



Isolation and Characterization of the Fungi From Dandruff-Afflicted Human Scalp and Evaluation of Anti-Dandruff Shampoo

KEYWORDS

Dandruff, fungi, Malassezia, ZnPTO, Tea tree oil.

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ABSTRACT

The human scalp provides a favourable environment for the growth of a number of microorganisms. Dandruff, a common scalp disorder, is known as a causative factor of a mild seborrheic dermatitis as well as Pityriasis versicolor and atopic dermatitis. A total of 35 samples were collected from the scalp of the individuals with dandruff. The age group of the participants was in the range of 18-30 years. Out of 35 samples collected a total of 39 isolates were obtained in this study before the use of anti-dandruff shampoo. These included *Malassezia furfur* -11(31%), *Candida albicans*-4(11%), other *Candida spp*-1(3%), *Aspergillus niger*-8(23%), *Aspergillus flavus*-3(8%), *Aspergillus fumigatus*-2(6%), *Penicillium spp*-2(6%), *Microsporium spp*-5(14%) and *Trichophyton spp*-3(8%). After the use of anti-dandruff shampoo 18 isolates were obtained. These included *Malassezia furfur*-5(14%), *Microsporium spp*-2(6%), *Aspergillus niger*-6(17%), *Aspergillus flavus* -2(6%), and *Candida albicans*-1(3%), other *Candida spp*-2 (6%). Anti-dandruff activity of shampoo with tea tree oil showed higher inhibitory activity followed by shampoo with ZnPTO and with Ketoconazole. Shampoo with tea tree oil gave a zone size of more than 35mm in diameter.

Introduction

Dandruff is a non-inflammatory condition of the scalp that is characterized by scaling and is considered to be a form of mild seborrheic dermatitis. Dandruff is a common scalp disorder affecting almost half of the post pubertal population regardless of ethnicity and gender and has several putative causes including non-microbial and microbial factors (1-3). The microbial etiopathology that is most widely accepted is the presence of lipophilic yeast belonging to the genus *Malassezia* (4). The scalp has a biotic community of which the known components are *Staphylococcus spp*, *Propionibacterium spp*, and *Malassezia spp*. (5). On the dandruff afflicted scalp, the levels of *Malassezia* increase by 1.5 to 2 times their normal level (6). *Malassezia* species are also known as a causative factor in pityriasis versicolor, seborrheic dermatitis (SD), and atopic dermatitis (AD) (7, 8).

Malassezia species are lipid-dependent microorganisms that adapt to the narrow niche provided by sebum rich skin. The lipophilic dimorphic yeast *Malassezia furfur* is one of the common causative microorganisms causing dandruff along with *M.restricta* and *M.globosa*. *Malassezia furfur* requires lipid as an essential source for its growth and feeds on the dermal lipids and proteins and facilitates lipase activity, causing dermal inflammation and tissue damage. *M.globosa* lacks the ability to synthesize fatty acids. It is highly adaptive but niche dependent and is commonly found on the scalp, back, face and chest where the highest levels of sebum are produced. *Malassezia* metabolism results in increased oleic acid levels that lead to the symptoms of dandruff and other forms of dermatitis in some individuals (9).

The potential of essential oils as antimicrobial agents is well established. Essential oils are mixtures of volatile secondary metabolites isolated from different parts of aromatic plants (10). Tea tree oil is widely used as natural anti-dandruff agents in many marketed formulations like anti-dandruff shampoos, ointments, creams and lotions.

Nenoff et al., 1996 demonstrated that the essential oil of tea tree inhibits the growth of *M.furfur* (11). An anti-dandruff shampoo containing 5% tea tree oil was reported to be effective in the treatment of mild to moderate dandruff (12).

Materials and Methods

Flakes or scales were collected by partitioning the hair with a sterile comb and scrapping approximately one inch area using blunt scalpel. Two such samples were collected from each person, one before shampooing and another after using anti-dandruff shampoo. The samples were inoculated as per the standard protocols over the surface of SDA (Sabouraud Dextrose Agar) which was incorporated with chloramphenicol to avoid bacterial contaminants. The plates were incubated at room temperature (25°C) for three days and then, up to a week (13). The microscopic examination of fungal growth was observed with lactophenol cotton blue stain. Nature of mycelium and conidia formation (macro and micro conidia) helped to differentiate various genera and species. Budding yeast cells of *Candida* spp. were identified microscopically. *Candida* species were classified as *albicans* and non-*albicans* group by the production of the chlamydo spores on corn meal agar and germ tube formation. Olive oil (2%) was over laid on the media for the isolation of *Malassezia* spp. Carbohydrate fermentation test was done for yeast for the identification of species. (D-glucose, D-sucrose, D-lactose, D-maltose).

Anti-dandruff activity of shampoo by disc diffusion method

Anti-dandruff activity of the shampoo was determined by disc diffusion method against three different shampoos-shampoo-A with ketoconazole, shampoo-B with 1% ZnPTO (Zinc pyrithione) and shampoo-C with tea tree oil. The 48 hours broth cultures were uniformly swabbed onto the surface of the Sabouraud's medium. The shampoos were dissolved in their respective solvents at 10 mg/ml and 100 mg/ml concentration. The disc was dispensed with different concentration of anti-dandruff shampoos and placed

on the media inoculated with organisms. The plates were incubated at 25°C for 72 hours and the zone of inhibition was measured (14).

