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Isolation and Characterization of dandruff causing fungi & effect of some plant extracts on it

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ABSTRACT

Yeast called *Malassezia* is a causative agent of Seborrheic dermatitis or dandruff. It was isolated from the scalp of human individuals and investigated further. Suitable biochemical tests were performed for the characterization of the organism focusing on its ability to produce Catalase, Urease, utilization of tweens, liquefaction of gelatin and assimilation of different carbohydrates. The organism after its isolation and characterization was further investigated for its susceptibility towards 20 different plant extracts.

Keywords: Seborrheic dermatitis, *Malasezzia*, mDixons agar, antifungal activity, medicinal plants.

INTRODUCTION

Fungi are ancient troublemakers, but the suspicion that they might cause disease is about 100 years old. Seborrheic dermatitis /dandruff is a condition where flaky skin of scalp sheds off. Louis Charles Malassez first saw yeast like substance lurking in the scales of a patient with Seborrheic dermatitis. *Malassezia furfur (Pitryosporum ovale),* is the fungus which plays an important role in Seborrhoeic dermatitis (Faergemann et al., 1996). The genus *Malassezia* comprises lipophilic yeasts found in the normal flora of human skin and other mammals. These yeasts were described as being associated with Pityriasis Versicolor (PV) lesions. The taxonomy and nomenclature of the genus *Malassezia* was controversial for many decades. Nine of the 13 species within the genus, *M. furfur*, *M. sympodialis*, *M. globosa*, *M. restricta*, *M. slooffiae*, *M. obtusa*, *M. dermatis*, *M. japonica*, and *M. yamatoensis*, are associated with normal human flora and pathologies. Four species, *M. pachydermatis*, *M. nana*, *M. equina*, and *M. caprae*, are associated with

animals (Cabanes et al., 2007, Khosravi et al., 2008). *Malassezia* species have been associated with diverse dermatological pathologies, including PV, Seborrheic dermatitis (dandruff), Atopic dermatitis, Folliculitis, Psoriasis, Onychomycosis, and Blepharitis.

Despite the difficulty in isolating, maintaining and identifying these yeasts, different characteristics of the genus, such as macroscopic and microscopic morphology and some physiological aspects (e.g., the presence/absence of catalase, urease) gelatin hydrolysis, sugar assimiliation (sorbitol, mannitol, xylose, dextrose, lactose, glucose, sucrose), assimilation of Tween 20 and 80 by *M. furfur*, *M. sympodialis*, allow them to be differentiated as yeasts. *M. furfur (Pitryosporum ovale)*, a lipophilic fungus affects the hair and causes dandruff. Dandruff is a condition which causes small white flakes of skin that separates and fall from the scalp. People who suffer from dandruff have over-active sebaceous glands, which make their scalp oily.

Plant extracts are promising sources for new natural antifungal agents even though they have relatively mild effect against human pathogenic fungi compared with commercial synthetic antifungal drugs (Rukayadi et al., 2006). Plant extracts and essential oils (Pawar and Thaker, 2006, 2008) have been reported to show antifungal activity against a wide range of fungi. The medicinal plants have been used for several purposes including antibacterial as well as antifungal effects. Some Indian medicinal plants have been used widely in treating a variety of skin diseases by the Ayurvedic physicians (Prusti et al., 2008).

In the present research work the characterization of the fungus was performed using different biochemical tests like Catalase, Urease, Gelatin Hydrolysis and Sugar Assimilation. The influence of twenty different plant extracts namely, Shikakai (*Acacia concinna),* curry tree (*Murraya koenigii*), *Tulsi (Ocimum sp).* Black pepper (*Piper nigrum*), Bael (*Aegle marmelos), Hibiscus rosa sinensis*, Betel (*Piper betle*), Cumin seed (*Cuminum cyminum),* Amla (*Emblica officinalis),* Brahmi *(Bacopa monnieri*), Tridax *(Tridax procumbens),* Ajwain (*Trachyspermum ammi*), Reetha *(Sapindus saponaria),* Pudina (M*entha sp.),* Bavanchi (*Psoralea corylifolia*), Horseweed *(Conyza canadensis),* Calendula *(Calendula officinalis),* Kalanchoe *(Kalanchoe luciae),* Aloe *(Aloe vera),* Neem (*Azadirachta indica*), on the growth of dandruff causing organism has also been studied.

