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RESEARCH ARTICLE

Free Radical Scavenging and Antimicrobial Activities of Three *Geranium* Species Growing in Turkey

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Summary

Antimicrobial and free radical scavenging activities of EtOAc, n-BuOH and the remaining H_2O extracts of aerial parts of Geranium glaberrimum Boiss et Heldr. Geranium stepporum Davis and Geranium psilostemon Ledeb were investigated in this study. Broth microdilution and spectrophotometric DPPH methods were used to test antimicrobial and free radical scavenging activity, respectively. All tested extracts showed free radical scavenging activity. The EtOAc extracts of all studied plants were found to possess the highest antimicrobial and free radical scavenging activity. Among the tested extracts, the EtOAc extract of G. psilostemon is considered the most active against the tested microorganisms. All EtOAc extracts were shown to possess higher free radical scavenging activity than that of ascorbic acid at 50 µg/mL.

Key Words: *Geranium, Geranium glaberrimum, Geranium stepporum, Geranium psilostemon, Geraniaceae, antimicrobial activity, free radical scavenging activity.*

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Türkiye'de Yetişen Üç Geranium Turunun Serbest Radikal Supurucu ve Antimikrobiyal Aktiviteleri

Özet

Bu çalışmada, Geranium glaberrimum Boiss et Heldr. Geranium stepporum Davis and Geranium psilostemon Ledeb.' in toprak üstü kısımlarından hazırlanan EtOAc, n-BuOH ve kalan H_2O ekstrelerinin antimikrobiyal ve serbest radikal süpürücü aktiviteleri araştırılmıştır. Antimikrobiyal aktivite testi için sıvı mikrodilüsyon yöntemi, serbest radikal süpürücü aktivite tayini için spektrofotometrik DPPH yöntemi kullanılmıştır. Tüm ekstreler serbest radikal süpürücü aktivite göstermiştir. Araştırılan ekstrelerin EtOAc fazları en yüksek antimikrobiyal ve serbest radikal süpürücü aktivite göstermişlerdir. Test edilen ekstreler arasında G. psilostemon' un EtOAc ekstresi mikroorganizmalara karşı en yüksek etkili ekstre olarak belirlenmiştir. Tüm EtOAc ekstreleri 50 µg/mL' da askorbik asitten daha yüksek serbest radikal süpürücü aktiviteye sahiptir.

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

INTRODUCTION

The genus *Geranium* L. comprises almost 400 species in temperate areas and tropical mountains throughout most of the world (1). Some *Geranium* species are used in traditional medicine as antidiabetic, hemostatic, antihemorrhoidal, antidiarrheic and as a remedy for tonsillitis, cough, whooping cough, urticaria, dysentery, kidney pain, and gastrointestinal ailments (2-4). There are evidences indicating various properties of *Geranium* plants such as antiprotozoal, antileishmanial, antiinflammatory, α-glucosidase and HIV reverse transcriptase inhibitory, antioxidant, and antibacterial; they have also shown antiviral activity against influenza A and B viruses (3-11). Leaves of some *Geranium* species are consumed as food in western Anatolia (12). Phytochemical investigations in our laboratory on *G. stepporum* resulted in the isolation of some flavonoid glycosides (13). Based on the traditional usage and previous research on *Geranium* species in Turkey and worldwide, the present activity screening study was performed on the extracts prepared with ethy(l) acetate (EtOAc), *n*-butanol (*n*-BuOH), and water (H_2O) from the aerial parts of *G. glaberrimum, G. stepporum* and *G. psilostemon* in order to evaluate their possible antimicrobial

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and free radical scavenging activities. Broth microdilution method recommended by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) was used to determine antimicrobial activity(14,15). DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was used as radical for free radical scavenging activity (16).

MATERIALS and METHODS

Plant Material

G. glaberrimum Boiss et Heldr. was collected between Akseki and Seydişehir, 36 km from Akseki, 1700-1750 m, in May 2006. *G. stepporum* Davis was collected from Eğrisöğüt, Pınarbaşı, Kayseri in May 2006 by D. Şöhretoğlu. *G. psilostemon Ledeb* was collected in Hamsiköy, Trabzon in August 2006 by D. Şöhretoğlu. A voucher specimen (H. Duman, 9661) of G. glaberrimum has been deposited at the Herbarium of the Department of Biology, Faculty of Science and Literature, University of Gazi, Ankara, Turkey (GAZI). Voucher specimens of *G. stepporum* and *G. psilostemon* were deposited in the Herbarium of Hacettepe University Faculty of Pharmacy, Ankara, Turkey (HUEF 06001, 06003, respectively).

Preparation of Extracts

Air-dried and powdered aerial parts of the plant material (20 g) were extracted with MeOH:H_2O (8:2) mixture (3x 200 mL) for 5 h at 40°C 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°C) (PE) (3x 100 ml), EtOAc (x 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. The yielded extracts are given in Table 1.

