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Original article

EVALUATION OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF *Geranium pyrenaicum* L.

Berrin ÖZÇELİK[1*], Selda ÖZGEN[1], Seda ÖZTÜRK[2], Şenay KÜSMENOĞLU[2]

[1*]Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, 06330, Etiler-Ankara,TURKEY
[2]Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, 06330, Etiler-Ankara,TURKEY

Abstract

In this study, the ethanolic extracts were prepared from the roots and aerial parts of Geranium pyrenaicum growing in Turkey, their antibacterial and antifungal effects were tested using microdilution method in vitro and compared with the herbal drug that contains Pelargonium sidoides and P. reniforme root extract.

In our study, G. pyreniacum has been found to have antibacterial and antifungal activities, which are similar to those of the herbal drug that contains P. sidoides and P. reniforme root extract.

Key words: *Geranium pyrenaicum, Geraniaceae, Pelargonium, Antibacterial, Antifungal*

Geranium pyrenaicum'un Antibakteriyal ve Antifungal Etkilerinin Değerlendirilmesi

Bu çalışmada; Türkiye'de yetişen Geranium pyrenaicum'un kök ve herbasından etanol ekstreleri hazırlanmış, antibakteriyel ve antifungal etkileri in vitro mikrodilüsyon metodu kullanılarak test edilmiş ve Pelargonium sidoides ve Pelargonium reniforme kök ekstresi içeren bitkisel ilaçlar ile kıyaslanmıştır.

Çalışmamızda, G. pyrenaicum'un P. sidoides ve P. reniforme kök ekstresi içeren bitkisel ilaçlardakine benzer antibakteriyel ve antifungal etkilere sahip olduğu bulunmuştur.

Anahtar kelimeler: *Geranium pyrenaicum, Geraniaceae, Pelargonium, Antibakteriyel, Antifungal*

***Correspondence**: E-mail: berrin@gazi.edu.tr, microberr@yahoo.com

INTRODUCTION

Geranium (Cranesbil) species (Geraniaceae) are known as "Turnagagası" in Turkish and the aerial parts of the plant are used as antidiarrheic, diuretic, tonic, hemostatic, stomachic, and antidiabetic (1). Although *Geranium* and *Pelargonium* belong to different genera of Geraniaceae family, they are often confused because of their morphological similarity. Species which belong to these two genera contain similar chemical constituents and their biological effects in the literature are also very similar (1-5). *Pelargonium* species including; *P. antidysentericum*, *P. rapaceum*, *P. triste*, *P. reniforme* and *P. sidoides*, which are well documented in some traditional medicine. The species of *P. reniforme* and *P. sidoides* have been reported with antituberculosis activity and their potentials as a remedy for ear, nose and throat disorders as well as respiratory tract infections. Following the well-documented therapeutic benefits in these conditions, a modern formulation, EPss 7630, has been elaborated from the traditional herbal medicine and successfully introduced in modern phytotherapy (Umckaloabos, marketed by Spitzner Arzneimittel, Ettlingen, Germany) (6-8). Although accumulating evidence suggests *P. sidoides* to form the origin of the popular traditional herbal medicine (9), commonly termed "umckaloabo", it is most likely that some medicinal records apply to mixtures prepared from both species. This and the earlier taxonomic ambiguity prompted a parallel study on the pharmacological profile of botanically defined plant material of the titled *Pelargonium* species in order to provide a rationale for the therapeutic activity, which has been demonstrated in a number of clinical studies (10-15). The claimed curative effects related to hepatic disorders may tentatively be explicable on the basis of the radical scavenging activities of the broad range of phenolic compounds. Flavonoids and hydrolysable tannins isolated from *P. reniforme* showed marked antioxidant effects using a DPPH radical-generating system and a luminal-dependent chemiluminescence assay (16). Despite of the documented information on the medicinal use of the species, there is little information on its antibacterial, antifungal and antiviral activities. The samples exhibited merely moderate direct antibacterial capabilities against a spectrum of gram-positive and gram-negative bacteria. Along with the appearance of resistance of bacteria and viruses to chemotherapeutic agents, the search for microbial inhibition for plant origin becomes a promising approach in the development of new drugs. A considerably number of extracts and pure substances have shown antimicrobial and antiviral activities (17). It is reported that *in vitro* antimicrobial screening has provided the preliminary observation necessary to select among crude plant extracts, those with potentially useful properties for further chemical and pharmacological investigations (18).

