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HERBAL EXTRACTS AND THEIR ANTIFUNGAL ACTIVITY AGAINST *MALASSEZIA FURFUR*

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ABSTRACT

Malassezia furfur, is a dermatophilic fungi that utilizes the secretions of the sebaceous glands for its growth and causes infections like dandruff and associated secondary infections. Herbal based treatments for dandruff are now gaining more significance over the conventional synthetic anti-dandruff remedies. In our study, efficacy of 23 medicinal plants for their antifungal activity was evaluated .Aqueous and ethanolic herbal extracts were used for the assay. The Minimum Inhibitory Concentration or MIC was determined as per guidelines laid down by Clinical and Laboratory Standards Institute and the antifungal activity was assayed by well diffusion method.In our study *Malassezia furfur* was found to be most susceptible to the aqueous extracts of *Eugenia jambolana* and least susceptible to *Terminalia paniculata* and among the ethanolic extracts it was most susceptible to *Quisqualis indica* and least susceptible to *Terminalia paniculata.*

KEYWORDS: **Dandruff, *Malassezia* furfur, lipophilic, well diffusion, Mc Farland.**

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INTRODUCTION

Dermatophytic infections have been one of the major crises prevalent all over the world. Dermatophytes feed majorly on the secretions of the sebaceous glands present below the skin. Dandruff has worldwide occurrence. Its frequency is variable and depends on different climatic, occupational and socio-economic conditions.It is reported that approximately 30% of dermatophilic infections are due to the lipophilic yeasts[1].Hereditary factors also play a major role in transmission of the disease [2].It was observed that the main symptom of dandruff infection was the appearance of patches of discoloured skin with sharp borders and fine scales. The patches were dark reddish-tan in colour. The most common sites were found to be the back, head, underarms, upper arms, chest and neck.Larger lesions were multihued and had relatively sharp irregular margins and smaller lesions were circular or oval[3]. *Malassezia furfur* is a fungus that belongs to the class exobasidiomycetes and is generally characterized by globose, oblong-ellipsoidal to cylindrical yeast cells, but it can also grow in a mycelial phase. *M. furfur* is lipophilic yeast.Therefore *in-vitro* growth must be stimulated by addition of oils or other lipid substances to the growth medium. The most common media used is Sabouraud's dextrose agar containing cycloheximide (actidione) with olive oil or alternatively a more specialized media like Dixon's agar which contains glycerol mono-oleate can be used. On such media, colonies were observed to be cream to yellowish, smooth or lightly wrinkled, glistening or dull, with the margin being either entire or lobate. The incubation temperature of this fungus was between 30-35°C and the duration was for 48 hours[4].In our study the media used for isolation and culturing of the fungus was Leeming Notman's medium which contained 1% peptone, 1% glucose, 0.2% yeast extract, 0.8% oxbile, 1% glycerol (v/v),0.5% tween 60 (v/v), 1.5% agar and overlaid with olive oil as a lipid supplement to support their growth at 30°C for 48 hours.Anti-dandruff shampoos and oils are most commonly used to treat dandruff problems. They are of synthetic as well as herbal origin. Many synthetic compounds used for topical application are found in shampoos like azole antimycotics, ciclopiroxolamine,zinc pyrithione, or sulfur-containing substances. Synthetic shampoos use a combination of ingredients to control dandruff. Salicylic acid (used in Sebulex) removes dead skin cells from the scalp and decrease the rate at which these cells are created. Zinc Pyrithione is used to control mould, mildew, yeasts, gram positive and gram negative bacteria[5].In recent years rare cases of systemic infections and fungemias caused by *Malassezia* have been reported[6].Consequently, drugs of herbal origin are also used to treat *Malassezia* infections. Antifungal activity of herbal extracts was observed by using the combination of *Wrightia tinctoria* and *Hibiscus rosa sinensis in vitro* against the isolates of *Malassezia furfur* recovered from dandruff. Other plant extracts namely *Aloe vera, Eucalyptus globulus, Phyllanthus emblica* and *Wrightia tinctoria* (leaf extracts)were tested for the antimycotic activity. They showed antifungal property as these extracts progressively inhibited the growth of *M. furfur* on SDA media i.e., Sabouraud's dextrose agar medium. *Eucalyptus globulus* and *Aloe vera* were more effective than other species[7]. The oils of plants such as *Melaleuca alternifolia*, *Rosmarinus officinalis*,*Coleus forskohlii*, *Syzigium aromaticum*, *Piper nigrum*, *Azadirachta indica* and *Ocimum basilicum* also showed anti-fungal activity[8].In our study, the extracts of 23 plants were assayed for their anti-fungal activity. The plants belonged to the families Apiaceae, Combretaceae, Anacardiaceae,Lamiaceae, Moraceae, Meliaceae, Fabaceae, Lamiaceae ,Myrtaceae, Apocyanaceae,Annonaceae, Phyllanthaceae, Rosaceae, Basellaceae, Acoraceae, Acanthaceae and Asteraceae. Out of these families, plants belonging to the family Myrtaceae, Combretaceae and Apiaceae showed considerable anti-fungal activity.