MATERIAL AND METHODS

Isolation and culture of the fungus

Total of 12 samples were examined for the occurrence of dandruff causing organism. The sampling was accomplished from 12 human individual's viz., 7 females and 5 males suffering from dandruff. Cotton swab technique was used for the collection from scalp and the samples were cultured on Sabouraud dextrose agar. They were incubated at 36 ± 1°C for 5 days after which the growth of the organisms was observed. The desired colonies were selected and sub cultured on PDA (potato dextrose agar), SDA (Sabouraud dextrose Agar) and mDixon's agar (Kindo et al., 2004). Coconut oil was added to PDA and SDA in the medium and similarly, Olive oil was added to mDixon's agar for the lipid requirement of the organism. The mDixon's agar was modified with olive oil; Tween 80 was used instead of Tween 20 and bile salt was not added into the medium. All the plates were incubated at 32 ± 4°C for 5 days. The organism showing desired cultural characteristics were chosen for direct microscopy with KOH 20% and methylene blue, crystal violet, lacto phenol staining. All slides were examined under 4x, 10x, 40x, 100x magnification.

Biochemical Characterization

Catalase test: It was determined by using method given by Kindo et al., 2004

Urease test: To perform this method Christensen's Urease medium was used (Cox et al., 2000)

Gelatin Hydrolysis Test: The medium contained Meat extract, Peptone, NaCl and Gelatin. All the above ingredients were dissolved in distilled water, adjusted to pH 7.6, and filtered. It was autoclaved for 10 mins at 120 ^{o}C, removed and cooled at 55 ^{o}C, when pre filtered sterilized freshly prepared Ferrous Chloride (10 % -5 ml) solution was added to it. The medium was tubed in narrow tubes and sealed with corks impregnated with paraffin wax. With the help of straight wire, the isolates were swabbed inside the gelatin tube and incubated at 20 ^{o}C for at least 7 days and further observed for results.

Sugar Assimilation Tests: This test was performed to check the utilization of 8 different carbon sources by the isolates. Eight different sugars included: Sucrose, Glucose, Dextrose, Xylose, Lactose, Mannitol, Sorbitol, and Glycerol. The medium was prepared as (Peptone: 3.6g, Sugar: 0.1g, Phenol red: 0.2%) for 100 ml. Culture suspension was added to each sugar and kept for incubation for the assimilation of sugars for 4-7 days.

Preparation of Plant extracts:
Twenty different plants (*A. concinna, M. koenigii, Ocimum sp., P. nigrum, A. marmelos, Hibiscus, P.betle, C.cyminum, E. officinalis, B. monnieri, T. procumbens, T. ammi, S. saponaria,* M*entha sp., P. corylifolia, C. canadensis, C.officinalis, K. luciae, A. vera and A. indica*) were collected from in and around botanical garden of K.T.H.M College, Nashik. The plant parts were washed thoroughly in tap water followed by distilled water and ground by using mortar and pestle. Five grams of plant material was dissolved in 100 ml of distilled water. These samples were refluxed for 2 h at 30-40 ^{o}C and supernatant was collected later. These extracts were oven dried and the dried powder was weighed and used for antifungal activity.

Antifungal assay (disc diffusion method)
The broth culture of the fungal isolates was swabbed over the Dixon's agar by using sterile cotton buds. Sterile 5mm diameter discs prepared from Whatman filter paper no.1 were placed equidistantly (3cm apart) round the margin of the plates. The absorption capacity of paper discs was found to be 5 µl per discs and therefore each sample was calculated as per 5 µl. Three replicates were maintained. The plates were incubated at 30 ± 4 ^{o}C and the zone of inhibition was observed after 2 days. Control was maintained with filter paper discs dipped in distilled water.

