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COMPARISON OF ANTIFUNGAL ACTIVITY OF DIFFERENT PLANT EXTRACTS AGAINST DANDRUFF CAUSING ORGANISM *MALASSEZIA* SPECIES

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ABSTRACT Dandruff is a common scalp disorder affecting most of the population at the pre-maturity age which involves excessive shedding of dead skin cells from the scalp nearly affecting a person irrespective of age. The clinical importance of causative agents of dandruff is significantly increasing. Malassezia sp., is the most predominant lipophilic fungus which is the causative agent of dandruff. This study involves the collection, isolation and identification of dandruff-causing pathogens from the infected person by pure culture technique using an SDA agar plate enriched with olive oil and chloramphenicol. The isolate was identified by various biochemical tests and microscopic examination. The Agar well diffusion method was used to determine antifungal activity. A comparative study of the effect of natural plant materials such as Psidium guajava, Azadirachta indica, Glycyrrhiza glabra and Trigonella foenum-graecum leads to the conclusion that the activity against dandruff-causing pathogen.

KEYWORDS : Dandruff, *Malassezia* sp., Biochemical tests, Antifungal activity

INTRODUCTION

The word dandruff is a combination of "tan" meaning "tetter" and "drof" meaning dirty thus the dandruff is the itchy dirt [3]. Dandruff is a common scalp disorder affecting most of the population at the pre-maturity age [13]. It is characterized by flaky white to yellowish scales, along with itching. The *Malassezia* sp., yeast is responsible for dandruff which feeds on scalp oils. Some people's bodies view this oil breakdown as an irritant, so the scalp speeds up skin cell renewal, which results in dandruff [11]. Seven different varieties including *M. globosa, M. furfur, and M. pachydermatis*, have been linked to the development of dandruff. *Malassezia* flourishes in an atmosphere that is warm and humid, crowded and has poor personal hygiene standards. Men get dandruff more frequently than women do [7].

The guava (*Psidium guajava* L.) tree was used for many years as it contains anti-dandruff action and is a traditional plant used to treat certain respiratory and to increase platelets in patients suffering from dengue fever. *Azadirachta indica* commonly known as Neem has lots of medicinal properties such as antibacterial activity, anti-inflammatory and also helps in the treatment of parasitic diseases, tumor etc., *Glycyrrhiza glabra* is used in the formulation of sunscreens and also for facial cleansers etc., Fenugreek (*Trigonella foenum-graecum* L.) has lots of medicinal properties such as anticarcinogenic, antidiabetic etc., It contains nicotinic acid which is one of the top remedies to control dandruff naturally.

This study aimed to identify the *Malassezia* species isolated from a dandruff patient by using various techniques and to compare the antifungal activity against different plant extracts.

MATERIALS AND METHODS

Collection And Extraction Of Plant Materials

Fresh leaves of Guava, Neem, roots of Adhimadhuram and Fenugreek seeds were procured from in and around the Tirupur region and washed under running water to remove contaminants and authenticated from the Department of Botany, LRG Government College for Women, Tirupur. All the ingredients were shade-dried converted into coarse powders and stored in an airtight container [4]. All the powdered herbs were macerated both individually and in different ratios (1:2:3:4, 2:1:4:3, 3:4:1:2, 4:3:2:1) using 100ml of distilled water, ethanol and chloroform for 48 hours. The extract was centrifuged at 2000 rpm. The supernatant was collected and stored for further processing [5].

Collection Of Dandruff Sample

Dandruff samples were obtained from the patients by scrapping the scalp surface with sterile combs [8].

Isolation Of The Isolate

The collected samples were enriched using 5 ml Sabouraud Dextrose Broth consisting of olive oil and chloramphenicol and incubated overnight with shaking and spread on Sabouraud Dextrose Agar and incubated at 32ºC for three days. By looking at colony morphology the suspected colonies were identified by further analysis [2].

Identification Of The Isolate

Lactophenol Cotton Blue Staining

The colony morphology of the isolate was studied by making stained smears using LPCB stain [6].

KOH wet mount

The isolates were mixed with a drop of 10% Potassium Hydroxide and observed under 40x [10].

Catalase Test

A drop of 3% hydrogen peroxide was placed on a glass slide. The isolates were transferred and mixed well [2].

Tween Assimilation Test

2 ml of the isolate was mixed with SDA with olive oil overlay containing chloramphenicol and plated. Four wells were made and filled with 5 µl Tween 20, 40, 60 and 80 and incubated for 1 week at 32°C [12].

Bile Esculin Splitting Test

An esculin agar tube was used to determine glucosidase activity. The isolates were deeply inoculated into the agar and incubated at 32°C for 7 days with daily observations [1].