MATERIALS AND METHODS

Test Organism

The organism used in this study was *Malassezia furfur* (strain MTCC 1374) obtained

from Microbial Type Culture Collection , Institute of Microbial Technology, Chandigarh, India.

Media Used

Leeming Notmann's agar and broth were used to the culture the fungus *Malassezia furfur* [9].

Standard- Zinc Pyrithione (ZPTO)

Standard solution was prepared by dissolving 5mg or 0.005g of Zinc pyrithione (ZPTO) in 50 mL Dimethyl sulphoxide to make up the final concentration to 0.01 %.

Plants used

Plant	Family	Part used
Anethum graveolens	Apiaceae	Leaves
Foeniculum vulgare	Apiaceae	Fruit
Trachyspermum ammi	Apiaceae	Leaves and seeds
Terminalia myriocarpa	Combretaceae	Leaves
Terminalia paniculata	Combretaceae	Leaves
Calycopteris floribunda	Combretaceae	Leaves
Quisqualis indica	Combretaceae	Inflorescence
Pimenta dioica	Myrtaceae	Leaves
Eugenia jambolana	Myrtaceae	Leaves
Anacardium occidentale	Anacardiaceae	Leaves
Mangifera indica	Anacardiaceae	Bark
Ficus reliogiosa	Moraceae	Leaves
Prosopis glandulosa	Fabaceae	Fruit
Rosa chinensis	Rosaceae	Leaves
Annona squamosa	Annonaceae	Seeds
Swietenia mahogany	Meliaceae	Bark
Sauropus androgynous	Phyllanthaceae	Leaves
Artemissia vulgaris	Asteraceae	Leaves
Basella alba	Basselaceae	Leaves
Tabernaemontanum coronarium	Apocyanaceae	Flowers and Leaves
Acorus calamus	Acoraceae	Leaves
Hemigraphis colorata	Acanthaceae	Leaves
Clerodendrum thomsoniae	Lamiaceae	Leaves

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory concentration was determined by broth dilution method based on Clinical Laboratory Standards guidelines [11].

Preparation of plant extracts

The solvent for preparation of plant extract was water and ethanol(absolute). 1g of powdered plant sample was added to 10mL of the respective solvents taken in boiling tubes. They were then placed in preheated water bath at 65^0C for 10 minutes. Then the contents from each of the boiling tubes were emptied into separate centrifuge tubes and centrifuged at 2000 rpm for 20 min and the supernatant was used as plant extract.

Preparation of McFarland Standard

McFarland Standard was prepared as per the guidelines described in the National Committee for Clinical Laboratory Standards[10].

Collection of plant samples

Plant samples were collected from the Horticulture Society of India (Kolkata), natural habitat in Arunanchal Pradesh, Gandhi Krishi Vignyan Kendra (Bangalore), Institute of Ayurveda and Integrative Medicine (Bangalore).

Assaying the antifungal activity by well-diffusion method

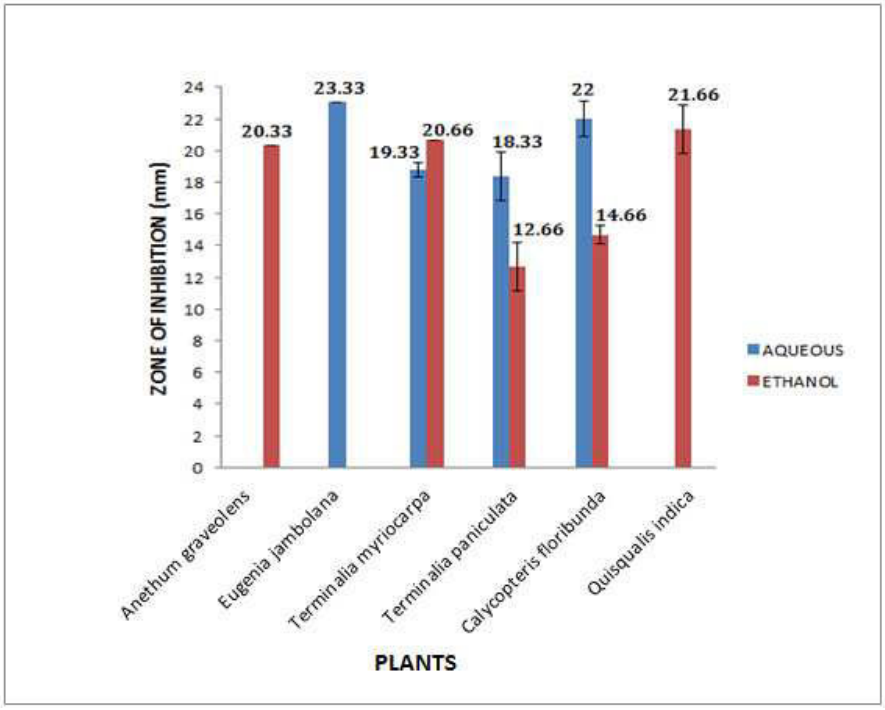
For plating, Leeming Notman's agar was prepared, autoclaved and dispensed into sterilized petri plates. After solidification of the agar, 50µL of *Malassezia furfur* inoculum from broth culture was added in the centre of the petri plate using a micropipette and spread evenly on the agar using a sterilized glass spreader. Three wells were bored into the agar at three corners of the plate taking care that

