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Research Article

In Vitro Evaluation of Phenolics Content, Antioxidant and Antimicrobial Activities of Three *Centaurea* L. Species from Iran

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ABSTRACT

Background: Members of the genus *Centaurea* L. (Astraceae) are used in some countries for betterment of various ailments in the popular medicine. The main group of secondary metabolites in plants are phenolic compounds and flavonoids which are the main subset of phenolic compounds, possess a lot of biological properties such as antioxidant activity. The aim of present research is to determine *in vitro* total phenol and flavonoid contents, antioxidant potency and also antibacterial activity of the methanolic extracts of capitulla and aerial parts of three *Centaurea* species, namely *C. sosnovsky* Grossh., *C. irritans* Wagenitz and *C. kandavanesis* Wagenitz. In addition, any relationships between the phenolics content and their antioxidant properties were discussed.

Methods: To determine total phenol and flavonoid contents of the methanolic extracts, Folin-Ciocalteu and aluminum chloride methods were used, respectively. To evaluate antioxidant activity three different test systems, namely 2, 2-diphenyl-1-picrylhydrazyl free radical scavenging (DPPH), Cu-chelating and ß-carotene/linoleic acid bleaching were used. In addition, disc diffusion method was used to assess antibacterial activity of the extracts against 6 gram positive and negative bacteria.

Results: Results indicated that the highest total phenol content (98.91 ± 1.87mg GAE/g of dry extract) represented by the aerial part extract of *C. kandavanensis*. The highest total flavonoid content (5.382 ± 0.231mg GAE/g of dry extract) and also DPPH radical scavenging activity (IC_{50}; 0.20 ± 2.02 mg/ml) were exhibited by the capitulla extract of *C. sosnovsky*. However, the aerial part extract of *C. irritans* had the most potency for β-carotene bleaching (74.74%) among the studied samples. In addition, the extracts had acceptable antibacterial activity against both gram-positive and gram-negative bacteria tested.

Conclusion: Overall, high content of phenolic compounds and proper antioxidant activity of the examined *Centaurea* extracts may suggest them as potent antioxidants for special use in future.

Introduction

One source of medicine in virtually all cultures are medicinal plants.[1] According to WHO, plant medicines are crucial agent for the health of about 80% of the world's population, especially developing countries. Therefore, it is important to develop the research on new drugs and natural products from plants, microorganisms, fungi and animals as sources of innovative and powerful therapeutic agents for newer and affordable medicines.[2] In various pathological conditions such as tissue injury, inflammation process and neurodegenerative diseases, reactive oxygen species (ROS), play a vital function. However humans body is protected against ROS by antioxidants.[3] ROS compounds can easily ruin phospholipids, nucleic acids, cell membranes and polypeptides chain and play crusial role in development of different diseases.[4] Scavenger agents like antioxidants will prevent biological systems from the free radical damages.[5,6] Synthetic antioxidants like Butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) were previously used in food industries, but because of their toxic effects on human body using of BHA and BHT are recently restricted.[7] Tocopherols and tocotrienols, Fat-soluble antioxidants (vitamin E), folic acid (vitamin B9), L-ascorbic acid (vitamin C), flavonoids , carotenoids, phenyl acrylic acids and phenyl methanoic acids are the important antioxidants

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which exist in plants.[8] The genus *Centaurea* L. belongs to the family Asteraceae and includes 400-700 herbaceous thistle-like flowering species are mainly distributed in Middle East. Ethnopharmacological studies have shown that many *Centaurea* species are used for treatment of diverse ailments in the popular medicine in some countries. For example, in Scottish medicine, *Centaurea cyanus* and *Centaurea scabiosa* are used as diuretic agents.[9] However; there are few reports to previous works on biological activity of other *Centaurea* species. The goal of current study is to evaluate *in vitro* antioxidant properties and total phenolics content of the methanolic extracts of three *Centaurea* species (*C. sosnovsky*, *C. irritans* and *C. kandavanensis*) in both capitulla and aerial parts. Besides, correlation among total phenol and flavonoid contents and antioxidant properties is determined.

