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Formulation of Poly Herbal Hair Oils and Evaluation of its Anti-Dandruff Activity against the Fungus *Malassezia (Malassezia furfur)*

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Abstract: In the present study, hair formulations were prepared using *Trigonella foenum-graecum*, *Ocimum tenuiflorum*, *Phyllanthus emblica*, *Murraya koenigii*, *Rosa damascena*, *Azadirachta indica*, *Citrus limon*, *Hibiscus rosa-sinensis*, *Mentha arvensis*, *Jasminum sambac*, *Aloe barbedensis*, *Camellia sinensis*. Seven formulations were made using definite ratios of extracts. The antifungal activity of formulated polyherbal hair oils was evaluated by the agar well diffusion method against the fungus *Malassezia furfur*, a causative agent of the dandruff problem. Among the seven different combination samples, Sample II and Sample VI showed better inhibition effect against *M.furfur*. Hence, the formulated herbal oils are eco-friendly, natural and can be used as an alternative for synthetic anti-dandruff products.

Keywords: Anti-fungal, formulations, *Malassezia furfur*, agar well diffusion, inhibition, anti-dandruff.

I. INTRODUCTION

Dandruff is a common fungal infection found in most human beings' scalps and is characterized by itching, drying, redness, and scaling in the scalp.

Dandruff can be caused by three factors- *Malassezia* fungi, sebaceous secretions or individual sensitivity. *Malassezia* fungi are ubiquitous skin residents in humans and other warm-blooded animals. It can cause diseases such as seborrheic dermatitis and dandruff, which together affect more than 50% of human beings. The genus *Malassezia* is divided into many different species - *M.globosa*, *M.obtusa*, *M.furfur*, *M.resticta*, *M.sloofia*, *M.symphodialis*, *M.pachydermatitis*. Among all the *Malassezia* species, *M.furfur* is one of the main causative agents of dandruff. Dandruff itself is not a fungus, although the presence of *Malassezia*spp causes it. Dandruff is caused as this fungus breaks down oils on the scalp called sebum. This process produces Oleic acid, to which some people are sensitive.

To get rid of dandruff, most people use anti-dandruff oils and shampoos containing chemical ingredients, which will lead to more side effects such as dryness of scalp and hair, irritation of scalp, hair-fall and discoloration of hair. On the other side, herbal medicines are gaining more importance in the current scenario for treating many diseases due to their significant effects and lesser or nil side effects compared to chemicals or allopathic medicines. A wide range of herbs has been found to possess better anti-dandruff activity.

Polyherbal hair oil formulation is one of the oldest and most commonly practiced methods in India and other countries. A polyherbal formulation is the combination of two or more plant extracts in definite ratios. It is known that plants have different phytoconstituents responsible for various curable properties attributed to them, and when they are combined, it may show better activity compared to the individual extract.

Various types of oils like coconut oil, mustard oil, castor oil, olive oil etc., are used as an oil base to prepare herbal tonics. Of all these, coconut oils are the best suitable oil base due to its effective activity and economical compared to other oils. To obtain polyherbal hair tonics, coconut oil is extracted initially and simultaneously, and the required crude drugs are collected and dried.

The present work was framed to prepare poly herbal oils using the herbs -*Trigonella foenum-graecum*(*Fabaceae*), *Ocimumtenuiflorum*(*Lamiaceae*), *Phyllanthus emblica*(*Phyllanthaceae*), *Murraya koenigii*(*Rutaceae*), *Rosa damascene*(*Rosaceae*), *Azadirachta indica*(*Meliaceae*), *Citrus limon*(*Rutaceae*), *Hibiscus rosa-sinensis*(*Malvaceae*), *Mentha arvensis*(*Menthaceae*), *Jasminum sambac*(*Oleaceae*), *Aloe barbedensis*(*Asphodelaceae*), *Camellia sinensis*(*Theaceae*). At this juncture, the present study was designed to determine the prepared hair oils' antifungal activity against *Malassezia furfur*.

