

IN VITRO ANTIFUNGAL ACTIVITY OF FLOWER EXTRACT OF *PANDANUS ODORATISSIMUS* AGAINST DERMATOPHYTIC FUNGI

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ABSTRACT

Objective: The present study aimed to evaluate the antifungal activity of *Pandanus odoratissimus* oil against dermatophytic fungi, and it was compared against the two commonly used antifungal agent's fluconazole and griseofulvin.

Methods: A total of seven strains of dermatophytes were tested for antifungal activity using oil extracted from the flower of *P. odoratissimus* by using agar-well diffusion method and the zone of inhibition was compared with antifungal agent's fluconazole and griseofulvin. Minimum inhibitory concentration (MIC) was determined using the tube-dilution method.

Results and Conclusion: The zone of inhibition varied from 16.32 to 19.76 mm for fluconazole, 12.12–18.16 mm for griseofulvin, and 2.5–9.59 mm and 7.63–12.88 mm for 2.5 mg/ml and 5 mg/ml of *P. odoratissimus* extract, respectively. Epidermophyton *floccosum* and *Trichophyton violaceum* showed the lowest MIC value of 0.15 mg/ml. The results of our study have shown that the extract from *P. odoratissimus* can work significantly better against fungal diseases caused by dermatophytes. It was also found that it acts as a perfect alternative to the currently available antifungals such as fluconazole and griseofulvin.

Keywords: Dermatophytes, Antifungals, Agar-well diffusion method, Anti-dermatophytic activity, Medicinal plant, *Pandanus odoratissimus*.

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INTRODUCTION

Human fungal infections pose serious medical issues. Up to now, more than a hundred thousand fungal species are considered pathogenic to humans [1]. Dermatophytosis, a major superficial fungal infection that affects the keratinized layers of epidermis, nails, subcutaneous tissue, and hair mainly caused by three genera of filamentous fungi *Epidermophyton*, *Trichophyton*, and *Microsporum* affect millions of people worldwide. The incidence of dermatophytic infection is increasing with more than 20%–25% of the population getting affected worldwide [2]. The proteolytic enzymes keratinase produced by dermatophytes hydrolyze keratin present in hair, skin, and nails. Infection caused by dermatophytes varies from mild-to-severe form based on the host immune response [3,4]. The enzyme required to hydrolyze the macromolecules such as keratin, collagen, and elastin, which constitute 25% of the mass is therefore considered essential for the virulence of dermatophytes. The severity of infection caused by dermatophytes is mainly related to the host response and other factors such as virulence of the stains, anatomical site, and environmental factors [5]. Medicinal plants are a wide source of chemicals, which can be used for the development of new antifungal agents. The currently available antifungals are of the narrow spectrum with less fungicidal efficacy and have various side effects. The plant-derived products are considered to be safe and have very less side effects [6]. Clinicians, researchers, and pharmaceutical companies are in great consent in need of effective secure, potent, and new antifungal drugs [7]. Historically, most of the substances have been a part of natural products. Hence, it is logical to include plant extracts in search for novel antifungals. In formulating the new prototype antifungal agents, it is essential that products with specific properties and structure are to be identified from those drugs currently available. In such cases, higher plants are the only choice, mainly due to a vast variety of newer molecules, often referred to secondary metabolites [8]. Monoterpenes

are the secondary metabolites found in most of the medicinal plants, reported to have antifungal activity but only a few have been evaluated for their activity against fungi [9]. Conventionally, in Indian ayurvedic medicine, *Pandanus odoratissimus* Linn. family (Pandaneaceae) has been recommended for various ailments. Ayurvedic science has used kewda oil, the main constituent isolated from the flower of *Pandanus* in treating debility, earache, rheumatism, spasms, headache, smallpox, giddiness, and arthritis. The essential oil obtained from inflorescences of *P. odoratissimus* (kewda) contains the following chemicals 7.5% 2-phenylethyl alcohol, 18.6% terpene-4-ol, 8.3% germacrene-B, 8.3% α -terpineol, 8.8% viridine, 37.7% ether, and 11% benzyl benzoate along with lesser amount of benzyl acetate, benzyl salicylate, and benzyl alcohol on gas chromatography. The phytochemical screening of aqueous and methanolic extracts of *Pandanus* have shown the presence of proteins, lignans, mucilage, coumestrol, alkaloids, isoflavones, glycosides, phenolic compounds, carbohydrates, tannins, steroids, flavonoids, sterols, saponins, terpenes, gums, vitamins, and amino acids [10,11]. Mainly due to the increase in resistance of dermatophytes to the azoles and other antifungal drugs and due to the side effects of long-term usage of currently available antifungals, new antifungal agents are to be explored. Therefore, a new prototype antimicrobial agent from a natural source is needed to address this situation [12]. Hence, the current study was conducted to evaluate the antifungal activity of *P. odoratissimus* oil against dermatophytic fungi and compared with commonly used antifungal agents fluconazole and griseofulvin.

