Original Article

Insignificant antifungal activity of plant extracts on *Malassezia furfur*

Sreelatha Gopalakrishnan
Lalitha,
Lakshmeesha Thimappa
Ramachandrappa,
Soumya Krishnamurthy,
Jayashree Basavaraju,
Sharmila Thirumale

Department of Microbiology and
Biotechnology, Jnanabharathi
Campus, Bangalore University,
Bengaluru, Karnataka, India

Abstract

Objective: The aim of present study was to investigate the antimalassezial potential of the selected plant extracts known to possess medicinal values. **Materials and Methods:** The crude extracts of five botanicals were evaluated against *Malassezia furfur*. The dried and pulverized plant material was extracted with water and also successively extracted with hexane, chloroform, and methanol, using the Soxhlet apparatus. The antimicrobial activity assay was done by disk diffusion and 96-well plate microdilution method. **Results:** The results point toward the fact that all the tested extracts extracted in solvents of different polarities exhibited no antifungal activity. **Conclusion:** The tested extracts did not have, or had too little, antifungal activity to be used as a promising source of novel antimicrobial substances against *M. furfur*.

Key words: Disk diffusion, *Malassezia furfur*, microdilution, plant extracts

INTRODUCTION

Pityriasis capitis, commonly known as dandruff, is a most common physiological condition causing desquamation of the skin surface due to the imbalance caused to the stratum corneum.^[1] Seborrheic dermatitis is yet another condition that is closely related to dandruff, characterized by highly pruritic chronic inflammatory lesions on the head.^[2] These pathologic conditions are considered to be related to three etiologic factors: *Malassezia* yeasts, secretions of the sebaceous glands, and susceptibility to infections in individuals. It has been generally accepted and through the literature inferred by studies, indicating a strong link to said conditions caused by *Malassezia* yeasts, especially *Malassezia furfur* and, established later, its two species forms *Malassezia globosa* and *Malassezia restricta*.^[3] Other conditions associated with this species are pityriasis

versicolor, folliculitis, atopic dermatitis, psoriasis, and confluent and reticulate papillomatosis.^[4]

The hydrolytic enzyme lipase secreted by *Malassezia* species residing on the surface of the scalp and in the follicular infundibulum cleaves sebaceous triglycerides into free fatty acids and glycerol into the extracellular milieu. The *Malassezia* consumes necessary saturated fatty acids required for their proliferation and leave behind an increased quantity of irritating unsaturated fatty acids. The unsaturated fatty acids penetrate the stratum corneum, and in susceptible individuals, due to the nonuniform structure, breach the skin barrier function. This barrier breach induces an irritation response leading to scalp hyperproliferation, ultimately leading to pathological conditions such as dandruff and seborrheic dermatitis.^[5]

Treatment strategies include synthetic drug-based formulations of ketoconazole, zinc pyrithione, selenium sulfide, ciclopirox, etc., which reduce visible symptoms

Address for correspondence:

Dr. Sharmila Thirumale, Department of Microbiology and
Biotechnology, Jnanabharathi Campus, Bangalore University,
Bengaluru - 560 056, Karnataka, India.
E-mail: dr.sharmilathirumale@yahoo.com

Access this article online

Quick Response Code:

Website:

www.pnrjournal.com

DOI:

10.4103/0976-9234.177055

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Lalitha SG, Ramachandrappa LT, Krishnamurthy S, Basavaraju J, Thirumale S. Insignificant antifungal activity of plant extracts on *Malassezia furfur*. J Pharm Negative Results 2016;7:16-20.

of flaking and restore the normalcy of the skin.^[6] In fact, most medicated formulations carry risks of side effects such as dryness of hair/skin, associated cytostasis, and eczema, and also frequent reoccurrence made therapy costlier. So, it is imperative to search for drugs that are safe, cost-effective, and eco-friendly. Thus, there was a call to return to nature and botanical remedies became a part of the green revolution.

