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An investigation on dandruff and development of a herbal antidandruff agent

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Abstract
Dandruff is a major cosmetic concern in the world despite the availability of numerous antidandruff medications and products. It is a clinical condition caused by the lipophilic fungus *Malassezia furfur*. The present study is an honest attempt to isolate and identify the causative agent of dandruff, to test the efficacy of petroleum ether, ethanol and aqueous extracts of four medicinal plants-*Wrightia tinctoria, Elephantopus scaber, Naregamia alata* and *Azadiracta indica* in inhibiting the growth of the fungus, to compare the effectiveness of five widely used commercial antidandruff shampoos and finally to formulate a shampoo from the most effective plant extracts among these. Aqueous and ethanolic extracts of *Wrightia tinctoria* leaf has notable potential in inhibiting the fungal growth and is used to formulate a herbal shampoo. The physico-chemical parameters, conditioning effect and the antifungal properties of the shampoo have been evaluated. The herbal shampoo exhibited better antimycotic activity compared to commercial antidandruff shampoos. The results suggest the potential of *W.tinctoria* leaf extract as an effective antidandruff agent.

Keywords: *Malassezia furfur*; dandruff; antifungal activity; *Wrightia tinctoria*

Introduction
Plant based medicines and herbal products act as important therapeutic weapons to cure human diseases and cosmetic concerns. Formulation of herbal medications has surged in recent research endeavors and has been a prime investigation objective. Dandruff is a clinical condition, an ultimate remedy for which is still a question. Being a scalp condition with white flaky breakoffs and irritancy ranging from moderate to severe, dandruff remains a major scalp health concern worldwide [1]. It represents 25% of all scalp disorders and is present in an estimated 50% of the adult population. The pathogenesis of dandruff is manifested by the hyper proliferation of the fungus under the scalp skin leading to deregulation of keratinization. Salicylic acid and sulfur compounds are keratolytic agents constituting most anti dandruff shampoos [2]. They act by softening, dissolving and releasing the attachments between the corneocytes and allow them to be washed away with shampooing, which gives a temporary relief but fail to eliminate the chances of reoccurrence of the condition. *M. furfur* is a scalp commensal yeast that feeds on lipophilic exudates. It is a notable member of the genus Malassezia which belongs to the division Basidiomycota, class Exobasidiomycetes and the order Malasseziales of the kingdom Fungi. During extensive proliferation of the fungus, the natural renewal of the scalp cells is hindered and results in dandruff. The fungal exudates cause irritation and inflammation of the skin. Formulations with active anti-fungal principles rather than keratolytic agents are required to effectively cure dandruff. The commercial anti dandruff shampoos have proven side effects including dryness, oiliness and irritation of scalp, skin and mucous membranes of eyes, discoloration and loss of hair. This is where a reliable herbal remedy becomes a necessity. *Azadiracta indica* and *Wrightia tinctoria* are known to have anti-fungal properties long before. *Naregamia alata* is rich in essential oils and *Elephantopus scaber* is being investigated for its medicinal properties. Apart from the antimicrobial activity, the essential oils present in these plants helps in enhancing the texture and growth of hair. A shampoo formulation that incorporates the anti-dandruff and hair follicle enhancing properties of plants can be an effective substitute to the currently available products [3].

Materials and methods
Sample collection, isolation and selection of *Malassezia furfur*
Fungal samples were collected by scraping the dandruff flakes from the scalp using a sterile cotton swab and were dissolved in distilled water. The supernatant was collected and inoculated into SDA broth (Sabouraud Dextrose Agar) in a sterile culture tube and was incubated overnight. This culture was then kept in refrigerator for further studies [7] .Culture plates were made by swabbing the inoculum from this fungal culture over petri plates. Olive oil was spread above the medium as a lipid source to enhance the fungal growth. Dextrose in the medium served as the source of carbohydrate [12]. The pH was adjusted to 6-optimum for the fungal growth. The plates were incubated for 2 days at room temperature [4]. The colonies formed were smooth, pasty and yeast like, characteristic of *M. furfur*. A sample obtained from these colonies was used for further identification.