In the present study, the root and aerial extracts of *G. pyrenaicum* were tested against standard and isolated strains of *Escherichia coli*, *Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Acinetobacter baumannii, Staphylococcus aureus*, and *Enterococcus faecalis* for the determination of antibacterial activity, as well as against *C. albicans* and *C. parapsilosis* for establishing antifungal activity.

EXPERIMENTAL

Plant material

G. pyrenaicum was collected from Kargasekmez locality, 5 km to Kızılcahamam , Ankara in May, 2007. The dried parts of the whole plant were grounded separately into root and aerial parts. Voucher specimens are deposited in the Herbarium of Gazi
University, Faculty of Pharmacy (GUE 2590).

Microbiological studies

Test materials

The water and ethanol extracts of the root and herba of *G. pyrenaicum* were dissolved in dimethylsulphoxide (DMSO) solution at a final concentration of 128 μg mL^{-1} and sterilized by filtration using 0.22 μm Millipore (MA 01730, USA) and used as the stock solutions.

Reference antibacterial agents of ampicillin (AMP, Faco), ofloxacin (OFX, Hoechst Marion Roussel), levofloxacin (LVX, Faco) as well as reference antifungal agents of ketoconazole (KET, Bilim) and fluconazole (FLU, Pfizer) were obtained from their respective manufacturers and dissolved in phosphate buffer solution (ampicillin, pH: 8.0; 0.1 mol mL^{-1}), DMSO (ketoconazole), or in water (fluconazole, ofloxocin, levofloxacin). The stock solutions of the agents were prepared in medium according to the Clinical and Laboratory Standards Institute (CLSI) (19).

Microorganisms and inoculum preparation

Antibacterial activity test was carried out against standard (ATCC; American type culture collection, RSKK; Culture collection of Refik Saydam Central Hygiene Institute) and isolated (clinical isolate and obtained from Department of Microbiology, Faculty of Medicine, Gazi University), gram-negative strains; *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 10145, *Proteus mirabilis* ATCC 7002, *Klebsiella pneumoniae* RSKK 574 *Acinetobacter baumannii* RSKK 02026, and as standard and isolated gram-positive strains *Staphylococcus aureus* ATCC 25923, and *Enterococcus faecalis* ATCC 29212 were used for the determination of antibacterial activity. *Candida albicans* ATCC 10231 and *C. parapsilosis* ATCC 22019 were used for the determination of antifungal activity. Mueller Hinton Broth (MHB; Difco) and Mueller Hinton Agar (MHA; Oxoid) were applied for growing and diluting of the bacteria suspensions. The synthetic medium RPMI-1640 with L-glutamine was buffered to pH: 7 with 3-[*N*-morpholino]-propansulfonic acid (MOPS) and culture suspensions were prepared through the guideline of CLSI (20). The microorganism suspensions used for inoculation were prepared at 10^5 CFU (colony forming unite mL^{-1}) by diluting fresh cultures at McFarland 0.5 density (10^8 CFU mL^{-1}). Suspensions of bacteria and fungi were added in each well of the diluted extracts, density of 10^5 CFU/ mL for fungi, and for bacteria. The bacterial suspensions used for inoculation were prepared at 10^5CFU mL^{-1} by diluting fresh cultures at McFarland 0.5 density (10^8 CFU/mL). The fungi suspension was prepared by the spectrophotometric method of inoculum preparation at a final culture suspension of $2.5x10^3$ CFU/mL (19).

