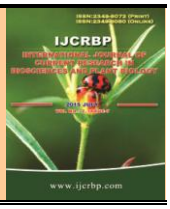
Int. J. Curr. Res. Biosci. Plant Biol. 2015, 2(7): 206-211

EXCELLENT PUBLISHERS

International Journal of Current Research in Biosciences and Plant Biology

ISSN: 2349-8080 Volume 2 Number 7 (July-2015) pp. 206-211

www.ijcrbp.com



Original Research Article

Studies on Antifungal Activities of Certain Plant Extracts against Dandruff-Causing Fungus, *Malassezia*

Surabhi Pisal and Vaishali Mane*

Department of Microbiology, Karmaveer Bahurao Patil College, Vashi, Navi Mumbai-400 703, India

**Corresponding author.*

Abstract

Dandruff is a clinical condition caused by *Malassezia* species. In the present study, anti dandruff activity of various plant extracts were studied. The plant sources selected were reetha, shikhakai, amla, *Aloe vera*, *Hibiscus* flower, neem leaves and tea tree oil. Dandruff sample was isolated from 15 to 20 people using scotch tape method and transferred to sterile Dixon agar for the isolation of dandruff causing fungus. Macroscopic and Microscopic characterization of *Malassezia* fungus was done which was further confirmed by 18srDNA sequencing. Alcoholic and butanoic plant extracts showed effective anti-fungal activity against the isolated Uncultured *Malassezia* fungus using agar well diffusion Method. Especially neem and amla plant extracts showed greater anti-fungal activity against uncultured *Malassezia* fungus. Thus all the above plant extracts can be effectively used in formulation of natural anti-dandruff shampoo.

Keywords

Antifungal activity

Dandruff

Malassezia fungus

Plant extracts

Introduction

Dandruff is nothing but scaling off of the dead skin cells of the scalp. Mild flaking is normal but severe flaking along with inflammation and redness of scalp may need treatment. *Malassezia* is an opportunistic dandruff causing fungus which grows well in sebum (secreted by sebaceous glands on the scalp) (Ro and Dawson, 2005; DeAngelis et al., 2005). Eradication of dandruff causing fungus would facilitate effective treatment of dandruff. Many treatments are available to treat dandruff whose aim is to inhibit dandruff causing fungus. Commercially available anti dandruff products comprises of scalp lotions, creams, anti-dandruff shampoos and conditioners. They consist of various chemical based anti fungal agents like clotrimazole, amphotericim B, miconazole, ZPTO, nystatin, etc. along with foaming and stabilizing agents (sulphates, parabens, silicones, etc.) with their individual harmful effects (Pierard et al., 1996).

Various natural plant extracts are known for their anti-dandruff properties. Evaluation of anti-fungal properties of such plant extracts can be done and they can be used effectively as an alternative to chemical agents in various anti dandruff formulations. Along with anti-fungal properties, plant extracts are also known for their conditioning properties which will be fruitful in maintaining the overall health of scalp and hair (Balakrishnan et al., 2011). In the present study, the extracts of the plants such as, Reetha (*Sapindus mukorossi*), shikakai (*Acacia concinna*), hibiscus flower

(*Hibiscus sabdariffa*), aloe (*Aloe vera arbadensis*), amla (*Emblica officinalis*), neem (*Azadirachta indica*) and tea tree oil (*Melaleuca alternifolia*).

Materials and methods

Isolation of dandruff causing fungus

About 20 people were taken into consideration. Scrapings of dandruff were collected from people with visible flakes whereas Scotch tape/Cello tape method was used to collect dandruff from people with no visible flakes. In Scotch tape/Cello tape method, scotch tape was affixed to the scalp skin and the sticky side was transferred on Sterile Dixon agar and incubated at 32ºc for 3-5 days (Vijayakumar et al., 2006)

Identification of dandruff causing fungus

Microscopic and Macroscopic identification was done to specifically identify the fungus. Microscopic identification was done by Loffeler's Methylene Blue staining and wet mounting, whereas macroscopic identification was done by Tween assimilation test, urease test, catalase test and esculin hydrolysis test. Sequencing of 18srDNA was done to further confirm the isolated fungal species (Saghazadeh et al., 2010).

Molecular identification of isolated fungus using partial 18srDNA Sequencing

PCR and sequencing of fungal sample was carried out by isolating fungal Genomic DNA using Chromous Fungal Genomic DNA Isolation Kit. PCR amplification of Genomic DNA was done using Universal 18srDNA primers. Further Gel extraction of PCR products was carried out and Sequencing of the PCR product was done using ABI sequencing machine**.** FASTA sequence of 18srDNA was used for BLAST Analysis for identification of fungus.