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

Chemicals

TLC Plates: Silica gel 60 F_{254} 20x20 Merck (Art. 5554); Organic Solvents: Methanol, Chloroform: Carlo Erba, Petroleum Ether, *n*-Butanol, Ethyl acetate: Merck; DPPH: Fluka; Ampicillin: Mustafa Nevzat; Fluconazole: Pfizer.

Table 1. Yield of extracts

	Extract	Yield (%)
G. glaberrimum	PE (GG-PE)	0.92
	EtOAc (GG- EtOAc)	3.50
	n-BuOH (GG- n-BuOH)	3.75
	H_2O (GG- H_2O)	14.21
G. stepporum	PE (GS-PE)	0.85
	EtOAc (GS- EtOAc)	2.85
	n-BuOH (GS- n-BuOH)	5.43
	H_2O (GS- H_2O)	16.32
G. psilostemon	PE (GP-PE)	0.91
	EtOAc (GP- EtOAc)	3.92
	n-BuOH (GP- n-BuOH)	3.22
	H_2O (GP- H_2O)	12.24

Thin Layer Chromatography

TLC analyses were carried out on pre-coated aluminium sheets (Merck Art. 5554). $CHCl_3$-MeOH-H_2O (80:20:2, 70:30:3; 61:32:7) were used for the development of the plates. Plates were examined by UV fluorescence and spraying 10% H_2SO_4 or 3% $FeCl_3$/MeOH, followed by heating at 100°C for 1-2 min.

Antimicrobial Activity

Test organisms: Plant extracts were tested against two standard Gram-positive bacteria: *Staphylococcus aureus* ATCC 29213 (American Type Culture Collection) and *Enterococcus faecalis* ATCC 29212; two standard Gram-negative bacteria: *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853; and three fungi: *Candida albicans* ATCC 90028, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019.

Antimicrobial activity test: Broth microdilution method recommended by the CLSI was used to determine the antimicrobial activity (14,15). Antibacterial activity test was performed in Mueller-Hinton broth (MHB, Difco Laboratories, Detroit, MI, USA); for antifungal test, RPMI-1640 medium with L-glutamine (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 plant extract 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 fungi. After the incubation period, minimum inhibitory concentration (MIC) values were defined as the lowest concentration of the extracts that inhibits the visible growth of the microorganisms.

DPPH radical scavenging activity: Experiments were carried out according to slightly modified procedure (16). Briefly, 3 mL solutions of the proper extract dilution (25, 50, 100 and 200 µg/mL) were added to a 1 mL solution of 1.5 x 10^{-5} M DPPH radical solution in methanol. The absorbance was measured at 517 nm after 30 min of incubation at room temperature. Decreased DPPH solution absorbance indicates an increase in the DPPH radical scavenging activity. The percent of radical scavenging activity was calculated as the ratio of the absorption 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

The DPPH solution without extract was used as control solution. Ascorbic acid was used as reference compound.

RESULTS and DISCUSSION

Preliminary TLC analysis of the tested extracts of the titled *Geranium* species revealed the presence of tannins in all extracts, and EtOAc and *n*-BuOH extracts also contain flavonoids.

Table 2 summarizes the antimicrobial activity of the investigated extracts. According to previous studies, a greater number of medicinal plants have been found to be more active against Gram-positive than Gram-negative bacteria (17). Moreover, the extracts were found to have lower MIC values against Gram-positive than Gram-negative bacteria and fungi in this study, which is in good agreement with earlier reports. In general, EtOAc extracts of the title plants showed the highest antimicrobial activity for almost all microorganisms, thus indicating that EtOAc fractions may contain components with antimicrobial activity. Among the tested extracts, the EtOAc extract of G. psilostemon can be considered as the most active against almost all tested Gram-positive bacteria and fungi. While none of the tested extracts showed activity against *E. coli, S. aureus* was the most susceptible organism among those tested at the tested concentrations. GP-EtOAc was the most effective against *C. krusei* among the tested extracts.

Table 2. Antimicrobial activities of different extracts of *Geranium glaberrimum, G. stepporum* and *G. psilostemon*

Test material	MIC (µg/mL)						
	Bacteria				Fungi		
	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
GG-EtOAc	32	256	≥1024	>1024	512	512	1024
GG-n-BuOH	≥1024	1024	1024	1024	512	512	1024
GG-H2O	≥1024	1024	≥1024	≥1024	1024	1024	1024
GS-EtOAc	64	512	≥1024	≥1024	1024	1024	≥1024
GS-n-BuOH	≥1024	≥1024	≥1024	≥1024	≥1024	≥1024	≥1024
GS-H2O	≥1024	512	≥1024	512	512	512	512
GP-EtOAc	32	512	≥1024	≥1024	256	128	512
GP-n-BuOH	512	≥1024	≥1024	≥1024	1024	1024	1024
GP-H2O	1024	≥1024	≥1024	≥1024	256	256	1024
Ampicillin	1	8	2	-	-	-	-
Fluconazole	-	-	-	-	1	64	8

The DPPH radical scavenging activities of the tested *Geranium* species were found to be comparable to those of the reference compound, ascorbic acid (Figures 1-3). The EtOAc extracts of all the tested species possessed the strongest activity, in all tested concentrations, whereas the H_2O extracts of *G. glaberrimum* and *G. psilostemon* did not show any radical scavenging activity at 25 µg/mL. The EtOAc extracts of all species showed higher activity than ascorbic acid at 50 µg/mL. The free radical scavenging activity of the extracts increases in the order: H_2O < *n*-BuOH < EtOAc. *G. glaberrimum* EtOAc extract showed two times higher activity than that of ascorbic acid at 25 µg/mL.