In vitro identification of *Malassezia furfur*
Malassezia furfur was identified done by adopting the standard procedures, wherein both morphological and biochemical tests were involved as detailed below

- Morphological test

Few drops of methylene blue stain was added over a heat fixed smear of fungus on a clean glass slide [25], kept for 5 minutes, washed with distilled water and examined under

high power oil immersion microscope and the characters were recorded.

- Biochemical test (Catalase test)

3 ml of 3% hydrogen peroxide solution was poured into a test tube and isolated fungal colonies were immersed into the test tube using a sterile glass rod. Active bubbling was observed, signifying the release of Oxygen from H_2O_2.

Preparation of plant extracts

The plant parts were thoroughly washed and dried for 3 weeks in shade, powdered and stored in sterile bottles. The plant extracts were prepared by immersing 10 g each of the powdered plants bound in Whatmans' filter paper in 30 ml each of ethanol, petroleum ether and water. The extraction was done overnight.

Anti-mycotic assay (Disc diffusion method)

To carry out anti-mycotic assay, culture plates were prepared by swabbing the broth culture of *M. furfur* across the plates with SDA medium. Paper discs with a diameter of 0.5 cm were cut out from Whatman No.2 filter paper. 1 ml each of the extracts was diluted with 1 ml distilled water and paper discs dipped in the extracts were placed over the plates. The plates were incubated at room temperature for one day and the zone of inhibition formed was measured.

Comparative study of commercial shampoos

The antidandruff efficacy of five commercial shampoos was studied by disc diffusion method. 2 mg each of the shampoos were weighed out and diluted with 1 ml of distilled water in eppendorf tubes and tested for anti-mycotic activity and the zone of inhibition formed was measured. Ketoconazole was used as the control.

Formulation of shampoo using the most effective plant extracts

The plant extract that showed the maximum zone of inhibition was selected to formulate the shampoo. Taking into account the essential factors required for an appealing cosmetic product, the formulation was developed [7]. The constituents of the formulation are as follows:

Sodium lauryl sulphate (20%), citric acid (1%), Sodium chloride (2%), hibiscus juice (15%), honey (5%), herbal extract (2%), essential oil (2%) and distilled water (q.s).

Evaluation of the properties of the shampoo formulation

Simple, reproducible and quantitative determination of various properties of the shampoo formulation was done [10].

- pH **:**The pH of the formulation was determined at 25^0C by using digital pH meter.
- Dirt dispersion test**:** To two drops of the shampoo formulation taken in a test tube, 10 ml of distilled water and 1 drop of ink was added. The sealed test tube was shaken thoroughly 10 times. The amount of ink in the foam was estimated and the observations were recorded. The presence of ink in the foam indicates poor dirt dispersion property of the shampoo. Therefore, the ink remaining in the water portion is the expected result.
- Foam formation**:** 50 ml of 1% solution of the shampoo was taken in a graduated cylinder and shaken ten times. The volume of the foam formed was recorded.
- Foam retention**:** The reduction in the volume of foam after the shake test was noted at 1 minute intervals for 4 minutes.
- Wetting time (Drave's test**):** A weighed ball of cotton was allowed to sink through the shampoo solution taken in a graduated cylinder. The time taken for sinking was considered as the wetting efficiency, the least being the best [3].

Comparison of the Herbal Formulation with Commercial Shampoos

A comprehensive comparative study of the anti-dandruff potential of the herbal shampoo and the commercial shampoo was done. The zone of inhibition was measured using the software Image J to obtain precise values.

Results and Discussion

Identification of M. furfur

- *Morphological Identification***:** The fungus was confirmed to be *M. furfur* by the bottle shaped appearance of the cells on microscopic observation after Methylene blue staining.
- *Biochemical Identification***:** The active bubbling in the Catalase test showed that the test fungus was catalase positive. Bubbling indicates the release of O_2 from H_2O_2 as a result of catalase production. Thus the pathogen was identified as *M. furfur*, which is the only catalase positive *Malassezia* species.

