

Evaluation of Anti-Dandruff Activity of Poly Herbal Hair Oil against the Fungus *Malassezia Furfur*

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Abstract: Poly herbal hair oil formulations was prepared using *Ricinus communis* L., *Phyla nodiflora* Mich., *Cyamopsis tetragonoloba* L., *Wrightia tinctoria* R.Br., *Vernonia cinerea* Less. leaf crude extracts and *Magnolia champaca* (L.) Baill. ex Pierre flower oil. Totally five formulations were prepared using definite ratios of extracts. The antidandruff activity of formulated poly herbal hair oil was evaluated by agar well diffusion and broth dilution assay against dandruff causal agent *Malassezia furfur*. Among the five different combinations the F3 formulation showed better activity against *M. furfur*.

Keywords: Poly herbal hair oil, antidandruff activity, *Malassezia furfur* and dandruff

Date of Submission: 06-01-2019

Date of acceptance: 21-01-2019

I. Introduction

Dandruff is a common fungal infection found in the scalp and can be an embarrassing condition characterized by scaling, itching and redness of the scalp. Dandruff results from at least three etiologic factors: *Malassezia* fungi, sebaceous secretions, and individual sensitivity.^{1, 2} *Malassezia* fungi are ubiquitous skin residents of humans and other warm-blooded animals. It's involved in disorders including dandruff and seborrheic dermatitis, which together affect >50% of humans.³ It is commonly called as lipophilic yeast, widely accepted to play a role in dandruff.⁴ Taxonomically the genus *Malassezia* is divided into seven different species. They are *M. globosa*, *M. restricta*, *M. obtusa*, *M. sloofia*, *M. sympodialis*, *M. furfur* and *M. pachydermatitis*.⁵ Among these, *M. furfur* is the main causative agent of dandruff. The exact mechanism of dandruff formation is now believed to be the result of the formation of enzymes called lipases. The *Malassezia* fungus uses these enzymes to break down sebum to oleic acid. The oleic acid then penetrates the top layer of skin and causes increased skin cell turnover in susceptible people.

Nowadays most of the people are using anti-dandruff products having chemical ingredients for controlling the dandruff infection. It will lead to more side effects such as dryness of scalp & hair, irritation of scalp, discoloration of hair and hair fall rather than the cure. Herbal medicine, in the current scenario is gaining importance for treating many diseases due to their significant effect with lesser side effects when compared with allopathic medicines. A wide range of herbs have been documented to have good antidandruff activity.

Poly herbal formulation is one of the branch in traditional and alternative medicine which is practiced very commonly in the developing and developed countries. A poly herbal formulation is the combination of more than two to three plants at a definite ratio. It is known that plants have different phytoconstituents (i.e) phytochemicals which are responsible for the various curable properties that are attributed to them and when a combination of plants with these constituents are combined together it may show better activity when compared to the individual extract.

The present work was framed to prepare poly herbal leaf extracts of *Ricinus communis* L. (Euphorbiaceae), *Phyla nodiflora* (L.) Greene (Lamiaceae), *Cyamopsis tetragonoloba* (L.) Taub. (Fabaceae), *Wrightia tinctoria* R.Br. (Apocynaceae) and *Vernonia cinerea* L. (Asteraceae). To formulate anti-dandruff poly herbal hair oil; the essential oil was isolated from the flowers of *Magnolia champaca* (L.) Bail. ex Pierre belongs to the family Magnoliaceae. In this juncture the present study is designed to determine the anti-dandruff activity of hair oil based poly herbal formulations against *Malassezia furfur*.

II. Materials And Methods

Collection of Plant Materials and Preparation extracts

The leaves of the selected species was collected, washed and cleaned to remove the dust particles and subsequently they were dried under shade. After the plant materials were powdered using pulverizer. The methanolic crude extracts were prepared using soxhlet extraction method.

Magnolia champaca flower oil extraction

Fresh flowers were harvested from healthy and well grown plants. The samples were subjected to hydro distillation using a cleverger apparatus for 3 - 4 h for isolation of oil. The oil samples were stored in clean dry air tight container at 0⁰ C.

Formulation of anti-dandruff hair oil

The leaf crude extracts of study plants were dissolved at various concentrations in 1 ml coconut oil in separate containers and were stirred at high speed using magnetic stirrer then 1ml of *M. champaca* flower oil was added and kept for further use. Prepared poly herbal hair oil was subjected to their anti-dandruff activity against the causal organism *M. furfur*.

