Journal of Basic and Clinical Pharmacy

www.jbclinpharm.com

Bioefficacy of methanolic root extract of *Piper longum* L. against isolated strains of Keratinophilic fungi

Kodagikere Palakshappa Prassanna[1], Raja Naika[1] and Pasura Subbaiah Sujan Ganapathy*[2,3]

[1]Department of P.G. Studies and Research in Applied Botany, Kuvempu University, Shankaraghatta, 577451, Karnataka, India.
[2]Department of P.G. Studies and Research in Biotechnology & Bioinformatics, Kuvempu University, Shankaraghatta 577451, Karnataka, India.
[3]Centre for Vrikshayurveda, A Division of Centre for Advanced Studies in Biosciences, Jain University, Chamrajpet- 560019, Karnataka, India.

ABSTRACT

A total of 9 species belonging to 3 genera of keratinophillic fungi were recovered from twelve soil samples collected from different sites in shivamogga using the hair-baiting technique. Most of the fungal species isolated are known to be agents of human and animal infection. The methanolic root extract of *Piper longum* was evaluated for antifungal activity against the isolated strains to determine the active. It was observed that the extract was effective in inhibiting species with zone of inhibition ranging between 3 mm and 11 mm but the extract showed no zone of inhibition for *Chrysosporium keratirophilum*. The results indicate that the methanolic root extract of *Piper longum* might be exploited as natural drug for the treatment of several infection caused by these organisms.

KEY WORDS: Keratinophilic fungi, soil, *Piper longum*, antifungal.

received on 14-04-2011
accepted on 13-05-2011
available online 15-11-2011
www.jbclinpharm.com

INTRODUCTION

Keratinophilic mycoflora grow and reproduce on keratin materials such as skin, hair, nail, fur, feather, horn, hoof, beak etc., they utilize keratin as carbon source [1]. Keratinophilic fungi are important ecologically and present in the environment with variable distribution patterns and cause human and animal mycoses [2] Most cutaneous infections are the work of homogeneous group of keratinophilic fungi known as dermatophytes [3]. The dermatophytes have the capacity to invade keratinized tissue of the body including skin, hair and nails [4].

Piper longum L. is an aromatic climber with stout roots, jointed stems, and ovate leaves belongs to the family Piperaceae, which is very sparsely distributed in forests of the Western Ghats, India [5]. In Indian system of medicine 'Ayurveda', the plant is popularly known as Pippali. The root have been used as stomachic, thermogenic, aphrodisiac, carminative, expectorant, laxative, digestive, emollient, antigiardias, antiamoebic, anti-asthmatic, antiseptic and also active against bacterial diseases [6-7]. The plant also finds folkloric usage in the treatment of constipation cardiac disease, piles, liver disorders, and urinary disorders [5].

The main objective was to report the prevalence of keratinophilic fungi in this region and to investigate the effect of methanolic root extract of *Piper longum* on these keratinophilic fungi.

MATERIALS AND METHODS

Plant Material:

The dried roots of *Piper longum* were collected from Somavarpet region of Coorg district, India. The plant material was authenticated by Dr. Raja Naika, Department of Applied Botany, Kuvempu University, Shankaraghatta (Voucher specimen number PL.AB.214).

Extraction:

Dried roots of *Piper longum* were powdered using a mechanical grinder. 100 g of dried root powder was soaked in 1000 ml of methanol (LR grade, Merck, India) and kept on a rotary shaker for 24 h. The extract was filtered under vacuum through a Whatman No. 1 filter paper and the process repeated until all soluble compounds has been extracted. Extraction was considered to be complete when the filtrate had a faint colour. The extracts were evaporated to dryness under reduced pressure using a Rotavapor (Buchi Flawil, Switzerland). A portion of the residue was used for the antifungal assay.

Collection of Keratinophilic fungi:

A total of 12 soil samples of 250 g were collected from different sites in Shivamogga city (garden soil, animal enclosures, hair dumping areas, chicken farms, bus stands and markets). The soil samples were examined for the presence of keratinophilic fungi employing Hair baiting technique [8].

For this purpose, sterile Petri-dishes were half-filled with the soil samples, moistened with sterile water and baited with sterile human hairs. The plates were wrapped in papers, incubated at 25°C, and examined over 8-10 weeks at periodic intervals for the development of fungal growth on the hairs. When growth occurred, baits of hairs were removed with sterile forceps, and fungi were cultured on Sabouraud's dextrose agar medium (SDA) supplemented with chloramphenicol 50 mg/l and streptomycin sulfate 500 mg/l. Petri dishes were incubated for 2 weeks at 28± 2°C and the developed colonies were identified by following keys proposed by Carmichael [9], Chabasse [10] along with other standard mycology manuals [11-13].

The isolates were maintained in cold (40 C) on sabouraud dextrose agar (SDA) slants until further use . The organisms were subcultured once in every fifteen days and the purity of the cultures was checked regularly under microscope.

