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Comparison of antifungal activity of plant extracts and shampoos against dandruff causing organism Malassezia Species

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ABSTRACT

Dandruff is a scalp disorder whose characteristic feature is excessive shedding of skin cells from the scalp. It is a common problem faced by people of all age groups. It is one of the major cosmetic problems as it ultimately leads to hair fall. Malassezia furfur, a lipophilic basidiomycetous fungus is a causative organism for dandruff. As dandruff is globally prevalent, it needs an effective therapeutic remedy. Presently people are depending on commercial shampoos as a treatment for dandruff. However, plant products contain various compounds like alkaloids, flavonoids, tannins, terpenoids etc. which have efficient antifungal activity. A comparative study of the effect of commercial antidandruff shampoos and natural plant products to evaluate their anti-fungal efficacy lead to the conclusion that the activity of some of the natural extracts was equivalent to that of the commercially available branded shampoos. As crude herbal drugs have been included in traditional medicine and household remedies for a long time, regular usage of these tested plant extracts can reduce the incidence of dandruff.

Keywords— *Dandruff, Malassezia furfur, Causative organism, Therapeutic remedy, Shampoos, Plant extracts*

1. INTRODUCTION

Dandruff is a skin condition that mainly affects the scalp. Flaking and sometimes mild itchiness are the main symptoms of dandruff. It is a common scalp disorder and also a major cosmetic problem as it leads to hair fall problems (Ravichandran, G. and Shivaram Kolhapur, S.A., 2004). It can result in social or self-esteem problems. It is a common problem faced by people of all age groups.

The underlying mechanism of dandruff involves the overgrowth of skin cells. As the epidermal layer continuously replaces itself, the cells are pushed outwards, where they gradually 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 greyish flakes on the scalp, skin and clothes (De Angelis Y. M, *et al*., 2005).

Yeast like lipophilic basidiomycetous fungus *Malassezia furfur* earlier known as *Pittosporum ovale* is the causative organism for dandruff (Arora, P., *et al*., 2011). Dandruff is medically described as Pityriasis capitis caused by Malassezia species like *M.furfur, M.globosa, M.restricta* (Shuster, S., 1984). Malassezia species formerly known as Pityrosporum is a lipophilic, dimorphic opportunistic yeast causing skin and hair infections like pityriasis versicular, seborrheic dermatitis and dandruff (Gupta, A.K. *et al*., 2004 and Vijayakumar, R. *et al*., 2006)

It is a known fact that there is no complete cure for this disease. As this disease is known to occur all over the world it needs effective therapeutic remedy. There are natural effective remedies to control dandruff in Ayurveda (Sonica Krishnan, 2011), but nowadays people are depending on commercial shampoos containing antifungal compounds like miconazole, ketoconazole, selenium sulphide etc. Plant products contain various compounds like alkaloids, flavonoids, tannins, terpenoids etc which have efficient antifungal activity [Agrawal DP, 2001] [Saneesh Kumar, 2013]. The present work was a comparative study of the effect of commercial antidandruff shampoos and natural plant products to evaluate their antifungal efficacy.

2. METHODOLOGY

- Isolation of culture: Scrapping of dandruff was collected from people with the help of moist swab and was streaked on the Sabouraud's dextrose agar plates. The plates were incubated at 25℃ for 5 – 7 days. The isolate was maintained on Sabouraud's media slants and stored in a refrigerator at 40°C for one month.

- Growth and Identification: The isolate was screened by plating the scalp swab on Sabouraud's media enriched with 2 % lipid source like olive oil. The organism was identified based on cultural, microscopic and biochemical methods.
  - Cultural: Growth pattern and colony morphology was observed on Sabouraud's media enriched with a lipid source like olive oil/ butter (Kaw Bing Chau, *et al*., 2005)
  - Microscopic: Wet mount of the fungal culture was prepared and flooded with lacto phenol cotton blue stain and was examined under a microscope using 10 x objective lens.
  - Biochemical: The organism was biochemically tested by using gelatin hydrolysis test, litmus milk reaction, fermentation of carbohydrates like dextrose, xylose, rhamnose, raffinose and mannitol and the results were recorded (Nakabayashi, A., 2000).
- Inoculum preparation: The inoculum of *Malassezia furfur* was prepared by inoculating in 5ml of Sabouraud's broth and incubated at 25°C such that there are 10^6 cell/ ml (Nakamura, Y., 2000).
- Dilution of shampoos: The commercially available shampoos as mentioned in (Table 1) were diluted with sterile distilled water to get 10 fold dilutions. These were used for antifungal assays.

