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ORIGINAL RESEARCH

In vitro evaluation of antioxidant and antimicrobial activities of some *Centaurea* L. species

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ABSTRACT: The purpose of this study was to investigate the antioxidant, antimicrobial activities and total phenolic contents of ten methanol extracts obtained by maceration from capitulums and aerial parts (except for capitulum) of five *Centaurea* species (*C.stenolepis, C.kilaea, C.cuneifolia, C.iberica, C.solstitialis* subsp. *solstitialis*). Free radical scavenging activities and total phenolic contents of the species were assayed by DPPH method and Folin–Ciocalteu method, respectively. The antimicrobial activity of the extracts were tested by the micro-broth dilution method against seven microbial species. In the DPPH radical scavenging assay, the IC_{50} values of tested ten methanol extracts were in range 1.767-4.665 mg while the total phenol contents of four extracts were found to range between 4.825 and 12.460 mg GAE/g dry material. Four extract showed moderate activity against *Pseudomonas aeruginosa* (MIC: 312 µg/ml), while six out of ten extracts exhibited moderate activity against *Candida albicans* (MIC: 312 µg/ml). The methanol extract prepared from the aerial parts of *C.cuneifolia* possessed weak activity against *Staphylococcus aureus* (MIC: 625 µg/ml).

KEYWORDS: *Centaurea* species, antioxidant activity, antimicrobial activity

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INTRODUCTION

Oxidation is vital to most living organisms. It is required to produce the energy which fuels the biological processes. However, oxygen-centred free radicals and other reactive oxygen species (ROS) produced continuously by most living organisms cause to cell death and tissue damage. Therefore, there is an interest in natural antioxidants such as polyphenols which are found in medicinal and dietary plants so as to prevent oxidative damage (1).

Some pathogens are resistant against firstly discovered effective antimicrobial drugs. New compounds inhibiting microorganisms such as benzoin and emetine have been isolated from plants. Contrary to presently used antimicrobial drugs, antimicrobial compounds in plants might inhibit bacterial growth by different mechanisms and may be used as antibiotic against resistant microbial strains. Thus there is a need to find new bioactive compounds of plant origin which can be used in the treatment of resistant microbial strains (2).

The genus *Centaurea* L.(Asteraceae) is represented by 205 taxon in Turkey (3,4,5). In traditional medicine, they are used for fever, menstrual disorders, vaginal candidiasis ,the treatment of liver, kidney and ulcer diseases, as antidiarrheal, stomachic, tonic, appetitive, antidiabetic, antipyretic, also as a diuretic and expectorant (6,7).

MATERIALS AND METHODS

Plant Material

Plant samples were collected in the flowering periods from the different regions of Istanbul in 2009 and were identified by Dr.Gizem BULUT. Voucher specimens were deposited in the Her-

TABLE 1. List of plants used in this work.

	Botanical name	Place of collection	Endemic	MARE No	Time of collection
1.	*Centaurea stenolepis* Kerner	Çatalca	-	11651	July, 2009
2.	*Centaurea kilaea* Boiss.	Çatalca	Endemic	11712	July, 2009
3.	*Centaurea. cuneifolia* Sm.	Çatalca	-	11690	July, 2009
4.	*Centaurea iberica* Trev.ex Sprengel	Şile	-	11966	July, 2009
5.	*Centaurea solstitialis L.* subsp. *solstitialis*	Haydarpaşa	-	11965	July, 2009

TABLE 2. The extraction yields of *Centaurea* species.

Extracts	CSC	CSA	CKC	CKA	CCC	CCA	CIC	CIA	CSSC	CSSA
% Yield	13,224	13,542	11,331	12,916	12,693	10,603	10,750	12,439	10,901	8,139

Abbreviations: CSC, Capitulums of *Centaurea stenolepis*; **CSA,** Aerial parts of *C. stenolepis*; **CKC,** Capitulums of *C.kilaea*; **CKA,** Aerial parts of *C. kilaea*; **CCC,** Capitulums of *C. cuneifolia*; **CCA,** Aerial parts of *C. cuneifolia*; **CIC,** Capitulums of *C. iberica;* **CIA**, Aerial parts of *C. iberica;* **CSSC**, Capitulums of *C. solstitialis* subsp. *solstitialis;* **CSSA,** Aerial parts of *C. solstitialis* subsp. *solstitialis*

barium of the Faculty of Pharmacy, Marmara University (MARE) (Table 1).

Plant extraction

The dried and powdered capitulums and aerial parts (except for capitulum) of *Centaurea* species were extracted by maceration with MeOH three times (24h×180ml) at room temperature. All extracts were filtered, dried under vacuum and stored under refrigeration for further analysis.