the wells did not lie in close proximity to the edges of the petri plate or to each other. Each of the three wells represented the sample, standard and control respectively. Using a micropipette 50 µL of plant extract (filtrate) was added to the sample well. Similarly 50 µ L of Distilled water or ethanol (depending on type of the plant extract) was added to the well representing control. In the well representing the standard 50 µL of 0.01 % Zinc Pyrithione solution was added. The plates are placed in a refrigerator for 30 min and then transferred to an incubator set at 30^0C. After 48 hrs of incubation, the zone of inhibition was measured (in mm) using the antibiotic scale provided by Hi-Media. The experiment was conducted in triplicates for each sample.

RESULTS AND DISCUSSION

Medicinal plants are increasingly of interest as antimicrobial agents and have been widely used in traditional medicine[12]. Our study revealed the following results :

Zone of inhibition of aqueous and ethanolic plant extracts



Our results revealed that out of the 23 plants assayed, 6 plants showed positive results. It was observed that among the aqueous extracts (10% concentration), *Malassezia furfur* was most susceptible to *Eugenia jambolana* with a zone of inhibition of 23.33mm and least susceptible to *Terminalia paniculata* with a zone of inhibition of 18.33mm. It was also inferred that *Anethum graveolens* and *Quisqualis indica* showed no zone of inhibition in their respective aqueous extracts. Among the ethanolic extracts (10% concentration), *Malassezia furfur* was most susceptible to *Quisqualis indica* with a zone of inhibition of 21.66mm and least susceptible to *Terminalia paniculata* with a zone of inhibition of 12.66mm. However it was observed that *Eugenia jambolana* did not show any zone of inhibition in its respective ethanolic extracts.

Figures

A) Zone of inhibition of the aqueous extract of Eugenia jambolana and Terminalia paniculata. The well with sample contains the plant extract.

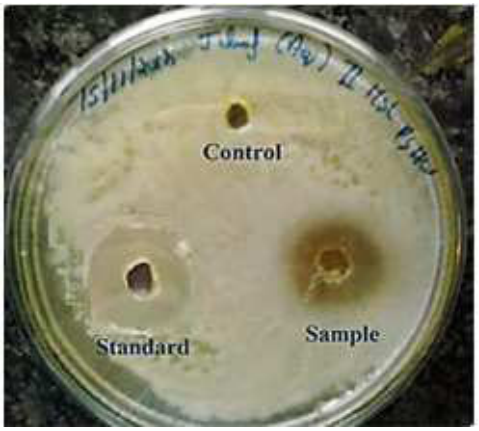


Figure 1
Aqueous extract of Eugenia jambolana

Figure 2
Aqueous extract of T.paniculata

B) Zone of inhibition of the ethanolic extract of Quisqualis indica and Terminalia paniculata.The well with sample contains the plant extract.

Figure 3
Ethanolic extract of Quisqualis indica

Figure4
Ethanolic extract of T.paniculata

SCOPE

Over the years, consumers have become aware of advantages of using herbal based cosmetics. Unlike those of synthetic origin, there are no side effects caused by the usage of herbal based products. This attributes to the growing demand for herbal products in urban and in semi urban areas. Presently the market research shows upwards trend in the herbal cosmetic industry playing major role in fuelling this worldwide demand for herbals. The recent interest of consumers in herbal cosmetics has been stimulated due the following reasons namely decline in faith of modern synthetic cosmetics, the belief that plant remedies were natural and thereby superior to man – made synthetic cosmetics, and the reference to successful historical use by different cultures. These reasons have contributed to the increased acceptance as well as manufacture of herbal cosmetics [13].

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