METHODS

Plant collection

The flowers of *P. odoratissimus* were collected from the natural habitat within Pondicherry. Samples of collected *P. odoratissimus* flowers were prepared, packed, and stored according to the herbarium rules and

regulations. It was identified as *P. odoratissimus* and authenticated by the plant Taxonomist at Pondicherry University and voucher specimens deposited at the university herbarium.

Essential oil extraction

The flowers of *P. odoratissimus* were shade dried and chopped into small pieces. The powdered flower was weighed and loaded into the still of a flat-bottomed distillation tank that formed part of the modified Clevenger-type apparatus. Sufficient distilled water was poured into the tank and the lid secured tightly. The essential oil was obtained by subjecting to steam distillation, and the process was continued for 5–8 hrs. The volatile oil collected was dried using anhydrous sodium sulfate (Na₂SO₄), and it was filtered by Whatman filter paper (No. 1). Finally, weighed and collected to 3 ml airtight glass vials. The essential oils were then stored at –20°C in a freezer [13].

Microbial culture and growth conditions

Microsporium gypseum (2819), *Epidermophyton floccosum* (MTCC 613), *Trichophyton mentagrophytes* (MTCC 7687), *Microsporium canis* (MTCC 2820), *Trichophyton rubrum* (MTCC 296), *Trichophyton verrucosum* (ATCC 52319), and *Trichophyton violaceum* (ATCC11902) were used as test microorganisms. The above strains were cultured onto Sabourauds dextrose broth (HiMedia) for 48 h at 28°C and maintained on agar slants at 4°C [13,14].

Inoculum preparation

Stock inoculums of above organisms were obtained by culturing on potato dextrose agar (PDA) for 10 days at 28°C to induce sporulation. About 5 ml of sterile saline solution (0.85% NaCl) was added to fungal colonies and then scraped gently with a sterile loop. The fungal units thus obtained were transferred to a sterile tube. The turbidity of the final inoculum was compared with McFarland standard 0.5 tube and adjusted to 10⁶ CFU/ml. The inoculum quantification was done by culturing 0.01 ml of inoculum on to Sabourauds dextrose agar (SDA) and incubated at 28°C and observed daily for visible fungal colonies [14,15].

Agar-well diffusion method

The assay was performed using the agar-well diffusion method. Five-day-old culture was used for preparing fungal lawn. The fungal suspension was prepared by adding saline (0.85% NaCl). The turbidity was compared with McFarland standard 0.5 tube and adjusted to 10⁶ CFU/ml. About 1 ml of the fungal suspension was spread on PDA using a sterile glass rod. Wells of 4-mm diameter were punctured using sterile borer. 20 µl of serially diluted extract (2.5 and 5 mg/ml) was added to the wells. For diffusion of extracts, the plates were kept inside the refrigerator for 1 h and then incubated at 28°C. After 48 h, the plates were observed for the zone of inhibition, and the zone diameter was measured. The experiment was done in triplicates. Dimethyl formamide (DMF) was used as a negative control [15,16]. Fluconazole (25 µg) and griseofulvin (25 µg) were used as reference controls.

Minimum inhibitory concentration (MIC)

Seven rows of eight sterile screw-capped tubes were taken. 1 ml of sterile SDA was added to all tubes. 1 ml of the extract was added to

tube 1 of all the rows. Serial dilution was carried out. After mixing the contents well, 1 ml was transferred from tube 1 to tube 2 and repeated until tube 6. The final concentration of the extract was ranged from 0.15 to 05 mg/ml. 50 µl of inoculum was added to all tubes and mixed well. Tube 7 acts as a medium control (no drug and no inoculum) and tube 8 acts as inoculum control (no drug). The tubes were incubated for 96 h at 30°C, and fungal growth was evaluated depending on the turbidity. The lowest concentration of the extract at which the turbidity of the medium was the same, as the media controls were taken as MIC [17].

Statistical analysis

Data were presented as mean±standard error of the mean. All experiments were conducted in triplicate, and statistical analysis of the data was performed by analysis of variance using GraphPad software version 3. A P<0.05 was considered to denote statistically significant.