Materials and Methods

Plant materials

Three *Centaurea* species (*Centaurea sosnovsky* Grossh., *Centaurea irritans* Wagenitz and *Centaurea kandavanensis* Wagenitz) were collected from their natural distribution areas; then their aerial and capitulla parts were dissected (Table 1). The Voucher pattern of the species representing these collections have been deposited at the Bu Ali Sina University Herbarium (BASU), Hamedan, Iran.

Extract preparation

Briefly, powdered parts of aerial and capitulla of the species (25 g) were extracted by 250 ml absolute methanol for 12 hat 50 ºC using a Soxhlet apparatus and solvents were removed under the vacuum evaporator (Lab Tech, Ev 311, Italy) at temperature below 60 °C to yield a waxy material. Finally, the extracts were lyophilized and kept in 4 °C until used.

Total phenol content estimation

Folin-Ciocalteu reagent was used to determine total phenol content.[10] In brief, 0.5 ml each of the extract (1:10 g/ml) or gallic acid was admixed with 5 ml Folin-Ciocalteu reagent (1:10 distilled water) and 4 ml aqueous Na_2CO_3 (1 M). After 15 min at room temperature the total phenol content of the mixture was determined by colorimetric method at 765 nm (Perkin Elmer UV/visible spectrophotometer, USA). The calibration curve (y = 0.002 x + 0.0177, R^2 = 0.978) was prepared using gallic acid solution at different concentrations of 0-250 µg/ml in 50% methanol. Total phenol content was calculated from calibration curve and expressed as gallic acid equivalent (mg GAE/g).

Total flavonoid content Estimation

The $AlCl_3$ method was used for estimating total flavonoid content.[11] Briefly, 0.5 ml each of extract (1:10 g/ml) in methanol were mixed with 1.5 ml methanol, 0.1 ml $AlCl_3$ (10%), 0.1 ml KCH_3COO (1 M) and 2.8 ml distilled water and left at room temperature for 30 min. Absorbance of the mixtures was measured at 415 nm (Perkin Elmer UV/visible spectrophotometer, USA). The calibration curve (y = 0.0091 x + 0.0206, R^2 = 0.995) was prepared using quercetin solution at different concentrations of 12.5-100 µg/ml in methanol. Then, the total flavonoid content was calculated from calibration curve and expressed as quercetin equivalent (mg QE/g extract).

Evaluation of antioxidant activity

Free radical scavenging

The method of Mensor et al was used for measuring free radical scavenging activity of the extracts.[12] In order to obtain dilutions, different extract concentrations were prepared in methanol (0.2-1 mg/ml) and 2.5 ml each of concentration was added to 1 ml DPPH alcohol solution (0.3 mM). After completion the samples were first kept in a dark place at room temperature for 30 min and bleaching of DPPH was read at 517 nm against a blank (methanol). The inhibition in percent for each concentration was calculated according to the following formula:

$$DPPH\ free\ radical\ scavenging(\%) = [1 - (As - Ab)/Ac)] * 100 \quad \text{Eq.(1)}$$

Where, As is absorbance of the reaction mixture containing 2.5 ml of extracts + 1 ml DPPH, Ab is absorbance of the reaction mixture containing 2.5 ml of extracts + 1 ml methanol and Ac is absorbance of control sample containing 1 ml DPPH + 2.5 ml methanol. Also IC_{50} value, which represented the concentration of the sample that caused 50% inhibition, was determined. Tests were carried out in triplicate and the positive control was ascorbic acid.

Table 1. The collections of investigated *Centaurea* species.

Species	Voucher number/locality	Date	Altitude (m)
C. sosnovsky	BASU 28813/Lorestan	10.5.2015	2100
C. irritans	BASU 28814/Kermanshah	10.5.2015	2100
C. kandavanensis	BASU 29197/Mazandaran	15.7.2015	2140

Cupric ion reducing antioxidant capacity (CUPRAC assay)

The cupric ion reducing capacity was measured due to the method of Apak et al.[13] 1 ml $CuCl_2$ (10 mM), 1 ml neocuproine (7.5 mM), and 1ml NH_4AC buffer (1M, pH 7.0) solutions were added into a test tube. Then, 0.5 ml of extract at different concentrations was mixed and total volume was brought up to 4.1 ml with deionized water. The mixture absorbance was recorded against a blank at 450 nm after 30 min incubation at room temperature.

