Comparison Of Anti-Dandruff Activity Of Synthetic Shampoos And Crude Plant Extracts On Dandruff Causing Isolates

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Abstract:Dandruffis very rampant and the market loaded with antidandruff shampoos and other skin care products, There is need to assume resourceful research into this area, To check the antidandruff activity of such antidandruff products, experiments was carried out on Malassezia furfur the causal organism for dandruff which was isolated using SDA with olive oil. The anti-dandruff activities of the synthetic shampoos and plant extracts were compared. Among the plant extracts tested, Lemon showed the highest zone of inhibition followed by lime, Neem plant and aloe vera while among the synthetic antidandruff shampoo venoz showed the highest zone of inhibition followed by Adal A hair shampoo, vintage and 24 hours shampoo. Comparatively the plant extract shampoos showed a high zone of inhibition than the synthetic shampoos. Medicinal plants have some natural Antimicrobial property and therefore such combination could be a potential antidandruff activity.

Keywords: Dandruff, Synthetic products, Shampoo, Plant extractand Malassezia furfur.

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I. Introduction

Dandruff is a scalp disorder which is characterized by excessive shedding of skin cells from the scalp. It is a common problem faced by people of all age groups. As the epidermal layer continually replaces itself, cells are pushed outward where they eventually die and flake off. For most individuals, these flakes of skin are too small to be visible. However, certain conditions cause cell turnover to be unusually rapid, especially in the scalp. It is hypothesized that for people with dandruff, skin cells may mature and be shed in 2–7 days, as opposed to around a month in people without dandruff. The result is that dead skin cells are shed in large, oily clumps, which appear as white or grayish flakes on the scalp, skin and clothes [1].Yeast like lipophilic basidiomyceteous fungus *Malassezia furfur* [*Pytirosporum ovale*] is the causative organism for dandruff [2].

Malassezia converts the sebum lipid into fatty acids and triglycerides, which accelerate hyperproliferation of keratinoytes [3]. The treatment options [ointments, lotions, shampoos] currently available for management of dandruff have zinc pyrithione, salicylic acid, imidazole derivatives, selenium sulphide, tar derivatives, ketocanazole etc. as key ingredients[4]. These synthetic treatment options have certain limitations, which may be due to poor efficacies or due to compliance issues[4]. These are unable to prevent reoccurrence of dandruff with side effects that cannot be neglected. The best approach to treat dandruff is to use plants and herbal formulations which possess antidandruff properties. Studies evaluating antifungal effect of essential oils have also been reported [5],[6]Antifungal activity of different plant extracts against Malassezia furfur is carried out in this work. Various natural plant extracts are known for their antidandruff properties. Evaluation of antifungal properties of such plant extracts can be done and they can be used effectively as an alternative to chemical agents in various antidandruff formulations. Along with anti-fungal properties, plant extracts are also known for their conditioning properties which will be fruitful in maintaining the overall health of scalp and hair [7], Older literature cites the fungus Malasseziaglobosa (previously known as Pityrosporumovale) as the cause of dandruff. While this species does occur naturally on the skin surface of both healthy people and those with dandruff, in 2007 it was discovered that the responsible agent is a scalp specific fungus, Malassezia furfur that metabolizes triglycerides present in sebum by the expression of lipase, resulting in a lipid by-product oleic acid. The increasing antimicrobial resistance exhibited by microorganisms causing dandruff infections has led to extensive research on the therapeutic potential of anti dandruff herbal plants. This work is aimed at investigating the organism causing dandruff and comparing the effectiveness of synthetic antidandruff shampoo and various natural plants extracts which are known for their antidandruff properties.

II. Materials And Methods

Different plant extracts that possess various active compounds which have antifungal activity were used and some synthetic shampoo was used. The inhibitory action was studied using agar well diffusion assay and the results indicated in percentage of inhibition.

Sample Collection:

The organism was isolated from scalp of persons suffering from Dandruff Flakes or scales, collected by partitioning the hair with a sterile comb and scrapping approximately one inch area using a sterile blunt scalpel. The specimen was then transferred into a dark sampling paper to prevent exposure to sunlight. The samples was inoculated over the surface of Sabouraud Dextrose Agar (SDA) plus olive oil which was incorporated with chloramphenicol to avoid bacterial contaminants. The plateswas then incubated at 30°C for 7 days and close observation was employed regularly.