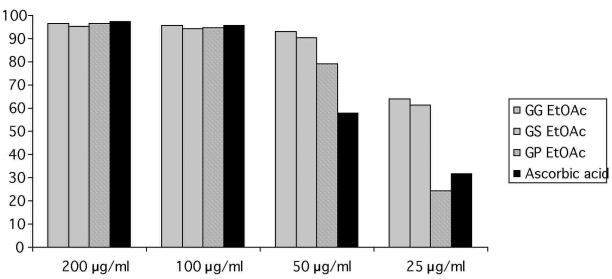

Figure 1 : Comparison of DPPH radical scavenging activities of EtOAC extracts of *Geranium glaberrimum, G. stepporum* and *G. psilostemon*.

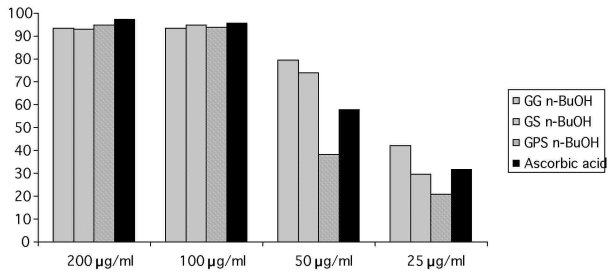

Figure 2 : Comparison of DPPH radical scavenging activities of n-BuOH extracts of *Geranium glaberrimum, G. stepporum* and *G. psilostemon*

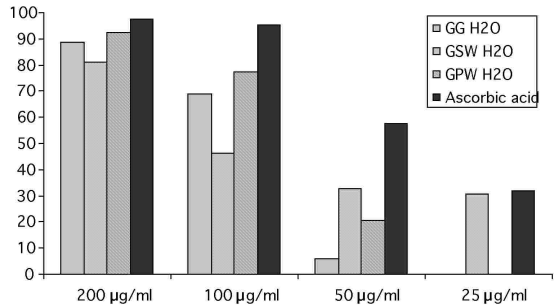

Figure 3 : Comparison of DPPH radical scavenging activities of H2O extracts of *Geranium glaberrimum, G. stepporum* and *G. psilostemon.*

The wide spectrum of therapeutic activities of the *Geranium* species may be explained by their polyphenolic contents(4-6,9,11). According to the literature, none of the studied *Geranium* species has been reported to have biological activity to date. Preliminary TLC analysis of the different extracts of the *Geranium* species revealed the presence of tannins in all extracts, whereas flavonoids were detected only in EtOAc and *n*-BuOH extracts. The antimicrobial activity of the polyphenolic compounds has been reported in the literature(18,19). In this study, we found that EtOAc extracts of *Geranium glaberrimum, G. stepporum* and *G. psilostemon* had polyphenolic components. It was concluded that the antimicrobial activity of the EtOAc extracts may be due to their polyphenolic components.

Antioxidant and free radical activities of the medicinal plants are mainly attributed to their polyphenolic contents like flavonoids and tannins. In a previous study, free radical scavenging activity of some isolated flavonoids from *G. pratense* subsp. *fitinimum* was tested against electrolysis-induced impairment of endothelium-dependent relaxation in aortic rings isolated from rats. It was stated that quercetin-3-O-(2"-*O*-galloyl)-β-D-glucopyranoside and quercetin-3-O-(2"-O-galloyl)-β-D-galactopyranoside mixture and quercetin-3-O-β-D-glucopyranoside and quercetin-3-O-β-galactopyranoside mixture possessed free radical scavenging activity(9). In another report, Miliauskas et al.(20) investigated radical scavenging activities of different extracts of G. macrorrhizum, and they determined gallic acid, ellagic acid, 4-galloyl quinic acid, quercetin, quercetin-3-O-β-glucopyranoside, quercetin-4'-O-β-glucopyranoside and quercetin-3-O-β-galactopyranoside as the compounds responsible for this activity. Flavonoids and tannins are well-known classes of compounds exhibiting high free radical scavenging activity(21,22). Thus, the high free radical scavenging potential of the studied plants might be related to their polyphenolic content.

CONCLUSION

This study presents the antimicrobial and free radical scavenging activities of *G. glaberrimum, G. stepporum* and *G. psilostemon*. However, the chemical composition of extracts was investigated only by TLC. Further research is required in order to identify the bioactive components that showed antimicrobial and free scavenging activities in this study.

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