ß-Carotene linoleic acid bleaching

According to the method of Barriere et al, antioxidant capacity is assessed by measuring the inhibition of the volatile organic compounds and the conjugated dienehydroperoxides arising from linoleic acid oxidation.[14] A stock solution of *ß*-carotene/linoleic acid was prepared as follows: first, 0.5 mg *ß*-carotene was dissolved in1 ml chloroform, then 25 µl linoleic acid and 200 mg Tween 40 were added. The chloroform was subsequently evaporated using a vacuum evaporator. Then 100 ml distilled water saturated with oxygen (30 min at 100 ml/min) were added with vigorous shaking. Aliquots (2.5 ml) of this reaction mixture were transferred to test tubes, and 350 µl portions of the extracts (2 g/l in ethanol) were added before incubating for 48 h at room temperature. The same procedure was repeated with BHT at the same concentration and a blank containing only 350 µl ethanol. After the incubation period, the absorbance of the mixtures was measured at 490 nm. Antioxidant capacity of the samples was compared with that of BHT and blank.

Antibacterial activity assay

The plant extracts were dissolved in DMSO to a final concentration of 2 mg/ml. Antibacterial activity of the extracts at 4 different concentrations (100, 50, 25 and 12.5 mg/ml of extract) was measured against 6 gram positive and negative bacteria, namely *Serratia marcescens* (PTCC 1111), *Enterabacter aerogenes* (PTCC 1221), *Salmonella enteric* (PTCC 1236), *Bacillus thuringiensis* (Wild), *Staphylococcus aureus* (Wild) and *Bacillus megaterium (*PTCC 1017) by disc diffusion method.[15] The extracts were dissolved in DMSO to make a 100 mg/ml solution and other concentrations make from this concentration, and then apply on the blank paper discs. Dried discs were placed onto Muller-Hinton agar medium that previously inoculated with a bacterial suspension (1.5×10^8 of bacteria/ml). The cultures were incubated at 37 °C for 24 h. The antibacterial activity against each test organism was quantified by determining mean zone of inhibition. Gentamycin, Penicillin, Nitroflantoin and Neomycin were also used as positive controls.

Statistical analysis

All data are the average of triplicate analyses. Statistical analysis of variance was performed using Student's t-test by SPSS program and p value < 0.05 was regarded as significant. Data are expressed as means ± standard deviation.

Results

Determination of total phenol and flavonoid contents

Results proved that the content of total phenols was different in the studied species (from 19.34 ± 3.83 to 98.91 ±1.87 mg GAE/g of dry extract). The highest total phenol content was found for the aerial part extract of *C. kandavanensis* and the lowest one for the capitulla extract of *C. irritans*. The capitulla extract of *C. sosnovsky* had the highest flavonoid content (Table 2).

DPPH Free radical scavenging

Results from assessment of free radical scavenging capacity of the corresponding extracts are shown in Table 2 and Figure1. All extracts showed a weaker potency than ascorbic acid for scavenging DPPH free radicals. A lower IC_{50} represented the higher antioxidant activity.

Table 2. Total phenol and flavonoid contents and DPPH radical scavenging activity of the *Centaurea* species.

Extract	Total phenol (mg/g dw)	Total flavonoids (mg/g dw)	ß-Carotene/linoleic acid	DPPH radical scavenging	
				IC_{50} (mg/ml)	(%)
C. sosnovsky (aerial)	56.33^{e} ±1.89	4.558^{d} ±0.467	49.19^{bc} ±2.55	0.75^{g}±2.33	44.40
C. sosnovsky (capitulla)	69.85^{de} ±7.10	5.382^{b} ±0.231	47.50^{bc} ±3.97	0.2^{b}±2.02	78.26
C. irritans (aerial)	86.05^{bc} ±5.26	2.410^{e} ±0.065	74.74^{a} ±4.85	0.29^{bc}±2.9	80.46
C. irritans (capitulla)	19.34^{f} ±3.83	1.413^{f} ±0.011	58.76^{b}±4.33	0.39^{cd}±0.88	67.80
C. kandavanensis (aerial)	98.91^{ab} ±1.87	5.083^{bcd} ±0.231	49.07^{bc} ±6.05	0.33^{c}±2.95	77.43
C. kandavanensis (capitulla)	66.35^{e} ±7.12	5.240^{bc} ±0.338	50.95^{bc}±8.66	0.44^{d}±3.2	65.91
Ascorbic acid	-	-	-	0.125^{a}±0.8	80.61

Experiment was performed in triplicate and expressed as mean ± SD. Values in each column with different superscripts are significantly different (P < 0.05).