RESULTS

Amongst the samples cultured on SDA, PDA and mDixon's agar, 3 isolates were selected which were morphologically similar to dandruff causing organism as isolate 1, isolate 2 and isolate 3. In the present research work it was observed that mDixon's agar with the addition of olive oil as a lipid source proved better for the growth of the organism followed by SDA and PDA with coconut oil. And also it was established that Tween 80 and temperature 32 ± 4°C was suitable for the growth of organism.

Morphological characterization:
The organisms were developed as dirty white colored, smooth and pasty in appearance over the medium. Microscopy revealed that the cells were bottle shaped.

Biochemical Characterization:
Catalase test: The test was performed in duplicates and was found to be positive in all the 3 isolates.
Urease test: The test was performed in duplicates and was found to be positive in all the 3 isolates.
Gelatin hydrolysis test: The test was performed in duplicates and was found to be negative in all the 3 isolates.

Sugar assimilation test: The test was performed in duplicates for the 3 isolates and observed for the assimilation of sugars after 4 days of incubation (Table 1.). Amongst the three isolates viz., isolate 1 did not assimilate sugar; in isolate 2 only glucose was assimilated and in isolate 3 all the sugars were assimilated. Thus on the basis of sugar assimilation isolate 3 was selected for further investigation.

Table 1: Sugar assimilation test (*yellow colour shows utilization of the sugar)

No.	Sucrose	Glucose	Dextrose	Xylose	Lactose	Mannitol	Sorbitol	Glycerol
Control	Red	Red	red	red	red	red	Red	red
Isolate 1.	Red	Red	red	red	red	red	Red	red
Isolate 2.	Red	Yellow	red	red	red	red	Red	red
Isolate 3.	Yellow	Yellow	yellow	yellow	yellow	yellow	yellow	yellow

Table 2: Antifungal activity exhibited by various plants studied

Sr. No.	Plant	Zone of inhibition (mm)	Standard deviation
1	*A. concinna*	17	0.4
2	*M. koenigii*	-	-
3	*Ocimum sp.*	9	0.2
4	*P. nigrum*	-	-
5	*A. marmelos*	8	0.5
6	*Hibiscus*	11	0.3
7	*P. betle*	10	0.5
8	*C.cyminum*	-	-
9	*E. officinalis*	22	0.8
10	*B. monnieri*	-	-
11	*T. procumbens*	-	-
12	*T. ammi*	-	-
13	*S. saponaria*	10	0.2
14	*Mentha sp.*	-	-
15	*P.coryfolia*	-	-
16	*C. canadensis*	-	-
17	*C. officinalis*	12	0
18	*K.luciae*	-	-
19	*A.vera*	-	-
20	*A.indica*	-	-

Antifungal assay:

Amongst the twenty plant extracts tested, *E. officinalis* and *A. coccinna* were found to be most effective than other species. Similarly *Ocimum sp., A. marmelos, P.betle, Hibiscus, Sapindus sp. and C. officinalis* were also found to be active against the tested fungal isolate (Table 1).

DISCUSSION

The dandruff causing organism is yeast like fungus. The cells are oval and budding form similar in appearance to that of *Malassezia.* The *Malassezia* species are difficult micro-organisms to identify and maintain in culture. The present research work showed that the organism grew well at pH 5 ± 1, temperature 32 ± 4°C. The dandruff causing organism could not be grown without the use of lipids. Commonly, Sabouraud's agar is used is used for culturing of dermatophytes (Khosravi et al., 2009). Sabouraud's agar and Potato dextrose agar with coconut oil showed poor growth while mDixon's agar (Guillot et al., 1998) with olive oil improved growth. Although the morphological characteristics (colony and microscopic examination) for *Malasezzia* yeast is used for primary identification; but they do not provide sufficient information for specific identification of isolates (Khosravi et al., 2009). Thus for the specific identification of organism characterization by urease test, gelatin hydrolysis test, sugar assimilation test and Catalase test respectively was carried out in the present study. The sugar assimilation test was found positive for one of the three isolates which was further selected for antifungal activity.

