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Original Article

Pharmacological investigation of leaves of *Polypodium decumanum* for anti-bacterial activity against gram-positive and gram-negative bacteria

Pankaj Singh Niranjan*[1], ChandrulKoushal[1] and S.K. Jain [2]

[1]Institute of Pharmacy, Shri Venkateshwara University, Gajroula, Uttar Pradesh, India
[2] Institute of Pharmacy, Bundelkhand University Jhansi, Uttar Pradesh, India

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ABSTRACT: The widespread use of antibiotics has led to an increase in multidrug-resistant bacteria, which represent a serious risk of infection, in view of this problem the aim of this research was performed to characterize the antibacterial effect of ethanolic and petroleum ether extract of *Polypodium decumanum* leaves against three gram-positive bacteria(*Staphylococcus aureus, Bacillus subtilius, and Streptococcus pyogenes*) and three gram-negative bacteria(*Pseudomonas aeruginosa, Escherichia coli, and Pseudomonas alcaligenes*) *in vitro* by disc-diffusion method. In this study, we have taken 3 concentrations of *Polypodium decumanum*plant extract (ethanolic and petroleum ether) viz. 50 mg/ml, 100 mg/ml, and 200 mg/ml, and observed that the ethanolic extract of *Polypodium decumanum*of the concentration of 200 mg/ml showed higher antibacterial effect compare to other concentration against all 3 gram-positive bacteria and all 3 gram-negative bacteria, but not higher than standard drug Ampicillin.

⇑ Corresponding author at:
Pankaj Singh Niranjan, Institute of Pharmacy, Shri Venkateshwara University, Gajroula, Uttar Pradesh, India
E-mail address:niranjanpankaj@yahoo.co.in

INTRODUCTION

Medicinal plants contain chemical substances and have been reported to possess many useful properties including anti-inflammatory activity, oestrogenic activity, enzyme inhibition, antimicrobial activity[1,2,], anti-allergic activity, antioxidant activity vascular activity and cytotoxic anti-tumor activity[3]. These plants can serve as a possible source for new antimicrobial to which pathogen strain are not resistant[4,5]. Traditional medicine is an important source of product for developing countries in treating common infection bacteria. The emergence of multiple drug resistant infectious bacteria, high cost of synthetic compounds as well as undesirable side effect of certain drugs insists on pharmaceutical companies to look for new therapeutic agents from other alternative sources including medicinal plants[6].

Recently there has been a renewed interest in improving health and fitness using more natural products. Herbs and spices are an important part of the human diet. Indian subcontinent is a rich territory of medicinal plants which is use in medicinal treatment and forms a rich source of knowledge[7,8]. The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plants to treat different ailments[9].

Polypodium is a genus of between 75-100 species of true ferns, widely distributed throughout the world, with the highest species diversity in the tropics. Polypodies have use in herbalism, but are today most important in horticulture where several species, hybrids, and their cultivars like *Polypodium*. Green Waves are commonly used as ornamental plant for shady location.

The Polypody family contains 3 quarters of all ferns over 6,000 species of plants, mostly native to the tropics of both hemispheres. There are 75 species of plants in the *Polypodium* genus, many of which have been used medicinally for centuries. *Polypodium*has the ability to regulate and support the growth of various cells in the body including the skin and brain. It's most commonly used in traditional medical systems to treat skin disorders and upper respiratory infections (especially those with a cough), however, newer research has discovered an even more useful ability. That is to protect the brain cells from damage. Other uses for *Polypodium* includes treatment for vitiligo, kidney disease, cleansing the blood, an addition to detox formulations, improving and modulating the immune system, arthritis, skin disorders, and cancer.

Poplypodium contains flavonoids, alkaloids and lipids[10]. It is a rich source of lipids and fatty acids. The main plant chemicals identified in *Poplypodium* includes adenosine, alkaloids, arachidonic acid, arabinopyranosides, calagualine, ecdysone, ecdysterone, eicosapentaenoic acid, elaidic acid, juglanin, kaempferols, linoleic acids, linolenic acids, melilotoside, oleic acid, ferulic acid, polypodaureine, ricinoleic acid, rutin, selligueain, and sulphoquinovosyldiacylglycerols[11].

Many of existing synthetic drugs cause various side effects. Hence, drug development from plant based compounds could be useful in meeting this demand for newer drugs with minimal side effect. Antibacterial active principles isolated from higher plants appear to be one of the most important alternative approaches to contain antibiotic resistance and the management of diseases[12]. Plant metabolites appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to the synthetic pesticides.

The aim of this research was to identify the antibacterial effect of the *Polypodium decumanum*plant extract obtained from the leaves of the plant.

MATERIALS AND METHODS

The *Polypodium decumanum* used in this study was procured from local market in Indore and authenticated at College of Agriculture, ICAR division Indore.