Preparation of plant extracts

Reetha (*Sapindus mukorossi*), shikakai (*Acacia concinna*), hibiscus flower (*Hibiscus sabdariffa*), aloe (*Aloe vera arbadensis*), amla (*Emblica officinalis*), neem (*Azadirachta indica*) and tea tree oil (*Melaleuca alternifolia*) were selected.

Reetha, shikakai, amla and neem powders were obtained from Patanjali, Koperkhairane, Navi Mumbai. Hibiscus powder was prepared by drying Hibiscus flower and grinding it to fine powder. Similar procedure was followed for *Aloe vera* leaves. Tea tree oil was bought commercially.

In this assay, 2.5g of each powder was added in 25ml of 95% Alcohol and Butanol respectively and covered with aluminium foil with occasional stirring for 2 days for all the samples except Tea Tree oil. It was further filtered using Whatman filter paper and dried to get powdered plant extract. The dried powder extract can be dissolved in sterile distilled water for further use. The extract can also be used directly for anti-fungal assay keeping solvent as control (Balakrishnan et al., 2011).

Antifungal activity

To check the Minimum Inhibitory Concentration (MIC) of various plant extracts, different concentrations of alcoholic and butanoic plant extracts were made in the range of 10µg/ml, 100µ/ml and 200µ/ml and their antifungal activities were checked using Agar Well Diffusion method. Tea Tree Oil was diluted using DMSO. Agar well diffusion method was done to determine the zone of inhibition of extracts against dandruff causing *Malassezia* fungus.

Results and discussion

Collection of dandruff sample and isolation of dandruff causing fungus

Collected dandruff sample was inoculated on sterile Dixon agar plates. Typical white growth around the scotch tape is indicative of dandruff-causing fungus. Dandruff scrapings gave white colonies around dandruff flakes. Isolated fungus was further used for microbiological analysis.

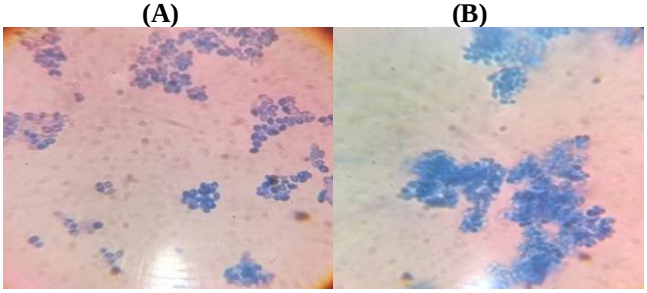
Microbiological analysis of isolated fungus

Microscopic identification

Staining: Isolated fungus was stained using Loeffler's Methylene Blue stain and observed under oil immersion lens. Fig.1 (A) shows the typical blue coloured bottle-shaped cells of dandruff causing *Malassezia* fungus. Wet mounting of the isolated fungus was done using Lactophenol cotton blue stain. Fig. 1 (B) shows typical bottle-shape blue coloured cells were observed.

Fig. 1: (A) Bottle-shaped cells of isolated fungus using of Loeffler's Methylene Blue stain; (B) Wet mount of isolated fungal culture using lactophenol cotton blue stain.

(A) **(B)**



Macroscopic identification: The isolated fungus was streaked on sterile Dixon agar plates to get isolated colonies. Table 1 shows detailed Colony characteristics of isolated *Malassezia* fungus grown on sterile Dixon agar plate after incubating at 32ºC for 5 days. Fig. 2 indicates whitish colonies of *Malassezia* with creamy consistency.

Fig. 2: Isolated colonies of fungus on sterile Dixon agar plate.

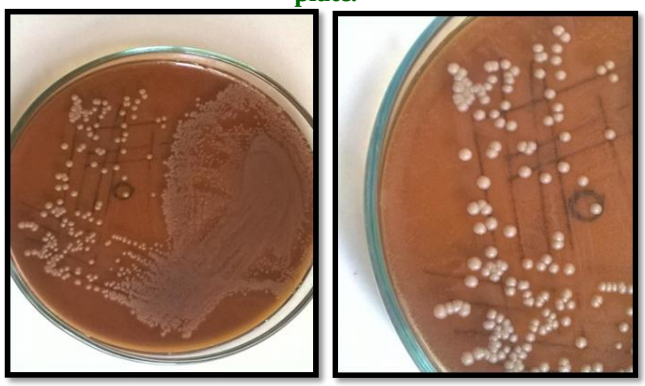


Table 1. Colony characteristics of the isolated fungus.

