Original Research

DOI: doi.org/10.47363/AMR/2016(4)142

In vitro antimicrobial potential of *potentilla polyphylla* Wall. ex Lehm. taproot extract against human pathogens

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Received: January 14, 2016
Accepted: March 22, 2016
Published: March 30, 2016

ABSTRACT

Background: The whole plant of *Potentilla polyphylla* Wall. ex Lehm. (Rosaceae) is valued for its ethno-medicinal properties. It has been used as a folk remedy against a variety of ailments. **Aim:** This study aimed to assess the antimicrobial potential of *P. polyphylla* taproot extract against human pathogens. **Methods:** The antimicrobial activity of the aqueous methanol extract (1:4) against bacterial strains and clinically important yeast were assessed using the agar diffusion assay and minimum inhibitory concentration studies. Its effect was compared with some standard antibiotics. **Results:** The extract showed inhibition of 17.5-30.0 mm and minimum inhibitory concentrations of 0.01 mg/ml and 0.4 mg/ml against *Streptococcus mutans* and *Candida albicans*, respectively. Tested pathogens, except *P. mirabilis,* were found susceptible to standard antibiotics. **Conclusions:** Results showed that *P. polyphylla* possesses broad-spectrum antimicrobial properties and hence merits detailed phytochemical investigations for its pharmaceutical applications.

KEY WORDS: *Potentilla polyphylla;* Antimicrobial activity; *Streptococcus mutans; Candida albicans*

INTRODUCTION

The use of medicinal plants has a long history throughout the world. The use of herbal preparations, in various forms, including extracts, is found in pharmacopoeia of many countries [1]. At least one third of the herbal remedies are for skin and wounds. However, only limited percentage (1-3%) of drugs listed in the Western pharmacopoeia are intended for use on the skin and wounds [2]. Many traditional practitioners across the world, particularly in countries like India and China with age old traditional practices, have valuable information of many lesser-known hitherto unknown wild plants used by the traditional healers for treating cuts, wounds and burns. The claims of some of these plants have been validated scientifically, however, most of which remain unexplored.

Microorganisms such as *Escherichia coli*, *Staphyllococcus aureus*, *Streptococcus mutans*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella enterica serovar typhii* and *Candida albicans* were reported to cause wound infections and also diseases like diarrhoea, typhoid, etc. Therefore, the need arise to screen plants which may have effect on these organisms, which will impact the effective use of medicinal plants against the diseases caused by the mentioned pathogens. Further, an increasing acceptance of traditional/herbal medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics have led researchers to investigate the antimicrobial activity of herbal extracts.

Potentilla species have been used for a long time in traditional medicines. Fuchs [3] mentioned five Potentilla species in his 'New Kreiiterbuch" comprising *Potentilla alba* L., *Potentilla retans* L., *Potentilla neumanniana* RCHB, *Potentilla anerium* L. and *Potentilla erecta*. Extracts prepared with water, milk, honey and alcohol were used for the treatment of toothache, throat inflammations, wound healing, jaundice, mouth ulcers, dysentery and as a homeostatic. Moreover, as many as three hundred species of the genus *Potentilla* Linn. are used in Ayurvedic, Unani, Siddha, Chinese and Tibetan systems of medicines [4,5,6,7], due to high content of polyphenols in their aerial and underground parts. Polyphenol rich plants are gaining significance in maintaining good health [8] due to their antioxidant and radical scavenging capacities [9]. These polyphenols form stable complexes with metal ions, protein and polysaccharides. Polyphenols help in healing of wounds, burns, inflammations, membrane-stabilizing and radical-antagonizing actions and thereby protect the underlying mucosa from toxins and irritants, control dental caries and ameliorate degenerative diseases. Medicinally important chemical constituents such as polyphenols, tannins, flavonoids and triterpenoids are already reported in the genus. *P. polyphylla* Wall. ex Lehm., one of the species of the genus *Potentilla*, is found at an altitude of ~1800 meters above sea level. It is found in the northeast region of India. Ethnic tribes in this region, such as Poumai, Mao, Kuki, etc., in Senapati District of Manipur, use the taproot of this herb for the treatment of burns and wounds. Chopped piece of the taproot is chewed as such in case of dental, diabetic and gastritis problems. In view of this, the present investigation was designed to evaluate its antimicrobial activity against potential wound, dental caries and other pathogens which may pave the way in

finding the active principles responsible for the presence of its efficacy.