II. MATERIALS AND METHODS

A. Plant Materials

For the preparation of herbal hair oils, various plant materials were collected. Fenugreek seeds, Tulsi leaves, Curry leaves, Amla fruits, Rose petals, Neem leaves, Lemon peel, Hibiscus petals, Mint leaves, Jasmine flowers, Aloe vera leaves and Green tea leaves from various herbal gardens and stores of Hyderabad and were adequately authenticated by Dr. Vijay BhaskarReddy, Dept. of Botany, Nizam College.

B. Equipment and Apparatus

Measuring cylinder, borosilicate beakers, mortar and pestle, weighing balance, stirrer, hot plate, muslin cloth, test tubes, test tube stand, conical flasks.

C. The Procedure Followed For The Preparation Of Polyherbal Hair Tonic

- 1) *Collection of Herbs:* Herbs of Neem, Hibiscus, Fenugreek, Amla, Curry Leaves, Rose, Lemon, Mint, Jasmine, Green Tea, Aloe vera were collected.
- 2) *Drying Of Herbs:* The collected herbs were dried under shade, and proper aeration is provided to hasten the drying process. Drying under shade will retain the active constituents. Hence shade drying is preferred over artificial drying.
- 3) *Formulating Herbal Hair Tonic:* Pure coconut oil extracted from *Cocos nucifera* is used. Initially, the oil is heated under a low flame. Herbs in required quantities were added to this hot oil and directly boiled with continuous stirring and heated until the drug will wholly be extracted in the oil base. Thus, the active ingredients of the plant herbs will get absorbed into the hot coconut oil. Later the hot oil is cooled, and the filtration process removes any traces of crude drug powders.

The oils have a pleasant smell with rejuvenating activity for hair growth. The prepared hair oils are exclusively obtained from natural drugs, so they have no side effects.

The formulated oils are stored under cool conditions. This keeps the oil stable for a more extended period without undergoing rancidity or saponification.

Table :1
Formulation of polyherbal hair tonics

Samples	Ingredients	Quantity	Base	Boiling Time
I	Fenugreek Seeds + Ocimum Leaves	25gms + 25gms	Coconut Oil (100 ml.)	25 mins
II	Amla Fruits + Curry Leaves	25gms + 25gms		25 mins
III	Rose Petals + Amla Fruits	25gms + 25gms		20 mins
IV	Neem Leaves + Amla Fruits	25gms + 25gms		25 mins
V	Lemon Peel + Hibiscus Petals	25gms + 25gms		20 mins
VI	Mentha Leaves + Jasmine Flowers	25gms + 25gms		20 mins
VII	Aloe Vera + Green Tea Leaves	25gms + 25gms		25 mins

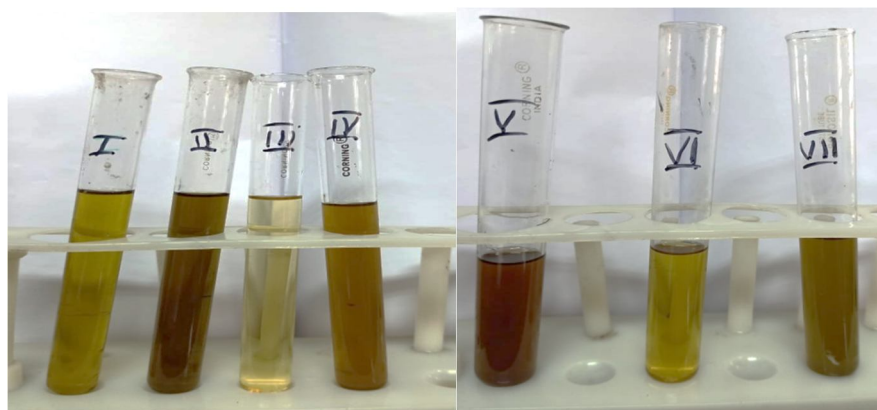


Figure:1 Images of oils prepared

D. Test for Antifungal Activity

Dandruff causing agent *Malassezia furfur* was collected by scraping the patient's scalp and stored in sterile containers at cool temperature until use. Petri plates with Potato Dextrose Agar (PDA) medium were then inoculated with *Malassezia furfur*'s causative organism by placing it in the center of each Petri plate. Each 7mm in size, three small holes were punched in the Petri plates to pour the oil sample. The same process is repeated with all seven oil samples. One Petri plate as a control treatment was also maintained without adding any oil sample to the medium. These petri plates were incubated for nine days at $\pm 25^{\circ}\text{C}$ to ensure that no contamination had taken place. After incubation for nine days at room temperature, radial growth was measured when fungus attained maximum growth in control plates. The fungal culture was stained with lactophenol cotton blue stain and is examined under a high power microscope for identification.

