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Free Radical Scavenging and Antimicrobial Activities of Some *Geranium* Species

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Introduction

There are 35 *Geranium* (Geraniaceae) species recorded in the Flora of Turkey[1,2]. Some *Geranium* (Geraniaceae) species are used as antidiabetic, hemostatic, antihemorrhoidal, antidiarrheic and for the treatment of pain, fevers and gastrointestinal ailments[3]. Leaves of some *Geranium* species are consumed as food in Anatolia[4]. Furthermore, tubers of *G. tuberosum* in Erzurum and aerial parts of *G. purpureum* in Kocaeli are used as vegetable[4,5]. Plants from the genus *Geranium* are known to contain flavonoids[6,7], tannins[7,8], lignans[9] and essential oils[10]. Recent papers reported that flavonoids and tannins isolated from this genus have different biological activities such as antileishmanial[7], antiinflammatory[11], antiprotozoal[12], antiinfluenza[13] antioxidant [6,8,14], antiproliferative[14]. Phytochemical investigations on *G. tuberosum* subsp. *tuberosum* and *G. lasiopus* resulted in the isolation of flavonoids, simple phenolic compounds and tannins[15,16] in our laboratory. In the present study, antimicrobial and antioxidant activities of *Geranium tuberosum* L. subsp. *tuberosum, G. purpureum* Vill. and *G. lasiopus* Boiss&Heldr. are investigated. In order to evaluate the possible efficacy of antioxidant

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and antimicrobial activities of title plants, ethyl acetate, *n*-butanol and water extracts of aerial parts of these plants were tested through radical scavenging activity with the DPPH method[17] and antimicrobial activity tested by using broth microdilution method[18,19].

Material and Methods

Plant Materials

Geranium tuberosum L. subsp. *tuberosum* was collected from Atatürk Forest Farm, Ankara, on 6th of May 2005. *G. purpureum* Vill. was collected from Borabay Lake, Amasya on 30th of June 2004. *G. lasiopus* Boiss&Heldr. was collected from Akseki, Güzelsu province, Antalya on 30th of May 2006. Voucher specimens of *G. tuberosum* subsp. *tuberosum* and *G. purpureum* were deposited in the Herbarium of Hacettepe University Faculty of Pharmacy, Ankara, Turkey (HUEF 05003, 04161 respectively). A voucher specimen (H. Duman, 9662) of *G. lasiopus* has been deposited at the Herbarium of Department of Biology, Faculty of Science and Literature, University of Gazi, Ankara, Turkey (GAZI).

Preparation of Extracts

Air-dried and powdered aerial parts of the plant material (20 g) was extracted with MeOH:H_2O (8:2) mixture (3x200 mL) for 5 h at 40 ºC and concentrated to dryness under reduced pressure. An aliquot of the concentrated crude extract was suspended in H_2O (100 mL) and partitioned with petroleum ether (40-60 ºC) (PE) (3x 100 ml), EtOAc (5x 100 mL) and *n*-BuOH (5x 100 ml), individually. PE, EtOAc, and *n*-BuOH extracts, as well as the remaining H_2O phase were concentrated to dryness and lyophilized in *vacuo*. Yield extracts were given in Table I.

Crude extracts were first subject to phytochemical analysis by thin layer chromatography (TLC) and then applied to activity tests.

General experimental procedures

The UV spectra were recorded in MeOH using a *Bio-Tek Instruments, M-Quant Biomolecular* spectrophotometer. TLC Plates:

Silica gel 60 F_{254} 20x20 Merck; Organic Solvents: Sigma-Aldrich, Merck; DPPH (2,2-diphenyl-1-picrylhydrazyl): Fluka; Ampicillin: Mustafa Nevzat; Fluconazole: Pfizer.

TABLE I

Yield values of extracts prepared with G. tuberosum subsp. tuberosum, G. purpureum and G. lasiopus

Extract	Yield (%)
G. tuberosum subsp. *tuberosum*–EtOAc (GT-EtOAC)	2.65
G. tuberosum subsp. *tuberosum*-*n*-BuOH (GT-*n*-BuOH)	3.15
G. tuberosum subsp. *tuberosum*-H_2O (GT-H_2O)	13.8
G. purpureum-EtOAc (GP-EtOAc)	2.50
G. purpureum-*n*-BuOH (GP-*n*-BuOH)	2.95
G. purpureum-H_2O (GP-H_2O)	11.2
G. lasiopus-EtOAc (GL-EtOAc)	2.86
G. lasiopus-*n*-BuOH (GL-*n*-BuOH)	3.45
G. lasiopus- H_2O (GL-H_2O)	16.2

Thin Layer Chromatography

TLC analyses were carried out on pre-coated aluminium sheets (Merck). $CHCl_3$-MeOH-H_2O (80:20:2, 70:30:3; 61:32:7) and EtOAc-Acetic acid- H_2O (90:5:5) were used for the development of the plates. Plates were examined by UV fluorescence and spraying 10 % H_2SO_4 or 3 % $FeCl_3$ in MeOH, followed by heating at 100 °C for 1-2 min.

