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Antimicrobial and Antioxidant Activities of Some Species of *Centaurea* Collected from Turkey

ISMET UYSAL[1,*], SEZGIN ÇELIK[2], HÜSNIYE SAGLAM[3] and KIYMET GUVEN[4]

[1]Department of Biology, Faculty of Science and Arts, Canakkale Onsekiz Mart University, TR-17020 Canakkale, Turkey
[2]Department of Elemantary Education, Faculty of Education, Kirikkale University, TR-71451 Kirikkale, Turkey
[3]Department of Pharmacognosy, Faculty of Pharmacy, Ege University, TR-35100 Izmir, Turkey
[4]Department of Biology, Faculty of Science, Anadolu University, TR-26470 Eskisehir, Turkey

*Corresponding author: Fax: +90 286 218 0533; Tel: +90 286 2180018/1868; E-mail: ismetuysal@gmail.com

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The antimicrobial activity of the ethanol, acetone, ethyl acetate and chloroform extracts from *Centaurea persica* Boiss., *C. polyclada* DC. and *C. consanguinea* DC. (Asteraceae) were investigated by agar-well diffusion assay. All of the extracts exhibited an antimicrobial effect against most of the bacteria and all of the yeasts tested. The plant extracts were also screened for radical scavenging potential using a 1,1-diphenyl-2-picryl-hydrazyl (DPPH) *in vitro* model system for antioxidant activity. Ethyl acetate extracts showed the most significant inhibitory activity. Therefore, the minimal inhibitory concentration (MIC) of only ethyl acetate extracts of the samples was determined and *C. consanguinea* showed the highest antimicrobial effect. However, the ethanol extract of *C. polyclada* at 0.5 mg/mL concentration displayed the highest antioxidant activity. This activity was followed by ethanol extracts of. *C. persica*, ethyl acetate extracts of *C. polclada*, *C. persica* and *C. consanguinea* respectively.

Key Words: Antimicrobial and antioxidant activity, *Centaurea persica*, *C. polyclada* and *C. Consanguinea*.

INTRODUCTION

Medicinal components from plants play an important role in conventional medicine. People on all continents have long applied poultices and imbibed infusions of thousands of plants, dating back to prehistory[1]. Since their discovery, antimicrobial drugs have remarkably proved to be effective for the control of bacterial infections. The indiscriminative use of antibiotics resulted in the emergence of a number of resistant bacterial strains and the antimicrobial compounds from plants may inhibit bacteria by a different mechanism than the antibiotics presently used and may have clinical value in the treatment of resistant microbial strains[2].

On the other hand, research in herbal medicine has increased in developing countries as a way to rescue ancient traditions as well as an alternative solution to health problem in cities. Therefore, with the increasing acceptance of traditional medicine as an alternative way of health care, the screening of plants for active compounds has become very important. Many species of genus *Centaurea* L. (Asteraceae) have traditionally been used for the treatment of various ailments[3,4]. The genus *Centaurea* has been the subject of many antimicrobial and antioxidant activity properties[5-17].

The plants possess potent bioactive compounds capable of preventing and treating most oxidative related diseases. Natural antioxidants are known to exhibit a wide range of biological effects, including antiviral, antibacterial, antiinflammatory, antiallergic and antitrombotic activity. Therefore natural antioxidants have become a major area of scientific searches. The importance of searching for and exploiting natural antioxidants especially those of plant origin has increased remarkably in recent years[18,19].

In this study, antimicrobial properties of ethanol, acetone, ethyl acetate and chloroform extracts of 3 types *Centaurea* taxa namely, *Centaurea persica* Boiss., *C. polyclada* DC. and *C. consanguinea* DC., which were collected from Turkey were investigated against both clinical and food borne microorganisms (bacteria and yeasts) by using agar-well diffusion assay. Another objective of the present study was to assess the antioxidant potential of endemic *Centaurea* species as a source of antioxidants for nutritional and therapeutic purposes. *In vitro* antioxidant assay (DPPH scavenging activity) was performed for *C. consanguinea*, *C. polyclada* and *C. persica* extracts prepared with solvents having different polarities.

EXPERIMENTAL

Plant materials and extraction procedure: Three endemic *Centaurea* taxa of Turkish samples[20,21] were collected from original (typical) localities in Turkey by Celik and Uysal.