Colony characteristics	Observations
Size	1mm
Shape	Circular
Color	Whitish
Margin	Entire
Elevation	Convex
Opacity	Opaque
Consistency	Creamy
Texture	Smooth
Morphology	Bottle-neck shaped cells
Loeffler's Methylene Blue Staining	Bottle-neck shaped fungus

Biochemical characterization of isolated fungus

Tween assimilation test: Tween assimilation test was performed to observe growth pattern of the isolated fungus in Tween 60 and Tween 80. After incubation at 32ºC for 4-5 days, Zone of exhibition was observed around both the Tween compounds indicating positive Tween 60 and Tween 80 assimilation. Detailed Zone of Exhibition results are shown on Table 2.

Table 2. Tween assimilation test of isolated fungus.

Sr. no.	Compound	Zones measured (mm)
1	Tween 60	47 mm
2	Tween 80	51 mm

As shown in Table 3, the results of various biochemical tests showed positive results indicative of *Malassezia* fungus.

Table 3. Other biochemical tests for *Malassezia* fungus.

Sr. no.	Biochemical tests	Results
1	Urease test	+
2	Catalase test	+
3	Esculin hydrolysis test	+
Key: + - Positive test		

Molecular identification of isolated fungus using partial 18srDNA Sequencing

FASTA sequence of 18srDNA was used for BLAST analysis for identification of fungus. From the results of partial 18srDNA sequencing thus it can be concluded that isolated fungus was found to be uncultured *Malassezia*. The details of sequence alignment of uncultured *Malassezia* are as follows.

Uncultured *Malassezia* clone H-9 18S ribosomal RNA gene

Partial sequence
Sequence ID: gb|FJ393445.1|

MIC of plant extracts

Agar well diffusion assay was carried out using different concentrations of plant extracts (10µg/ml, 100µg/ml and 200µg/ml). According to Fig. 3, tea tree oil showed inhibition at the concentration of 100µg/ml (DMSO) whereas rest of the plant extracts showed inhibition at the concentration of 200µg/ml and the detailed results are shown in Table 4.

Table 4. Minimum Inhibitory Concentration of plant extracts against *Malassezia* fungus.

Sr. no.	Plant material	Concentration of extracts			Alcoholic extract/DMSO	Butanolic extract/PEG
		10µg/ml	100µg/ml	200µg/ml		
1	Amla	-	-	+	20mm	18mm
2	Reetha	-	-	+	13mm	16mm
3	*Aloe vera*	-	-	+	14mm	22mm
4	Hibiscus	-	-	+	No inhibition	23mm
5	Neem	-	-	+	No inhibition	21mm
6	Shikakai	-	-	+	11mm	23mm
7	Tea tree oil	-	+	-	29mm	No inhibition
Key: + - Positive, - - negative, DMSO- Dimethyl Sulfoxide, PEG- Polyethylene Glycol						

Fig. 3: Minimum Inhibitory Concentration of plant extracts against fungal isolates.

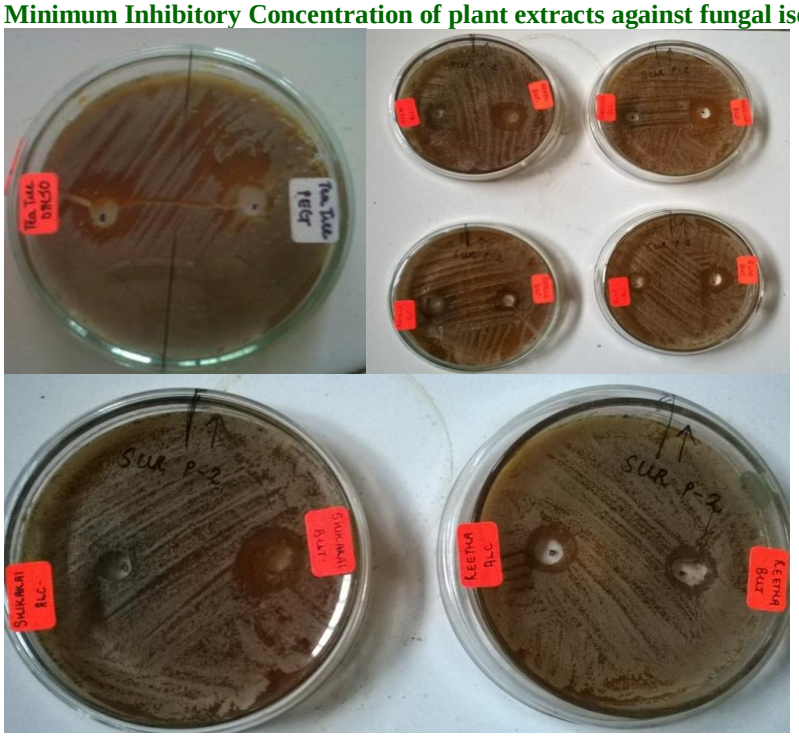


Table 5. Determination of Zone of Inhibition (antifungal activity) of plant extracts against isolated dandruff-causing fungus.

