compared with BHT (Table 3). The extracts showed concentration dependent radical scavenging effect. The radical scavenging activities of both *Centaurea* species (31.80%–47.36%) were significantly lower than that of BHT (92.15%) at 66.6 μg/mL ($P < 0.05$). For both *Centaurea* species, DPPH radical scavenging activities were following order: SE > UE > ME. Furthermore, the scavenging effects of *C. amaena* extracts were significantly higher than that of *C. aksoyi* extracts obtained by same extraction technique ($P < 0.05$) (Table 3).

3.5. β-Carotene bleaching activity

The antioxidant effects of the extracts were determined by β-carotene–linoleate model and compared with that of BHT. The ME, SE and UE of *C. aksoyi* showed 4.76%, 10.88% and 7.64% inhibition, respectively. In the case of the *C. amaena*, inhibition rates of ME, SE and UE were found as 9.07%, 16.43% and 14.06%, respectively. The inhibition rates of extracts obtained from both species were significantly lower than that of BHT (84.26%) ($P < 0.05$).

3.6. Antimicrobial activity

The antimicrobial activities of *C. aksoyi* and *C. amaena* were examined against nine Gram (−), four Gram (+) bacterial strains, one yeast and fungus mentioned above by the agar well

Table 3
% Inhibition rates of *Centaurea* extracts obtained by classical, Soxhlet and ultrasound extraction techniques on DPPH assay.

Plant conc. (μg/mL)	*C. aksoyi*			*C. amaena*			BHT
	ME	SE	UE	ME	SE	UE	
6.66	1.04 ± 0.44	4.03 ± 0.13	2.65 ± 0.00	2.88 ± 0.22	3.05 ± 0.00	3.93 ± 0.000	55.80 ± 0.21
8.30	4.04 ± 0.27	6.96 ± 0.15	5.84 ± 0.07	6.80 ± 0.19	7.62 ± 0.00	7.39 ± 0.19	89.01 ± 0.13
16.60	10.88 ± 0.63	11.07 ± 0.00	11.77 ± 0.07	12.14 ± 0.63	15.21 ± 0.19	14.83 ± 0.13	89.13 ± 0.32
33.30	19.13 ± 0.34	21.01 ± 0.00	20.55 ± 0.13	25.37 ± 0.63	29.78 ± 0.19	28.43 ± 0.15	91.47 ± 0.21
66.60	31.80 ± 0.45	34.97 ± 0.45	34.09 ± 0.32	45.56 ± 0.66	52.31 ± 0.21	47.36 ± 0.19	92.15 ± 0.12
100.00	47.28 ± 0.51	47.32 ± 0.19	47.27 ± 0.07	58.74 ± 0.21	74.46 ± 0.00	63.84 ± 0.00	–
133.30	58.74 ± 0.48	59.33 ± 0.84	58.87 ± 0.26	89.68 ± 0.19	90.39 ± 0.00	76.65 ± 0.02	–

–: Not detected.
All values expressed are mean ± standard deviation of three experiments.

Table 4
The antimicrobial activities of two *Centaurea* species and standard antibiotics.

