In-vitro evaluation of herbal and chemical agents in the management of dandruff

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ABSTRACT

Dandruff, a universal non-inflammatory scaling condition restricted to the scalp is caused by the lipophilic yeasts of the genus, Malassezia. This study investigated in-in-vitro, the anti-microbial claims of some of the current chemical and herbal products in the market. A standard isolate of Malassezia furfur was used for the in-vitro study and maintained in Sabouraud’s dextrose agar supplemented with corn oil. Multiple agents utilised in over-the-counter products claiming anti-dandruff activity were selected based on literature for this study. Among the shampoos tested, Zinc Pyrithione based shampoo’s showed a zone of inhibition of 37mm and shampoo’s containing tea tree oil showed a zone of inhibition of 39mm. The Minimum inhibitory concentration of the shampoo actives against Malassezia furfur were studied in-vitro by agar dilution method, where 10mg/ml of Zinc Pyrithione showed effective inhibition followed by 100mg/ml for tea tree oil. Zinc Pyrithione and Ketaconazole based shampoos recorded good antidandruff activity among synthetic ingredients with an ability to reduce the growth of the test organism by 67% and 44% respectively. Teatree oil scored good activity among herbal ingredients with the recorded 78% reduction in microbial growth. Synthetic drugs have the shortcomings of poor efficacy and recurrence, so herbal agents can be an effective alternative.

Keywords: Dandruff, Malassezia furfur, Chemical agents, Herbal agents.

INTRODUCTION

Dandruff is a universal scalp disorder affecting almost half of the post pubertal population of any ethnicity and gender. The exact nature and etiology of dandruff has always been controversial since the time of the Greeks, through Sabouraud’s era in the late nineteenth century till to-date [1]. Dandruff represents 25% of all scalp disorders [2] and is present in an estimated 15-20% of the total population and more than 50% of adult population [3]. Dandruff is characterized by scaling of the scalp, and is frequently associated with seborrhea, and seborrhea is the precursor of seborrhic dermatitis [5]. The lipophilic dimorphic yeast *Malassezia furfur* is one of the causative microorganisms of dandruff and it is a member of normal fungal flora of the human skin, because it may be isolated from the scalp and from the upper part of the human body in 97% of the healthy human population [6]. *Malassezia furfur* requires lipids 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 [7]. The present available treatment options in the management of dandruff include therapeutic use of zinc pyrithione, salicylic acid, imidazole derivatives, glycolic acid, steroids, sulphur and tar derivatives. However, these agents have certain limitations, either due to poor efficacies or due to compliance issues. Mostly, they do not prevent recurrence of infection [8]. Besides the chemical substance, currently herbal treatments are available. Herbal cosmetics are widely used when compared to synthetic cosmetics. In hair cosmetics, synthetic cosmetics lead to various side effects such as toxicity to eye, over drying of hair and deposition of salt on hair shaft [9]. Ayurveda has numerous natural medications wherein the most common herbs include Neem, Kapoor (naphthalene), and Henna, Hrida, Behada, and Amalaki, Magic nut, Bringaraj, Rosary Pea, Sweet Flag, Cashmere tree and Mandor found to have anti dandruff activities [10]. There are also wide range of herbal ingredients like pepper extract, basil extract, neem extract, rosemary oil, basil oil, clove oil, coleus oil, teatree oil which have been documented to have good anti pityrosporum or antidandruff activity [11].
MATERIALS AND METHODS

Standard strain used for the study
A Standard isolate of *Malassezia furfur* procured from Institute of Microbial Technology, Chandigarh, India (strain No. MTCC 1374) was used for the *in vitro* study. The isolate was maintained in Sabouraud’s dextrose agar (SDA) supplemented with 10% of corn oil. The morphology of the lipophilic yeast cells were studied by Gram stained smears of the isolates from Sabouraud’s dextrose agar after 3-4 days of incubation at 37°C. The organism was biochemically analysed for its nitrate reduction ability, where the catalase reaction was determined by application of 2-3 drops of 3% hydrogen peroxide onto a portion of a colony on a glass slide, or directly on colonies on the culture media and the organism was physiologically tested for its individual Tween utilization ability.