Sample Analysis:

Culture media preparation

The culture media used was Sabouraud Dextrose Agar (SDA) which was prepared according to manufacturer's specification and sterilized in an autoclave at 121°C at 15psi for 15mins.

Direct microscopy: A drop of 10% KOH was added onto a clean slide containing the smear of sample and the smear was covered with a cover slip. The slides were viewed under 40X objective lens[8].

Culture: The collected samples were cultured on SDA which was incorporated with chloramphenicol to discourage the growth of bacterial contaminants. Small amounts of the samples collected were introduced into petri dishes containing the media using sterile forceps. The petri dishes were labelled accordingly and incubated at 30 $^{\circ}$ C for 7 days [9].

Biochemical Tests

Catalase Test: Catalase test was carried out to ascertain the presence of *Malasseziaspecies* as it is catalase positive, except M. *restricta* which is catalase negative. 3 mL of 3% hydrogen peroxide (H_2O_2) solution was poured into a test tube. Several colonies of the isolated fungal colonies were immersed into the test tube using a sterile glass rod. The gas bubbles observed is the breakdown of hydrogen peroxide to oxygen and water by enzyme catalaze.

Esculin Hydrolysis Test: The medium used was bile esculin agar slant which is a nutrient agar-based medium containing 0.1% esculin and 10% bile salts, and allowed to solidify as a slant. The bile salt inhibits some bacteria, and also shows the ability to grow in the presence of bile salts represents a second test use for the medium. Inoculums from a pure culture was aseptically transferred into a sterile tube of bile esculin agar and streaked along the slant. The inoculated tube was incubated at 30°C for 24 h and the result was determined.

Staining with Lactophenol cottonBlue

A drop of lactophenol cotton blue was introduced at the centre of a clean slide. fragment of the fungus colony 2-3mm from the colony was collected using wire loop and the fragment was droped on the stain and teased gently, a cover slip was applied. The preparation was examined under low and high magnifications. **Preparation of crude extract**:

Preparation of crude extract:

Collected plant material was washed with sterile distilled water and triturated individually by usingsterile mortar and pestle. Then the extract was filtered, centrifuged and the supernatant was used for antidandruff activity.

Antifungal activity of plant extracts

The antifungal activity of different extracts on *Malasseziafurfur* was investigated where the agar is poured into a petri dish and allowed to cool then the media was coated with a drop of olive oil and then the organism was spread uniformly over the agar surface. Wells were punched aseptically with cork borer round the margin of the plates equidistantly (3cm apart). In to each of these wells 50microlitres of extracted solutions were placed carefully. The plates were allowed to diffuse for 30 minutes and incubated at 37°C for 72 hrs. In all cases zones of inhibition may be observed, the diameter of the zones giving a rough indication of the relative activities of different anti microbial substances. After incubation zone of inhibitions were measured using a meter rule.

III. Results

Among the plant extracts tested Lemon showed the highest zone followed by lime, Neem plant and aloe vera while among the synthetic antidandruff shampoo venoz showed the highest zone followed by Adal A hair shampoo, vintage and 24 hours shampoo. Comparatively the plant extracts shampoos showed a high zone of inhibition than the shampoos. The inhibition zones of antidandruff shampoos almost matched with those of plant extracts but the sum effect of the later were greater.

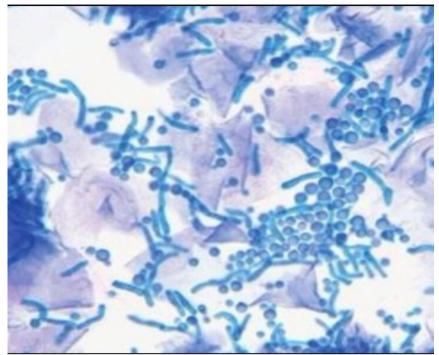


Figure 1: Microscopic view of fungal isolate

Table 1: Morphological and Biochemical Characteristic of Dandruff causing Iso	olate
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Colony characteristics	Observations
Size	1-3 mm
Shape	Circular
Colour	Whitish
Margin	Entire
Elevation	Convex
Opacity	Opaque
Consistency	Creamy
Texture	Smooth
Lactophenol cotton blue Staining	meat ball shaped fungus
Esculin Hydrolysis Test	positive

Table 2: Zone of Inhibition of Plant Extracts			
Name of plant	Zone of inhibition		
Lemon	28mm		
Aloe vera	11mm		
Lime	25mm		
Neem plant	13mm		