The following descending order showed the effectiveness of extracts as DPPH radical scavengers: ascorbic acid (IC_{50}; 0.143 ± 0.007 mg/ml) < *C.sosnovsky* (capitulla) (IC_{50}; 0.2 ± 2.02 mg/ml) < *C. irritans* (aerial parts) (IC_{50}; 0.21 ± 2.09 mg/ml) < *C. candavanensis* (aerial parts) (IC_{50}; 0.333 ± 2.95 mg/ml) < *C. irritans* (capitulla) (IC_{50}; 0.392 ± 0.88 mg/ml) < *C. kandavanensis* (capitulla) (IC_{50}; 0.444 ± 3.2 mg/ml) < *C. sosnovsky* (aerial parts) (IC_{50}; 0.754 ±2.33 mg/ml (Table 2).

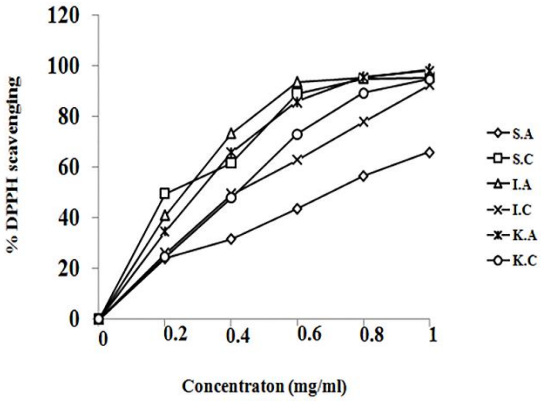

Figure 1. Ability of DPPH radical scavenging of the studied *Centaurea* species (S.A: *C. sosnovsky* aerial, S.C: *C. sosnovsky* capitulla, I.A: *C. irritans* aerial, I.C: *C. irritans* capitulla, K.A: *C. kandavanensis* aerial, K.C: *C. kandavanensis* capitulla).

Cupric ion reducing antioxidant capacity

Results from Cu chelating assay showed that the absorbance of extracts linearly depended on concentration (0.2 to 01 mg/ml) and increased by increasing concentration. The aerial part extract of *C. irritans* and capitulla extract of *C. sosnovsky* displayed the highest absorbance values (Figure 2).

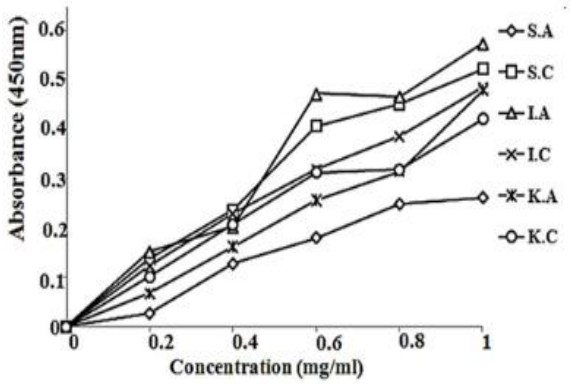

Figure 2. Cupric reducing antioxidant capacity of the studied *Centaurea* species at different concentrations (S.A: *C. sosnovsky* aerial, S.C: *C. sosnovsky* capitulla, I.A: *C. irritans* aerial, I.C: *C. irritans* capitulla, K.A: *C. kandavanensis* aerial, K.C: *C. kandavanensis* capitulla).

ß-Carotene linoleic acid bleaching

Results from antioxidant activity using ß-carotene/linoleic acid model system indicated that the aerial parts extract of *C. irritans* was more active (74.74 ± 4.85%) than other samples. However, no substantial difference ($P < 0.05$) was observed between other evaluated samples (Table 3).