Antidandruff activity of shampoo actives
The antidandruff activity was performed with a little modification from procedure followed by Prasanth (2011) [12]. The different dilution of the shampoo 1:20, 1:40, 1:80, 1:160 were incorporated in SD broth, then *Malassezia furfur* was inoculated in equal volume (0.1 ml) in each dilutions and incubated at 37°C for two days under aerobic conditions. The culture broth without shampoo was kept as the positive control and the un-inoculated shampoo with its lowest dilution was kept as the negative control to measure the turbidity of shampoo in the broth. The anti-dandruff effect was tested by measuring the OD at 490 nm.

MIC (Minimum Inhibitory Concentration) determination
MIC was determined by incorporating different concentrations (by weight) from 2.5 to 250mg/ml of anti-dandruff shampoos in 10 ml of Sabouraud’s medium. Broth inoculated with test organism without the addition of shampoo was maintained as positive control. The medium with the ingredients was emulsified thoroughly with the help of Tween 20 and the tubes were incubated at 37°C for 2-3 days. After incubation, culture from each tube was re-inoculated on individual SDA plates to check for growth. The MIC was determined as the lowest concentration of ingredient that inhibited the growth of the organism when compared to control which was considered as 100% [13].

Zone of inhibition study
Diffusion dependent activities of the anti-dandruff shampoos were studied by zone of inhibition. The 48 hours broth culture was uniformly swabbed onto the surface of the Sabouraud’s medium. All the active ingredients and the shampoo were dissolved in their respective solvents at 10 mg/ml and 100mg/ml concentration. The disc was dispensed in different concentration of anti-dandruff shampoos and placed on the Petri plate seeded with organisms. The plates were incubated at 37°C for 72 hours and the zone of inhibition was measured [14].

RESULTS

Characterization of *Malassezia furfur*
Thick walled spherical Bottle shaped yeast cells were observed in clusters under the oil immersion objective (Figure 1). The colony morphology of yeast culture on Sabouraud’s dextrose agar was studied as cream to yellowish, smooth, lightly wrinkled, dull with the lobate margin (Figure 2). The formation of red colour after the addition of sulphanilic acid and α-Naphthalamine indicated a positive reaction in the nitrate reduction test. The production of gas bubbles indicated a catalase positive reaction. The utilization of Tween was observed at different concentrations.
The degree of growth increased as the concentration of the Tween increases and was recorded most suitable when supplemented with Tween 80 (Figure 3).

**Antidandruff activity of shampoo actives**

The different dilution of the shampoo incorporated in culture broth inhibited the growth of the organism in various degrees. As the concentration of the shampoo decreases, the growth of the organism increases. The degree of growth was decreased by 33% in Zinc pyrithione (ZnPTO) based shampoo at 1:160th dilution when compared to other shampoos containing the synthetic agents ketoconazole and climbazole. So, the Zinc pyrithione shampoo proved to be best among synthetic ingredients used. Similar results were observed for Tea tree oil based shampoo with a 78% reduction in growth among four different herbal ingredients (Figure 4).

**MIC (Minimum Inhibitory Concentration) determination**

Agar dilution method was used for the evaluation of shampoos (Ranganathan et al., 1996). When different concentration of shampoos from 2.5mg to 150mg containing active ingredients were used as a culture medium, ZnPTO based shampoos recorded good antifungal activity among synthetic ingredients with inhibitory concentration of 10mg/ml. Tea tree oil recorded good activity with 100mg/ml as MIC among herbal ingredients (Table 1).