Isolation and culture preparation of Dandruff causing organism

Dandruff causal agent was collected by scraping of patient's scalp and stored in sterile containers and stored under refrigeration until use. The causal organism was inoculated in potato dextrose agar (PDA) media enriched with coconut oil. The inoculated plates were incubated at 37° C for 3 - 5 days. The fungal culture was stained with lactophenol cotton blue stain and examined under the high power objective microscope to identify the fungus.

Identification of *Malassezia furfur*

The *Malassezia furfur* species can be identified based on their macro/microscopic and Biochemical features are as follow. ⁶.

Macroscopy	Microscopy	Biochemical
Dull, smooth or slightly folded with convex elevations (average diameter 5mm); soft/friable texture	Large, oval, cylindrical or spherical cells, broad base bud	Assimilation of glycine: This is positive in <i>M. furfur</i> only

Anti-dandruff activity

The antidandruff activity of poly herbal hair oil was studied by well diffusion and broth dilution assay.

Well diffusion assay ^{7,8}

Isolates from the dandruff was inoculated by swabbing on the surface of gelled media plates. Wells of 6 mm in diameter was performed in the PDA media, and each well filled with 50 µl of various concentrations of poly herbal hair oil. The plates were kept in laminar air flow for 30 minutes for proper diffusion of the extract and thereafter incubated at 37° C for 3 - 5 days. The radius for the zone of inhibition was measured in millimeters and recorded against the corresponding concentration.

Broth dilution assay

Broth dilution assays are standard method used to compare the inhibition efficiency of the anti-dandruff agents. 5 ml of the potato dextrose broth, 0.1 ml of the culture (grown for 24 hrs) and the poly herbal hair oil was added in the culture tubes. The tubes were incubated at 37° C for 24 h. The optical densities were measured spectrometrically at 600 nm ⁹. The percentage of inhibition was calculated by using the following formula.

$$\% \text{ of inhibition} = \frac{\text{Control O.D} - \text{Test O.D}}{\text{Control O.D}} \times 100$$

O.D = Optical density

Data analysis

Results were averaged, and given as mean ± standard deviation, calculated by using the Microsoft excel.

III. Results

In the present investigation the causal organism *Malassezia furfur* was identified based on morphological and biochemical features (Figure 1). Poly herbal hair oil of various formulations was prepared using crude extracts of leaves of *Ricinus communis* L., *Phyla nodiflora* Mich., *Cyamopsis tetragonoloba* L., *Wrightia tinctoria* R.Br., and *Vernonia cinerea* Less. with 1 ml flower oil of *Magnolia champaca* (L.) Baill. ex Pierre and supplemented with 1 ml of coconut oil in each formulations with definite ratios are given in Table 1 and Figure 2.

The formulated Poly herbal hair oil was subjected to evaluate the anti-dandruff activity against *M. furfur* by agar well diffusion and broth dilution assay are given in Table 2 & 3 and Figure 3 - 6. The anti-dandruff activity by agar diffusion method was measured as diameter of zone of inhibition. All formulations are

Evaluation of Anti-Dandruff Activity of Poly Herbal Hair Oil against The Fungus *Malassezia Furfur*

exhibited good anti-dandruff activity against *M. furfur*. The maximum zone of inhibition was observed as 30 mm in F3 formulation and the minimum zone of inhibition was observed 23 mm in F1 formulation, whereas the lowest anti-dandruff activity was observed for commercially available antidandruff hair oil (13 mm). It is also confirmed by broth dilution assay the maximum percentage of inhibition (90.53 %) was observed in F3 formulations. In broth dilution assay the moderate percentage of inhibitions 57.51 and 58.35 % was observed in F4 and F5 formulations respectively.

Among the anti-dandruff hair oil formulations, F3 showed better response against *M. furfur* than other formulated hair oils. The F3 formulation contains the methanolic extracts of herbal plants in milligram viz. *Ricinus communis* - 30, *Phyla nodiflora* - 20, *Cyamopsis tetragonoloba* - 50, *Wrightia tinctoria* - 10, *Vernonia cinerea* - 40, with 1 ml of *Magnolia champaca* flower oil and 1 ml of coconut oil. However the hair oil formulation F3 was considered to be the best formulation for dandruff problem especially against the causal organism *M. furfur*.