E. Identification of Fungus(*M.furfur*)

The fungus is identified based on its macroscopic and microscopic features:

- 1) *Macroscopic*: Dull, smooth or slightly folded with creamy yellow to brown colour.
- 2) *Microscopic*: Large, oval, spherical or cylindrical cells. Mostly bottle-shaped with a protruded end.

III. RESULTS AND DISCUSSION

A. Physical and Biological Evaluation

Various parameters like colour, physical state, pH, and sensitivity test, were evaluated, as shown in Table 2.

- 1) *pH*: The pH was determined by using a digital pH meter. 20 ml of sample oil was taken in a beaker, and the bulb of the P.H. meter was dipped in hair tonic. The obtained pH values were noted down.
- 2) *Sensitivity Test*: The prepared herbal oils were applied on 1cm of the hand's skin and exposed to sunlight for 4-5 minutes. It was conducted to evaluate whether the formulated oils will cause any irritation on intact skin of humans. All of the prepared oils were tested, but none of the oil samples showed any erythema or edema, which indicates that the prepared formulations are non-irritant on humans' skin.
- 3) *Odour*: All the oil samples possess pleasant odour except for Neem and Amla oil, which had a somewhat bitter odour.

TABLE: 2
Physical And Biological Evaluation Of Oils

Sample	Oil	Colour	Ph	Physical State	Sensitivity Test
SAMPLE I	Fenugreek and Ocimum oil	Olive green	7.5	Greasy liquid	Non-irritant
SAMPLE II	Amla and Curry leaves oil	Muddy green	6.6	Greasy liquid	Non-irritant
SAMPLE III	Rose and Amla oil	Light yellow	6.5	Greasy liquid	Non-irritant
SAMPLE IV	Neem and Amla oil	Caramel colour	7.2	Greasy liquid	Non-irritant
SAMPLE V	Lemon and Hibiscus oil	Chocolate colour	6.0	Greasy liquid	Non-irritant
SAMPLE VI	Mint and Jasmine oil	Yellowish green	7.1	Greasy liquid	Non-irritant
SAMPLE VII	Aloe vera and Green tea oil	Muddy green	7.5	Greasy liquid	Non-irritant

The results obtained for the evaluation tests are under the specified limits and are according to the standard values.

B. Results of Antifungal Activity

The antifungal activity of all the seven oil samples against *Malassezia furfur* was observed in the present investigation. After nine days of incubation, the petri plates were observed. The results showed significant variability inefficacy of all the formulated oil samples in inhibiting the growth of *Malassezia furfur*. The antifungal activity of all the samples is depicted in Table 3. The growth reduction in each petri plate was taken into consideration and the inhibition effect was evaluated. Fungal growth was determined by quantifying colony diameter and percentage inhibition. The efficacies of oil samples were expressed as percent inhibition of mycelial growth over control, which was computed by applying the formula given by Vincent (1927).

$$I = \frac{(C-T)}{C} \times 100$$

Where I = Percent inhibition
 C = Colony diameter(mm) incontrol
 T = Colony diameter (mm) in treatment