Antimycotic assay of plant extracts

The average value of the zone of inhibition of growth obtained for each of the plant extract measured using the software Image J is shown in Fig.1

Antidandruff efficiency of commercial shampoos

The zones of inhibition obtained by the disc diffusion method of the 5 commercial shampoos are shown in Table 1.

Evaluation of the shampoo formulation

The result of the evaluation of various physico- chemical properties of the formulation are as follows:

pH - 5.5 at 25^0C, Dirt dispersion efficiency – Moderate, Foam formation - 5ml, Foam retention - 8 minutes and Wetting time - 6 minutes.

Comparison of Antidandruff Efficiency of the Herbal and Commercial Shampoo

The result obtained by the comparison of the antidandruff efficiency of the herbal shampoo formulation with the most effective commercial shampoo is shown in Table 2.

Many attempts have been made to understand the pathogenesis and pathogenicity of dandruff. The identification of the pathogen from the scalp scrapings using the Methylene blue and Catalase test in the present study has proved that *M. furfur* is the predominant causative agent of dandruff. This observation is in agreement with earlier findings [21]. The bottle shaped and yeast like appearance of the fungus corresponds to the earlier works done to identify the pathogen [22, 23]. The result of the catalase test that produced active bubbling indicating the release of Oxygen, characteristic of *M. furfur* is also consistent with the results obtained in earlier studies [24].

The present study reveals the potential of the aqueous and ethanolic extracts of *W. tinctoria* in eradication of dandruff and inhibition of keratinocyte proliferation which is in concordance with the observations of Krishna moorthy and Ranganathan [26]. The antifungal activity coupled with keratinocyte proliferation inhibition of *W. tinctoria* makes it very effective in the management of dandruff. Analysis of the zone of inhibition formed by the plant extracts has shown that *W.tinctoria* is highly potent in inhibiting fungal growth with inhibitory effect similar to that of commercially available anti dandruff products. *Naregamia alata* showed comparatively smaller inhibition zone but its high essential oil content makes it a desirable component in hair care products. Essential oils nourish the hair and keep the scalp healthy by getting rid of dry skin. *Azadiracta indica* showed very minimal zone of inhibition and hence was not chosen as a component in the herbal shampoo formulation. However it can be used against dandruff as it provides protection against relapse of dandruff for longer periods. The evaluation of shampoo formulation offered expected results in terms of foam formation, retention and wetting time. The comparable ZOI obtained indicates the potential of herbal formulations in effective treatment of dandruff. It can be concluded that better, safer and greener anti dandruff products can be formulated using potential plant extracts the performance of which can be enhanced by incorporating components that act synergistically to obtain the desired effect.