Preparation of Extract

In the continuous hot extraction method, the plant leaves extracted in Ethanol in 3 regular days at the temperature of 78- 80^{o}C. The mixture was subsequently filtered and concentrated under reduced pressure at 40^{o}C. The extract yield was 26% w/w.

Chemicals

All the chemicals and reagents used were from CDH, New Delhi. Glass wares used were from borosil. The media and broth used for chemical used for microbial culture were from MTCC, Chandigarh.

Culture media and Strain

The media used for antibacterial test was nutrient agar media of HI media Pvt. Bombay, India and potato dextrose agar/broth of HI media Pvt. Bombay, India. Pathological strains i.e. *Staphylococcus aureus* (MTCC 389), *Bacillus subtilus*(MTCC 121), *Streptococcus pyogenes* (MTCC 1924), *Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* (MTCC 390), *and Pseudomonas alcaligenes* (MTCC 493) were tested for antibacterial activity. These cultures of strain were collected form Microbial Tissue Culture Collection (MTCC) Chandigarh, India.

Disc-Diffusion method

The antibacterial activity of the extract was determined by disc diffusion method (NCCLS, 1997) in petri disc[13]. The paper discs (6 mm in diameter) were separately impregnated with 15 µL of extract placed on the agar which had previously been inoculated with the selected test microorganism.

Ampicillin was used as a positive reference for bacteria. Discs without samples were used as a negative control. The petri discs were kept in the refrigerator at 4^{o}C for 45 minutes for diffusion to take place. The plates were incubated at 37^{o}C for 24 h for bacteria. Antibacterial activity was assessed by measuring the diameter of the growth-inhibition zone in millimeters (including disc diameter of 6 mm) for the test organism comparing to the control[14,15].

Determination of Antibacterial activity

The extracts were individually tested against a panel of microorganism selected. Bacterial strains were cultured overnight at 37^{o}C in nutrient agar (NA)[16,17]. The paper disc (6 mm in diameter) was separately impregnated with 15 µL extract placed on the agar which had previously been inoculated with the selected test microorganism. The diameters of zone of inhibition observed are measured[18,19].

Determination of MIC

The antibacterial plant extract then after evaluated to determine MIC value. The broth dilution method was adopted by using N-saline for diluting the plant extract and was incubated for 48h. The minimum dilution of the plant extract that inhibits the growth of the organism was taken as MIC[20,21,22].

RESULTS AND DISCUSSION

The antibacterial activity observed by measuring the diameter of zone of inhibition recorded. The different extract of the plant *Polypodium decumanum*were found to have significant antibacterial activity. The results obtain in the evaluation of the antibacterial activity of the plant extract against gram positive bacteria *Staphylococcus aureus, Bacillus subtilus, Streptococcus pyogenes* and gram-negative bacteria *Pseudomonas aeruginosa, Escherichia coli, Pseudomonas alcaligenes*are shown in Table1.

Table 1: Antibacterial activity of *Polypodium decumanum* plant extract

S.No.	Extract used/standard drug	Conc.(mg/ml)	Zone of Inhibition (mm)					
			Gram positive bacteria			Gram negative bacteria		
			S. aureus	*B.subtilus*	*S. pyogenes*	*P. aeruginosa*	*E. coli*	*P.alcaligenes*
1	Ethanolic extract	50	4.5±0.15	5.7±0.11	3.5±0.11	1.1±0.05	0.9±0.05	NIL
2	Ethanolic extract	100	11.2±0.17	12.9±0.13	10.6±0.18	9.1±0.05	8.0±0.05	7.0±0.05
3	Ethanolic extract	200	16.7±0.17	15.6±0.12	13.7±0.18	11.5±0.12	12.1±0.15	10.2±0.15
4	Pet. ether extract	50	7.3±0.12	9.4±0.12	6.8±0.15	6.0±0.05	7.2±0.05	NIL
5	Pet. ether extract	100	9.4±0.12	11.7±0.15	8.0±0.15	7.1±0.01	8.2±0.01	3.2±0.01
6	Pet. ether extract	200	11.7±0.17	13.1±0.19	10.7±0.12	8.0±0.01	9.3±0.01	5.3±0.01
7	Ampicillin	20mg/10ml	23±0.00	24.7±0.00	20.9±0.00	18.7±0.00	19.5±0.00	17.5±0.00
8	Control		0.2	0.3	0.1	NIL	NIL	NIL

All values represent Mean ± SEM, n = 3 in each group, values are significantly differ from reference standard (Ampicillin), * $p < 0.05$, $^{**}p<0.01$

In this study, we found that the ethanolic extract of *Polypodium decumanum*at concentration of 200 mg ml^{-1} showing higher effect against all gram-positive bacteria as well as gram negative bacteria. We also found that the effect of plant extract of *Polypodium decumanum*(ethanolic and petroleum ether) showing higher antibacterial effect against gram positive bacteria i.e. *Staphylococcus aureus, Bacillus subtilus,* and*Streptococcus pyogenes* compare to gram negative bacteria *Pseudomonas aeruginosa, Escherichia coli, and Pseudomonas alcaligenes.*

CONCLUSION

It is reported that *Polypodium decumanum*contain phenol, alkaloids, flavonoids, rutin, calagualine etc., So based on the constituent present in the *Polypodium decumanum* it can be concluded that the antibacterial activity of the plant is due to the presence of these chemical constituent in the plant. We can also say that ethanolic extract of *Polypodium decumanum* showed a significant antibacterial effect against all 3 gram positive bacteria and 3 gram negative bacteria based on the zone of inhibition it. When compared with the antibacterial effect of standard drug Ampicillin, the plant extract showed only 20% lesser effect.