Microorganisms		*C. aksoyi* UE		*C. amaena* UE		*C. amaena* SE		*C. amaena* ME		Tetracycline		Oxacillin	
		IZ (mm)	MIC (mg/mL)	IZ (mm)	MIC (mg/mL)	IZ (mm)	MIC (mg/mL)	IZ (mm)	MIC (mg/mL)	IZ (mm)	MIC (μg/mL)	IZ (mm)	MIC (mg/mL)
Gram (−)	*A. hydrophila*	–	–	7.0 ± 0.0	7.50	–	–	–	–	19.0 ± 0.0	<7.81	22.0 ± 0.0	0.06
	E. coli	–	–	7.0 ± 0.0	1.88	–	–	–	–	21.0 ± 0.0	<7.81	12.0 ± 0.0	1.00
	E. coli O157	7.0 ± 0.0	3.75	7.0 ± 0.0	0.94	–	–	–	–	21.0 ± 0.0	<7.81	–	–
	K. pneumoniae	–	–	–	–	–	–	–	–	21.0 ± 0.0	<7.81	22.0 ± 0.0	0.50
	M. morganii	–	–	–	–	–	–	–	–	20.0 ± 0.0	31.25	21.0 ± 0.0	0.50
	P. mirabilis	–	–	8.0 ± 0.0	3.75	–	–	–	–	17.0 ± 0.0	31.25	17.0 ± 0.0	0.50
	P. aeruginosa	–	–	10.0 ± 0.0	3.75	7.0 ± 0.0	3.75	7.0 ± 0.0	3.75	18.0 ± 0.0	15.63	12.0 ± 0.0	0.50
	S. typhimurium	–	–	8.0 ± 0.0	3.75	–	–	–	–	16.0 ± 0.0	62.50	11.0 ± 0.0	1.00
	Y. enterocolitica	–	–	11.0 ± 0.0	7.50	9.0 ± 0.0	7.50	9.0 ± 0.0	7.50	20.0 ± 0.0	15.63	13.0 ± 0.0	0.13
Gram (+)	*B. cereus*	–	–	8.0 ± 0.0	7.50	–	–	–	–	26.0 ± 0.0	62.50	24.0 ± 0.0	0.50
	M. smegmatis	–	–	8.0 ± 0.0	3.75	–	–	–	–	18.0 ± 0.0	125.00	–	–
	L. monocytogenes	–	–	7.0 ± 0.0	0.94	–	–	–	–	20.0 ± 0.0	62.50	23.0 ± 0.0	0.50
	S. aureus	–	–	7.5 ± 0.7	0.94	–	–	–	–	23.0 ± 0.0	15.63	24.0 ± 0.0	0.50
Yeast and mold	*C. albicans*	–	–	8.0 ± 0.0	≥30.00	7.0 ± 0.0	≥30.00	7.0 ± 0.0	≥30.00	24.0 ± 0.0	62.50		
	A. parasiticus	14.0 ± 0.0	≥0.50	–	–	–	–	13.0 ± 0.0	≥0.50	15.0 ± 0.0	15.63		

IZ (Inhibition zone) values are expressed as mean ± standard deviation of two experiments. Inhibition zone include diameter of hole (6 mm). Sample amount is 50 μL. MIC: Minimal inhibitory concentration. UE: ultrasonicated extract; SE: Soxhlet extract; ME: macerated extract. –: Not active.

diffusion and micro-well dilution methods. Pure methanol (control) used as solvent had no inhibitory effects on the microorganism tested. The ME and SE of *C. aksoyi* showed no antimicrobial activity against all tested microorganisms except *A. parasiticus* (14 and 13 mm inhibition zones, respectively) (Table 4). The UE of *C. aksoyi* was only effective against *E. coli* O157:H7 932 (7 mm, MIC = 3.75 mg/mL) among the tested microorganisms. The UE was found to be most effective among the all *C. amaena* extracts. The ME and SE of *C. amaena* was only effective against *P. aeruginosa* and *Y. enterocolitica* among the tested bacteria. The UE of *C. amaena* showed antibacterial activity against all Gram (+) bacteria, it showed also no antibacterial activities against *K. pneumoniae* and *M. morganii* among the all Gram (−) bacteria. Among the extracts tested, the ME, SE and UE of *C. amaena* only effective against *C. albicans* (7–8 mm, MIC ≥ 30 mg/mL). All extracts of *C. aksoyi* had effective (13–14 mm) against *A. parasiticus*, while the ME was only effective (13 mm) among all of the *C. amaena* extracts.

Natamycin is an antibiotic used only against yeast and mold.