Table 1: List of the anti-dandruff shampoos used

—	**Shampoos**
—	Dove
—	Head and shoulders
—	All clear
—	Pantene

- Preparation of plant extracts: The different plant sources tested are mentioned in (Table 2) along with their generic names, appropriate plant part used and dosage (Saneesh Kumar, 2013). The plant part was collected from the plant source washed thoroughly, cut into smaller pieces and ground into a fine paste. The fine paste was made into a solution with sterile distilled water, centrifuged at 5000 rpm and the supernatant was used as a sample for anti fungal assays.

Table 2: List of different plant extracts used:

S. no	Name of the plant	Part used
1	*Acacia concinna*	Nuts
2	*Hibiscus rosasinensis*	Flower
3	*Hibiscus rosiness*	Leaves
4	*Lawsonia inermis*	Leaves
5	*Phyllanthus emblica*	Fruit
6	*Allium cepa*	Bulb
7	*Aloe vera* leaves	Leaves
8	*Azadirachta indica*	Leaves
9	*Azadirachta indica*	Fruit
10	*Citrus lemons*	Fruit
11	*Sapindus mukorossi*	Nuts
12	*Murraya koenigii*	Leaves
13	*Fenugreek*	Seeds

- Antifungal Assays: Antifungal activity of different plant extracts on *Malassezia furfur* was investigated by cup plate method or agar well diffusion method (Finn, R.K., 1959). The media was coated with a drop of olive oil and then the organism was spread uniformly over the agar surface. Wells have punched aseptically with cork borer round the margin of the plates equidistantly (3cm apart). In to each of these wells, 100 µl of extracted solutions were placed carefully. The plates were allowed to diffuse for 30 minutes and incubated at 24℃ for 48 hrs. After incubation, the zone of inhibition (in mm) was measured. The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimetres. Simultaneously, the antifungal activity of commercially available antidandruff shampoos was also determined.

3. RESULTS

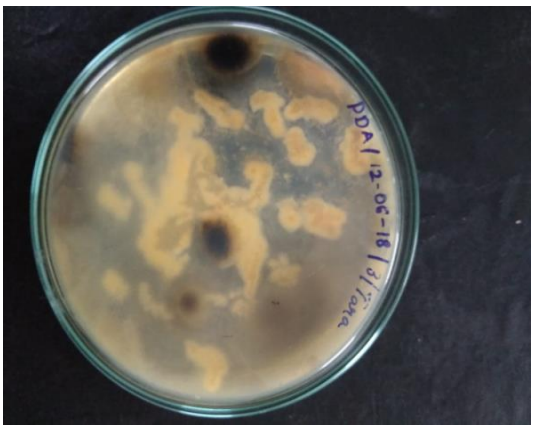

Fig. 1: Colony morphology

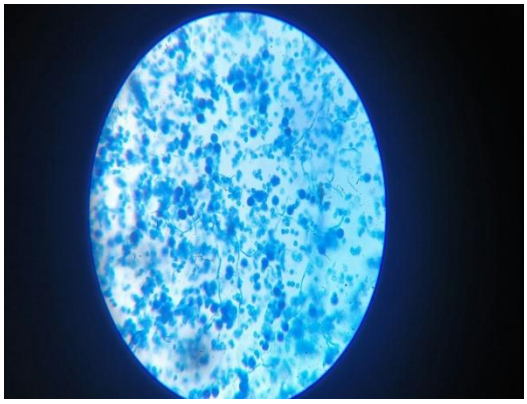

Fig. 2: Physical characterization of fungal strain using lacto phenol cotton blue

Malassezia furfur grew as white to the tan cream coloured colony with smooth pasty consistency on Sabouraud's media (Fig 1) and the cells appeared bottling shaped when observed microscopically (figure 2). The Biochemical studies indicated that fermentation of dextrose and xylose produced acid but no gas. Maltose, lactose, rhamnose, raffinose and mannitol were not fermented by *M. furfur*. Liquefaction of gelatin was observed and there was acidification of litmus milk.