Antibacterial and antifungal tests

The microdilution method was employed for antibacterial and antifungal activity tests. Media were placed into each 96 wells of the microplates. Extract solutions at 256 μg/ mL were added into first rows of microplates and two fold dilutions of the compounds (128-0.0312 μg/ mL) were made by dispensing the solutions to the remaining wells. 10μl culture suspensions were inoculated into all the wells. The sealed microplates were incubated at 35°C for 24 h and 48 h in humid chamber. The lowest concentration of the extracts that completely inhibit macroscopic growth was determined and minimum inhibitory concentrations (MICs) were reported (21, 22, 23).

RESULTS AND DISCUSSION

As seen in Table 1, the extracts of root and herba of *G. pyrenaicum* have shown more antibacterial activity against gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*), than gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Acinetobacter baumannii*) ones. Moreover, all the extracts screened herein have exerted a better inhibitory effect towards ATCC strains used as a control (extracts of *Pelargonium sidoides* & *P. reniforme*, ampicilline, ofloxacin, levofloxacin)

Table 1. Antimicrobial activity of *Geranium* extracts and references expressed as minimum

	Microorganisms *E. coli*		*P. aeruginosa*		*P.mirabilis*		*K. pneumonaei*		*A. baumannii*		*S. aureus*		*E. faecalis*		*B. subtilis*		*C. albicans*	*C. parapsilosis*
Extracts	ATCC 35218	Isolated strain	ATCC 10145	Isolated strain	ATCC 7002	Isolated strain	RSKK 574	Isolated Strain	RSKK 02026	Isolated strain	ATCC 25923	Isolated strain	ATCC 29212	Isolated strain	ATCC 6633	Isolated strain	ATCC 10231	ATCC 22019
Root extract of G. pyrenaicum	32	64	32	128	32	64	32	64	32	64	8	32	8	32	32	64	8	8
Herba extract of G. pyrenaicum	32	64	32	128	32	64	32	64	32	64	8	32	8	32	32	64	8	8
U	16	64	32	64	16	32	16	32	16	32	8	16	8	16	16	64	2	2
AMP	2	64	-	-	2	4	2	4	2	4	0.12	8	0.5	1	0.12	2	-	-
OFX	0.12	1	1	4	0.12	1	0.12	1	0.12	2	0.5	4	1	2	1	1	-	-
LVX	0.12	0.25	1	2	0.12	1	0.12	1	0.12	2	0.5	4	0.5	2	0.5	1	-	-
KET	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
FLU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	4

U: Extracts of *Pelargonium sidoides* and *P. reniforme*; AMP: ampicilline; OFX: ofloxacin; LVX: levofloxacin KET: ketoconazole; FLU: Fluconazole; -: No activity observed.

than the isolates strains. No significant differences were evident between the extracts regarding the antibacterial activity.

Among tested samples, significant antibacterial activities were found at 8-32 μg/mL; minimum inhibitory concentrations (MICs) against *S. aureus* and *E. faecalis*. Additionally, lower antibacterial effects were seen against *B. subtilis* at MICs values of 32-64μg/mL.

Similarly, in one study reported by Uzun et. al (24), the petroleum ether extract from *G. asphodeloides* was shown an antibacterial activity against *S. epidermidis* at a MICs of 78.1 μg/mL, while they were shown antibacterial activity against *S. aureus* at 312.5 μg/mL MIC, which were determined by using *in vitro* microdilution method. In another study, it was reported that the polyphenol extract of *G. sanguineum* had antibacterial activity against *S. aureus* (1000 μg/mL) and antifungal activity against *C. albicans* (125 μg/mL) by disc diffusion method (25).

In our study, the samples of root and the herba from *G. pyrenaicum*, which were shown antibacterial activity (32-64 μg/mL; MICs) against gram-negative bacteria, they were also shown significant antibacterial activity (64 μg/mL; MICs) against *E. coli* as compared with a control extracts of *Pelargonium sidoides* and *P. reniforme*.

Their antifungal activities were determined at 8 μg/mL MICs against both of *C. albicans* and *C. parapsilosis*. Also, it is reported that the essential oils from *Geranium* were demonstrated antibacterial activity using disc diffusion method against gram-positive (*S. aureus*, *B. subtilis*) and gram-negative (*K. pneumoniae*, *P. vulgaris*, and *E. coli*) bacteria at concentrations ranges of >12.8-1.6 μg/mL, >12.8-0.8 μg/mL, respectively (26).