Table 3. Inhibition percentages of the linoleic acid oxidation by two parts of three *Centaurea* species and BHT.

Extracts	Inhibition (%)
C. *sosnovsky* (aerial)	49.19bc ±2.55
C. *sosnovsky* (capitulla)	47.50bc ±3.97
C. *irritans* (aerial)	74.74a ±4.85
C. *irritans* (capitulla)	58.76 b±4.33
C. *kandavanensis* (aerial)	49.07bc ±6.05
C. *kandavanensis* (capitulla)	50.95bc±8.66
BHT	97.29a±1.69
Control -	13.39d±0.44

Experiment was performed in triplicate and expressed as mean ± SD. Values in each column with different superscripts are significantly different (P < 0.05).

Antibacterial activity assay

Results from antibacterial assay indicated that *C. sosnovsky* extract in both parts have strong antibacterial capacity against 6 gram-positive and negative bacteria. Then again, the aerial and capitulla parts of *C. kandavanensis* and also the capitulla part of *C. irritans* showed the weakest antibacterial function versus gram negative bacteria tested. The aerial part extract of *C. irritans* had no effect on *Bacillus thuringiensis*as a gram positive bacterium. *Bacillus megaterium* and *Staphylococcus aureus* had the highest sensitivity to the studied extracts (Tables 4-9).

Discussion

Phenolic compounds which act as free radical terminators, are a class of antioxidant agents, and their bioactivities may be related to their capabilities to chelate metals, inhibit lipoxygenase and scavenge free radicals.[16,17] Activity of multiple cytochrome P_{450} isoforms, lipoxygenases, cyclooxygenase and xanthine oxidasecan strongly inhibited by phenolic structures. They also play an important role in plant defense mechanisms to counteract reactive oxygen and nitrogen (ROS and RNS) and inhibit lipid peroxidation.[18-20] Antioxidant agents of phenolic compounds are hydroxyl groups that are power hydrogen donors. Flavonoids are big group of antioxidant, which have been classified into seven sub groups: flavanols, flavones, flavanones, isoflavones, catechins, anthocyanins and proanthocyanidins. Because of their potent antioxidant ability, flavonoids have positive effects on human health.[21] In current research, all extracts indicated a high total phenolic content. The highest content was observed in the aerial part of *C. kandavanensis* (98.91 ± 1.87 mg GAE/g of dry extract) and the lowest one in the capitulla of *C. irritans* (19.34 ± 3.83 mg GAE/g of dry extract).

Table 4. Average inhibition zone of evaluated bacterial strains against aerial parts extract of C. sosnovsky.

Bacterial species	Inhibition zone (mm) Concentration (mg/ml)				
	100	**50**	**25**	**12.5**	**DMSO**
Bacillus megaterium	7.83±5.3	7.33±2.5	7.33±1.58	7.67±2.33	NA
Staphylococcus aureus	NA	9.00±1.65	8.67±1.11	14.67±1.00	NA
Serratia marcescens	8.51±2.13	8.33±1.51	10.00±1.98	10.17±1.00	NA
Enterabacter aerogenes	6.83±2.65	7.00±2.47	7.33±1.87	8.33±1.15	NA
Salmonella enterica	10.56±1.65	10.00±1.45	8.33±1.65	7.67±2.00	NA
Bacillus thuringiensis	7.00±2.11	8.00±1.9	11.33±1.15	11.17±1.14	NA

NA, No Active.
Experiment was performed in triplicate and expressed as mean ± SD.

Table 5. Average inhibition zone of evaluated bacterial strains against capitulla extract of C. sosnovsky.

Bacterial species	Inhibition zone (mm) Concentration (mg/ml)				
	100	**50**	**25**	**12.5**	**DMSO**
Bacillus megaterium	7.87±1.22	7.33±0.85	7.33±1.514	7.78±1.45	NA
Staphylococcus aureus	8.33±1.85	9.40±1.50	8.93±1.30	13.67±1.54	NA
Serratia marcescens	7.51±1.45	7.33±1.12	9.00±1.80	9.17±1.78	NA
Enterabacter aerogenes	6.50±2.50	7.00±2.10	7.83±1.21	8.38±1.69	NA
Salmonella enterica	9.56±1.74	10.04±1.31	8.33±1.66	7.00±1.00	NA
Bacillus thuringiensis	6.00±2.00	7.30±1.05	9.53±0.95	10.11±1.81	NA

NA, No Active.
Experiment was performed in triplicate and expressed as mean ± SD.

