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Full Length Research Paper

Antimicrobial activity of *Pelargonium endlicherianum* Fenzl. (Geraniaceae) roots against some microorganisms

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***Pelargonium endlicherianum* Fenzl. (Geraniaceae) is one of the important species of *Pelargonium* growing in Turkey. The purpose of this study was to evaluate the antimicrobial activities of *P. endlicherianum* roots on medically important microorganisms. The antimicrobial activities of 11% ethanol and 70% methanol *P. endlicherianum* extracts against various bacteria and yeast were studied using the agar dilution method at concentrations ranging from 0.375 to 30.00 mg/ml. The minimum inhibitory concentrations (MICs) of *P. endlicherianum* extracts against some microorganisms was determined. The highest inhibition was exhibited against *Staphylococcus aureus* by the 70% methanol extract of *P. endlicherianum* at 1.38 ± 0.36 mg/ml. The results of this study suggest that *P. endlicherianum* extracts possess antimicrobial activity against some of the tested microorganisms.**

Key words: *Pelargonium endlicherianum*, antimicrobial activity, agar dilution method, susceptibility test.

INTRODUCTION

Pelargonium endlicherianum Fenzl. (Geraniaceae) belongs to the family Geraniaceae which has about 750 species scattered widely around the world growing as annuals. Members of the family occur mostly in temperate and subtropical climates and have many important medicinal features (Brendler and van Wyk, 2008). A standardized extract obtained from *P. sidoides* DC. roots is used and prescribed by physicians in the treatment of some illnesses such as acute rhinosinusitis and tonsillopharyngitis (Bachert et al., 2009; Bereznoy et al., 2003). The liquid preparation of this extract, which is known as UMCA® (Abdi Ibrahim, Turkey), is used for treating upper respiratory tract infections and coughs especially for children. Much scientific research had been conducted on the anti infective and immunomodulatory effects of *Pelargonium. sidoides* both *in vitro* and *in vivo* (Conrad and Frank, 2008; Conrad et al., 2007; Kim et al., 2009; Kolodziej H, 2008). *In vitro* studies of *P. sidoides* have investigated its antibacterial, antimycobacterial, antifungal, immunomodulator activities and effects on the mucosiliary system (Lewu et al., 2006; Lis-Balchin et al., 1998; Neugebauer et al., 2005; Janecki et al., 2007; Kayser et al., 2001; Kayser et al., 2003; Kolodziej et al., 2003). The crude extract and compounds derived from *P. sidoides, Pelargonium reniforme* and *Pelargonium radula* have been previously tested against Gram positive, Gram negative bacteria and yeasts (Kayser and Kolodziej, 1997; Papeljnjak et al., 2005).

Turkey has rich flora, and the *Pelargonium* species are being grown both in the wild and as cultivars (Davis, 1967). One of the wild species, *P. endlicherianum* (local name “solucanotu") and the decoction prepared from the roots and fresh flowers are used against intestinal parasites (Bozan et al., 1999).

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Table 1. The yields and amount of total phenol of *P. endlicherianum*.

Extract	Yield (%)	Amount of total phenol (mgGAE/gextre)
11% ethanol	1.5	20.92 ± 2.42
70% methanol	7.2	130.50 ± 2.49

However, until now, few studies have been conducted on the roots of *P. endlicherianum.* The aim of this study was to reveal the antimicrobial activity of *P. endlicherianum* collected from Turkey.

MATERIALS AND METHODS

Plant material and extract preparation

P. endlicherianum were collected in the month of March 2009 from Eskisehir, Turkey. The botanical identification of the plant was done at Department of Pharmaceutical Botany, Erciyes University. The roots were washed and dried at 40°C and powdered in a hammer-mill. The dry powdered root was extracted by soaking with 11% ethanol and 70% methanol for 24 h at 25°C in a shaking water bath. After this procedure, the extracts were filtered, and the residue was extracted by same procedure once more. The extracts were then evaporated in a vacuum room temperature. These extracts were sterilized by filtration through a membrane filter with a 0.45 µm diameter. They were then dissolved in water, and the final concentrations of the extracts were obtained in the medium ranging from 0.375 to 30.00 mg/ml.