4. Discussion

Many papers about the antioxidant and antimicrobial effects of the different extracts and compounds obtained from many *Centaurea* members can be found in the literature. However, no studies on the bioactivities of *C. aksoyi* and *C. amaena* were found in the literature. A literature search showed that no report on analysis of phenolic contents of both plants is available. Previous experiments on *Centaurea* have shown the presence of phenolic compounds, one coumarin glucoside and four flavon glycosides from *Centaurea ensiformis* [38], sesquiterpene lactones and lignans from *Centaurea ptosimopappa* [39], flavones, flavone glcuronopyranoside, phytosterol, phenylpropanoid glycoside and chlorogenic acid from *Centaurea depressa* [40], eudesmanolide, 4-*epi*-malacitenolide, flavonoid aglycons, nor-isoprenoid and sterol from *Centaurea grisebachii* ssp. *grisebachii* [41], sesquiterpene lactones, steroidal compound of *Centaurea iberica* [20], β-sitosterol 3-glucoside, protocatechuic acid, scopoletin, chlorogenic acid, jacein, centaurein, kaempferol-3-glucoside, quercetin 3-glucoside, arctiin and janerin from *Centaurea isaurica* [42], cnicin, 4′-O-acetylcnicin, flavonoid aglycons and lignans from *Centaurea orphanidea* [43], sesquiterpenes and sesquiterpene glycosides, flavones; hispidulin, flavon glycoside, flavonol glycoside, kaempferol from *Centaurea hierapolitana* [24], sesquiterpene lactones, eudesmanolide and elemane derivative from *Centaurea deusta* [44], sesquiterpene lactones, isoprenoids and flavonoids from *Centaurea omphalotricha* [9], sesquiterpene lactones from *Centaurea thessala* ssp. *drakiensis* and *Centaurea attica* ssp. *attica* [45], grosheimin and cynaropicrin sesquiterpenes from *Centaurea helenioides* [46]. Sixteen compounds including protocatechuic acid hexoside and ferulic acid were determined in the methanol extract of *Centaurea baseri* using LC/MS [17].

Aktümsek *et al* examined total phenolic, flavonoid concentrations and antioxidant potentials of five *Centaurea* species (*C. kurdica*, *C. rigida*, *C. amanicola*, *C. cheirolopha* and *C. ptosimopappoides*) from Turkey flora. They reported that all extracts had significant antioxidant activities [19]. Zengin *et al* reported that *C. pulchella* had the strong antioxidant activity. Total antioxidant activity and phenolics of three *Centaurea* species were found to be 41.30–67.89 mg AAE/g and 22.27–55.00 mg GAE/g, respectively [47].

Total phenolic contents of *Centaurea calcitrapa* subsp. *calcitrapa*, *C. ptosimopappa* and *C. spicata* were found ranged between 17.25 and 120.50 mg GAE/g and their IC_{50} values in DPPH assay were determined ranged between 237.81 and 1018.21 μg/mL [14]. The phenol, flavonoid content and IC_{50} value in DPPH of *C. depressa* were found 44.5 mg GAE/g, 42.25 mg QE/g and 0.23 mg/mL, respectively [48]. It has previously been reported that ethanol extract of *C. behens* roots possess significant DPPH radical scavenging activity with 19.21%–80.12% inhibition at 10–100 μg/mL [49]. In our present study, inhibition rates of all extracts of both *Centaurea* species were found in the rage of 4.04%–74.46% at 8.3–100.0 μg/mL. The DPPH scavenging effect (IC_{50} = 200 ± 7.0 μg/mL) of *Centaurea behen* methanol extract which had good total phenol contents have been detected previously by Esmaeili *et al* [50]. The antioxidant activities of *C. aucheri* and *C. albonites* were showed by DPPH assay and their IC_{50} values were determined as (103.8 ± 5.2) and (104.5 ± 7.6) μg/mL respectively [51]. Kaskoos detected that methanolic extract of *C. calcitrapa* showed strong DPPH scavenging activity with IC_{50} = 49.98 ± 3.78 μg/mL [25]. The DPPH scavenging effect and the linoleic acid oxidation inhibition rate of the methanol extract of *Centaurea mucronifera* were noted to be 67.8 μg/mL (IC_{50}) and 35.2%, respectively [28].

Shoeb *et al* reported that the methanol extract of the seeds of *Centaurea dealbata* exerted significant levels of antioxidant activities with an IC_{50} value 4.7×10^{-2} mg/mL, also their major bioactive components were found to be the lignans, arctigenin, arctiin and matairesinoside [52]. At another study of the same authors, matairesinoside, arctiin and matairesinol were found as major bioactive components of the methanol extracts of *Centaurea pamphylica*, which had significant antioxidant effects (IC_{50} = 47.3 × 10^{-2} mg/mL) [53]. A methanol crude extract and a sesquiterpene lactone, cynaropicrin, isolated from *Centaurea zuvandica* aerial parts, were tested for antioxidant and antimicrobial activity. Methanolic extract and cynaropicrin possessed high antioxidant effect and the extract had inhibitory effect on *S. aureus*, *B. cereus* and *E. coli* but had no inhibitory effects on *Streptococcus faecalis*, *P. aeruginosa* and *K. pneumonia* [6]. Dumlu *et al* showed that both the extract of *C. iberica* and the compound isolated showed significant antioxidant (84% and 80% inhibition in DPPH, respectively) and antimicrobial activities [20].