3.1 Antifungal activity

Agar well diffusion was carried out using 200µg/ml concentration of plant extract. The zone of inhibition was measured and recorded which is shown in table 3. Figures 3 to 7 show the antifungal activity of different plant extracts and shampoos against dandruff causing organism.

Table 3: Antifungal activity of plant extracts and shampoos

S. no	Plant extract (concentration of extract 200µg/ml)	Zone of inhibition (in mm)
01.	*Acacia concinna*	NIL
02.	*Hibiscus rosa sinesus (leaf)*	27
03.	*Phyllanthus Emblica*	16
04.	*Fenugreek seeds*	NIL
05.	*Allium cepa*	NIL
06.	*Aloe vera*	16
07.	*Sapindusmukorossi*	12
08.	*Lawsonia inermis*	13
09.	*Hibiscus rosa sinesis (Flower)*	NIL
10.	*Azardirachta indica (leaf)*	14
11.	*Citrus limon*	30
12.	*Murraya koenigii*	NIL
13.	*Azardirachta indica (fruit)*	13
14..	Dove	22
15.	Head and shoulders	23
16.	All clear	33
17.	Pantene	21

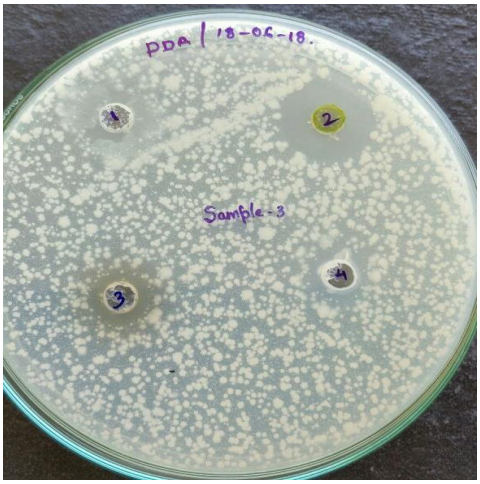

Figure 3: Antifungal activity of different plant extracts (a)

1. *Acacia concinna*
2. *Hibiscus rosasinensis*
3. *Phyllanthus emblica*
4. *Fenugreek seeds*

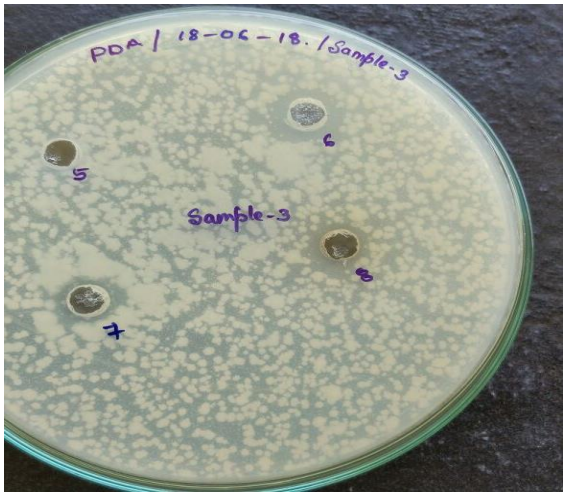

Fig. 4: Antifungal activity of different plant extracts (b)

5. *Allium sepa*
6. *Aloe vera*
7. *Sapindusmukorossi*
8. *Lawsoniainermis*

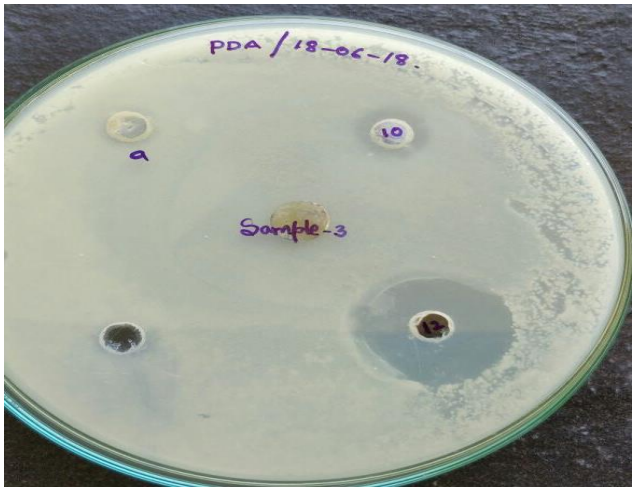

Fig. 5: Antifungal activity of different plant extracts (c)