Inoculum preparation:

Cultures were grown on sabouraud dextrose agar slants. Sterile saline solution (0.85%) was added to the slants and the culture was gently swabbed with a cotton-tipped applicator to dislodge conidia from the hyphal mat. The suspension was transferred to a sterile tube and the resulting suspension was used for the experiments.

Antifungal assay:

Antifungal activity of methanolic root extract of *Piper longum* was tested using agar well diffusion method. The extract was dissolved in 10% aqueous dimethylsulfoxide (DMSO) to a final concentration of 250, 500, 1000 µg/100µl. Pure DMSO was taken as the negative control.

*Corresponding Author E-mail: sujan.ganapathy@gmail.com

Table 1: Distribution of keratinophilic fungi in soils of public places

Sl. No.	Isolated species
01	Chrysosporium anum
02	Chrysosporium keratirophilum
03	Chrysosporium lobatum
04	Chrysosporium tropicum
05	Microsporum gypseum
06	Microsporum nanum
07	Trichophyton ajelloi
08	Trichophyton mentagrophytes
09	Trichophyton terrestre

200µl of inoculum was aseptically introduced on to the surface of sterile agar plates and sterilized cotton swabs were used for even distribution of the inoculum. A well of about 6.0mm diameter with sterile cork borer was aseptically punched on each agar plate. 100 µl of test and control compound was introduced in the well. The same procedure was used for all the strains. Plates were kept in laminar flow for 30 minutes for pre diffusion of extract to occur and then incubated at 37°C for 24 hours. Resulting zone of inhibition was measured using a scale.

RESULTS AND DISCUSSION

During this study, nine species of keratinophilic fungi belonging to three genera were isolated from twelve soil samples. The most dominant species (4 species) belong to the genus *chrysosporium*, followed by *Trichophyton* (3 species) and *Microsporum* (2 species) (Table 1). The distribution of these keratinophilic fungi among the different sites was not uniform, and this could be attributed to the difference in the organic matter content of the soil. It has been reported that the organic matter content of the soil is one of the major factors affecting the presence and distribution of the keratinophilic fungi in soils [14].

Among *Chrysosporium* species, *Chrysosporium keratinophilum* was isolated frequently from all places in Shivamogga city. The occurrence of *Chrysosporium keratinophilum* is considered noteworthy because of its tolerance to a wide range of temperatures [10, 15-20]. Among the other identified species, *Microsporum gypseum* and *Trichophyton mentagrophytes* are recognized to cause skin, hair and nails diseases in man and animals, and have worldwide distribution [21-23]. These fungi have survived several generations of therapeutic regimens; there is certainly no guarantee that they would not become resistant to the latest antifungals. Since multidrug resistance of microorganisms is a major medical concern, screening of natural products in a search for new antimicrobial agents that would be active against these microorganisms is the need of the hour [24].

In the present investigation, the antifungal activity of methanolic root extract of *Piper longum* was evaluated against 9 species of keratinophilic fungi. Data presented in Table 2 revealed that methanolic root extract of *P. longum* was effective in inhibiting eight species with zone of inhibition ranging between 0 mm and 11 mm but the extract showed no zone of inhibition for *Chrysosporium keratirophilum*. These observations may be attributed to two reasons; firstly, due to the nature of biologically active components (alkaloids, flavonoids, sterols, quinine, tannins etc.) which might be enhanced in the presence of methanol [25]. It has been documented that alkaloids, flavonoids and tannins are plants metabolites well known for their antimicrobial activity [26]. Secondly, the stronger extraction capacity of methanol could have produced a greater number of active constituents responsible for antimicrobial activity.

CONCLUSION

The present study reveals that the public places of shivamogga are rich in Keratinophilic fungi. The antifungal potential of methanolic root extract of *Piper longum*

Table 2: Antifungal activity of *Piper longum* methanolic root extract against test organisms.

Strains tested	Concentrations		
	250 µg	500 µg	1000 µg
Chrysosporium anum	0.00±0.00	3.33±0.18	4.87±0.52
Chrysosporium keratirophilum	0.00±0.00	0.00±0.00	0.00±0.00
Chrysosporium lobatum	4.33±0.07	4.60±0.12	6.80±0.12
Chrysosporium tropicum	5.73±0.27	7.33±0.67	11.00±0.00
Microsporum gypseum	4.33±0.33	6.60±0.12	9.13±0.24
Microsporum nanum	5.67±0.33	7.60±0.12	10.33±0.24
Trichophyton ajelloi	5.40±0.31	7.60±0.31	9.73±0.37
Trichophyton mentagrophytes	5.00±0.12	5.33±0.18	7.67±0.33
Trichophyton terrestre	4.20±0.12	6.40±0.12	8.53±0.24

The values are the mean of three experiments ± S.E.

against Keratinophilic species for which the problem of multidrug resistance may emerge thereby making them difficult to treat. Further *in vitro* and *in vivo* studies are required in order to prove the bioefficacy of the extract. The encouraging results indicate that this extract might be exploited as natural drug for the treatment of several diseases caused by these organisms and could be useful in understanding the relations between traditional cures and current medications.