Sr. no.	Plant material	Butanolic / DMSO extract			Alcoholic/PEG extract		
		20%	60%	100%	20%	60%	100%
1	Amla	13mm	21mm	30mm	12mm	14mm	22mm
2	Shikakai	12mm	20mm	26mm	13mm	14mm	20mm
3	Reetha	12mm	19mm	24mm	12mm	14mm	19mm
4	Aloe vera	20mm	24mm	26mm	12mm	13mm	20mm
5	Hibiscus	12mm	22mm	25mm	-	-	-
6	Neem	14mm	23mm	30mm	-	-	-
7	Tea tree oil	14mm	18mm	22mm	-	-	-
Control 1 (butanol)- 12mm							
Control 2 (alcohol)- 12mm							
Control 3 (DMSO)- 11mm							
Key: - - No Inhibition							

Fig. 4: (A). Antifungal activity shown by butanoic plant extracts (Zones of Inhibition).

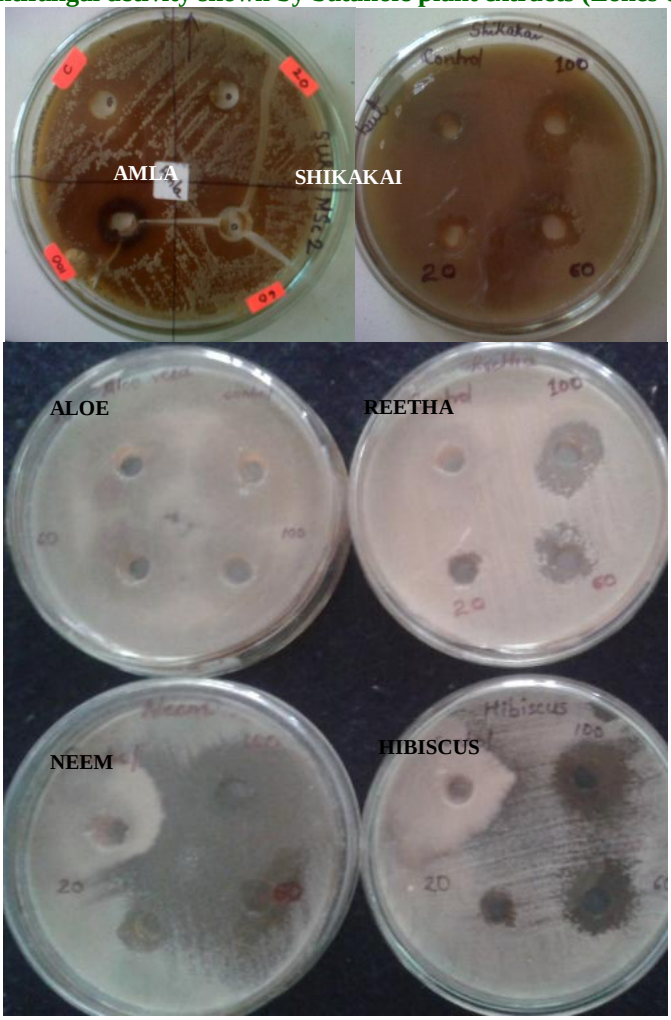
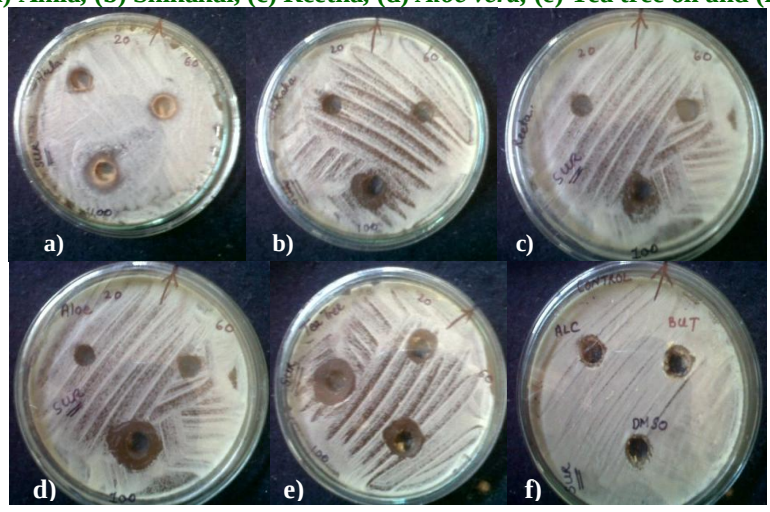


Fig. 4: (B). Antifungal activity shown by tea tree oil and alcoholic plant extracts (Zones of Inhibition). L to R: (a) Amla, (b) Shikakai, (c) Reetha, (d) *Aloe vera*, (e) Tea tree oil and (f) Control.