RESULTS

The *P. odoratissimus* oil used in this study was screened against dermatophytic fungi using agar-well diffusion method, and the zone of inhibition was compared with the control of antifungals griseofulvin and fluconazole. The results of antifungal activity are summarized in Table 1.

Table 1 shows the antifungal activity of *P. odoratissimus* flower extract against various dermatophytes. Two concentrations of *P. odoratissimus* flower extract (2.5 and 5 mg/ml) and two antifungal agents fluconazole and griseofulvin were tested against various dermatophytes such as *T. violaceum*, *M. canis*, *T. mentagrophytes*, *E. floccosum*, *T. rubrum*, *M. gypseum*, and *T. verrucosum*. DMF was used as a control, and the zone of inhibition was measured.

The zone of inhibition varied from 16.32 to 19.76 mm for fluconazole, 12.72–18.16 mm for griseofulvin, 2.58–9.59 mm for 2.5 mg/ml of *P. odoratissimus*, and 7.63–12.88 mm for 5 mg/ml of *P. odoratissimus*. No zone of inhibition or no activity was seen for DMF. The antibiogram revealed 2.5 mg/ml extract of *P. odoratissimus* had maximum activity against *E. floccosum* and *T. mentagrophytes* and was found to be significant in comparison to griseofulvin. The activity of 5 mg/ml extract of *P. odoratissimus* was found to be significantly similar to the control drug fluconazole against *T. mentagrophytes*, *T. verrucosum*, *E. floccosum*, and *T. violaceum*. The extract also showed similar activity to griseofulvin against the following dermatophytes *T. rubrum*, *T. mentagrophytes*, *T. verrucosum*, *E. floccosum*, and *T. violaceum*. The extract of *P. odoratissimus* was found to be ineffective or has minimum activity against *M. gypseum* and *M. canis* as shown by the zone of inhibition viz. The lowest MIC value was obtained for *E. floccosum* and *T. violaceum*, i.e., 0.15 mg/ml. The MIC value of 0.63 mg/ml was obtained for *T. rubrum*, and MIC value of *T. mentagrophytes* and *T. verrucosum* was 0.31 mg/ml (Table 2).

DISCUSSION AND CONCLUSION

The present study was therefore undertaken to determine the *in vitro* antifungal activity of oil extracted from the flower of *P. odoratissimus*, and its efficacy was compared with two commonly used antifungal

Table 1: Antifungal activity of *P. odoratissimus* flower extract against various dermatophytes

S. No	Test Substance	Zone of Inhibition (mm)						
		<i>E. floccosum</i>	<i>M. canis</i>	<i>M. gypseum</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	<i>T. verrucosum</i>	<i>T. violaceum</i>
1	Control (DMF)	No activity	No activity	No activity	No activity	No activity	No activity	No activity
2	Fluconazole (25 µg)	16.32±0.84	19.76±0.65	19.64±0.37	17.32±0.62	17.63±0.60	16.94±0.66	18.55±0.73
3	Griseofulvin (10 µg)	15.58±0.93	17.33±0.88	18.16±0.88	13.53±0.56	12.72±0.34	13.90±0.72	14.85±0.92
4	<i>P. odoratissimus</i> (2.5 mg/ml)	8.74±0.22**	4.61±0.31*****	2.58±0.12*****	9.59±0.63**	4.47±0.20*****	7.71±0.32*****	8.10±0.52*****
5	<i>P. odoratissimus</i> (5 mg/ml)	11.63±0.34**	8.73±0.92*****	7.63±0.65*****	10.42±0.94**	8.51±0.32**	11.42±0.84**	12.88±0.53**

Values are in Mean±SEM; *p<0.05, **p<0.01, and ***p<0.001 compared to Fluconazole, #p<0.05, ##p<0.01, and ###p<0.001 compared to Griseofulvin. DMF: Dimethyl formamide, *E. floccosum*: *Epidermophyton floccosum*, *M. canis*: *Microsporium canis*, *M. gypseum*: *Microsporium gypseum*, *T. mentagrophytes*: *Trichophyton mentagrophytes*, *T. rubrum*: *Trichophyton rubrum*, *T. verrucosum*: *Trichophyton verrucosum*, *T. violaceum*: *Trichophyton violaceum*, *P. odoratissimus*: *Pandanus odoratissimus*