Antibacterial activity of the extracts and isolated constitutes of *Pelargonium sidoides* and *P. reniforme* was evaluated with microdilution technique by Kayser et. al (27). Antibacterial activity was reported against gram-positive (*S. aureus*, *S. pneumoniae*, β-Hemolitic Streptococci) and gram-negative (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa, H. influenza)* bacteria at 5-7.5 μg/mL MIC ranges. It is also reported that no significant differences were evident between *P. sidoides* and *P. reniforme* extracts regarding the antibacterial activity.

Lewu et. al (18) investigated in acetone and methanol extracts of the shoot and root the *Pelargonium sidoides* by the dilution method on the solid agar medium. It is reported higher antibacterial activity against gram-positive bacteria (*S. aureus*, *B. subtilis*, *S. epidermidis*, *S. pyogenes*, *M. kristinae*) that gram-negative (*S. sonnei*, *K. pneumoniae*) bacteria at MIC value of 1-5 μg/mL. However, it is reported that no antibacterial activity was observed at tested concentrations ranges (0.1-10 mg/mL) against some of tested Gram negative bacteria (*E. coli, S. marcescens* ve *P. aeruginosa*).

In many studies, different biological activities have been reported in *Geranium* species such as antiviral activity, anti-protozoal activity, TNF-α releasing inhibitors effect, antioxidant properties, immunomodulatory activities, antinociceptive and anti-inflammatory activities (28-40). In one of them, it is reported that the root extract from *P. sidoides* have anti-adhesive activity against *Helicobacter pylori* and could therefore be a useful choice to avoid the first step of a bacterial infection (39). In another study, antibacterial properties of the aqueous and methanolic extracts of *G. mexicanum* (aerial parts) were tested against *Escherichia coli* and *Shigella sonnei* as enteropathogens and the result showed the highest antibacterial activity against *S. sonnei* with MIC values ranging from 1 to 4 μg/mL (35). The effects of essential oils on methicillin-resistant *S. aureus* (MRSA) were studied in *Geranium* using a dressing model by Edward-Jones et al. (37), as a result, the most effective of the tested oils against MRSA was reported in vapor phase. In conclusion, this is the first report showing that the antibacterial and antifungal activities of the extracts prepared from the root and aerial parts of *G. pyrenaicum*.