The MIC was determined as the lowest concentration of ingredient that inhibited the growth of the organism when compared to control. Diffusion dependent activities of the anti-dandruff shampoos were studied by zone of inhibition study.
Figure 5: Zone of Inhibition of Shampoo Actives

A1 - Base Shampoo, 0.1 Dilution
B1 – Ketoconazole, 0.1 Dilution
C1 – Shampoo A – 1% ZnPTO, 0.1 Dilution
D1 – Shampoo B – 1% ZnPTO, 0.1 Dilution
A2 – Base Shampoo, 0.01 Dilution
B2 – Ketoconazole, 0.01 Dilution
C2 – Shampoo A – 1% ZnPTO, 0.01 Dilution
D2 – Shampoo B – 1% ZnPTO, 0.01 Dilution

A1 - A2: First Column
B1 - D1: Second Column
C1 - D2: Third Column

Concentration of shampoos in different dilutions

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Table 1: Antidandruff activity of commercially available shampoo with synthetic and herbal active ingredients

<table>
<thead>
<tr>
<th>S.No</th>
<th>Shampoo with Actives</th>
<th>MIC (mg/ml)</th>
<th>ZOI Diameter(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Base Shampoo</td>
<td>250</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>Ketoconazole</td>
<td>15</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>Shampoo A with 1% ZnPTO</td>
<td>10</td>
<td>37</td>
</tr>
<tr>
<td>4</td>
<td>Shampoo B with 1% ZnPTO</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>Shampoo C with 1% ZnPTO &amp; Climbazole</td>
<td>10</td>
<td>37</td>
</tr>
<tr>
<td>6</td>
<td>Eucalyptus</td>
<td>150</td>
<td>22</td>
</tr>
<tr>
<td>7</td>
<td>Tea Tree Oil</td>
<td>100</td>
<td>39</td>
</tr>
<tr>
<td>8</td>
<td>Amla, Licorice</td>
<td>150</td>
<td>11</td>
</tr>
</tbody>
</table>

ZnPTO – Zinc Pyrithione; MIC – Minimum Inhibitory Concentration; ZOI – Zone of Inhibition

Zone of inhibition study
Among the shampoos, those containing ZnPTO and Ketoconazole recorded higher activity levels. Similar results were observed in Tea tree oil based shampoo among herbal ingredients (Figure 5 & 6). Zinc Pyrithione showed a zone of inhibition of 37mm and tea tree oil with a zone of 39mm diameter (Table 1). The lack of ZOI for the actives may be attributed to their inability to diffuse through the agar medium.

DISCUSSION
Dandruff, the visible desquamation of scalp, is the mildest manifestation of seborrhic 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]. *Pityrosporum* organisms are linked to T-cell depression, increased sebum levels and an
activation of the alternative complement pathway [16]. The components of a fungus play an important role in the interaction with the host immune cells [17]. The synthetic treatment options available have certain limitations, which may be either due to poor efficacies or due to compliance issues. Furthermore, these synthetic drugs are unable to prevent recurrence, the common problem associated with them [18]. Only in recent years it has been accepted that the human disease known as dandruff is actually caused by a yeast that in many instances is part of man’s normal scalp flora. Identifying yeast as the causative agent creates the potential for being able to control this condition with antifungal agents. It was observed that all of the assayed products were able to kill the yeast when used in high concentrations. The antifungal effects were found to be present in all of these products when diluted up to 1:1000. The experiments were designed in a way to determine which one of these shampoos had the greatest antifungal activity. To determine this, dilutions were made with the various commercially available hair shampoos to the point were the weaker ones showed no antifungal activity and the stronger ones were still effective. These findings show that these products have antifungal activity in vitro against the yeast which causes dandruff. The study confirmed that the shampoo’s containing ketoconazole and Zinc pyrithione were the most effective chemical agents against the Malassezia furfur, which correlated with the findings of Prabhamanju et al., (2009). These two drugs have been on the market for over two decades and their side effect profiles (safety) were good. Thus, it is an excellent and extremely safe product when used topically [19]. The herbal drugs prove to be an alternative for synthetic drugs which have the shortcomings of poor efficacy and recurrence [14]. The shampoo’s with most effective herbal agent against the yeast was Tea tree oil which is similar to the results obtained by Prabhamanju et al., (2009), where the tea tree oil activity was tested against Malassezia furfur. Hence this study aids in choosing the most suitable over-the-counter product available against dandruff in the Indian market and also helps in comparing while assessing the grade of the new product tested for antidandruff activity.

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REFERENCES

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