Antifungal Activity

The antifungal activity of various solvent extracts of the plants both individual and in different ratios was evaluated against the isolates by agar well diffusion method. Isolates were spread over the surface of SDA and 100µl of respective extracts were added to the wells. Ketoconazole (10µg) was maintained as a positive control [10]. The plates were incubated at 32ºC for 3 days and determined by measuring the diameter of the zone of inhibition in mm and classified by using a standard chart.

RESULT AND DISCUSSION

Collection And Extraction Of Plant Materials

Guava leaves, Neem leaves, Adhimadhuram and Fenugreek seeds were dried and powdered. Distilled water, Ethanol and Chloroform extracts of the plant materials were centrifuged at 2000 rpm and the supernatant was collected.

Collection Of Dandruff Sample

Between December and January 2023, a total of 10 samples were collected from the age group of 18-24.

Isolation Of The Isolate

The growth in olive oil overlay plates after 72 hours of incubation indicated that the isolate was not a lipid-independent species. Approximately 10 suspected isolates were taken for analysis. Among the 10 isolates, isolate 7 which appeared as a cocci-shaped smooth,

raised, cream-yellow colored colony was taken for further analysis as it was one of the most significant organisms causing dermatitis. Similar studies were carried out by Begum *et al*., 2019 in which a total of 35 suspected single colonies from 25 samples were isolated and amongst them, 15 isolates were identified as *Malassezia* sp.,.

Table 1: Colony Morphology Of The Isolates

Isolates	Colony morphology
1	Wrinkled white
2	Cream coloured
3	Lightly wrinkled
4	Dull white
5	Yellowish wrinkled
6	Cream wrinkled
7	Smooth raised creamy
8	Creamy wrinkled
9	Cream colored
10	Dull raised

Figure 1: Isolation Of The Isolate (spread Plate)identification Of The Isolate

Lactophenol Cotton Blue Staining

Under the 40x objective, the isolate appeared typical spherical shape associated with yeast spores. Similar result was reported by Gebrehiwot Gebremedhin *et al*., 2020 in which the *M. globosa* appeared round shape that is blue upon staining.

KOH Wet Mount

Alkali is applied to digest the keratin surrounding the fungi. The slides were observed under the 40X objective which generally exhibits the typical form of "spaghetti and meatballs".

Catalase Test

The production of gas bubbles which indicates catalase positive confirmed that the organism was not the catalase negative species. This was lined with the report of Gebrehiwot Gebremedhin *et al*., 2020 which showed a positive catalase reaction for *M. globosa.*

Tween Assimilation Test

The utilization of Tween was assessed by the degree of growth (precipitation) of the lipophilic yeasts around the wells. There is a dense growth of colonies around the wells filled with Tween 20, 40, 60 and 80 respectively. A similar result was obtained by Ishaku *et al*., 2021.

Bile Esculin Splitting Test

Splitting of esculin into esculetin and glucose is revealed by the darkening of ⅔ of the medium. Concerning the above tests, it was confirmed that the organism isolated was *Malassezia furfur.*

Antifungal Activity

In individuals, the Ethanol extract of *Psidium guajava* showed the highest zone of inhibition ranging 27mm against *M. furfur*. In Combinatorial all the four-plant ethanol extract (3:4:1:2) showed a maximum zone of inhibition against isolated dandruff causing organism range (21mm). The antifungal activity of guava aqueous crude extract was reported as a 15mm zone of inhibition against *Malassezia furfur* in a study by Keragala *et al*., 2020, whereas in our study, guava distilled water extract showed higher activity of 25mm zone against the isolated fungal pathogen. The antifungal activity of fenugreek was reported as 30mm for the *Malassezia furfur* in a study (Panicker *et al*., 2020), whereas in our study, fenugreek aqueous extract showed a zone of 11mm. Plant extracts have been found to exhibit antifungal activity, and these findings suggest that plant extracts could be a promising source of antifungal agents.

Table 2: Antifungal Activity Of Plant Extracts Against *Malassezia furfur*

Plant extracts	Dis.H_2O	Ethanol	Chloroform
G: N: A: F 1: 2: 3: 4	-	12mm	9mm
2: 1: 4: 3	-	13mm	9mm
3: 4: 1: 2	-	21mm	-
4: 3: 2: 1	-	17mm	-
Psidium guajava	25mm	27mm	14mm
Azadirachta indica	-	13mm	12mm
Glycyrrhiza glabra	-	23mm	15mm
Trigonella foenum-graecum	11mm	15mm	19mm
Ketokonazole disc (10µg)- PC	28mm		

CONCLUSION

Dandruff causing organism was isolated, identified and confirmed as *Malassezia* sp., The tested extracts showed significant antifungal activity against *Malassezia furfur*. Individually, Guava showed the best results and the combination where Guava was used in 3 parts showed maximum inhibition. Thus, these results can be used to prepare a combinational herbal mix either with any formulations to get better anti-dandruff activity. The compounds responsible for the antifungal activity can be further isolated and identified using bioassay-guided fractionations.

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