REFERENCES

1. **Baytop, A**., Farmasötik Botanik. İstanbul: İstanbul Üniversitesi Yayınları No: 3637, **1991**.
2. **Heywood, V.H**., Flowering Plants of the World: Oxford University Press, **1979**.
3. **Davis, P.H**., *Geranium* L. in: Flora of Turkey and the East Aegean Islands, Ed.: P.H. Davis, Vol. 2, 451-474, University Press, Edinburg, **1967**.
4. **Peter, K.V**., *Geranium*. Handbook of Herbs and Spices. Cambridge: Woodhead Publishing; **2004**.
5. **Baytop, T**., Türkiye'de Bitkiler ile Tedavi. İstanbul:Nobel Tıp Kitabevi; **1999**.
6. **Seidel, V., Taylor, P.W.,** "*In vitro* activity of extracts and constituents of *Pelargonium* against rapidly growing Mycobacteria" *Int. J. Antimicrob. Agents.*, 23: 613-619, **2004**.
7. **Kolodziej, H., Kiderlen, A.F.,** "*In vitro* evaluation of antibacterial and immunomodulatory activities of *Pelargonium reniforme*, *Pelargonium sidoides* and the, related herbal drug preparation EPss 7630" *Phytomed.*, 14: 18-26, **2007**.
8. **Kolodziej, H., Kayser, O., Radtke, O.A., Kiderlen, A.F., Koch, E**., "Pharmacological profile of extracts of *Pelargonium sidoides* and their constituents" *Phytomed.*, 10, 18-24, **2003**.
9. **Kolodziej, H., Kayser, O.,** "*Pelargonium sidoides* DC. –Neueste Erkenntnisse zum Verständnis des Phytotherapeutikums Umckaloabo" *Z. Phytother.*, 19, 141-151, **1998**.
10. **Heil, Ch., Reitermann, U.,** "Atemwegs-und HNO Infektionen: Therapeutische Erfahrungen mit dem Phytotherapeutikum Umckaloabos" *Pädiatrie,* 7, 523-525, **1994**.
11. **Dome, L., Schuster, R.,** "Umckaloabo-eine phytotherapeutische Alternative bei akuter Bronchitis im Kindesalter" *Arztezeitschr. Naturheilv* ., 37,216-222, **1996**.
12. **Haidvogl, M., Schuster, R., Heger, M.,** "Akute Bronchitis im Kindesalter-Multizenter-Studie zur Wirksamkeit und Vertraglichkeit des Phytotherapeutikums Umckaloabos" *Z Phytother.*, 17, 300-313, **1996**.
13. **Blochin, B., Heger, M.,** "Umckaloabos versus symptomatische Therapie in der Behandlung der Angina catarrhalis" *Päd*, 6,2-8, **2000.**
14. **Blochin, B., Haidvogl, M., Heger, M.,** "Umckaloabos im Vergleich mit Acetylcystein bei Kindern mit akuter Bronchitis" *Der Kassenarzt.*, 49, 46-49, **1999**.
15. **Khalaj, L., Farzin, D.,** "Evaluation of antidepressant activities of rose oil and *Geranium* oil in the forced swim test in mouse" *Eur. Neuropsychopharmacol.*,16(4), 334-335, **2006**.
16. **Latte, K.P., Kolodziej, H., "**Antioxidant properties of phenolic compounds from *Pelargonium reniforme*" *J. Agric. Food. Chem.*, 52, 4899-4902, **2004**.
17. **Serkedjieva, J**., "Antiinfective activity of a plant preparation from *Geranium sanguineum* L." *Pharmazie,* 52(10), 799-802, **1997**.
18. **Lewu, F.B., Grierson, D.S., Afolyan, A.J.,** "Extracts from *Pelargonium sidoides* inhibit the growth of bacteria and fungi" *Pharm. Biol.,* 44, 279-282, **2006**.
19. Clinical and Laboratory Standards Institute (CLSI); (formerly National Committee for Clinical Laboratory Standards-NCCLS). Methods for dilution antimicrobial susceptibility tests bacteria that grow aerobically, 3rd edition, approved standard. NCCLS document M100-S12, Wayne, PA, USA, **2002**.
20. **Koca, U., Özçelik, B.,** "Antiviral Antibacterial, and antifungal activities of the lipophylic extracts of Evaluation of *Centaurea tchihatcheffii* extracts" *Turk J. Pharm. Sci.,* 6, (2), 125-134, **2009**.
21. **Kan, Y., Orhan, İ., Koca, U., Özçelik, B., Aslan, S., Kartal, M., Küsmenoğlu; Ş**., "Fatty Acid Profile and antimicrobial effect of the seed oils of *Urtica dioica* and *U. pilulifera*" *Turk J. Pharm. Sci.,* 6(1),21-31, **2009**.
22. **Özçelik, B., Gürbüz, İ., Karaoglu, T., Yeşilada, E.,** "Antiviral and antimicrobial activities of three sesquiterpene lactones from *Centaurea solstitialis* L. ssp. s*olstitialis* extract" *Microbiol. Res.*, 164, 545-552, **2009**.