Determination of total phenolics

Total phenols were estimated as gallic acid equivalents (GAE), expressed as mg gallic acid/g extract (Singleton et al., 1999). To ca. 6.0 ml H_2O, 100 µl sample was transferred in a 10.0 ml volumetric flask, to which 500 µl undiluted Folin-Ciocalteu reagent was added subsequently. After 1 min, 1.5 ml 20% (w/v) Na_2CO_3 was added and the volume was made up to 10.0 ml with H_2O. After 2 h incubation at 25°C, the absorbance was measured at 760 nm and compared to a gallic acid calibration curve. The data are presented as the average of triplicate analyses.

Microorganisms and antimicrobial activity test

The 14 bacterial cultures and 2 yeast cultures used for screening were: *Staphylococcus aureus* American type culture collection (ATCC) 25923, *Streptococcus pyogenes* ATCC 19615, *Streptococcus agalactia* ATCC 12401, *Streptococcus pneumoniae* ATCC 6303, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Lactobacillus acidophilus* ATCC 11975, *Candida albicans* ATCC 90028, *Candida glabrata* Refik Saydam Culture Collection (RSKK) 04019, *Streptococcus sanguinis* DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) 20567, and clinical strains such as *Klebsiella pneumoniae, Streptococcus mutans, Staphylococcus epidermidis, Staphylococcus caprae, Proteus mirabilis.*

Antimicrobial activity testing was performed by the agar dilution method according to CLSI protocol standards (CLSI M7-A7., 2006). By adjusting density standards (Phoenix Spec Nephelometer, Becton Dickinson, USA), the microorganism suspensions were prepared to equal the density of a 0.5 McFarland standard (1×10^8 CFU/ml for bacteria, 1 to 5 × 10^6 CFU/ml for yeast). Proper media for the microorganisms were prepared by mixing them with sterile Mueller Hinton Agar to give final extract concentrations of between 0.375 and 30.00 mg/ml. Blood was added for some microorganisms such as *S. sanguinis, S. pneumoniae, S. agalactia, S. mutans, S. pyogenes, E. faecalis* and *L. acidophilus* in medium to optimum growth conditions. The microorganisms were inoculated on the plate which included different concentrations of *P. endlicherianum* extracts. Inoculations were performed by means of spots containing a 1×10^4 CFU/spot from each of adjusted microorganism inoculums, and the plates were incubated under the proper conditions for each microorganism. Control samples included: growth control and solvent control. Streptomycin and fluconazole were used as positive reference standards for bacterial and yeasts strains, respectively. The MIC was defined as the lowest concentration of plant extract that did not result in any visible growth of the microorganisms when compared with growth control. All determinations were performed in six times.

RESULTS

Two crude extracts used in this study were obtained from the roots of *P. endlicherianum*. The extractions were carried out using 11% ethanol and 70% methanol solvents. The ethanol and methanol extracts of *P. endlicherianum* roots gave a high yield of 1.5 and 7.2% (% concentration w/v) respectively (Table 1). The results of our investigations showed that the 11% ethanol and 70% methanol extracts of *P. endlicherianum* roots exhibited antimicrobial activities against some of the test microorganisms. The results of the general screening for antimicrobial activity are shown in Table 2. In this study, the highest inhibition was exhibited by the 70% methanol *P. endlicherianum* extract against *S. aureus,* the lowest inhibition was exhibited by the 11% ethanol *P. endlicherianum* extract against *L. acidophilus*. In addition, the concentrations of both *P. endlicherianum* extracts also showed no effect against microorganisms such as *K. pneumoniae, P. mirabilis, C. albicans, C. glabrata.* This study showed that *P. endlicherianum* has broad-spectrum antibacterial activity but no antifungal activity.