It has been reported that arctiin from *Centaurea sclerolepis* did not show any significant antibacterial activity against *S. aureus*, *Micrococcus luteus*, *B. cereus*, *E. coli* and *P. aeruginosa* [54]. At the study of Cansaran *et al*, it has been showed that the flower extracts of *Centaurea cankiriense* had significant inhibitory effect on tested strains, while hexane extracts from both flower and stem hah no inhibitory effect on two Gram (−) bacteria. But, methanol extract inhibited the bacteria except for *E. coli* and *K. pneumonia* [13]. Similar to our results, *E. coli* and *M. morganii* was found as the most resistant bacteria to extracts. The dichloromethane and methanol extracts of *Centaurea polyclada* showed free radical scavenging effect (IC_{50} values 1.17 and 0.015 mg/mL, respectively) and dichloromethane extract showed important antibacterial effect against all nine test strains except *E. coli* (MIC = 1.25–2.50 mg/mL) [22].

The different extracts of *C. ensiformis* had antibacterial effect but had no effect on the yeasts [12]. Same authors reported the antibacterial effect of the chloroform extract of *Centaurea austro-anatolica* [26] and *Centaurea cariensis* subsp. *niveo-tomentosa* [27]. Ozsoy *et al* reported that the total phenol and flavonoid content of *Centaurea antiochia* var. *praealta* extract were found as 3.68 mg GAE/g and 1.94 mg catechin/g, respectively [55]. In this study, *C. antiochia* var. *praealta* extract was found as DPPH radical scavenger (IC_{50} = 5.10 mg/mL) and antimicrobial agent against. Güven *et al* showed that the extracts of five *Centaurea* species had antibacterial effects against most of the bacteria, but no antifungal activity [56]. In a previous study on antimicrobial effects of extracts of *Centaurea tchihatcheffii*, it has been dedicated that all the extracts appear to have antibacterial effect against standard Gram (−) and Gram (+) bacteria ranging from MIC of 2–16 μg/mL, besides they have antibacterial activity at 32–128 μg/mL concentrations to isolated strains. Notable activity was observed with the extracts against *C. albicans* and *C. parapsilosis* fungi at 4 and 8 μg/mL concentrations, respectively [57]. The antimicrobial activity against twelve microorganisms of the methanol soluble fraction of *Centaurea diffusa* was reported by Skliar *et al* [58]. The antibacterial effect of the methanol extracts of twelve *Centaurea* against *E. coli*, *B. cereus, Salmonella enteritidis*, *S. aureus* were screened by Tekeli *et al* [15] and determined that eight *Centaurea* species inhibited most of the microorganisms tested. The antimicrobial activities of the extracts of *C. pseudoscabiosa* ssp. *glechnii*, *C. spicata*, *C. glastifolia*, *C. salonitana*, *C. balsamita* and *C. behen* were displayed by Uysal *et al* [59]. The antimicrobial effects of the extracts from *C. persica*, *C. polyclada* and *C. consanguinea* were demonstrated by Uysal *et al* [60]. It has been reported that the extract of *C. baseri* showed strong antifungal effect against *Candida utilis* (MIC = 60 μg/mL) [17].

The phenolics, antioxidant and antimicrobial effects of the methanol extracts of *C. amaena* and *C. aksoyi* have not been previously reported. This is the first report on their phenolic contents and biological activities. Obtained results suggest that methanolic extracts of both species may be potential sources of natural antioxidant and antimicrobial substances. *C. amaena* extracts are more active than the *C. aksoyi* extracts. The extracts obtained by the ultrasonic extraction contained larger amount of extractive substances and showed stronger antioxidant and antimicrobial activity than the extracts obtained from other techniques, as well as. This study confirms ultrasonic extraction to be suitable method to yield extractive substances which have antioxidant and antimicrobial activity.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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