Tables and Figures

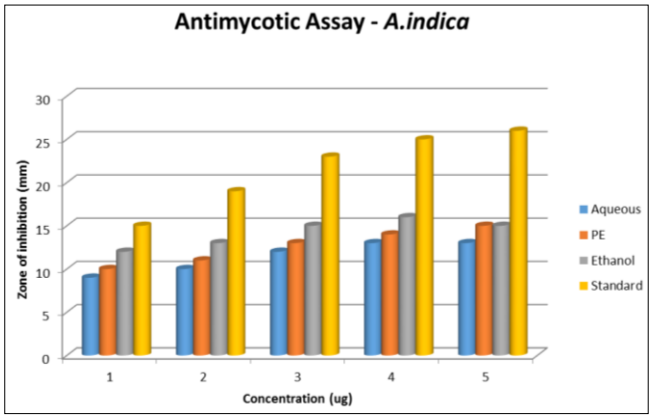

Fig 1: Antimycotic assay of *Azadirachta indica*

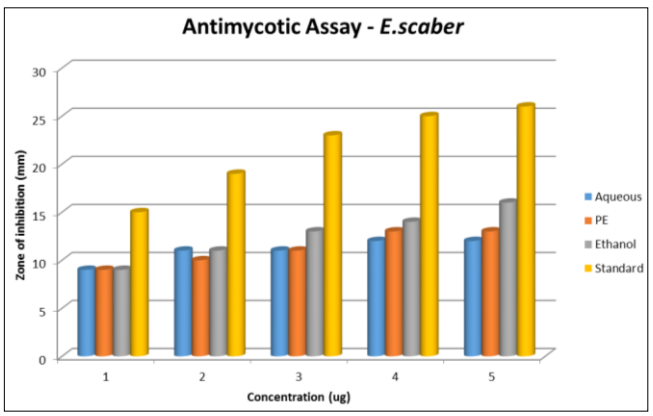

Fig 2: Antimycotic Assay of *Elephantopus scaber*

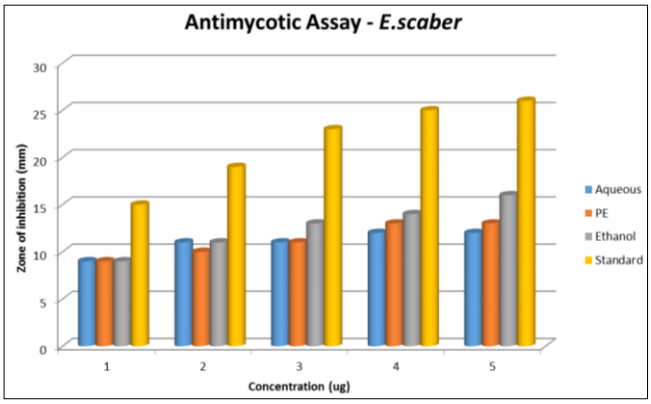

Fig 3: Antimycotic Assay of *Naregamia alata*

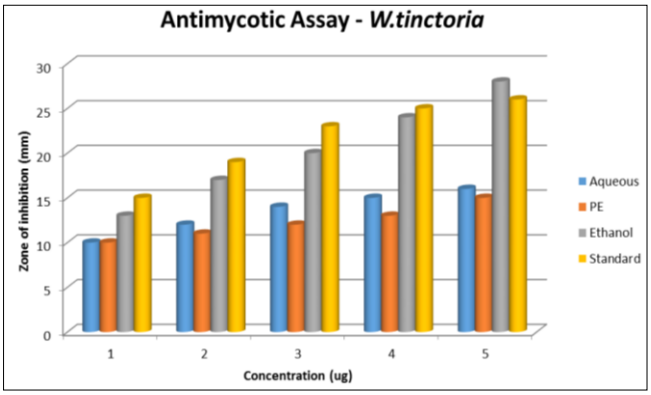

Fig 4: Antimycotic Assay of Wrightia tinctoria

Table 1: Zone of inhibition of Commercial shampoos (in mm)

No.	Name	ZOI
1.	Pantene	20.011± 0.013
2.	Clear	20.172± 0.002
3.	Head &Shoulders	20.197± 0.016
4.	Fructis	10.70± 0.002
5.	Dheedhi	01.33± 0.011
	Standard- Ketoconazole	20.153±0.001

Table 2: Comparison of the Formulation and Commercial Shampoo (in mm)

No.	Sample	ZOI
1.	Formulation	20.196± 0.012
2.	H&S	20.172± 0.016

Conclusion

The search for remedies for human ailments ends in the bounty of nature. Despite the availability of uncountable antidandruff medications, dandruff still remains an unsolved puzzle. This humble study has successfully thrown light to the potential of *Wrightia tinctoria* leaf extract in inhibiting the Malassezia furfur. The comparable and/or better inhibition obtained by the plant extract to the synthetic counterparts offers optimistic results in further studies. The usefulness of a medicine lies in its method of application as well. Hence formulating a ready to use shampoo is relevant in the present day context. The present study concludes the potential of *Wrightia tinctoria* leaf extract as an effective alternative to synthetic antidandruff agents and its suitability in being a component of shampoo formulations.

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