Results

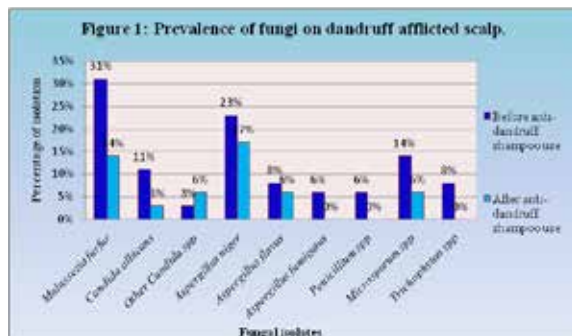
A total of 35 samples were collected from the scalp of the individuals with dandruff. The age group of the participants was in the range of 18-30 years.

Before Using Anti-Dandruff Shampoo

Out of 35 samples collected a total of 39 isolates were obtained in this study before the use of anti-dandruff shampoo. These included *Malassezia furfur*-11(31%), *Candida albicans*-4(11%), other *Candida spp*-1(3%), *Aspergillus niger*-8(23%), *Aspergillus flavus*-3(8%), *Aspergillus fumigatus*-2(6%), *Penicillium spp*-2(6%), *Microsporum spp*-5(14%) and *Trichophyton spp*-3(8%).

After Using Anti-dandruff Shampoo

After using anti-dandruff shampoo 18 isolates were obtained in this study. These included *Malassezia furfur*-5 (14%), *Microsporum spp*-2 (6%), *Aspergillus niger*-6 (17%), *Aspergillus flavus* -2 (6%) and *Candida albicans*-1 (3%), other *Candida spp*-2 (6%). (Figure 1)



Anti-dandruff activity of shampoo by disc diffusion method

Among the shampoos tested, shampoo with tea tree oil showed higher inhibitory activity followed by shampoo with ZnPTO and with Ketoconazole. Shampoo with tea tree oil gave a zone size of more than 35mm in diameter.

Discussion

Dandruff, the visible desquamation of scalp, is the mildest manifestation of seborrheic dermatitis combined with multiple host factors. The age of onset suggests that an androgenic influence may be responsible, when the level of sebaceous activity is at its peak. Dandruff is commonly aggravated by changes in humidity, trauma, season and emotional stress (15).

In the present study a total of 35 samples were collected from the scalp of the individuals with dandruff. The age group of the participants was in the range of 18-30 years. Out of 35 samples collected a total of 39 isolates were obtained in this study before the use of anti-dandruff shampoo. These included *Malassezia furfur*-11(31%), *Candida albicans*-4(11%), other *Candida spp*-1(3%), *Aspergillus niger*-8(23%), *Aspergillus flavus*-3(8%), *Aspergillus fumigatus*-2(6%), *Penicillium spp*-2(6%), *Microsporum spp*-5(14%) and *Trichophyton spp*-3(8%). After using anti-dandruff shampoo 18 isolates were obtained in this study. These included *Malassezia furfur*-5(14%), *Microsporum spp*-2 (6%), *Aspergillus niger*-6(17%), *Aspergillus flavus* -2(6%) and *Candida albicans*-1 (3%), other *Candida spp*-2(6%).

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Malassezia furfur is an important causal factor for dandruff. Several studies on the prevalence of dandruff across the world have shown a prevalence of dandruff of up to 50% in the general populations. *Malassezia furfur* was the most common organism isolated in our study both before and after the use of anti-dandruff shampoo. This was found to be in agreement with the studies of De Angelis et al.,2007 and Ranganathan & Mukhopadhyay, 2010 (16,17).

Significant number of *Aspergillus* was obtained from the individuals in this study. This exhibits the higher chances of pathogenicity and toxicosis in these individuals. Invasive sinus Aspergillosis due to *A. flavus* and massive intracranial infections also has been reported in several studies. Chances of secondary ill effects such as carcinoma is also at higher probability for such individuals. Hence significant consideration should be given for treating the mycotic infection by this fungus and presence of these fungi should be prescreened for such individuals to prevent such infections to develop and proceed at later stage (18). The presence of these non-dermatophytes (particularly *Aspergillus* and *Penicillium* species) may be due to the ubiquitous nature of their spores in our environment, carried transiently on scalp. In our study this organism was recovered mixed with other pathogens and its recovery from hair-scalp should be considered significant. This was found to be in agreement with Oyeka et al.,2002 and Okuda et al.,1989 (19, 20).

The present study confirmed that the shampoo containing tea tree oil was most effective against the fungi isolated from the scalp followed by shampoo containing zinc pyrithione and ketoconazole which correlated with the findings of Prabhmanju et al., (2009) (21). This study highlights the use of herbal shampoo with tea tree oil over chemical shampoo which is safe for topical application.

Conclusion

The present study shows mycological examination of the dandruff afflicted scalp of individuals. *Malassezia furfur* was the most common yeast isolated. Herbal shampoo with tea tree oil was found to be the most effective shampoo compared to shampoo with chemicals like zinc pyrithione and ketoconazole.

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