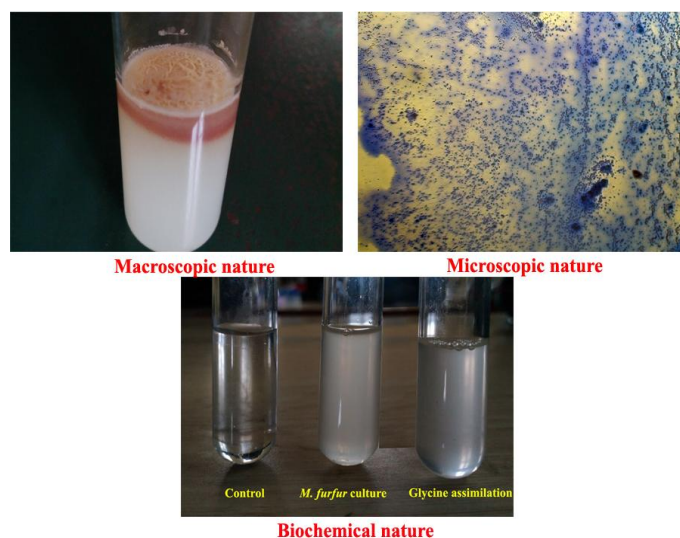


Figure 1 Identification of *Malassezia furfur* by macro/microscopic and biochemical nature

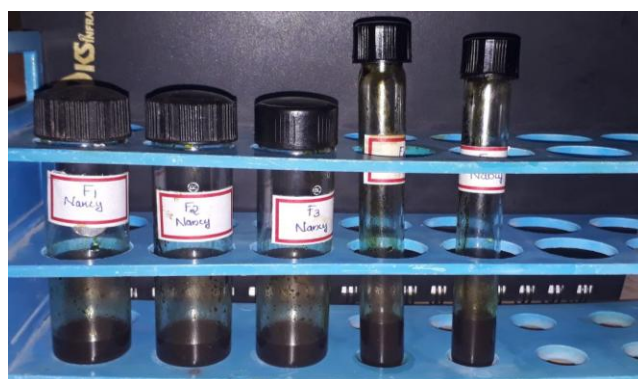


Figure 2 Formulated antidandruff poly herbal hair oil

Table 1 Formulations and composition of anti-dandruff poly herbal hair oil

Ingredients	Formulation of extracts in different concentration (mg)				
	F1	F2	F3	F4	F5
<i>Ricinus communis</i>	10	20	30	40	50
<i>Phyla nodiflora</i>	50	40	20	30	10
<i>Cyamopsis tetragonoloba</i>	20	30	50	10	40
<i>Wrightia tinctoria</i>	40	50	10	20	30
<i>Vernonia cinerea</i>	30	10	40	50	20
<i>Magnolia champaca</i> flower oil	1 ml	1 ml	1 ml	1ml	1ml
Coconut oil	1ml	1ml	1ml	1ml	1ml

F – Formulation

Table 2 Anti-dandruff activity of poly herbal hair oil against *M. furfur* by agar well diffusion

S.No	Poly herbal hair oil formulations	Zone of inhibition (mm)
1	F1	23 ± 0.58
2	F2	25 ± 0.58
3	F3	30 ± 1.00
4	F4	24 ± 1.00
5	F5	28 ± 0.57
6	Commercial anti-dandruff oil (Standard)	13 ± 0.57

Values are expressed as mean ± Standard Deviation (SD)

Table 3 Anti-dandruff activity of poly herbal hair oil against *M. furfur* by broth dilution Assay

S.No	Poly herbal hair oil formulations	% of inhibition
1	F1	13.36 ± 0.20
2	F2	25.41 ± 0.99
3	F3	90.53 ± 1.04
4	F4	57.51 ± 0.34
5	F5	58.35 ± 0.35
6	Commercial anti-dandruff oil (Standard)	15.37 ± 0.47

Values are expressed as mean ± Standard Deviation (SD)

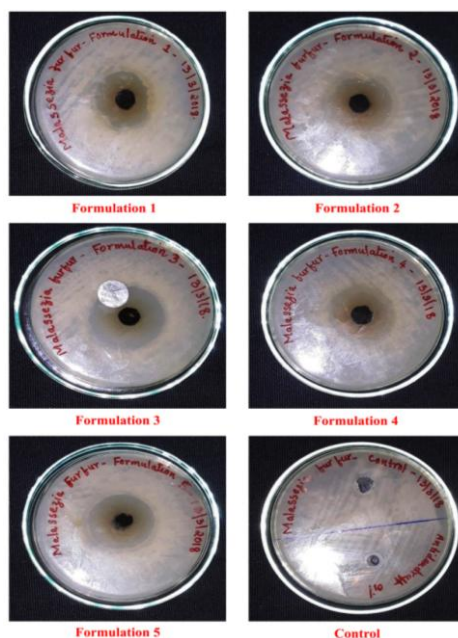


Figure 3 Anti-dandruff activity of poly herbal hair oil formulation against *M. furfur* by agar well diffusion