Table 2: MIC results of the *P. odoratissimus* flower extract against test fungi

S. No	Test organism	MIC value in mg/ml
1	<i>E. floccosum</i>	0.15
2	<i>M. canis</i>	1.25
3	<i>M. gypseum</i>	-
4	<i>T. mentagrophytes</i>	0.31
5	<i>T. rubrum</i>	0.63
6	<i>T. verrucosum</i>	0.31
7	<i>T. violaceum</i>	0.15

MIC: Minimum inhibitory concentration, *P. odoratissimus*: *Pandanus odoratissimus*, *E. floccosum*: *Epidermophyton floccosum*, *M. canis*: *Microsporium canis*, *M. gypseum*: *Microsporium gypseum*, *T. mentagrophytes*: *Trichophyton mentagrophytes*, *T. rubrum*: *Trichophyton rubrum*, *T. verrucosum*: *Trichophyton verrucosum*, *T. violaceum*: *Trichophyton violaceum*

agents against seven species of dermatophytes. Significantly, the antifungal activity of *P. odoratissimus* was found to be similar to the control of antifungal drugs griseofulvin and fluconazole against most of the dermatophytic test fungi. The results of our study were in concurrent to an earlier study which has proved *P. odoratissimus* (kweda) was most effective against the keratinophilic fungi, mainly due to the presence of active components such as phenols, aliphatic acid, aldehydes, terpenes, and alcohols in it [18,19]. Screening by using agar-well diffusion method revealed that *P. odoratissimus* showed excellent antidermatophytic activity against *E. floccosum* and *T. violaceum*. These results were found similar to the studies by Prafulla Adkar and Bhaskar 2014, in which *P. odoratissimus* extract was most effective against *E. floccosum* [11]. In our study, it was found that antidermatophytic activity of *P. odoratissimus* oil was comparatively satisfactory to the control antifungal drugs fluconazole and griseofulvin against *E. floccosum*, *T. verrucosum*, *T. violaceum*, and *T. mentagrophytes*. Several studies have shown that *P. odoratissimus* has antiviral, antiplatelet, anti-inflammatory, antioxidant, antiallergy, and anticancer activity [20-25]. Although there is not much literature on the antifungal activity of *P. odoratissimus*, a study by Singh et al., 2011, has shown that antidermatophytic activity of *P. odoratissimus* was better than fluconazole [19]. Findings of the present study provided that oil extracted from the flower of *P. odoratissimus* exhibited good antifungal activity against most of the dermatophytes which could serve as a better alternative against the currently available antifungal agents and can be used in the future against the prevention and treatment of infections caused by the dermatophytes. More studies are required on the mechanism of action of such plants, and such studies could lead the way into the discovery of new agents with better distinctive pharmacological properties. Moreover, medicinal plants are environmentally safe and can serve as a perfect alternative to the current drugs. Herbal medicinal plants are a storehouse for newer antimicrobial products, which are yet to be explored, and they are now becoming an integral part of health care [26]. As suggested at this point of time, attention toward plant extracts with good antifungal activity used in traditional medicine to treat infections caused by pathogenic fungi is needed to combat the resistance toward current antifungal drugs [27].

AUTHOR'S CONTRIBUTIONS

Geethavani Babu: Study concept and design, acquisition of data, and drafting of the manuscript. Balamuruganvelu Singaravelu: Administrative, technical and material support, study supervision, analysis, and interpretation of data. Sreenivasalu Reddy Vallapu: Critical revision of the manuscript for important intellectual content. Srikumar Ramasundaram: Statistical analysis, analysis, and interpretation of data.

CONFLICT OF INTEREST STATEMENT

Nil.