Table 6. Average inhibition zone of evaluated bacterial strains against aerial parts extract of C. irritans.

Bacterial species	Inhibition zone (mm) Concentration (mg/ml)				
	100	**50**	**25**	**12.5**	**DMSO**
Bacillus megaterium	6.51±1.8	6.87±1.63	7.43±1.12	7.88±2.00	NA
Staphylococcus aureus	5.65±2.12	5.83±2.68	6.70±2.22	7.33±2.5	NA
Serratia marcescens	NA	NA	NA	NA	NA
Enterabacter aerogenes	NA	NA	NA	NA	NA
Salmonella enterica	NA	NA	NA	NA	NA
Bacillus thuringiensis	NA	NA	NA	NA	NA

NA, No Active.
Experiment was performed in triplicate and expressed as mean ± SD.

Table 7. Average inhibition zone of evaluated bacterial strains against capitulla extract of C. irritans.

Bacterial species	Inhibition zone (mm) Concentration (mg/ml)				
	100	**50**	**25**	**12.5**	**DMSO**
Bacillus megaterium	NA	NA	7.00±1.58	7.50±2.15	NA
Staphylococcus aureus	7.00±2.54	8.17±2.15	10.83±1.50	10.88±2.85	NA
Serratia marcescens	7.17±1.50	7.50±3.58	9.83±1.12	8.83±2.20	NA
Enterabacter aerogenes	NA	6.83±2.8	7.83±2.18	12.00±2.50	NA
Salmonella enterica	NA	NA	7.33±1.00	8.67±1.98	NA
Bacillus thuringiensis	7.17±0.55	8.83±1.20	9.33±0.54	14.33±1.50	NA

NA, No Active.
Experiment was performed in triplicate and expressed as mean ± SD.

Table 8. Average inhibition zone of evaluated bacterial strains against aerial parts extract of C. kandavanensis.

Bacterial species	Inhibition zone (mm) Concentration (mg/ml)				
	100	**50**	**25**	**12.5**	**DMSO**
Bacillus megaterium	13.83±1.15	9.33±2.14	6.83±2.50	6.67±2.80	NA
Staphylococcus aureus	8.67±1.33	5.83±2.68	6.70±2.22	7.33±2.5	NA
Serratia marcescens	NA	NA	NA	NA	NA
Enterabacter aerogenes	NA	NA	NA	NA	NA
Salmonella enterica	NA	NA	NA	NA	NA
Bacillus thuringiensis	7.00±1.20	8.17±1.45	8.83±1.6	6.67±1.32	NA

NA, No Active.
Experiment was performed in triplicate and expressed as mean ± SD.

Table 9. Average inhibition zone of evaluated bacterial strains against capitulla extract of C. kandavanensis.

Bacterial species	Inhibition zone (mm) Concentration (mg/ml)				
	100	**50**	**25**	**12.5**	**DMSO**
Bacillus megaterium	12.33±1.12	11.60±1.61	9.00±1.00	8.54±1.54	NA
Staphylococcus aureus	9.34±1.10	6.15±1.10	6.98±2.80	7.50±2.65	NA
Serratia marcescens	NA	NA	NA	NA	NA
Enterabacter aerogenes	NA	NA	NA	NA	NA
Salmonella enterica	NA	NA	NA	NA	NA
Bacillus thuringiensis	7.50±1.50	7.50±1.70	8.00±1.81	8.00±1.20	NA

NA, No Active.
Experiment was performed in triplicate and expressed as mean ± SD.

In another study the phenolic content of some *Centaurea* species, which was assessed varied from 82.273 mg GAE/g in *C. ptosimopappoides* to 175.404 mg GAE/g in *C. cheirolopha*.[22] It is popular that phenolic compounds providing health beneficial effects by modifying color, taste, aroma, and flavor. They also play a crucial role in protecting cells from damages of microorganisms, insects, and herbivores.[23] Our results indicated that *C. kandavanensis* in both of aerial and capitulla parts and *C. sosnovsky* in the capitulla part were not notable different ($P < 0.05$) in total flavonoid content. However, the aerial parts of *C. sosnovsky* represented a higher amount of total flavonoids (5.382 ± 0.231 mg QES/g of dry extract). The capitulla extract of *C. irritans* had the lowest content of total flavonoids in comparison with other samples.