The term “botanicals” refers to the preparations derived from herbs, spices, roots, stems, and other materials of botanical origin.^[7] Plants that are rich in a wide array of antifungal secondary metabolites are gaining importance as a primary source of commercial medicines and drug leads, and have been used for the treatment of skin disorders for centuries.^[8] Herbal drug technology has picked up strongly in terms of the extraction and characterization of active compounds and also the processing of herbs into medicine, considering the fact that modern medicine is not capable of providing a “cure all” solution for the pathologies mentioned above. Herbal therapy is considered as a therapeutic alternative, as a safer choice than synthetic medicines, or sometimes even as the only successful therapeutic way left to treat these disorders.^[9] It is important to document their uses and their distinct modes of action because such information can help in obtaining maximum benefits from these resources, and this increases the possibility of their safe and efficient use in the future. Traditionally, many botanicals tend to possess anti-dandruff activity, but scientific validation is needed to justify their role in this activity.^[10]

In this present scenario, a developing country such as India must focus on documentation of medicinal plants and development of herbal formulations to combat skin infections. The plants used in the present investigation, such as *Punica granatum* L. (Lythraceae), *Illicium verum* Hook (Schisandraceae), *Nyctanthes arbor-tristis* L. (Oleaceae), *Thespesia populnea* L. (Malvaceae), and *Piper betle* L. (Piperaceae) were used to treat cutaneous infections such as psoriasis, tinea infection, and eczema.^[11-15] Thus, the present study was conducted with particular emphasis on aforementioned medicinal plants used traditionally against the dermatologically prevalent yeast, *M. furfur*.

MATERIALS AND METHODS

Collection and maintenance of test microorganism

Malassezia furfur (strain no. 1374) was obtained from the Microbial Type Culture Collection and Genebank (MTCC), Chandigarh, India. The culture was maintained in Leeming-Notman (LN) agar, modified Emmons

agar medium, and Sabouraud's dextrose agar (SDA) supplemented with milk. LN agar medium was used for carrying out antimicrobial studies.^[16]

Plant materials

Plant materials used were selected based on their topical therapeutic properties according to the Ayurvedic system of medicine.^[17] Authenticated plants were collected from Gandhi Krishi Vignan Kendra, Bengaluru, Karnataka, India. The voucher specimens of all the plants were maintained at the Department of Microbiology and Biotechnology, Bangalore University, Bengaluru.

Preparation of plant extracts

Fresh plant materials were blot-dried and macerated with sterile, distilled water in a blender (1:3 w/v) for 10 min. The macerate was first filtered through double-layered muslin cloth and centrifuged at 8000 rpm for 10 min. The supernatant was filtered through Whatman no. 1 filter paper and sterilized at 120°C for 30 min, and this served as the mother extract. All the extracts were concentrated in water bath and stored at 4°C for further use.^[18]

For solvent extraction, 60 g of shade-dried plant material was pulverized, placed to fill the thimble of a Soxhlet extractor, and extracted with 250 mL of different solvents (sequentially using hexane, chloroform, and methanol). The process of extraction continued until the solvent in the siphon of the extractor become colorless. The extracts were concentrated using rotary evaporator and the dried extract was kept in the refrigerator at 4°C until further use.^[19,20]

Antimicrobial susceptibility test

As an initial step toward confirming preliminary antifungal activity, the disk diffusion assay was done to screen the antifungal activity of all the extracts. Inoculum suspension previously adjusted to 0.5 McFarland standard was swabbed evenly over the sterile LN agar medium plates set for the disk diffusion assay. Sterile 6 mm diameter disks were placed equidistantly round the margin of the petri dish. Of the test sample drug, 10 µL (25 mg mL⁻¹) was dispensed on the disks. The inoculated plates were incubated at 32 ± 2°C for 48 h. Each test was performed in triplicate. After the incubation period, the antimicrobial activity was recorded as the width (diameter of inhibition zone plus diameter of the disk) of the inhibition zone. Minimum inhibitory concentrations (MICs) were determined in liquid culture by a 96-well microtiter plate assay recommended by the National Committee for Clinical Laboratory Standards (NCCLS) with minor modification.^[21] Further, minimal yeast-cidal concentration (MYC) has been evaluated by streaking all treated samples on agar medium and assessing the

concentration that inhibits complete yeast growth after incubation.^[22]

Statistical analysis

Statistical analysis was performed using SPSS software version 16.0 (Armonk, NY, IBM Corporation). The results were expressed as mean $\pm$ (standard deviation) SD ($n = 3$). Univariate analysis followed by Tukey's honestly significant difference (HSD) *post hoc* test was applied for statistical analysis, with the level of significance set at $P < 0.05$. Results with $P < 0.05$ were considered to be statistically significant.