9. *Hibiscus rosasinensis*
10. *Azadirachtaindica*
11.*Citrus limon*

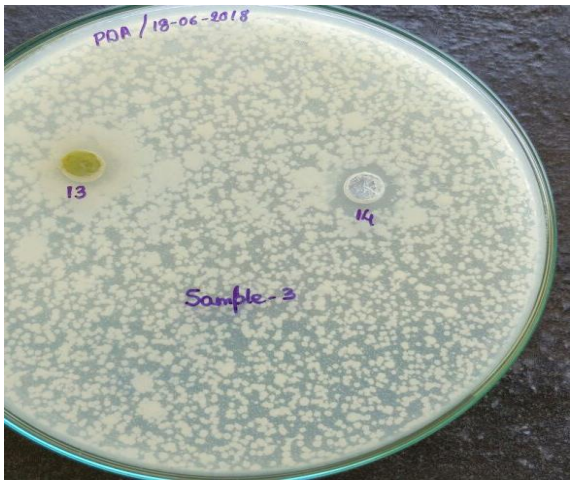

Fig. 6: Antifungal activity of different plant extracts (d)

13. *Murrayakoenigii*
14. *Azadirachtaindica*

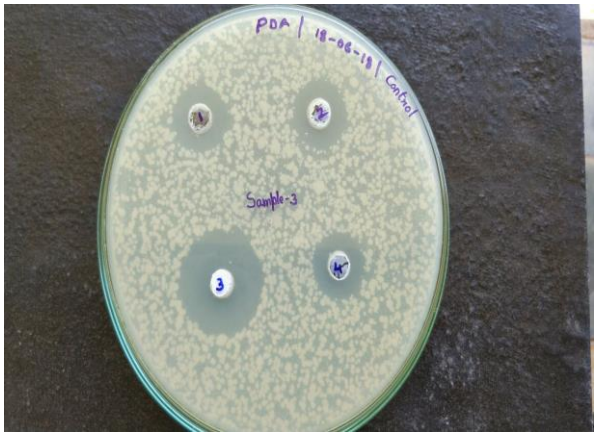

Fig. 7: Antifungal activity of shampoos

1. Dove
2. Head and shoulders
3. All clear
4. Pantene

4. DISCUSSION

Crude herbal drugs have been included in traditional medicine and household remedies for a long time. Not all herbal preparations have been scientifically tested. Many studies are reported on the antifungal activity of plant essential oils against dandruff causing fungi Malassezia furfur. There are meagre studies on the effect of plant extracts on these fungi. In an attempt to determine the benefits of various herbal extracts, the effect of different plant extracts against *Malassezia furfur*, the most common cause of dandruff. The Plants were selected based upon their usage as traditional medicine for treating dandruff. Both crude and powdered extracts were prepared and tested against Malassezia furfur by disc diffusion method and zone of inhibitions was measured.

Out of the selected plant parts, Lemon juice showed maximum activity. Next to lemon extracts a good activity was observed with Hibiscus leaf, Amla, Aloe vera, Henna, Neem leaf, Neem fruit &Shikakai extracts. Lemon, hibiscus leaf, amla, shikakai and neem had good antifungal activity as compared to other plant extracts which were made and checked for their synergistic activity against *Malassezia furfur*.

Antidandruff activities of four 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 considerable activity against dandruff causing organism *Malassezia furfur* and these extracts can be used for the treatment of dandruff without any side effects.

5. CONCLUSION

Of all the tested extracts, Lemon and Hibiscus leaf showed maximum antifungal activity against ***Malassezia species,*** the causative agent of dandruff. The activity was equivalent to that of the commercially available branded shampoos.
From the above results, we conclude that regular usage of plant extracts can reduce the incidence of dandruff.

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