Determination of DPPH radical scavenging activity

Free radical scavenging capacity of methanol extracts of *Centaurea* species and standart were evaluated according to the previously reported procedure using the stable DPPH (1). Briefly, extracts and standart solution (0.1 ml) in MeOH at different concentrations (5-0.3125 mg) were added to 3,9 ml (6×10^{-5} M) methanol solution of DPPH. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 30 min. Absorbance readings were taken at 517 nm. The percent radical scavenging activity of extracts and standard against DPPH were calculated according to the following:

DPPH radical-scavenging activity (%) $= [(A_0 - A_1)/A_0] \times 100$

where A_0 is the absorbance of the control (containing all reagents except the test compounds), and A_1 is the absorbance of the extracts/standard. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extracts concentration. Tests were carried out in triplicate. Ascorbic acid (AA) was used as positive control.

Determination of Total Phenolic Contents (TPC)

Total phenolic contents of methanol extracts of *Centaurea* species were measured using Folin–Ciocalteau reagent (8). 0.1 mL of extracts in various concentrations (5, 2.5, 1.25 mg/ml) were mixed with 0.2 mL Folin-Ciocalteu reagent (Sigma), 2 mL of H_2O, and 1 mL of 15% Na_2CO_3, and the absorbance was measured at 765 nm after 2 h incubation at room temperature. Gallic acid was used as a standard and the total phenolics were expressed as mg GAE / g dry plant.

Determination of antimicrobial activity

The antimicrobial activity of the extracts were tested against six bacteria (*Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 14153) and one yeast (*Candida albicans* ATCC 10231) by the microbroth dilutions technique strictly following the recommendations of Clinical Laboratory Standards Institute (CLSI) (9,10). Ciprofloxacin and fluconazole were used as the reference compounds for bacteria and fungi, respectively.

Statistical analysis

The data were reported as means±standard deviations and analysed by one-way analysis of variance (ANOVA) followed by the Tukey's multiple comparison tests using GraphPad Prism 5. Differences between means at $p<0.05$ level were considered significant.

RESULTS AND DISCUSSION

Extraction yields

The extraction yields of *Centaurea* species were found to range between 8-13.5 % (Table 2).

DPPH radical scavenging activities of Centaurea species

A low IC_{50} value (the concentration of extract, which is required to scavenge 50% of DPPH free radical) is an indication of strong antioxidant activity. All extracts showed low antioxidant activity when compared with standard ($p<0,05$). Also, Most of aerial parts of *Centaurea* species showed stronger anti-

TABLE 3. IC50 values (mg/ml) of extracts.

Capitulums	CSC	CKC	CCC	CIC	CSSC
IC_{50}	$2,051 \pm 0,244^{b}$	$3,617 \pm 0,386^{c}$	$3,827 \pm 0,491^{cd}$	$3,691 \pm 0,539^{c}$	$2,189 \pm 0,281^{b}$
Aerial parts	**CSA**	**CKA**	**CCA**	**CIA**	**CSSA**
IC_{50}	$1,767 \pm 0,094^{b}$	$2,121 \pm 0,179^{b}$	$2,627 \pm 0,231^{b}$	$4,665 \pm 0,640^{d}$	$2,227 \pm 0,245^{b}$
Standard	**Ascorbic acid**		**IC_{50}**	0.088 ± 0.008^{a}	

- Each value in the table is represented as mean ± SD (n = 3)
- Different letter superscripts in the same row or column indicate significant differences ($P < 0.05$)

TABLE 4. Total phenolic content of the methanol extracts obtained from *Centaurea* species.

Capitulums	CSC	CKC	CCC	CIC	CSSC
mg GAE/g dry plant	12,190±1,379[a]	9,523±1,139[bcd]	10,830±1,380[abc]	8,994±1,016[cde]	8,166±0,537[de]
Aerial parts	**CSA**	**CKA**	**CCA**	**CIA**	**CSSA**
mg GAE/g dry plant	12,460±1,234[a]	11,330±1,178[ab]	7,324±0,586[e]	8,705±1,063[de]	4,825±0,391[f]

- Each value in the table is represented as mean ± SD (n = 3)
- Different letter superscripts in the same row or column indicate significant differences ($P < 0.05$).

oxidant activity than their capitula. The antioxidant activities of the plant extracts are in the following order: CSA>CSC>CKA>CSSC>CSSA>CCA>CKC>CIC>CCC>CIA. Results are presented as IC_{50} values in the Table 3.