Due to the injurious role of free radicals in foods and biological systems, radical scavenging activity is very important.[24] Free radicals are generated as byproducts of biological reactions. *In vitro* antioxidant content of the phenolic compounds on a molar basis is more than vitamins E and C.[25] DPPH scavenging activity of the extracts and ascorbic acid as a synthetic antioxidant was assessed by using IC_{50}. There is no significant announcement to previous works on the antioxidant activity (as DPPH free radical scavenging) of *C. sosnovsky*, *C. irritans*and *C. kandavanensis*. The studied extracts here showed more amount of IC_{50} than ascorbic acid that means their lower antioxidant activity. The capitulla extract of *C. sosnovsky* and the aerial part of *C. irritans* represented the lowest IC_{50} values. To the best of our knowledge, several studies were fulfilled on radical scavenging activity of some members of the genus *Centaurea*, such as *C. cyanus*, *C. scabiosa*,[26] *C. calolepis* and *C. cadmea*.[27,28] However, the methods for DPPH assay, which were used in these studies was different, so their results are no directly comparable to ours.

Many reports revealed that there is a direct interaction between antioxidant activity and reducing power of plant extract.[29,30] Cupric reducing capacity procedure, is depended on the assessment of absorbance at 450nm by the organization of the reliable complex between neocuproine and copper**(**I). The latter is formed by the reduction of copper(II) in the presence of neocuproine.[13] According to our results, there was no relative relevance between cupric reducing capacity of the extracts and total phenol or flavonoid content. The aerial part extract of *C. irritans* and the capitulla extract of *C.sosnovsky* represented the highest absorbance and the lowest IC_{50} values in DPPH scavenging activity.

Discoloration of β-carotene by antioxidants is the base of ß-Carotene/linoleic acid test system. the extent of β-carotene degradation can be hindered by neutralizing the linoleate free radical and any other

free radicals formed within the system.[31] The aerial parts extract of *C. irritans* was effectively inhibited the oxidation of linoleic acid (74.74 ± 4.85%), followed by the capitulla extract of *C. irritans* (58.76 ± 4.33%). In addition, *C. kandavanensis* and *C. sosnovsky* in both of aerial and capitulla parts were not remarkable distinct ($P < 0.05$) (Table 2). There was a moderate correlation ($R^2 > 0.56$) between total flavonoid amount of the extracts and β-carotene bleaching inhibition potential, offering that up to 56% of β-carotene bleaching inhibition can be related to flavonoid compounds. Based on our results the studied *Centaurea* extracts had high flavonoid content and represented good antioxidant activity.

Our outcomes demonstrated that all tested bacteria the aerial parts extract of *C. sosnovsky* had a strong antibacterial activity against all tested bacteria. Also, the aerial and capitulla extracts of *C. kandavanensis* showed the weakest antibacterial function chiefly versus gram negative bacteria. Our results showed that the studied extracts have high antibacterial function especially against gram positive bacteria that is in parallel with Cansaran et al.[32] It could be due to the presence of multilayer membrane, which covered each gram-negative bacteria cell, whereas, gram-positive bacteria consist of a single layer. It is the reason why gram-negative bacteria are more resistant to antibacterial than gram-positive bacteria.[33]

Conclusion

Our current research prepared usable knowledge on phenolic content and antioxidant activity of three *Centaurea* species by ordinary, simple and reliable protocols. Our observations mentioned that difference in the antioxidant activity between the extracts cannot adequately describe by quantitative difference in their total phenolic contents. Hence, the methanolic extracts of *Centaurea* species contain major phytochemicals that may be candidates to develop new drugs, which might be practical in betterment of diseases caused by different free radicals. However, more researches are needed to figure out the *in vivo* antioxidant potential of these extracts in animal models.

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Conflict of interests

The authors claim that there is no conflict of interest.

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