RESULTS

The study examined the antimalassezial activity of selected extracts on the dermatologically prevalent yeast *M. furfur* using the *in vitro* technique, which is reliable, sensitive, and widely used. The hexane, chloroform, methanol, and water extracts of the selected plants yielded 0.48-18.15% of their dry weight. The yield was not constant with any particular solvent or plant part. Antimicrobial activity assessed in terms of zone of inhibition in mm of the ten extracts of five plants with different solvents and tested against *M. furfur* was recorded and can be seen in Table 1. In the current study, a total of ten extracts of selected plants were tested for their antifungal activity, of which all the extracts showed no activity or very little activity against *M. furfur* at the tested concentration of 25 mg mL⁻¹. Nevertheless, the highest antimicrobial activities were recorded for *N. arbor-tristis*

in methanol and hexane, with zones of inhibition of 8.33 ± 0.57 mm and 7.73 ± 0.46 mm, respectively. In case of the hexane extract of *I. verum*, the zone of inhibition observed was 7.66 ± 0.57 mm and the methanol extract of *P. granatum* showed the inhibition zone of 7.33 ± 0.57 mm, thus manifesting limited antifungal potential against *M. furfur*. The extracts that showed activity in disk diffusion assay were evaluated for their MIC and MYC values by the 96-well broth dilution method and are tabulated in Table 2. The MIC value of the extracts that showed confined activity was 25 mg mL⁻¹, while the MYC value was 50 mg/mL⁻¹. The remaining extracts exhibited no activity.

DISCUSSION

Antimicrobials of plant origin are in great demand due to their widespread biological activities providing a source for the discovery of many types of effective bioactive compounds. Of these, very few successful drugs are now available for the treatment of fungal infections, especially for superficial mycoses.^[23] Ever since the importance of the distribution of pharmacologically active principles in higher plants was understood and acknowledged, the importance of such plant-derived medicines in modern therapeutic practice has paved the way for the development of new drug leads that are safe, cost-effective, and eco-friendly. Taking this into account, the present study was conducted to assess the antifungal potential of medicinal plants, particularly to target the dandruff-causing yeast *M. furfur*. The present findings

Table 1: Antifungal activity of different plant extracts of *Malassezia furfur*

Name of the plant	Part used	Solvent used for extraction	Yield of extract (%)	IZ in mm
<i>Punica granatum</i> (L.)	Fruit rind	Chloroform	2.06	0.0 $\pm$ 0.0
		Hexane	1.12	0.0 $\pm$ 0.0
		Methanol	7.26	7.33 $\pm$ 0.57 ^b
		Water	7.8	0.0 $\pm$ 0.0
<i>Illicium verum</i> (Hook)	Fruits	Chloroform	0.56	0.0 $\pm$ 0.0
		Hexane	1.4	7.66 $\pm$ 0.57 ^{b,c}
		Methanol	1.28	0.0 $\pm$ 0.0
		Water	2.71	0.0 $\pm$ 0.0
<i>Nyctanthes arbor-tristis</i> (L.)	Leaves	Chloroform	2.0	0.0 $\pm$ 0.0
		Hexane	1.33	7.73 $\pm$ 0.46 ^{b,c}
		Methanol	2.733	8.33 $\pm$ 0.57 ^c
		Water	4.783	0.0 $\pm$ 0.0
<i>Thespesia populnea</i> (L.)	Leaves	Chloroform	9.345	0.0 $\pm$ 0.0
		Hexane	1.9	0.0 $\pm$ 0.0
		Methanol	12.4	0.0 $\pm$ 0.0
		Water	18.15	0.0 $\pm$ 0.0
<i>Piper betle</i> (L.)	Leaves	Chloroform	1.32	0.0 $\pm$ 0.0
		Hexane	0.48	0.0 $\pm$ 0.0
		Methanol	2.6	0.0 $\pm$ 0.0
		Water	3.4	0.0 $\pm$ 0.0