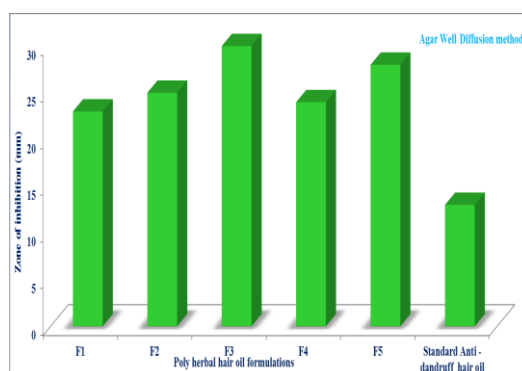


Figure 4 Zone of inhibition (mm) of poly herbal hair oil against *M. furfur* by agar well diffusion

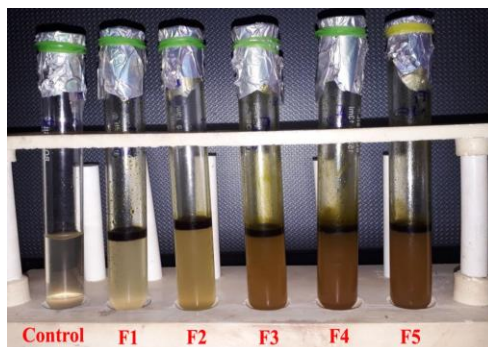


Figure 5 Anti-dandruff activity of poly herbal hair oil formulation against *M.furfur* by broth dilution assay

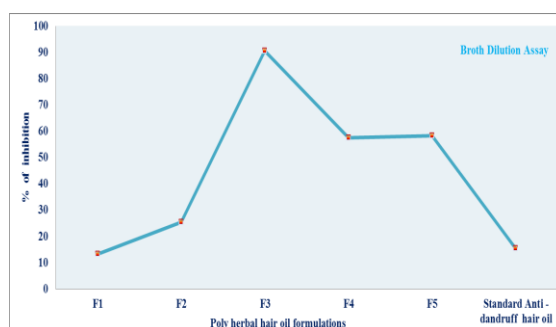


Figure 6 Percentage of inhibition of poly herbal hair oil against *M. furfur* by broth dilution assay

IV. Discussion

Herbal plants are the richest sources of antioxidants like vitamin A, vitamin C, vitamin E and other components like gallic acid, saponins, amino acids, elemental sulphur, enzymes, mucilages, flavanoids, tannins, essential oils and polysaccharides. In traditional knowledge itself number of plant parts was used in skin and hair care preparation like cucumber, burdock, marigold, watercress, daisy flower, witch hazel, hops, birch, gentian, fir, Indian cress, rosemary, sage, horsetail and thyme. Natural products in the form of herbal formulations are available on the market and are used as hair tonic, hair growth promoter, hair conditioner, hair-cleansing agent, antidandruff agents, as well as for the treatment of alopecia and lice infection.¹⁰

The present investigations agreed with the potential anti-dandruff activity of poly herbal hair oil contains extracts of *Hibiscus rosa sinensis*, *Centella asiatica*, *Eclipta alba*, *Embllica officinalis* and *Terminalia bellerica*.¹¹ It is clearly indicated that the combination of herbal plant extract having better response against dandruff than the single plant extract. Our results observed that the formulated poly herbal hair oil possess maximum anti-dandruff activity and minimum activity in commercially available synthetic antidandruff hair oil. Similarly the potency of antifungal action according to¹² the different plant extracts and commercially available anti-dandruff shampoos tested against a human dandruff isolate of *M. furfur*. The results confirmed that all plant extracts have possessed significant antifungal effect on growth of *M. furfur* than the commercial hair shampoos. Hence the herbal products are proving to be eco-friendly, alternative for synthetic anti-dandruff products. In order to avoid the harmful effects of synthetic products for controlling dandruff, it is suggested to choose herbal based anti-dandruff products for its control.

V. Conclusion

Hence it is confirmed that the formulation F3 was suitable to control dandruff organisms in humans. Further the formulations can be developed in to a complete commercial product.

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Evaluation of Anti-Dandruff Activity of Poly Herbal Hair Oil against The Fungus Malassezia Furfur

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IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) is UGC approved Journal with Sl. No. 4033, Journal no. 44202.

Sathishkumar P. " Evaluation of Anti-Dandruff Activity of Poly Herbal Hair Oil against The Fungus Malassezia Furfur." IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) 5.1 (2019): 01-06.