22. **Özçelik, B., Aslan, M., Orhan İ., Karaoglu T.,** "Antibacterial, antifungal, and antiviral activities of the lipophylic extracts of *Pistacia vera* L." *Microbiol. Res.*, 160, 159-164, **2005**.
23. **Özçelik, B., Orhan, İ., Toker G.** "Antiviral and antimicrobial assessment of some flavonoid type of compounds" *Z. Naturforsch.*, 61c, 632-635, **2006**.
24. **Uzun, E., Sarıyar, G., Andersen, A., Karakoc, B., Otuk G., Oktayoglu, E., Pırıldar, S.,** "Traditional medicine in Sakarya province (Turkey) and antimicrobial activities of selected species" *J. Ethnopharmacol.*, 95, 287-296, **2004**.
26. **Prabuseenivasan, S., Jayakumar, M., Ignacimuthu, S.,** "*In vitro* antibacterial activity of some plant essential oils BMC Complement" *Altern. Med.*, 6, 39, **2006.**
27. **Kayser O., Kolodziej, H.,** "Antibacterial activity of extracts and constituents of *Pelargonium sidoides* and *Pelargonium reniforme*" *Planta Med.*, 63, 508-510, **1997**.
28. **Kolodziej, H.,** "Fascinating metabolic pools of *Pelargonium sidoides* and *Pelargonium reniforme*, traditional and phytomedicinal sources of the herbal medicine Umckaloabo" *Phytomed.*, 6, 9-17, **2007**.
29. **Serkedjieva, J., Ivancheva, S.,** "Anti-herpes virus activity of extracts from the medicinal plant *Geranium sanguineum* L." *J. Ethnopharmacol,* 64, 59-68, **1999**.
30. **Serkedjieva, J., Hay, A.J.,** "*In vitro* anti-influenza virus activity of a plant preperation from *Geranium sanguineum* L." *Antiviral Res.*, 37, 121-130, **1998**.
31. **Sokmen, M., Angelova, M., Krumova, E., Pashova, S., Ivancheva, S., Sokmen, A., Serkedjieva, J.,** "*In vitro* antioxidant activitiy of polyphenol extracts with antiviral properties from *Geranium sanguineum* L." *Life Sci.*, 76, 2981-2993, **2005**.
32. **Kupeli, E., Tatli, II, Akdemir, Z.S., Yeşilada, E.,** "Estimation of antinociceptive and anti-inflammatory activity on *Geranium pratense* subsp. *finitimum* and its phenolic compounds" *J. Ethnopharmacol.*, 114, 234-240, **2007**.
33. **Kolodziej, H., Kiderlen, A.F.,** "*In vitro* evaluation of antibacterial and immunomodulatory activities of *Pelargonium reniforme*, *Pelargonium sidoides* and the related herbal drug preparation EPs 7630" *Phytomed.*, 14, 18-26, **2007**.
34. **Serkedjieva, J., Manolova, N.,** "Antiviral effect of a polyphenolic complexisolated from the medicinal plant *Geranium sanguineum* L. V. Mechanism of the anti-influenzal effect *in vitro*" *Acta. Microbiol. Bulg.*, 21, 66-71, **1987**.
35. **Alanis, A.D., Calzada, F., Cervantes, J.A., Torres, J., Ceballos, G.M.**, "Antibacterial properties of some plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders" *J. Ethnopharmacol.*, 100, 153-157, **2005**.
36. **Calzada, F., Yepez-Mulia, L., Tapia-Contreras, A.,** "Effect of Mexican medicinal plant used to treat trichomoniasis on *Trichomonas vaginalis* trophozoites" *J. Ethnopharmacol.*, 113, 248-251, **2007**.
37. **Edwards-Jones, V., Buck, R., Shawcross, S.G., Dawson, M.M., Dunn, K.**, "The effect of essential oils on methicillin-resistant *Staphylococcus aureus* using a dressing model" *Burns*, 30(8), 772-777, **2004**.
38. **Latte, K.P., Kolodziej, H.**, "Antioxidant properties of phenolic compounds from *Pelargonium reniforme*" *J. Agric. Food. Chem.*, 52, 4899-4902, **2004**.
39. **Beil W., Kilian P.** "EPs 7630, an extract from *Pelargonium sidoides* roots inhibits adherence of *Helicobacter pylori* to gastric epithelial cells" *Phytomed.*, 14, 5-8, **2007**.
40. **Conrad, A., Jung, I., Tioua, D., Lallemand, C., Carrapatoso, F., Engels, I., Daschner, F.D., Frank, U.,** "Extract of *Pelargonium sidoides* (EPs 7630) inhibits the interactions of group A streptococci and host epithelia *in vitro*" *Phytomed.*, 14, 52-59, **2007**.

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