Determining the Zone of Inhibition of plant extracts against dandruff-causing *Malassezia* fungus

Agar well diffusion assay was done to determine the zone of inhibition of extracts against isolated dandruff-causing fungus. Fig. 4 shows Effective anti-fungal activity of both alcoholic and butanoic extracts for all the concentrations of plant extracts used in this study. Zone of inhibition of the alcoholic extract of hibiscus and neem and PEG extract of tea tree oil was not determined as they did not show any activity in MIC test. Detailed results for Zone of Inhibition in mm are given in Table 5.

A recent review by Potluri et al. (2013) the causes, synthetic chemical, various herbs and the evaluation parameters for the anti-dandruff shampoo with a focus on the utilization of medicinal herbs. Chandran et al. (2013) developed herbal based anti-dandruff shampoo and their report shows that herbal shampoo prepared with *Sida cordifolia* leaf extract and soap nut and shikakai were found to be effective. These results are in line with the results of the present study. The isolation and identification of dandruff causing fungus was carried out successfully using scotch tape method and macroscopic/microscopic identification and 18srDNA sequencing respectively. All the above tests results showed that isolated fungus belongs to Uncultured *Malassezia*. Alcoholic and butanoic plant extracts showed effective anti-fungal activity against the isolated uncultured *Malassezia* fungus using agar well diffusion method. Especially neem and amla plant extracts showed greater anti-fungal activity against uncultured *Malassezia* fungus. Thus it can be concluded that all the above plant extracts can be effectively used in formulation of natural anti-dandruff shampoo. In addition, comparative analysis of effectiveness of formulated natural anti-dandruff shampoo with commercial herbal anti-dandruff shampoo could be developed with the tested plant materials in the present study.

Acknowledgement

Hereby authors would like to express heartfelt gratitude to Dr. D. B. Thakare, Head Department of Microbiology for his guidance and technical support. Also, we would like to thank teaching and non-teaching staff of Department of Microbiology, K.B.P College, Vashi for endless support in the completion of this research work.

References

Balakrishnan, K.P., Nithya, N., Soosamma, M., Karuna, G., 2011. Evaluation of some medicinal plants for their dandruff control properties. Int. J. Pharm. Bio Sci. 2(4), 16-22.

Chandran, S., Vipin, K. V., Augusthy, A.N., Lindumol, K. V., Shirwaikar, A., 2013. Development and evaluation of antidandruff shampoo based on natural sources. J. Pharm. Phytother. 1(4), 10-14.

DeAngelis, Y.M., Gemmer, C.M., Kaczvinsky, J.R., Kenneally, D.C., Schwartz, J.R., et al., 2005. Three etiologic facets of dandruff and seborrheic dermatitis: *Malassezia* fungi, sebaceous lipids, and individual sensitivity. J. Investig. Dermatol. Symp. Proc. 10, 295-297.

Pierard, G.E., Pierard, F.C., Oudjin, N.T., Nikkeis, A.F., Saint Legar, D., 1996. Improvement in the inflammatory aspect of androgenic alopecia: A pilot study with an antimicrobial lotion. J. Dermatol. Treat. 7, 153–157.

Potluri, A., Asma Shaheda, S.K., Rallapally, N., Durrivel, S., Harish, G., 2013. A review on herbs used on anti-dandruff shampoo and its evaluation parameters. Indo American J. Pharm. Res. 3(4), 3266- 3278.

Ro, B.I., Dawson, T.L., 2005. The role of sebaceous gland activity and scalp microfloral metabolism in the etiology of seborrheic dermatitis and dandruff. J. Investig. Dermatol. Symp. Proc. 10, 194-197.

Saghazadeh, M., Farshi, S., Hashemi, J., Mansouri, P., Khosravi, A.R., 2010, Identification of *Malassezia* species isolated from patients with seborrheic dermatitis, atopic dermatitis, and normal subjects. J. de Mycologie Medicale 20(4), 279-282.

Vijayakumar, R., Muthukumar, C., Kumar, T., Saravanamuthu, R., 2014. Characterization of *Malassezia furfur* and its control by using plant extracts. Ind. J. Dermatol. 51(2) 145-148.