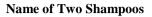
Table 3: Zone of Inhibition of Anti Dandruff Shampoos

Name of shampoo	Zone of inhibition
Venoz	20mm
24 hair shampoo	8mm
Adal A hair shampoo	19mm
Vintage	13mm

Table 4 : Zone of Inhibition of Combination of Two Plant Extracts

Name of Plant extracts	Zones of inhibition	
Lemon and Aloe vera	18mm	
Lime and Neem Plant	12	
Lime and Neem Plant	13mm	

Table 5: Zone of Inhibition of Combination of Shampoos





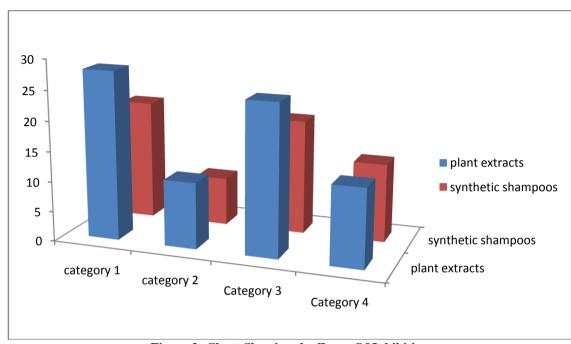


Figure 2: Chart Showing the Zones Of Inhibition

IV. Discussion

Dandruff is a common disease caused by *Malassezia* species especially *Malasseziafurfur*. The lipolytic activity of these organisms induces hydrolysis of human sebum triglycerides in to free fatty acids that cause both hair loss and scalp. Medically significant fungi are known to grow on Sabouraud's agar medium. The present isolate being lipolytic grew well on olive oil this is in accordance with other reports on growth of *Malassezia species*. All the antidandruff shampoos had good antifungal activity but there is considerable variation in the potency of their antifungal activity depending on the active compound. This work corresponds to the work of previous author [10], who reported that the causative organism isolated as *Malasseziafurfur* and further studied the antifungal activity of various plant extracts and synthetic shampoo on the organism and found out that the more zone of inhibition was shown by plant extracts than synthetic shampoo with sun flower (30mm using water as extract) having the highest zone of inhibition followed by lemon (27mm), aloe vera (17mm), lime (15mm), hibiscus plant and neem plant (10mm) while the best antidandruff shampoo was Vivel Ultra Pro (20mm) as it contains Ketocanozole which is reported to be anti-malassezial agent. This was followed by Dove (14mm) and Head and Shoulders (10mm) as they contain antifungal compounds like Zinc Pyrithione therefore comparatively the plant extracts showed a high zone of inhibition than the shampoos. The inhibition zones of antidandruff shampoos at low concentrations almost matched with those of plant extracts.

Also, according to[11] both crude and powdered extracts were prepared and tested against *Malasseziafurfur* and zone of inhibitions was measured. Out of the selected plant parts, lemon juice & lemon peel powder showed maximum activity. Next to lemon extracts a good activity was observed with Amla, shikakai, henna, aloe vera extracts. Lemon, aloe vera had good antifungal activity as compared to other plant extracts. Lemon juice (2.4cm), henna extract (2cm) and amla (1.8cm) combinations showed best activity as compared to other combinations. Antidandruff activities of three different branded antidandruff shampoos were also studied and their zone of inhibitions noted. These results were considered as standard reference and compared the results of the extracts with that of the shampoos. On comparison one can say that the plant extracts showed a considerable activity against dandruff causing organism *Malasseziafurfur* and can be used to treat dandruff which causes no side effects.

we can widely use the plant extracts for the anti dandruff activity against *Malasseziaspecies* and it is cheap and less side effect due to its herbal property but [12]discovered that Herbal anti-dandruff shampoos were also found to be effective but their anti-dandruff effect was less compared to synthetic ones. Further this research work can help educate patients on the use of natural plants for the treatment of dandruff infection and use of natural plant products is not only cost effective but also negligible side effects and synergistic mixtures of plant extracts could help eliminate dandruff which is a major cosmetic problem, faster and effectively.

V. Conclusion

This study has proved that plant extracts could be used to combantdandruffs and their efficacies was not questionable .The Findings and knowledge got from the research could be explored in the production of polyherbal mixture that could be incorporated in hair oil or shampoos for better anti-dandruff activity or even encourage the use of whole plant extract for treatment rather than synthetic products which could have long term effects on the users.

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