The amount of total phenol in 11% ethanol and 70% methanol extract *P. endlicherianum* was found 20.92 mgGAE/gextre ± 2.42 and 130.50 mgGAE/gextre ± 2.49 respectively (Table 1).

The methanol extract of *P. endlicherianum* displayed a higher inhibition against some microorganisms [*S. aureus*($p<0.019$), *S. agalactia* ($p<0.026$), *S. mutans* ($p<0.003$), *E. faecalis* ($p<0.003$), *E. coli* ($p<0.006$), *P. aeruginosa* ($p<0.009$)].

DISCUSSION

P. sidoides is traditionally used to cure tuberculosis in areas of Southern Africa (Brendler and van Wyk, 2008). The antimicrobial activities of *P. sidoides* extract are known, and a commercialized extract derived from the roots of *P. sidoides* is also used to treat acute bronchitis

Table 2. MIC values (mg/ml) of the extracts of *P. endlicherianum*

Microorganisms	MIC±standard deviation		
	P. endlicherianum **11% ethanol**	***P. endlicherianum*** **70% methanol**	**RD**
S. aureus ATCC 25923	2.83 ± 1.29	1.38 ± 0.36	0.004 ± 0.000
Streptococcus pyogenes ATCC 19615	12.92 ± 3.32	9.17 ± 1.29	0.002 ± 0.008
S. agalactia ATCC 12401	24.17 ± 2.04	20.00 ± 3.16	0.004 ± 0.000
S. pneumoniae ATCC 6303	25.00 ± 3.16	24.17 ± 2.04	0.003 ± 0.001
Streptococcus mutans (clinical isolate)	23.33 ± 2.58	10.42 ± 2.46	0.012 ± 0.001
S. sanguinis DSM 20567	24.17 ± 2.04	23.33 ± 2.59	0.009 ± 0.003
E. faecalis ATCC 29212	9.58 ± 1.02	5.08 ± 1.43	0.009 ± 0.003
Staphylococcus epidermidis (clinical isolate)	28,33 ± 2,58	27.50 ± 2.73	0.014 ± 0.005
Staphylococcus caprae (clinical isolate)	27.50 ± 2.73	28.33 ± 2.58	0.009 ± 0.003
L. acidophilus ATCC 11975	28.33 ± 2.58	29.17 ± 2.04	0.029 ± 0.007
E. coli ATCC 25922	14.17 ± 2.04	9.17 ± 1.29	0.004 ± 0.002
P. aeruginosa ATCC 27853	7.92 ± 1.02	4.75 ± 1.67	0.005 ± 0.002
Klebsiella pneumoniae (clinical isolate)	No inhibition	No inhibition	0.002 ± 0.001
Proteus mirabilis (clinical isolate)	No inhibition	No inhibition	0.005 ± 0.002
C. albicans ATCC 90028	No inhibition	No inhibition	0.004 ± 0.001
C. glabrata RSKK 04019	No inhibition	No inhibition	0.004 ± 0.003

RD: Reference drugs (streptomycin for bacteria, fluconazole for fungi). Values are mean± standard deviation of six experiments in replicate.

(Agbabiaka et al., 2008). *P. sidoides* does not grow in Turkey. However, *P. endlicherianum* is among the species of *Pelargonium* growing in Turkey (Sezik et al., 2001). In this study, we investigated the antimicrobial activities of *P. endlicherianum* because of its similarities to *P. sidoides*.

It had been shown that aqueous alcohol is a better extraction solvent for plant than water to extraction phenolic compounds (Ahmad et al., 1998). Standardized extract of *P. sidoides*, commonly known as EPs 7630, is produced by the extraction of milled roots with 11% (w/w) aqueous ethanol (Schoetz et al., 2008). However, in this study, we also used 11% ethanol extract and 70% methanol extract which is a standard method (Naczk and Shahidi, 2004).