REFERENCES

1. Cooke RC. Fungi Man and Environment. London: Longman Group Ltd.; 1980. p. 17-19.
2. Mohamed S. Ali-Shtayeh and Rana M.F. Jamous. Keratinophilic fungi and related dermatophytes in polluted soil water habitats. In: Biology of dermatophytes and other keratinophilic fungi. Kushawaha RKS, Guarro J, eds. Spain: Revista Iberoamericana de Micologia; 2000. p. 51-59.
3. Elewski BE. Onychomycosis, Pathogenosis, Diagnosis and Management. Clinical Microbiology Reviews. 1998; 2(3): 415-429.
4. Irene W, Summerbell RC. The Dermatophytes. Clinical Microbiology Reviews. 1995; 8(2): 240-259.
5. Yoganarasimhan SN. Medicinal plants of India. Bangalore, India: Interline publishers; 1996. p. 366.
6. Kirtikar KR, Basu BD. Indian Medicinal Plants. New Delhi, India: Periodical Expert Book Agency; 1984.
7. Warrier PK, Nambiar VPK, Raman KC. Indian Medicinal Plants. Madras, India: Orient Longman Ltd.; 1995.
8. Benedek T. Some historical remarks on the development of hair-baiting of Towa-Karling- Vandreusegheum (The To-Ka-Va hair-baiting method). Mycopathologia. 1962; 16: 104.
9. Carmichael JW. Chrysosporium and some other aleuriosporic hyphomycetes. Canadian Journal of Botany. 1962; 40: 113–173.
10. Chabasse D. Taxonomic study of keratinophilic fungi isolated from soil and some mammals in France. Mycopathologia. 1988; 101: 133–140.
11. Gilman JC. A Manual of Soil Fungi. USA: The Collegiate Press; 1945.
12. Onion AHS, Allsopp D, Eggins HOW. Introduction to Mycology. Smith's eds. London: Edward Arnold Publishers; 1981.
13. Barnett HL, Hunter BB. Illustrated Genera of Imperfect Fungi. 3rd ed. Minneapolis, Minnesota: Burgess Publ Co.; 1972.
14. Chmel L, Hasilikowa A, Hraslo J, et al. The influence of some ecological factors on keratinophilic fungi in soil. Sabouraudia. 1972; 10: 26-36.
15. Abdel-Fattah HM, Moubasher AH, Maghazy SM. Keratinophilic fungi in Egyptian soils. Mycopathologia. 1982; 79: 49–53.
16. Caretta C, Piontelli E. Microsporum megallanicum and Cunninghamella antartica, new species isolated from Australia and Antartic soil of Chile. Sabouraudia. 1977; 15:1–10.
17. Carg AK. Isolation of dermatophytes and other Keratinophilic fungi from soil in India. Sabouraudia. 1966; 4: 259–264.
18. Kaitzis G. Zierpflanzen-Hydrokulturen im Krankenhaus also mogliches Reservoir fur Hospitalismus-Nasskeiem. Mitt Osterr Sanitatsverwaltung. 1977; 78: 193–197.
19. Schlichting HE. Ejection of microalgae into the air via bursting bubbles. Journal of Allergy and Clinical Immunology. 1974; 53: 185– 188.
20. Smith V, Streifel A, Rhame FS, et al. Potted plant fungal spore shedding (abstract). Annual Meeting American Society for Microbiology. 1988; 414.
21. Frey D, Oldfield RJ, Bridger RC. A Colour Atlas of Pathogenic Fungi. London: Wolf Medical Publications Ltd.; 1979.
22. Rippon JW. Medical Mycology. The Pathogenic Fungi and Pathogenic Actinomycetes. Philadelphia: WB Saunders Co.; 1982.
23. Wolf PL, Russel B, Shimode A. Practical Clinical Microbiology and Mycology: Technique and Interpretations. New York: John Wiley and Sons Publishers; 1975.
24. Zgoda JR, Porter JR. A convenient microdilution method screening natural products against bacteria and fungi. Pharmaceutical Biology. 2001; 39: 221–225.
25. Kabir OA, Olukayode O, Chidi EO, et al. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. BMC Complementary and Alternative Medicine. 2005; 5 (6): 1-7.
26. Tschesche R. Advances in the chemistry of antibiotics substances from higher plants. In: Pharmacognosy and phytochemistry. Wagner H, Horharmmer L, eds. Proceeding of the 1st International Congress; 1970; Berlin: Springer-Verlag publishers; 1971. p. 274-289.