Antifungal activity of plant extracts was tested *in vitro*. In the present research work among the twenty plant extracts; the extract of *E. officinalis and A. concinna* were found to be most effective against the tested fungus. Similarly *Ocimum sp., A. marmelos, P.betle, Hibiscus, Sapindus sp. and C. officinalis* were also found to reduced the growth of organism. Thus it is suggested that by making use of all combination of these plants, more effective results could be obtained as it is better to use natural anti-fungal agents as chemical anti-fungal agents possess lots of side-effects. The extractions of active principle from these plants and their assay against *M. furfur* have been suggested by Vijaykumar et al., 2006. The etiology of SD is poorly understood. Many studies have indicated that *Malassezia* yeasts play an important role in SD (Baysal et al., 2004). Many of these are treatment studies which describe the effectiveness of antimycotics.

Thus in the present research work, dandruff causing organism was isolated from the affected individuals and cultured. It's morphological, microscopical and biochemical characteristics were studied. Further on confirmation of the organism 20 plants extracts were tested for their antifungal activity. The extractions of active principle from these plants and their assay against the organism have been suggested as future course work..

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REFERENCES

Baysal V, Yildirim M, Ozcanli C and Ceyhan AM (2004) Itraconazole in the treatment of Seborrheic dermatitis: a new treatment modality. *Int. J Dermatol.,* 43(1):63-66.

Cabanes FJ, Theelen B, Castella G and Boekhout T (2007) Two new lipid-dependent *Malassezia* species from domestic animals. *FEMS Yeast Res.,* 7:1064–1076.

Cox GM, Mukherjee J and Cole GT, Casadevall A, Perfect J (2000) Urease as a virulence factor in experimental Cryptococcosis. *Infect. Immun.* 68(2): 443-448.

Faergemann J, Jones JC, Hettler O and Loria Y (1996) *Pityrosporum ovale (Malassezia furfur)* as the causative agent of Seborrhoeic dermatitis: new treatment options. *Br. J Dermatol.* 134 Suppl 46:12-15.

Guillot J, Breugnot C, de Barros M and Chermette R (1998) Usefulness of modified Dixon's for quantitative culture of *Malasezzia* species from Canine Skin. *J. Vet. Diagn. Invest.,* 10: 384-386.

Khosravi AR, Eidi S, Katiraee F, Ziglari T, Bayat M and Nissiani M (2009) Identification of different *Malassezia* species isolated from patients with *Malassezia* Infections. *World Journal of Zoology*, 4 (2): 85-89.

Khosravi AR, Eidi S, Ziglari T and Bayat M (2008) Isolation and differenciation of *Malasezzia* species isolated from healthy and affected animals, ear and skin. *World Journal of Zoology* 3 (2): 77-80.

Kindo AJ, Sophia SKC, Kalyani J and Anandan S (2004) Identification of *Malassezia* species. *Indian J. Med. Microbiol.,* 22 (3):179-181.

Pawar VC and Thaker VS (2006) *In vitro* efficacy of 75 essential oils against *Aspergillus niger*. *Mycoses*, 49: 316-323.

Pawar VC and Thaker VS (2007) Evaluation of anti *Fusarium oxysporum f.sp cicer* and anti *Alternaria porri* effect of some essential oils. *World Journal of Microbiology and Biotechnology*, 23: 1099-1106.

Prusti A, Mishra SR, Sahoo S and Mishra SK (2008). Antibacterial Activity of Some Indian Medicinal Plant. *Ethnobotanical Leaflets,* 12: 227-230.

Rukayadi Y, Yong D and Hwang JK (2006) *In vitro* anticandidal activity of xanthorrhizol isolated from *Curcuma xanthorrhiza* Roxb. *J Antimicrob Chemother.,* 57(6):1231-1234.

Vijaykumar R, Muthukumar C, Kumar T and Sarvanamuthu R (2006) Characterization of *Malasezzia furfur* and its control by plant extract. *Indian J Dermatol,* 51:145-148..