C. persica Boiss. was collected in the region stretching from Sirnak to Yüksekova (July 2009, 1950 m), *C. polyclada* DC. was collected from Çanakkale Intepe (June 2009, 50 m) and *C. consanguinea* DC. was collected in the region extending from Mus to Bingöl (June 2009, 1100 m). The specimens collected were identified with the help of Flora of Turkey and the East Aegean Islands[20]. Voucher specimens were deposited in the Herbarium of Faculty of Science and Arts, Canakkale Onsekiz Mart University (COMU). Collector numbers were the same numbers as the numbers given by Celik and Uysal. The plants previously were air-dried and then aerial parts (stem, leaf, flower and fruit) were ground with the help of a Waring blender. Ground samples (20 g) were extracted with 150 mL of ethanol, acetone, ethyl acetate and chloroform solvent (Merck, Darmstadt, Germany) for 24 h by using Soxhlet equipment. The extracts were filtered by using Whatman filter paper (No. 1) and then were concentrated *in vacuo* at 70 ºC. The residues were stored in a refrigerator until subsequent use. For the bioassay the extracts were suspended in the same solvents (ethanol, acetone, ethyl acetate and chloroform) at a concentration of 10 mg/mL for the bioassay.

Microorganism: A total of 29 microbial genera including 26 bacteria and 3 yeast species were used in this study. Table-1 shows the isolates and their source of origin used in the study.

Antimicrobial activity: The antimicrobial activities of the ethanol, acetone, ethyl acetate and chloroform extracts were evaluated by means of the agar-well diffusion assay[22,23] with some modifications. Twenty milliliters of the specified molten agar (45 ºC) was poured into 9 cm sterile Petri dishes. A suspension (100 µL) containing 10^8 cfu/mL bacteria and 10^6 cfu/mL yeasts was spread on the plates of nutrient agar (Merck) and Mueller-Hinton agar (Oxoid, Hampshire, UK) medium, respectively. Once the plates were dried aseptically, 6 mm wells were bored using a sterile cork borer. Extracts (50 µL) were placed into the wells and the plates were incubated for 37 ºC for 24 h for bacterial strains and 48 h for yeast. Chloramphenicol (10 mg/mL) for bacteria and ketakonozol (10 mg/mL) for yeast were used as standard antibiotics. The tests were carried out in triplicate. Antimicrobial activity was evaluated by measuring zone of inhibition against the test organism.

Microdilution assay: The minimal inhibition concentration (MIC) values were also studied for the microorganisms which were determined as sensitive to the extracts in agar-well diffusion assay[11,24,25]. Stock solution of the ethyl acetate extract which gave the best inhibition in the agar-well diffusion test was prepared in dimethyl sulfoxide (DMSO, Carlo-Erba, France). Dilution series using sterile distilled water were prepared from 2000-3.9 µg/mL in test tubes which were transferred to 96 well microtiter plates. Overnight grown bacterial and yeast suspensions in double strength Mueller Hinton Broth were standardized to 10^8 cfu/mL using McFarland No: 0.5 standart solution. Microorganism suspension (100 µL) was then added into the wells. The last-well chain without microorganism was used as a negative control. Sterile distilled water and the medium served as a positive growth control. After incubation at 37 ºC for 18 h the first well without turbidity was determined as the minimal inhibitory concentration (MIC). Chloramphenicol was used as standard antibacterial agent.