IZ: Inhibition zone in millimeter $\pm$ S.D. including disk diameter of 6 mm. Results are the mean of three replicates $\pm$ S.D. ($n=3$). The values sharing the different superscripts differ significantly when subjected to Tukey's HSD test, ^b $P < 0.01$ and ^c $P < 0.001$ compare to control

Table 2: MIC and MYC of crude extracts

Name of the plant	Solvent used for extraction	MIC mg mL ⁻¹	MYC mg mL ⁻¹
<i>Punica granatum</i> (L.)	Chloroform	-	-
	Hexane	-	-
	Methanol	25	50
	Water	-	-
<i>Illicium verum</i> (Hook.)	Chloroform	-	-
	Hexane	25	50
	Methanol	-	-
	Water	-	-
<i>Nyctanthes arbor-tristis</i> (L.)	Chloroform	-	-
	Hexane	25	50
	Methanol	25	50
	Water	-	-
<i>Thespesia populnea</i> (L.)	Chloroform	-	-
	Hexane	-	-
	Methanol	-	-
	Water	-	-
<i>Piper betle</i> (L.)	Chloroform	-	-
	Hexane	-	-
	Methanol	-	-
	Water	-	-

- : Activity not determined; MIC: Minimum inhibitory concentration, MYC: Minimum yeast-cidal concentration

were in accordance to the reports of Dikshit *et al.*, Lee *et al.*, and Hayouni *et al.*^[24-26] However, the studied extracts had relatively high MIC and MYC values and thus cannot be considered a good antifungal agent.

CONCLUSION

To conclude, it is imperative to emphasize the significance of more comprehensive studies that will detect new lead molecules underlying the action of crude extracts that allow for new discoveries. Essentially, the present study, which establishes the lack of significant antimicrobial activity, removes the need for any repetitive study with these extracts in the future.

Acknowledgment

The authors would like to extend their sincere thanks and appreciation to the Department of Microbiology and Biotechnology, Bangalore University for providing adequate lab facilities and the Himalaya Drug Co, Nelamangala, Bangalore for their magnanimous gesture in supporting research and providing the required plant extracts needed for the study.