Microorganisms

Two Gram positive bacteria [*Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212)], two Gram negative bacteria [*Eschericha coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC

27853)]; and three yeasts [*Candida albicans* (ATCC 90028), *C.krusei* (ATCC 6258) and *C. parapsilosis* (ATCC 22019)] were used in this study.

Antimicrobial activity test: Broth microdilution method recommended by Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS), was used to determine the antimicrobial activity[18,19]. Antibacterial activity test was performed in Mueller-Hinton broth (MHB, Difco Laboratories, Detroit, MI, USA) and for antifungal test RPMI-1640 medium with L-Glutamin (ICN-Flow, Aurora, OH, USA), buffered with MOPS buffer (ICN-Flow, Aurora, OH, USA) was used. The inoculum densities were approximately $5x10^5$ cfu/mL and $0.5\text{-}2.5x10^3$ cfu/mL for bacteria and fungi, respectively.

Each of plant extracts was dissolved in sterile distilled water. Final two fold concentrations were prepared in the wells of the microtiter plates, between 1024-1 µg/ml. Ampicillin and fluconazole were used as reference antibiotics for bacteria and fungi, respectively (64-0.0625 µg/ml). Microtiter plates were incubated at 35 °C for 18-24 h for bacteria and 48 h for yeast. After the incubation period, minimum inhibitory concentration (MIC) values were defined as the lowest concentration of the extracts that inhibits the visible growth of microorganisms.

DPPH (2,2-diphenyl-1-picrylhyrazyl) radical scavenging activity: This activity tested according to the method of Çakır et al.[17]. 3 ml MeOH solutions of each sample at various concentrations (25, 50, 100 and 200 µg/mL) and reference antioxidant, L-ascorbic acid, were added to a solution of $1.5x10^{-5}$ M DPPH radical in MeOH (1 ml), and the reaction mixture was shaken vigorously. After incubating at 37 °C for 30 minutes, the remaining DPPH was determined by spectrophotometry at 517 nm. The radical scavenging activity of each sample was expressed using the ratio of the absorbtion of DPPH (%) relative to the control DPPH solution (100 %) in the absence of the sample. The percent of radical scavenging activity was calculated as the ratio of the absorbtion of the sample relative to the control DPPH solution by the following equation. % DPPH Radical Scavenging = [CA – EA] / CA x 100

CA = Control absorbance EA= Extract absorbance

All tests were performed duplicate.

Results and Discussion

The results of antimicrobial activity screening are summarized in Table II. In general, EtOAc extracts of the all tested plants showed the most significant activity, thus indicating that EtOAc extracts of the title plants contain components with antimicrobial activity. The general order of antimicrobial activities of extracts would be EtOAc extracts>*n*-BuOH extracts> H_2O extracts. None of H_2O extracts of title plants has shown antimicrobial activity against tested microorganisms. In comparison with other *Geranium* species, *G. tuberosum* subsp. *tuberosum* was the most active plant. *S. aureus* is the most susceptible organism in the tested microorganisms. MIC values of the EtOAc extract of all the tested plants, were between 32-64 μg/ml.

TABLE II
Antimicrobial activities of different extracts of *Geranium tuberosum* subsp. *tuberosum, G. purpureum* and *G. lasiopus.*

Extracts	**Bacteria/MIC (μg/ml)**				**Yeast/MIC (μg/ml)**		
	S. aureus ATCC 29213	*E. faecalis* ATCC 29212	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*C. albicans* ATCC 90028	*C. krusei* ATCC 6258	*C. parapsilosis* ATCC 22019
GT EtOAc	32	512	1024	1024	512	1024	512
GT *n*-BuOH	≥1024	512	≥1024	1024	256	256	512
GT H_2O	≥1024	≥1024	≥1024	≥1024	512	512	512
GP EtOAc	32	512	≥1024	≥1024	1024	1024	≥1024
GP *n*-BuOH	≥1024	≥1024	≥1024	1024	1024	1024	1024
GP H_2O	≥1024	≥1024	≥1024	1024	512	≥1024	≥1024
GL EtOAc	64	512	≥1024	≥1024	1024	1024	1024
GL *n*-BuOH	≥1024	1024	≥1024	≥1024	1024	1024	1024
GL H_2O	≥1024	≥1024	≥1024	1024	1024	1024	≥1024
Ampicillin	1	8	2	-	-	-	-
Fluconazole	-	-	-	-	1	64	8

The free radical scavenging activities of the tested extracts determined by DPPH method were presented Table III. All the tested

extracts possessed remarkable activity. The free radical scavenging activities of all extracts were in the order: EtOAc>*n*-BuOH>H_2O. The EtOAc extracts, had the highest free radical scavenging activity. *G. tuberosum* subsp. *tuberosum* was the most active plant comparing to other tested plants.