REFERENCES

1. Kacaniova M. Feeding soybean colonization by microscopic fungi. Trakya Univ J Sci 2003;4 Suppl 2:165-8.

- Havlickova B, Czaika VA, Friedrich M. Epidemiological trends in skin mycoses worldwide. Mycoses 2008;51 Suppl 4:2-15.
- Bhatia VK, Sharma PC. Epidemiological studies on dermatophytosis in human patients in Himachal Pradesh, India. Springerplus 2014;3 Suppl 1:134.
- Akcaglar S, Ener B, Tokar SC, Ediz B, Tunali S, Tore O. A comparative study of dermatophyte infections in Bursa, Turkey. Med Mycol 2011;49 Suppl 6:602-7.
- Weitzman I, Balows A, Haurisler W, Lennette EH, editors. Dermatophytosis. Laboratory Diagnosis of Infectious Diseases. New York: Springer-Verlag Inc.; 1988. p. 632.
- Jyothilakshmi M, Jyothis M, Latha MS. Antidermatophytic and protease inhibiting activities of the rhizome of *Zingiber zerumbet* (L) roscoe ex j. E: Smith from central Kerala, India. Int J Pharm Pharm Sci 2015;7 Suppl 1:56-9.
- Selitrennikoff CP, Firkeftein D, Ball C, editors. Screening for antifungal drugs. In: Biotechnology of Filamentous Fungi. London: Butterworth; 1992. p. 189-217.
- Clark AM, Hufford CD, Kinghorn AD, Balandrin MF, editors. Discovery and development of novel prototype antibiotics for opportunistic infections related to acquired immunodeficiency syndrome. In: Human Medicinal Agents from Plants. American Chemical Symposium Series 534. New York: American Chemical Society; 1992. p. 229-41.
- Dias DA, Urban S, Roessner U. A historical overview of natural products in drug discovery. Metabolites 2012;2 Suppl 2:303-36.
- Kumar R, Padmalaxmi D, Udupa AL, Ojeh N, Patil V, Kodancha P, et al. Antioxidant activity of methanol extract of *Pandanus fascicularis* Lam. Pharmacol Online 2011;1:833-41.
- Adkar PP, Bhaskar VH. *Pandanus odoratissimus* (Kewda): A review on ethnopharmacology, phytochemistry and nutritional aspects. Adv Pharmacol Sci 2014;2014:120895.
- Sati SC, Joshi S. Antibacterial potential of leaf extracts of *Juniperus communis* L. from Kumaun Himalaya. Afr J Microbiol Res 2010;4 Suppl 12:1291-4.
- Mohanty RC, Ray P, Rath S. *In-vitro* antifungal efficacy study of plant leaf extracts against three dermatophytes. CIB Tech J Microbiol 2012;1:27-32.
- Santos DD, Barros ME, Hamdan JS. Establishing a method of inoculum preparation for susceptibility testing of *Trichophyton rubrum* and *Trichophyton mentagrophytes*. J Clin Microbiol 2006;44 Suppl 1:98-101.
- Hadacek F, Greger H. Testing of antifungal natural products: Methodologies, comparability of results and assay choice. Phytochemical analysis. Int J Plant Chem Biochem Tech 2000;11 Suppl 3:137-47.
- Magaldi S, Mata-Essayag S, De Capriles CH, Perez C, Colella MT, Olaiola C, et al. Well diffusion for antifungal susceptibility testing. Int J Infect Dis 2004;8 Suppl 1:39-45.
- Crickshank R, Duguid JP, Marmion BP, Suain RH. A tests for sensitivity to antimicrobial agents. In: Medical Microbiology. 12th ed., vol. 2. London and New York: Churchill Livingstone Edinburgh; 1975. p. 190-208.
- Maruzzella JC, Sicurella NA. Antibacterial activity of essential oil vapors. J Pharm Sci 1960;49 Suppl 11:692-4.
- Singh PR, Bundiwal RU, Dwivedi LK. *In-vitro* study of antifungal activity of various commercially available ITRA (Volatile plant oil) against the keratinophilic fungi isolated from soil. Int J Pharm Bio Sci 2011;2 Suppl 3:178-84.
- Council for Scientific and Industrial Research. Anonymous, the Wealth of India: Raw Materials, Publications and Information Directorate. Vol. 7. New Delhi, India: Council for Scientific and Industrial Research; 1966.
- Prajapati ND, Purohit SS, Sharmak A, Kumar T. A Handbook of Medicinal Plants. 1st ed. Jodhpur, India: Agrobios; 2003.
- Dorman HJ, Deans SG. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J Appl Microbiol 2000;88 Suppl 2:308-16.
- Inouye S, Takizawa T, Yamaguchi H. Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. J Antimicrob Chemother 2001;47 Suppl 5:565-73.
- Kalemba DA, Kunicka A. Antibacterial and antifungal properties of essential oils. Curr Med Chem 2003;10 Suppl 10:813-29.
- Reda FA, Hoda MA, Nabila MS, Nagwa HA. Insecticidal activity of four volatile oils on two museum insects pests. Egypt Acad J Biol Sci 2010;2 Suppl 2:57-66.
- Bapat UC, Mhapsekar DR. Phytochemical investigations and antimicrobial and anticancer activities of *Homonoia riparia* Lour. Int J Pharm Pharm Sci 2014;6 Suppl 11:237-43.
- Singh R, Allaie AH, Ganaie AA, Prakash R, Mishra MV, Ali HA. Antifungal activity of medicinal plants with special reference to antidermatophytic activity: A review. Pharm Innov J 2017; 6 Suppl 11:251-259.