The phenolic compounds often cited as antimicrobial and their antimicrobial effects may be associated with the presence of phenols in plant extracts as had been shown similarly in other studies (Cowan et al., 1999). In this study, analysis of these extracts showed the presence of phenols and the methanol extract of *P. endlicherianum* displayed a higher antimicrobial activity against some microorganisms. The reason for this activity could be due to the presence of large amounts of phenolic compounds in 70% metanol extract *P. endlicherianum.* Kayser et al. reported the antibacterial activity of *P. sidoides* against microorganisms such as *S. aureus*, *S. pneumoniae*, *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *H. influenza,* and the MICs were found to be between 200 to 2000 µg/ml (Kayser et al., 1997). The dried roots of *P. reniforme* and *P. sidoides* have been Taylor, 2004). Pepeljnjak et al. (2005) demonstrated the antimicrobial activity of *P. radula.* Until now, no detailed research regarding *P. endlicherianum* had been conducted (Pepeljnjak et al., 2005). We find only one study on the antimicrobial activity of *P. endlicherianum*. Cakal et al. (2009) showed that the antimicrobial activities of the 11% ethanol and 70% methanol extracts of *P. endlicherianum* were effective against some microorganisms such as *E. coli, S. aureus, P. aeruginosa, S. epidermidis*. In our study, antimicrobial activity of *P. endlicherianum* extracts against *E. coli, S. aureus, P. aeruginosa, S. epidermidis*, *S. pyogenes, S. agalactia, S. pneumoniae, S. mutans, S. sanguinis, S. caprae* and *L. acidophilus* was found. These microorganisms are medically important and responsible for human illness. This is the first report showing the antimicrobial efficacy of *P. endlicherianum* against bacteria such as *S. pyogenes, S. agalactia, S. pneumoniae, S. mutans, S. sanguinis, S. caprae* and *L. acidophilus*.

The results of this study suggest that *P. endlicherianum* extracts possess anti-bacterial activities against some of the tested microorganisms which are significant pathogens in humans. *P. endlicherianum* may to be a potential source of antimicrobial compounds which could be used to treat infections caused by these bacteria.

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REFERENCES

Agbabiaka TB, Guo R, Ernst E (2008). *Pelargonium sidoides* for acute bronchitis: A systematic review and meta-analysis. Phytomedicine, 15: 378-385.

Ahmad I, Mehmood Z, Mohammad F (1998). Screening of some Indian medicinal plants for their antimicrobial properties. J Ethanopharmacol., 62: 183-193.

Bachert C, Schapowal A, Funk P, Kieser M (2009). Treatment of acute rhinosinusitis with the preparation from *Pelargonium sidoides* EPs 7630: A randomized, double-blind, placebo-controlled trial. Rhinology, 47: 51-58.

Bereznoy VV, Riley DS, Wassmer G, Heger M (2003). Efficacy of extract of *Pelargonium sidoides* in children with acute non-group A beta-hemolytic *Streptococcus tonsillopharyngitis*: A randomized, double-blind, placebo-controlled trial. Altern. Ther. Health Med., 9: 68-79.

Brendler T, van Wyk BE (2008). A historical, scientific and commercial perspective on the medicinal use of *Pelargonium sidoides* (Geraniaceae). J. Ethnopharmacol., 119: 420-433.

Bozan B, Ozek T, Kurkcuoglu M, Kirimer N, Can Baser KH (1999). The analysis of essential oil and headspace volatiles of the flowers of *P. endlicherianum* used as an anthelmintic in folk medicine. Planta Med., 65: 781-782.

Clinical and Laboratory Standards Institute (2006). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard, 7th ed. CLSI document M7-A7, Wayne, PA, USA.

Conrad A, Frank U (2008). Extract of *Pelargonium sidoides* (EPs 7630) displays anti-infective properties by enhanced phagocytosis and differential modulation of host-bacteria interactions. Planta Med., 74: 682-685.

Conrad A, Hansmann C, Engels I, Daschner FD, Frank U (2007). Extract of *Pelargonium sidoides* (EPs 7630) improves phagocytosis, oxidative burst, and intracellular killing of human peripheral blood phagocytes *in vitro*. Phytomedicine, 6: 46-51.