Up to date, there is no information about free radical scavenger or antimicrobial activities of examined species. However, Guo et al. reported that some tannins and flavonoids isolated from *G. sibiricum* showed antibacterial activity and corilagin, which is a hydrolyzable tannin had strong activity against *S. aureus*[20]. Considering to antimicrobial activity of polyphenolic compounds stated in the literature[21, 22], it seems logical to attribute the antimicrobial activity of tested plants to their polyphenolic contents.

TABLE III
Free radical scavenging activities of different extracts of *Geranium tuberosum* subsp. *tuberosum, G. purpureum* and *G. lasiopus*.

Extract	inhibition %			
	200 (µg/ml)	**100 (µg/ml)**	**50 (µg/ml)**	**25 (µg/ml)**
GT EtOAc	95.633	93.847	92.821	49.520
GT *n*-BuOH	90.001	79.110	74.040	29.349
GT H_2O	88.761	82.385	28.407	25.430
GP EtOAc	94.362	91.424	88.761	63.107
GP *n*-BuOH	81.334	84.435	40.390	38.281
GP H_2O	93.560	78.200	33.708	16.225
GL EtOAc	94.362	90.026	80.143	27.497
GL *n*-BuOH	94.508	92.872	66.167	2.447
GL H_2O	92.539	54.498	2.447	1.240
Ascorbic acid	97.500	95.554	57.623	31.948

All test were carried out in duplicate.

DPPH radical scavenger activity of $CHCl_3$:MeOH (1:1) extract, $CHCl_3$ extract, as well as two tannins (Geranins A and D) isolated from *G. niveum* S. Watson has been reported by Maldonado et. al. They found that all tested materials were able to quench DPPH radical in a

concentration dependent manner. They also pointed out that $CHCl_3$:MeOH extract was more active than its main active components (Geranins A and D)[8]. In another study, it has been stated that MeOH extract of *G. sanguineum* L. exhibited better DPPH radical scavenging effect than the synthetic antioxidant agent BHT (butylated hydroxytoluene)[23].

Preliminary TLC analysis of different extracts of titled *Geranium* species have revealed the presence of tannins in all extracts, whereas flavonoids have been detected in EtOAc extracts. Phytochemical investigations of the EtOAc extracts from *G. tuberosum* subsp. *tuberosum* and *G. lasiopus* also revealed the presence of flavonoids (quercetin, kaempferol and their different glycosides), simple phenolic compounds and a hydrolysable tannin[15, 16]. These phenols are well known antioxidants and our results are in good agreement with previous reports[24].

Consequently, this study demonstrates antimicrobial and free radical scavenger activities of different extracts of *Geranium tuberosum* subsp. *tuberosum, G. purpureum* and *G. lasiopus*. The efficacy of each species in studied activities differs depending on the chemical profile of the plants. However, further studies are required in order to clarify the bioactive principles responsible for these activities.

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Summary

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Antimicrobial and free radical scavenging potential of different extracts of *Geranium tuberosum* L. subsp. *tuberosum* (GT), *G. purpureum* Vill. (GP), and *G. lasiopus* Boiss&Heldr. (GL) were evaulated in this study. The minimum inhibitory concentrations (MICs) of these extracts

against 2 Gram-positive bacteria, 2 Gram-negative bacteria and 3 yeasts determined using broth microdilution method. Among the tested microorganisms, *S. aureus* showed the most susceptibility. Radical scavenging activity tested with the DPPH method (25-200 µg/ml). All of the studied extracts showed free radical scavenging activity and among them, the EtOAc extracts of studied plants were found to be with the highest activity. GT EtOAc extract showed almost two times higher activitiy than of ascorbic acid at 50 µg/ml.

Keywords: Geraniaceae, *Geranium*, antimicrobial activity, free radical scavenger activity

Özet

Bazı *Geranium* Türlerinin Serbest Radikal Süpürücü ve Antimikrobiyal Aktiviteleri

Bu çalışmada, *Geranium tuberosum* L. subsp. *tuberosum* (GT), *G. purpureum* Vill. (GP) ve *G. lasiopus* Boiss&Heldr (GL)' in farklı ekstrelerinin antimikrobiyal ve serbest radikal süpürücü potansiyelleri araştırılmıştır. Bu ekstrelerin 2 Gram-pozitif bakteri, 2 Gram-negatif bakteri ve 3 maya mantarına karşı minimum inhibisyon konsantrasyonları (MİK) sıvı mikrodilüsyon metoduyla belirlenmiştir. Serbest radikal süpürücü aktivite DPPH metoduyla test edilmiştir (25-200 µg/ml). *S. aureus*, test edilen organizmalar arasında en duyarlı olandır. Tüm çalışılan ekstreler serbest radikal süpürücü etki göstermişlerdir ve çalışılan bitkilerin EtOAc ekstreleri en yüksek aktiviteyi göstermiştir. GT EtOAc ekstresi, 50 µg/ml konsantrasyonda askorbik asitten yaklaşık iki kat daha yüksek aktivite göstermiştir.

Anahtar Kelimeler: Geraniaceae, *Geranium*, antimikrobiyal aktivite, serbest radikal süpürücü aktivite

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