Financial support and sponsorship

UGC - BSR meritorious fellowship for Sreelatha G.L.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Turner GA, Hoptroff M, Harding CR. Stratum corneum dysfunction in dandruff. *Int J Cosmet Sci* 2012;34:298-306.
- Hay RJ. *Malassezia*, dandruff and seborrheic dermatitis: An overview. *Br J Dermatol* 2011;165(Suppl 2):2-8.
- DeAngelis YM, Gemmer CM, Kaczvinsky JR, Kenneally DC, Schwartz JR, Dawson TL Jr. Three etiologic facets of dandruff and seborrheic dermatitis: *Malassezia* fungi, sebaceous lipids, and individual sensitivity. *J Invest Dermatol Symp Proc* 2005;10:295-7.
- Gupta AK, Batra R, Bluhm R, Boekhout T, Dawson TL Jr. Skin diseases associated with *Malassezia* species. *J Am Acad Dermatol* 2004;51:785-98.
- Dawson TL Jr. *Malassezia globosa* and *restricta*: Breakthrough understanding of the etiology and treatment of dandruff and seborrheic dermatitis through whole-genome analysis. *J Invest Dermatol Symp Proc* 2007;12:15-9.
- Pierard GE, Arrese JE, Pierard-Franchimont C, DE Doncker P. Prolonged effects of antidandruff shampoos - time to recurrence of *Malassezia ovalis* colonization of skin. *Int J Cosmetic Sci* 1997;19:111-7.
- Reuter J, Merfort I, Schempp CM. Botanicals in dermatology: An evidence-based review. *Am J Clin Dermatol* 2010;11:247-67.
- Ali-Shtayeh MS, Abu Ghdeib SI. Antifungal activity of plant extracts against dermatophytes. *Mycoses* 1999;42:665-72.
- Joshi K, Chavan P, Warude D, Patwardhan B. Molecular markers in herbal drug technology. *Curr Sci* 2004;87:159-65.
- Cavero RY, Akerreta S, Calvo MI. Medicinal plants used for dermatological affections in Navarra and their pharmacological validation. *J Ethnopharmacol* 2013;149:533-42.
- Jurenka JS. Therapeutic applications of pomegranate (*Punica granatum* L.): A review. *Altern Med Rev* 2008;13:128-44.
- Matan N, Matan N. Antifungal activities of anise oil, lime oil, and tangerine oil against molds on rubberwood (*Hevea brasiliensis*). *Int Biodeter Biodegr* 2008;62:75-8.
- Rani C, Chawla S, Mangal M, Mangal AK, Khagla S, Dhawan AK. *Nyctanthes arbor-tristis* Linn. (Night Jasmine): A sacred ornamental plant with immense medicinal potentials. *Indian J Tradit Knowl* 2012;11:427-35.
- Kumar VP, Chauhan NS, Padh H, Rajani M. Search for antibacterial and antifungal agents from selected Indian medicinal plants. *J Ethnopharmacol* 2006;107:182-8.
- Trakranrungsie N, Chatchawanchontea A, Khunkitti W. Ethnoveterinary study for antidermatophytic activity of *Piper betle*, *Alpinia galanga* and *Allium ascalonicum* extracts *in vitro*. *Res Vet Sci* 2008;84:80-4.
- Leeming JP, Notman FH. Improved methods for isolation and enumeration of *Malassezia furfur* from human skin. *J Clin Microbiol* 1987;25:2017-9.
- Khare CP. *Indian Medicinal Plants: An Illustrated Dictionary*. India: Springer; 2007. p. 325-659.
- Mohana DC, Raveesha KA. Anti-fungal evaluation of some plant extracts against some plant pathogenic field and storage fungi. *J Agr Sci Tech* 2007;4:119-37.
- Sreelatha GL, Babu UV, Sharath Kumar LM, Soumya K, Sharmila T. Investigation on biochemical characterisation and *in vitro* antifungal efficacy of plant extracts on *Malassezia furfur*. *Int J Pharm Bio Sci* 2015;6:1027-41.
- Yadav RN, Agarwala M. Phytochemical analysis of some medicinal plants. *J Phytol* 2011;3:10-4.
- National Committee for Clinical Laboratory Standards. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. Approved Standard. Document M-27A. National Committee for Clinical Laboratory Standards. Wayne, Pennsylvania: National Committee for Clinical Laboratory Standards; 1997.
- Lakshmeesha TR, Sateesh MK, Prasad BD, Sharma SC, Kavyashree D, Chandrasekhar M, et al. Reactivity of crystalline ZnO superstructures against fungi and bacterial pathogens: Synthesized using *Nerium oleander* leaf extract. *Cryst Growth Des* 2014;14:4068-79.
- Afolayan AJ, Grierson DS, Mbeng WO. Ethnobotanical survey of

- medicinal plants used in the management of skin disorders among the Xhosa communities of the Amathole District, Eastern Cape, South Africa. J Ethnopharmacol 2014;153:220-32.
24. Dikshit A, Tiwari AK, Mishra RK, Kamran A, Pandey A, Kumar A, *et al.* Botanicals for the management of dandruff. Med Plants 2012;4:55-64.
25. Lee JH, Lee JS. Chemical composition and antifungal activity of plant essential oils against *Malassezia furfur*. Kor J Microbiol Biotechnol 2010;38:315-21.
26. Hayouni EA, Miled K, Boubaker S, Bellasfar Z, Abedrabba M, Iwaski H, *et al.* Hydroalcoholic extract based-ointment from *Punica granatum* L. peels with enhanced *in vivo* healing potential on dermal wounds. Phytomedicine 2011;18:976-84.