Cowan MM (1999). Plant Products as Antimicrobial Agents. Clin. Microbiol. Rev., 12: 564–582.

Cakal S (2009). Research into comparative investigation of *Pelargonium endlicherianum* Fenzl. (Geraniaceae) root extracts with Umca® preparation. Master's thesis, Anadolu University. Health Sciences Institute.

Davis PH (1967). *Pelargonium* L'Hérit. In: Flora of Turkey and the East Aegean Islands, PH Davis (Eds.), University Press, Edinburgh, UK, pp. 487-489.

Janecki AJ, Kiderlen AF, Kolodziej H (2007). NO-, TNF-, and IL-12-inducing Activity of Fractions of EPs® 7630. 55th International Congress and Annual Meeting of the Society for Medicinal Plant Research, Abstracts. Planta Medica, p. 73.

Kayser O, Kolodziej H, Kiderlen AF (2001). Immunomodulatory Principles of *Pelargonium sidoides*. Phytother. Res., 15: 122-126.

Kolodziej H, Kayser O, Radtke OA, Kiderlen AF, Koch E (2003). Pharmacological profile of extracts of *Pelargonium sidoides* and their constituents. Phytomedicine, 4: 18-24.

Kayser O, Masihi KNI, Kiderlen AF (2003). Natural Products and Synthetic Compounds as Immunomodulators. Expert Rev. Anti Infect. Ther., 1: 319-335.

Kayser O, Kolodziej H (1997). Antibacterial activity of extracts and constituents of *Pelargonium sidoides* and *Pelargonium reniforme*. Planta Med., 63: 508-510.

Kim CE, Griffiths WJ, Taylor PW (2009). Components derived from *Pelargonium* stimulate macrophage killing of Mycobacterium species. J. Appl. Microbiol., 106: 1184-1193.

Kolodziej H (2008). Aqueous ethanolic extract of the roots of *Pelargonium sidoides*-new scientific evidence for an old anti-infective phytopharmaceutical. Planta Med., 74: 661-666.

Lewu FB, Grierson DS, Afolayan AJ (2006). Extracts from *Pelargonium sidoides* inhibit the growth of bacteria and fungi. Pharmaceut. Biol., 44: 279-282.

Lis-Balchin M, Buchbauer G, Ribisch K, Wenger MT (1998). Comparative antibacterial effects of novel *Pelargonium* essential oils and solvent extracts. Lett. Appl. Microbiol., 27: 135-141.

Naczk M, Shahidi F (2004). Extraction and analysis of phenolics in food. J. Chromatogr. A., 1054: 95-111.

Neugebauer P, Mickenhagen A, Siefer O, Walger M (2005). A new aproach to pharmacological effects on ciliary beat frequency in cell culture-exemplary measurements under *Pelargonium sidoides* extract (EPs® 7630). Phytomedicine, 12: 46-51.

Pepeljnjak S, Kalodera Z, Zovko M (2005). Antimicrobial activity of flavonoids from *Pelargonium radula* (Cav.) L'Hérit. Acta Pharm., 55: 431-435.

Schoetz K, Erdelmeier C, Germer S, Hauer H (2008). A detailed view on the constituents of EPs 7630. Planta Med., 74: 667-674.

Seidel V, Taylor PW (2004). *In vitro* activity of extracts and constituents of *Pelargonium* against rapidly growing mycobacteria. Int. J. Antimicrob. Agents, 23: 613-619.

Sezik E, Yeşilada E, Honda G, Takaishi Y, Takeda Y, Tanaka T (2001). Traditional Medicine in Turkey X. Folk Medicine in Central Anatolia. J. Ethnopharmacol., 75: 95-115.

Singleton VL, Orthofer R, Lamuela-Ravento´s RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In Methods in Enzymology; Packer, L., Ed.; Academic Press: San Diego, CA, 299: 152-315.