Total Phenolic Contents of Centaurea species

The total phenolic contents of extracts were calculated using the equation obtained from the standard curve of gallic acid graph ($y = 0.0033x - 0.044$, $R^2 = 0.9987$). The methanol extract prepared from the aerials part of *C. solstitialis* subsp. *solstitialis* (CSSA) had the lowest total phenolic content among other *Centaurea* species ($p<0,05$). The total phenolic contents of the plant extracts are in the following order: CSA> CSC> CKA> CCC> CKC> CIC> CIA> CSSC> CCA> CSSA. However, there was no correlation between antioxidant activities and total phenolic contents of the extracts ($R^2 = 0.0197$). The results are presented in Table 4.

Antimicrobial activities of Centaurea species

All other extracts except CSC, CCC, CSSC and CSSA extracts, exhibited moderate activity against *Candida albicans*. Only CCA extract showed low activity against *Staphylococcus aureus*, while CKC, CKA, CIC and CSSA extracts possessed moderate activity against *Pseudomonas aeruginosa*. None of the extracts were active against *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Staphylococcus epidermidis*. The results are summarized in Table 5.

TABLE 5. The MIC (Minimum Inhibitory Concentration) values (μg/mL) of the methanol extracts obtained from *Centaurea* species.

Extracts / Standards	Microorganisms						
	E.c[Ψ]	K.p	P.a	P.m	S.a	S.e	C.a
CSC	-	-	-	-	-	-	-
CSA	-	-	-	-	-	-	312
CKC	-	-	312	-	-	-	312
CKA	-	-	312	-	-	-	312
CCC	-	-	-	-	-	-	-
CCA	-	-	-	-	625	-	312
CIC	-	-	312	-	-	-	312
CIA	-	-	-	-	-	-	312
CSSC	-	-	-	-	-	-	-
CSSA	-	-	312	-	-	-	-
Ciprofloxacin	†	†	1	†	0.25	†	†
Fluconazole	†	†	†	†	†	†	1

ΨE.c: *Escherichia coli* ATCC 25922, **K.p**: *Klebsiella pneumoniae* ATCC 4352, **P.a:** *Pseudomonas aeruginosa* ATCC 27853, **P.m:** *Proteus mirabilis* ATCC 14153, **S.a:** *Staphylococcus aureus* ATCC 6538, **S.e:** *Staphylococcus epidermidis* ATCC 12228 and **C.a:** *Candida albicans* ATCC 10231 **- :** not active (>=1250 μg/ml), **†:** not tested

The present study clearly shows that five *Centaurea* species have good free radical scavenging activity but further studies are needed to determine cytotoxic activities of these *Centaurea* species to be used safely instead of synthetic antioxidants. Moreover, the substantial effect most of the extracts have against *Candida albicans* confirms traditional uses of *Centaurea* species on vaginal candidiasis.

Bazı *Centaurea* L. türlerinin in vitro antioksidan ve antimikrobiyal aktivitelerinin değerlendirilmesi

ÖZET: Bu çalışmanın amacı, 5 *Centaurea* türünün (*C.stenolepis, C.kilaea, C.cuneifolia, C.iberica, C.solstitialis* subsp. *solstitialis*) kapitulum ve kapitulumsuz toprak üstü kısımlarından maserasyonla hazırlanan toplam 10 metanol ekstresinin antioksidan, antimikrobiyal aktivitelerini ve toplam fenol içeriklerini araştırmaktır. Türlerin serbest radikal süpürücü aktivite tayinleri DPPH metodu ile, toplam fenolik madde miktar tayini ise Folin–Ciocalteu metoduyla yapıldı. Ekstrelerin antimikrobiyal aktivitesi 7 mikroorganizma türüne karşı mikro dilüsyon yöntemiyle test edildi. DPPH radikal süpürücü aktivite tayini deneyinde test edilen 10 ekstrenin $İK_{50}$ değerlerinin 1.767-4.665 mg, toplam fenolik madde miktarlarının ise g kuru materyalde gallik asite eşdeğer olarak 4.825-12.460 mg aralığında değişkenlik gösterdiği görüldü. Dört ekstre *Pseudomonas aeruginosa* (MİK: 312 μg/ml)'ya, 7 ekstre *Candida albicans* (MİK: 312 μg/ml)'a karşı orta derecede, *C.cuneifolia*'nın toprak üstü kısmlarımlarından hazırlanan metanol ekstresi ise *Staphylococcus aureus*'a (MİK: 625 μg/ml) karşı zayıf bir antimikrobiyal aktivite göstermiştir.

ANAHTAR SÖZCÜKLER: *Centaurea* türleri, antioksidan aktivite, antimikrobiyal aktivite

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