MATERIALS AND METHODS

Chemicals

Amphotericin, cefuroxime, fluconazole, gentamicin, nutrient agar (NA), Sabouraud dextrose agar (SDA), Sabouraud dextrose broth (SDB) and Muller Hinton broth were obtained from Himedia, Mumbai, India. Other reagents and solvents of analytical grade were purchased from Himedia and Merck, Mumbai, India.

Plant material

Fresh plants of *P. polyphylla* Wall. ex Lehm. were bought from Iewduh (Bara Bazar), Shillong, Meghalaya, India during October, 2012. Voucher specimen was identified with the help of Botanical Survey of India, Shillong (No. BSI/ERC/2010/Plant identification/477).

Preparation of extract

Aqueous methanol (1:4) was used as solvent for extraction. Taproot of *P. polyphylla* was washed thoroughly with tap water, rinsed with distilled water, shade-dried and powdered [10]. Briefly, about 50 g of ground sample was soaked in the solvent for 48 h with intermittent shaking. The extract was filtered using Whatman No.1 filter paper and the collected filtrate was concentrated under reduced pressure in a rotary vacuum evaporator (RV10 Control, IKA, Germany). Concentrated extract was air-dried to a constant weight at room temperature and stored at -20°C till further use.

Test concentration

10% (w/v) extract solution was prepared by dissolving the extract in an equivalent amount of DMSO (dimethyl sulphoxide) that gives 1% of the final solution. The solution was sterilized by filtering through a Millipore (0.2 µm) filter and stored. Test solutions of different concentrations were prepared from stock solution. 100 µl each per well were loaded for the activity determination.

In vitro antimicrobial activity

Microorganisms used

Seven pathogenic strains of bacteria and one fungal strain obtained from the Institute of Microbial Technology, Chandigarh, India were used to assess the antimicrobial property of the extract. The bacterial strains include: *Staphylococcus aureus* MTCC338, *Streptococcus mutans* MTCC497, *Escherichia coli* MTCC40, *Klebsiella pneumoniae* MTCC109, *Proteus mirabilis MTCC743*, *Pseudomonas aeruginosa* MTCC741, *Salmonella enterica serovar typhii* MTCC733 and yeast, *Candida albicans* MTCC854. Microbial strains preserved in nutrient agar at 4°C, were revivified in nutrient broth solution and incubated at 37 ± 1°C for 18–24 h. Nutrient agar (NA) and Potato dextrose agar (PDA) were used for antibacterial and antifungal activity determination. Antibacterial standards used were Gentamicin and Cefuroxine. Amphotercin and Fluconazole were used as antifungal standards.

Determination of antimicrobial activity and minimum inhibitory concentrations

The antimicrobial activity of the extract was determined by measuring the diameter of the zone of inhibition (ZI) in mm around the well according to Indian Pharmacopeia Commission, 2007[11]. Percentage inhibition was calculated according to Vyas et al. [12] and tabulated in Table 1 , where the control growth is 80 mm (diameter of the petriplate used). Culture broth and 1% DMSO (100 µl each) were inoculated into agar wells as a negative control. The susceptibility of the test pathogenic microorganisms to known antibiotics was tested as a positive control. Agar dilution method was used to determine the minimum inhibitory concentrations (MICs). The experiments were performed in triplicate.

Table 1. Antimicrobial activity of *P. polyphylla* taproot extract against test human pathogens

Microorganism	ZI (mm)	Percent Inhibition	MIC (mg/ml)
Staphyllococcus aureus	17.5	21.93	5
Streptococcus mutans	30	37.50	< 0.05
Escherichia coli	23	28.75	10
Klebsiella pneumonia	NA		
Proteus mirabilis	NA		
Pseudomonas aeruginosa	NA		
Salmonella enterica serovar typhii	NA		
Candida albicans	20	25	< 0.5

Values represent an average of triplicate, NA: No activity

RESULTS

Eight human pathogens have been investigated in the present study. The antimicrobial activity of *P. polyphylla* is represented in Table 1. Susceptibility profile of test pathogenic microorganisms against known antibiotics is presented in Table 2. TThe diameter of the zone of inhibition are presented in mm and minimum of inhibition in mg/ml.