TABLE-1
STRAINS USED IN THE STUDY AND THEIR SOURCE

Strain	Source
Bacillus cereus NRRL B-3711 *Bacillus subtilis* NRRL 744 *Salmonella typhimurium* NRRL B-4420 *Micrococcus luteus* NRRL B-4375 *Proteus vulgaris* NRRL B-123 *Xanthomonas campestris* pv. *campestris* NRRL-B1459 *Enterobacter aerogenes* NRRL B-3567 *Streptococcocus feacalis* NRRL B-14617 *Rhodotorula rubra* NRRL Y-2505	USDA, Agricultural Research Service, Peoria, IL.US
Escherichia coli ATCC 25922 *Staphylococcus aureus* ATTC 6538 T	Ege University, Faculty of Science, Dept. of Biology, Izmir, Turkey
Aeromonas hydrophila *Listeria monocytogenes* *Klebsiella pneumoniae* *Yersinia enterocolitica*	Ankara University, Faculty of Veterinary, Ankara, Turkey
Pseudomonas fluorescens B 130 *Bacillus pumilus* B122 *Pseudomonas syringae* pv. *tomato* 32 *Pseudomonas tobacco* 8 *Pseudomonas lachrymans* *Pseudomonas syringae*-1	J. B. Jones, University of Florida, IFAS, Plant Pathology Department
Pseudomonas syringae pv. *glycine* PG1-T	Vidaver, A. K. University of Nebraska Lincoln, Institute of Agricultural and Natural Resources
Pseudomonas syringae pv. *phaseolicola* NCPPB 52 *Pseudomonas gingeri* 3146 *Pseudomonas gladioli* pv. *agricola* RR3	J. G: Turner, Univ. of East Anglia, UK
Brucella spp. *Pseudomonas maltophila* *Candida albicans* *Candida globrata*	Osmangazi University Medical Faculty

Antioxidant activity (DPPH radical scavenging activity): The plant extracts were screened for radical scavenging potential using a 1,1-diphenyl-2-picryl-hydrazyl (DPPH) *in vitro* model system. The chloroform, acetone, ethyl acetate and ethanol extracts of *Centaurea consanguinea*, *C. polyclada* and *C. persica* was prepared at a concentration of 0.5 mg/mL. 1000 µL of each sample was added to 4 mL of 0.004 % methanol solution of DPPH. After a 0.5 h incubation period at room temperature in darkness, the absorbance of mixture was measured at 517 nm against methanol as blank using a UNICAM 8625 UV/VIS spectrophotometer. Free radical scavenging activity of α-tocopherol as reference compound was also determined. The inhibition percentage of the tested samples were evaluated by comparison with a control. Each sample was measured in triplicate and averaged.

The inhibition of free radical by DPPH in percent (I %) was calculated using the formula:

$$I\,(\%) = \left(\frac{A_c - A_s}{A_c}\right) \times 100$$

where A_c is the absorbance of the control and A_s is the absorbance of the tested sample after 0.5 h.

The calibration curve with α-tocopherol had a concentration ranging between 2.0-3.6 mg/mL and α-tocopherol equivalent antioxidant activities of samples were calculated from the following calibration curve:

$$y = 12.88x - 24.451 \ (r^2 = 0.9985)$$

The present study followed principles as described in the Declaration of Helsinki and all requirements was carried out and approved by the University Research Institution.

RESULTS AND DISCUSSION

The antimicrobial activities of ethanol, acetone, ethyl acetate and chloroform extracts of *Centaurea persica*; *C. polyclada* and *C. consanguinea* against microorganisms examined in the present study and their potency were qualitatively assessed by the presence or absence of inhibition zones and zone diameter (Table-2).

The microorganisms tested included clinical and food-borne pathogens and plant pathogens. The results showed that the ethyl acetate, acetone, chloroform and ethanol extracts of *C. persica*, *C. polyclada* and *C. consanguinea* had significant antimicrobial activities against many microorganisms tested. All of the isolates tested were susceptible to the ethyl acetate, acetone and ethanol extracts. Only the chloroform extracts out of the the three *Centaurea* spp. tested did not inhibit the growth of some bacteria and the yeast *R. rubra* (Table-2).

Although the ethyl acetate extract of *C. consanguinea* had greater antimicrobial activity than the other extracts of the plants, the antimicrobial activity of the plant extracts was not higher than the standard antibiotics of chloramphenicol and ketakonozol. Plant pathogenic *Pseudomonas syringae* pv. *phaseolicola*, *Pseudomonas syringae*, *Xanthomonas campestris* pv. *campestris* and *Pseudomonas gladioli* pv. *agricola* and human pathogenic *Yersinia enterocolitica* were the most susceptible to the ethyl acetate extract of *C. consanguinea* (Table-2).

Ethyl acetate extracts of the samples exhibited stronger and broader spectrum of antimicrobial activity as compared to ethanol, acetone and chloroform extracts. Therefore in the determination of minimal inhibitory concentration (MIC) only the ethyl acetate extracts of the three *Centaurea* sp. were used against some bacteria chosen from agar-well diffusion assay. MIC values of the extracts were observed between 250-125 µg/mL (Table-3).