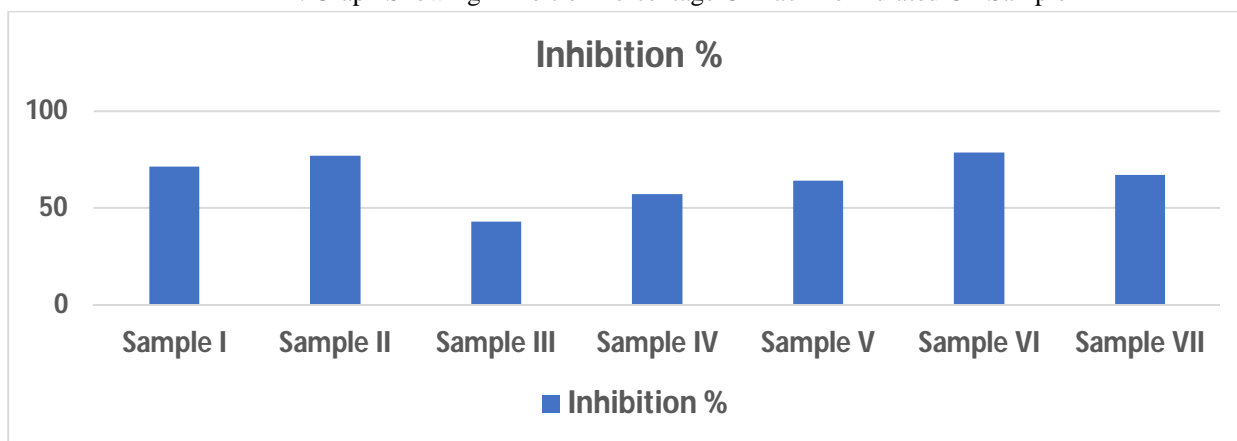
TABLE 3
 Inhibition Effect Of Oil Samples On The Growth Of M.Furfur

SAMPLE	OIL Vol(0.7ml)	CONTROL	TREATMENT	INHIBITION PERCENTAGE
SAMPLE I	Fenugreek+ Ocimum	70mm	20mm	71.4%
SAMPLE II	Amla+Curry leaves	70mm	16mm	77.1%
SAMPLE III	Rose+Amla	70mm	40mm	42.8%
SAMPLE IV	Neem+Amla	70mm	30mm	57.1%
SAMPLE V	Lemon+Hibiscus	70mm	25mm	64.2%
SAMPLE VI	Mint+Jasmine	70mm	15mm	78.5%
SAMPLE VII	Aloe vera+Green tea	70mm	23mm	67.1%

The inhibition percentage of Oil samples on the mycelial growth of *Malassezia furfur* was shown in table3. The rate of mycelial growth inhibition was shown to vary with different oil samples. Compared with the control, the petri plates treated with oil samples reduced the growth of *M.furfur*. Mycelial growth was reduced by 74.2% when treated with Sample I (Fenugreek and Ocimum oil), 77.1% mycelial reduction was seen when treated with Sample II (Amla and Curry leaves oil). Similarly, sample III (Rose and Amla oil) showed 42.8% reduction, Sample IV (Neem and Amla oil) showed 57.1% reduction, Sample V(Lemon and Hibiscus oil) showed 64.2% reduction, Sample VI(Mint and Jasmine oil) showed 78.5% reduction and Sample VII(Aloe vera and green tea oil) showed 67.1% reduction in the mycelial growth of *Malassezia furfur*.

Out of all the seven oil samples that were screened in the present investigation using the agar well diffusion method, the maximum inhibition in the growth rate of fungus was detected with Sample II(Amla and Curry leaves oil), i.e. 77.1% and Sample VI(Mint and Jasmine oil), i.e. 78.5%. In contrast, Sample III(Rose and Amla oil) showed the least inhibition effect, i.e. 42.8%. Samples I, IV, V and VII showed a moderate effect with inhibition percentages of 71.4, 57.1, 64.2 and 67.1, respectively.

TABLE 4. Graph Showing Inhibition Percentage Of Each Formulated Oil Sample



IV. CONCLUSION

Results from the current study showed that among all the formulated herbal oil samples, sample II (Amla and Curry leaves oil) and Sample VI (Mint and Jasmine oil) showed the highest antifungal activity (inhibition effect) against the growth of *Malassezia furfur*. Hence, it is verified that Amla fruits, Curry leaves, Mint leaves, and Jasmine flowers showed the highest antifungal activity among all herbs. All these herbs not only showed great activity but are also devoid of any side effects. As these herbs showed good antifungal activity, the oil formulated from these herbs is suitable for controlling humans' dandruff problem. Further, the formulations can be developed into an entirely commercial product. Further evaluation of novel bioactive compounds of Amla, Jasmine, Curry leaves, and Mint is needed, which can reveal more and more new biological activities of these potent medicinal plants.

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