DISCUSSION

Bioactive components present in a plant are responsible for protecting the plant against microbial infections or infestations by pests or stress. Preliminary chemical analysis showed the presence of flavonoids and tannins in high amount while saponins, steroids and terpenoids are in moderate amount [data not given]. However,

Table 2. Susceptibility profile of test human pathogenic microorganisms against known antibiotics

Antibiotics (µg)	Diameter of zone of inhibition in mm						
	Bacteria						
	Gram (+)		Gram (-)				
	S. aureus	*S.mutans*	*E. coli*	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*	*P. enteriaca serovar typhii*
Gentamicin (10)	22.56	33.56	35.45	18.03	30.90	31.23	33. 07
Cefuroxime (30)	20.67	40.45	30.09	18.98	R	18.27	19.07
				Yeast			
				C. albicans			
Amphotericin (30)				13.00			
Fluconazole (10)				35.78			

Values are represented as mean of 3 replicates. R: resistant

alkaloids were not detected in the investigation [13]. In general, phenolics are predominant class of secondary metabolites in plants [14] and natural antioxidant mainly comes from this class of phenolic compounds such as flavonoid, phenolic acids, tocopherols, etc. [15]. Therefore, exhibited antioxidant in the extract of *P. polyphylla* could be due to the presence of flavonoids and tannins as reported [13]. Moreover, gram-positive bacterial strains are most sensitive to this class of compound [16]. Thus, the presence of which could be, at least partly, responsible for the measured antibacterial activity against *S. mutans*, *S. aureus*, *E. coli and C. albicans*. *S. mutans* was found most susceptible to extract with the highest ZI of 30 mm, amongst the test pathogens, which was comparable to Gentamicin. However, Cefuroxime exhibits stronger activity against *S. mutans*. Susceptibility of *S. aureus* to extract, Gentamicin and Cefurocime was comparable. Extract showed activity against *E.coli*, though less effective than the antibiotics. Extract was found highly active against *C. albicans* with ZI of 20 mm which was higher than Amphotericin with ZI 13 mm. Moreover, *C. albicans* is a clinically important pathogen and therefore this observation stands out amongst other observations. Gram negative bacteria such as *K. pneumonia*, *P. mirabilis*, *P. aeruginosa* and *S. enterica serovar typhii* were not susceptible to the extract under investigation. Except *P. mirabilis*, all the test pathogens were found susceptible to test antibiotics. Flavonoids and hydroxylated phenolics are known to be synthesized by plants in response to microbial infections. Therefore they are attributed with broad spectrum antimicrobial attributes *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall [17]. Tannins act as growth inhibitors towards many microorganisms including bacteria, yeasts and fungi by inhibiting the transport of nutrients into the cell and retard the growth of the organism. Tannins–protein or polysaccharide complexed formed are often irreversible, and this characteristic confers bactericidal and bacteriostatic properties. Terpenoids are reported to be antioxidant [18], antimicrobial [19], anti-inflammation [20], etc. Steroids have been reported to have antibacterial properties [21]. Antimicrobial activities of plants have been attributed to the presence of bioactive compounds [12, 13] and mechanism of action is generally considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport and coagulation of cell contents [22]. Therefore, it is possible that the phytochemicals detected in the extract exert their effects via some of the mechanisms mentioned above.

In conclusion, this study revealed that, *P. polyphylla* possesses broad-spectrum antimicrobial properties which could be effectively used against diseases which are caused by those susceptible test organisms. However, further studies should be carried out to identify and characterize the antimicrobial agent(s) present in the plant which can further be explored for possible pharmaceutical leads for pharmaceutical applications.

ACKNOWLEDGEMENT

Authors are grateful to Defence Research and Development Organisation, New Delhi for financial support.