Kumarasamy *et al.*[9] tested *Centaurea scabiosa* seeds for antimicrobial activity and found that the extracts were effective only to *Proteus mirabilis* bacterium. Kumarasamy *et al.*[3] found significant antimicrobial activity of serotonin conjugates from *C. nigra* against penicillin-resistant *E. coli*. However, little antimicrobial activity was observed against this bacterium in this study.

TABLE-2
ANTIMICROBIAL ACTIVITY OF *C. persica*, *C. polyclada* AND *C. consanguinea* EXTRACTS AGAINST THE BACTERIAL, YEAST AND FUNGAL STRAINS TESTED BASED ON AGAR WELL DIFFUSION METHOD

Microorganisms	Inhibiton zone in diameter (mm/sensitive strains)												
	C. persica				*C. polyclada*				*C.consanguinea*				Standard antibiotic
	A	B	C	D	A	B	C	D	A	B	C	D	
Klebsiella pneumoniae	15	19	8	12	8	8	8	12	17	16	8	9.5	29
Staphylococcus aureus	14	13	9	14	13	10	8	12	18	12	8	16	35
Bacillus pumilus	15	14	8	13	13	10	8	12	18	9	8	8	28
Aeromonas hydrophila	17	13	–	20	16	13	–	18	22	11	–	14	31
Bacillus cereus	17	14	13	17	20	10	9	17	21	22	–	16	33
Yersinia enterocolitica	13	17	10	22	16	15	–	12	26	15	–	24	29
Salmonella typhimurium	11	19	13	22	24	14	8	15	22	14	9	18	35
Listeria monocytogenes	13	18	–	12	10	9	8	10	12	12	–	9	32
Brucella spp.	15	8	7	8	14	12	7	8	19	16	8	13	30
Bacillus subtilis	14	12	7	9	16	11	9	12	20	12	9	15	39
Enterobacter aerogenes	17	11	–	8	14	14	9	14	18	15	8	10	34
Proteus vulgaris	12	16	7	12	8	13	8	12	18	10	–	11	25
Pseudomonas maltophila	15	9	7	10	13	10	10	10	20	15	8	9	37
Escherichia coli	15	8	8	9	12	11	8	9	17	14	11	10	39
Micrococcus luteus	16	12	8	12	17	10	–	9	22	18	10	12	44
Streptococcocus feacalis	8	10	7	8	8	9	–	9	20	14	8	8	29
Candida albicans	13	11	8	9	13	12	9	9	16	13	12	10	29
Candida globrata	11	8	8	9	16	10	8	8	18	11	9	11	29
Pseudomonas gladioli pv. agricola	11	14	8	11	13	12	9	14	22	14	8	11	28
Pseudomonas lachrymans	17	20	8	15	14	12	–	14	19	17	8	15	23
Xanthomonas campestris pv. campestris	17	14	8	15	20	13	–	15	23	19	11	13	38
Pseudomonas syringae	20	18	–	18	18	14	–	15	25	14	8	14	36
Pseudomonas gingeri	15	13	10	10	16	11	–	12	19	12	10	8	42
Pseudomonas syringae pv. glycine	19	18	–	19	8	14	–	22	19	15	–	12	39
Pseudomonas syringae pv. tomato	22	16	–	12	10	10	–	12	22	16	–	17	41
Pseudomonas tobacco	15	12	7	9	12	11	–	12	17	11	13	10	39
Pseudomonas syringae pv. phaseolicola	15	12	8	8	NT	NT	NT	NT	30	18	9	9	42
Pseudomonas fluorescens	10	18	7	9	13	11	–	9	17	11	18	11	26
Rhodotorula rubra	16	10	–	12	10	11	9	14	17	12	8	12	37

A: Ethyl acetate extract; B: Acetone extract; C: Chloroform extract and D: Ethanol extract. NT: Not tested.

TABLE-3
MIC VALUES OF *Centaurea persica*; *C. polyclada* AND *C. consanguinea* EXTRACTS AGAINST THE MICROORGANISMS TESTED IN MICRODILUTION ASSAY (MIC in µg/mL)

Microorganisms	Ethyl acetate extracts			
	C. persica	*C. polyclada*	*C. consanguinea*	Standard antibiotic
Pseudomonas lachrymans	250	250	250	250
Pseudomonas syringae	125	125	125	15.62
Bacillus cereus	250	250	250	125
Pseudomonas syringae pv. tomato	250	250	250	250
Pseudomonas syringae pv. glycine	125	125	125	15.62
Xanthomonas campestris pv. campestris	250	125	125	7.81
Pseudomonas syringae pv. phaseolicola	250	250	250	15.62

The essential oils of air-dried *Centaurea sessilis* and *Centaurea armena* were analyzed for antimicrobial activity and, moderate activity was obtained[12]. The extracts showed better antimicrobial activity against Gr (+) bacteria when compared to Gr (–) bacteria.