REFERENCES

1. Berahou A, Auhmani A, Fdil N, Benharref A, Jana M, and Gadhi CA, Antibacterial activity of *Quercus ilex* bark's extracts, *Journal of Ethnopharmacology*,2007;112(3):426-429.[View in PubMed]
2. Akrayi HFS: Antibacterial Effect of Seed Extract of Cardamom (*Elettaria cardamom*) against *Staphylococcus aureus* and *Proteus mirabilis. Tikrit J. Pure Sci*, 2012;17(2):14-18.
3. Alzoreky NS, Nakahara K. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. *Int J Food Microbiol*. 2003; 80:223–30.[View in PubMed]
4. Thiem B and Goślińska O, Antimicrobial activity of *Rubuschamaemorus* leaves, *Fitoterapia*,2007;75(1):93-95.[View in PubMed]
5. Jones FA. Herbs as useful plants: Their role in history and today, *European Journal of Gastroenterology & Hepatology*, 1996;8(12): 1227-31.
6. Hammer KA, Carson CF, Riley TV. *Antimicrobial activity of essential oils and other plant extracts. J ApplMicrobiol*. 1999;86(6):985–990.
7. Harborne J B, Baxter H. *The handbook of natural flavonoids*, Vol. 1 and 2. Chichester, UK: John Wiley and Sons; 1999.
8. Kumar N, Kshrisagar M D, Saini V, GC-MS Analysis of *Polypodium decumanum*" *International Research Journal of Pharmacy*,2011; 2(9):155-156.
9. Lino A, Deogracious O. The *in-vitro* antibacterial activity of *Annona senegalensis*, *Securidaccalongipendiculata*and *Steganotaeniaaraliaceae - Ugandan medicinal plants. Afr Health Sci*. 2006;6:31–35.
10. Cowan MM. Plant products as antimicrobial agents, *Clinical Microbiology Reviews*, 1999;12(4):564-82.[View in Pubmed]
11. Marjorie MC. Plant products as antimicrobial agents, *Clin. Microbiol Rev*, 1999; 12(4): 564-582.[View in PubMed]
12. Lee MH, Kwon HA, Kwon DY*et al*., Antibacterial activity of medicinal herb extracts against *Salmonella*, *International Journal of Food Microbiology*,2006;111(3):270-75.
13. NCCLS (National Committee for Clinical Laboratory Standards), "Performance standards for antimicrobial disc susceptibility test" 1997, 6th Ed. Approved Standard. M2-A6.
14. Pathak P,SarsawathyD,Vora A, etal, In vitro antimicrobial activity and phytochemical analysis of the leaves of *Annona muricata*. *Int J Pharma Res* Dev.2010; 2:1-6.
15. Prashanth KV, Chauhan NS, Padh H, Rajani M. Search for antibacterial antifungal agents from selected Indian medicinal plants. *J Ethnopharmacol*. 2006; 107:182–83.

16. Ramasamy S, Charles MA. Antibacterial effect of volatile components of selected medicinal plants against human pathogens. *Asian J Microbial Biotech Env*, 2009;6:209–10.
17. S. Naz, R. Siddiqi, S. Ahmad, S. A. Rasool, and S. A. Sayeed, Antibacterial activity and isolation of compounds from *Punicagranatum*, *Journal of Food Science*,2007;72(9):341-45.
18. Satish S, RaghavendraM.P., RaveeshaK.A., Evaluation of the Antibacterial potential of some plants against human pathogenic bacteria, *Advance in Biological Research*, 2008,2:44-48.
19. Shaik D, Malika FA, Rafi SM, Naqui B. Studies of antibacterial activity of ethanolic extract from *Nericumindicum* and *Hibiscus rosasinensis*. *J Islamic Acad Sci*. 1994; 7:167–8.
20. Towers GH, Lopez A,Hudson JB, Antiviral and antimicrobial activities of medicinal plants. *J Ethnopharmacol*. 2001; 77:189-96.[View in PubMed]
21. Gopala V.Krishnakone and Samy RP. Current status of herbal and their future perspective, *Nature Proceedings*, 2007,1-13.
22. WHO, "Regulatory situation of herbal medicine: A World-Wide Review," World HealthOrganization, 1998, Geneva.

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