REFERENCES

1. Hostettmann K, Marston A, Maillard M, Hamburger M. Phytochemistry of plants used in traditional medicines. Clarendon Press, Oxford (1995).
2. Balick M, Cox PA. Plants, People and Culture: the Science of Ethnobotany Scientific American Library, WH Freeman and Company, New York 1996.
3. Fuchs L, New Kreüterbuch, Baesel. Kapitel 98. Von Tormenill, 1543.
4. Delgado L, Galledo F, Rico E. Karyosystematic study of Potentilla L., subgenus Potentilla (Rosaceae) in the Iberian Peninsula. Bot J Linn Soc 2000; 132(3): 263-280.
5. Xue PF, Luo G, Zeng WZ, Zhao YY, Liang H. Secondary metabolites from *Potentilla multifida* Linn (Rosaceae). Biochem Syst Ecol 2005; 33 (7): 725-728.
6. Xue PF, Zhao YY, Wang B, Liang H. Secondary metabolites from *Potentilla discolor* Bunge (Rosaceae). Biochem Syst Ecol 2006; 34 (11): 825-828.

7. Zhao YL, Cai GM, Hong X, Shan LM, Xio XH. Antihepatitie B virus activities of triterpenoid saponin compound from *Potentilla anserine* L. Phytomedicine 2008; 15 (4): 253-258.
8. Scalbert A, Johnson IT, Saltmarsh M. Polyphenols: antioxidant and beyond. Am J Clin Nutr 2005; 81 (1): 2155-2175.
9. Haslam E. Natural phenols (Vegetative Tannins) as drugs: Possible mode of action J Nat Product 1996; 59 (2): 205- 215.
10. Harborne JB. *Phytochemical Methods*. 3rd edn. (Chapman and Hall, London), 1988, 7.
11. Gov. of India, Ministry of Health and Family Welfare, Indian Pharmacopeia Commission. Pharmacopoeia*,* Vol. 1, The Controller of Publication, New Delhi, 2007.
12. Vyas YK, Bhatnagar M, Sharma K. Antimicrobial activity of a herb, herbal based and synthetic dentrifices against oral microflora. J Cell and Tissue Res 2006; 6 (1): 639-642.
13. Nakhuru KS, Gogoi J, Pfoze NL, Chattopadhyay P, Veer V. Comparative Studies on Phytoconstituents, Total Phenolic Content and Free Radical Scavenging Potential of Some of the Traditionally Used Medicinal plants of North East India. Int. J. Pharm. Sci. Rev. Res., 2014, 29(1): 166-170.
14. Singh R, Singh SK, Arora S. Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis* A. Conn. Food Chem Toxicol 2007; 45: 1216-1223.
15. Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahuand A, Bora U. Indian medicinal herbs as a source of antioxidants. Food Res Int 2008; 41: 1-15.
16. Ríos JL, Recio MC. Medicinal plants and antimicrobial activity. J Ethnopharmacol 2005; 22:100 (1-2): 80-4.
17. Marjorie C. Plant products as antimicrobial agents. Clin Microbiol Rev 1996; 12: 564-582.
18. Grassmann J. Terpenoids as plant antioxidant. Vitam Horm 2005; 72: 505-535.
19. Milena P Popova, Ioanna B Chinou, Ilko N Marekov, Vassya S Bankova. Terpenes with antimicrobial activity from Cretan Propolis. Phytochem 2009; 70 (10): 1262–1271.
20. Navarro A, de las Heras B, Villar AM. Andalusol, a diterpenoid with anti-inflammatory activity from Siderits foetens Clemen. Z Naturforsch C 1997; 52: 844-849.
21. Raquel FE. Bacterial lipid composition and antimicrobial efficacy of cationic steroid compounds. Biochim Biophys Acta 2007; 1768(10): 2500-2509.
22. Kotzekidou P, Giannakidis P, Boulamatsis A. Antimicrobial activity of some plant extracts and essential oils against food borne pathogens *in vitro* and on the fate of inoculated pathogens in chocolate. Food Sci Technol 2008; 41:119- 127.

Source of Support: Nil, Confl ict of Interest: None declared