In the previous study[11] of antimicrobial activity of other *Centaurea* species namely, *C. ptosomipapapoides*, *C. odyssei*, *C. ptosomipappa* and *C. kurdica*, it was found that the extracts exhibited antibacterial and antiyeast effect and ethyl acetate extracts showed the most significant inhibitory activity. In this study, we obtained similar results with the earlier study[11] showing that ethyl acetate extracts of the plants were the most active.

This study was the first record of antimicrobial activity of aerial plant extracts of *Centaurea persica*, *C. polyclada* and *C. consanguinea* and a moderate antimicrobial activity was observed against the bacteria and yeasts tested.

The hydrogen atom or electron donation abilities of plant extracts were measured from bleaching of the purple coloured methanol solution of DPPH. The model of scavenging the stable DPPH radical is widely used to evaluate the antioxidant activities. DPPH is a stable free radical with a characteristic absorbtion at 517 nm and as the antioxidants donate protons to these radicals, the absorbtion decreases. The decrease in absorbtion is taken as a measure of radical scavenging activity.

Table-4 shows the antioxidant effect of solvent extracts of *Centaurea* species. The antioxidant activity was shown in terms of inhibiton per cent of DPPH and activity equivalent to antioxidant reference compound α-tocopherol. Different solvents of varying polarities were used for the extraction of *Centaurea* species. The results revealed that acetone, ethyl acetate, chloroform and ethanol extracts of *Centaurea consanguinea*, *C. polyclada* and *C. persica* exhibited varying scavenging capacities.

The ethanol extract of *C. polyclada* at 0.5 mg/mL concentration displayed the highest antioxidant activity with 43.23 % inhibiton and 5.26 mg/mL α-tocopherol equivalent activity. This activity was followed by ethanol extracts of *C. persica* (38.22 %), ethylacetate extracts of *C. polclada* (28.21 %), *C. persica* (28.21 %) and *C. consanguinea* (25.14 %), respectively. The lowest scavenging activity was exhibited by the chloroform extract of *C. polyclada* with 1.93 % free radical inhibiton and α-tocopherol equivalent activity as 2.04 mg/mL. In comparison with the other solvents, except for *C. consanguinea*, all the chloroform extracts of plant materials were found to have lowest antioxidant activity.

Numerous substances have beeen suggested to appear as antioxidants. The detailed investigations were so far concerned with reactions involving phenolic compounds ranging from polymer chemistry to biochemistry and food chemistry. It has been revealed that various phenolic antioxidants such as flavonoids, tannins, coumarins, procyanidins scavenge radicals dose-dependently. On the basis of the results of this study, the extraction of antioxidant compounds depends largely on type of solvent. To the best of our knowledge, this is the first report of the antioxidant activity of *Centaurea persica*, *C. polyclada* and *C. consanguinea*. The ethanol and ethyl acetate extraction could be recommended for being most suitable method for the isolation of compounds responsible for the antioxidant activity.

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TABLE-4
FREE RADICAL SCAVENGING ACTIVITY OF THE *Centaureae* EXTRACTS

Solvent	Plant extract (0.5 mg/mL)	DPPH % inhibition	α-Tocopherol equivalent antioxidant activity (µg/mL)
Ethyl acetate	*Centaurea persica*	28.21	4.09
	Centaurea polyclada	28.21	4.09
	Centaurea consanguinea	25.14	3.85
Acetone	*Centaurea persica*	7.96	2.52
	Centaurea polyclada	13.08	2.91
	Centaurea consanguinea	4.09	2.21
Chloroform	*Centaurea persica*	2.51	2.09
	Centaurea polyclada	1.93	2.04
	Centaurea consanguinea	16.84	3.21
Ethanol	*Centaurea persica*	38.22	4.87
	Centaurea polyclada	43.23	5.26
	Centaurea consanguinea	24.46	3.79

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