Development and Evaluation of Polyherbal Antidandruff Hair Oil

Hati Deepak*, Bhatnagar S. P.a, Sethi Kalyan Kumarb**

aDepartment of Pharmaceutical Sciences, Birla Institute of Technology, Mesra. Ranchi – 835 215, India
bGITAM Institute of Pharmacy, GITAM University, Rushikonda, Visakhapatnam, A.P., 530 045, India

* Corresponding Author: Deepak Hati (DH), E. Mail: deepak588@gmail.com, Phone No.: +91-9040362539;
Kalyan Kumar Sethi (KKS), E. Mail: kalyansethi@gmail.com, Phone No.: +91-9160636049.

ABSTRACT

The present study on formulated polyherbal Antidandruff hair oil is very effective in management of dandruff. However, during our experiment the formulation has proved to be excellent hair growth stimulators. Ideal base oil was selected by mixing of different proportions of two vegetative base oils. From the experiment it was reported that the volatile oils of Eucalyptus globules and Ocimum gratissimum along with the petroleum ether extract of Hibiscus rosa sinensis, Phyllanthus embelica, Tridax procumbens posses antifungal activity. Further the last two extracts have significant hair growth activity. The result of open pilot clinical trials of prepared formulation brought strength to our claim. During the stability study of the formulation, the physical and chemical parameters remained unchanged after one month of preparation.

INTRODUCTION

Dandruff is the common complaint and is suffered by as many as 50% of the population of world at sometime during their life. The condition is generally characterized by the presence of flakes on scalp and in the hair. The symptoms can vary and the severity can range from mild scaling to severe scaling. Its prevalence and severity is greatest in young men and children. The older individuals suffer less frequently to dry scalp and dandruff.[1, 2]

Among 800 male, 521 (65.1%) suffer with dandruff, whereas 279 (34.9%) don’t suffer, particularly the person of age group 21-40 suffer most with dandruff than other age groups.[3] There are number of factors that responsible for the dandruff like fungal infections, hormonal imbalance, cold, dry weather, poor hygiene habits, long-term stress and anxiety, infrequent shampooing or inadequate rinsing of the scalp and hair, poor diet etc.[4] The central dandruff hypothesis remains that the lipophilic yeast Malassezia furfur (fig. 1) previously known as Pityrosporum ovale is the causal agent of dandruff. It is also known as an opportunistic pathogen involved in pityriasis versicolor, seborrheic dermatitis, Pityrosporum folliculitis, confluent and reticulated papillomatosis (Gougerot-Carteaud) and some kinds of atopic dermatitis.[1, 2, 5–7]

The common topical preparation used to treat dandruff includes- ketoconazole, flucanazole, selenium sulphide, coal tar etc. [5] Both synthetic and natural drugs are dispensed in different formulation like shampoos, cream, lotions, emulsions, hair oils and other cosmetic formulation.[8] Along with synthetic treatment, natural elements are more preferred for the management of dandruff.

MATERIALS AND METHODS

General

Poly herbal hair oil is an herbal product, formulated specifically for all ages of men, women & children. Herbal antidandruff hair oil is a perfect blend of ancient herbs that have been used from centuries to provide dandruff free scalp giving sufficient strength and vitality to hair. This product also provides all the necessary nourishment to the root of hair and promotes the natural growth of hair. It also checks and controls hair-fall, dandruff, thinning of hair and more.
Taking mixture of ideal base oil, effective plant extract, some essential oils, color and perfumes help to formulate best antidandruff hair oil that can combat dandruff along with promotes hair growth\textsuperscript{9}.

Drugs of herbal origin have rapid demand for formulations. With rapid production of herbal formulations and coming up newer combinations of herbal drugs there is a need for standardization and evaluation for safer use. In this direction we have selected the formulated poly herbal hair oil for standardization both in analytically and pharmacologically.

**Chemicals**

0.5 alcoholic KOH, Wij’s solution, saturated potassium iodide solution, solution of glacial acetic acid, chloroform, Folin-Denis regent, of 0.1N sodium thiosulphate solution (Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3}), 0.5 N HCl, 0.1 N NaOH solution, phenolphthalein indicator, fresh starch indicator.

**Plant materials**

There are some plant extracts, volatile oils and isolated compound found to be effective for treatment and management of dandruff (table 1). There are number of plants that proved scientifically to be effective against Malassezia furfur\textsuperscript{10}. Eucalyptus globules Labil, Wrightia tinctoria, Aloe vera, Cassia alata, Melaleuca alternifolia (Tea tree oil), Azadirchta indica, Phyllanthus emblica L.. However, in Indian traditional medicines literature there are many plants which used traditionally for dandruff.\textsuperscript{11–13}

**Anti dandruff hair oil**

The antidandruff hair oil enriched with natural oils prevents dandruff by eliminating microbial infections of the scalp. In the formulation, the plant species have been selected those used in Ayurvedic hair oils for the treatment of ailments of the head and scalp conditions. Systematic standardization (quality control) from raw materials to the final product the plants are proven to contain constituents that can cause hair growth and removing dandruff.

**Base oil**

Base oil or carrier oil play an important role in carrying and diluting highly concentrated essential oils. By means of dilution they inhibit evaporation rate of essential oils and spreading easily and evenly over the skin encourage quick absorption into the skin dermal layers. One part of coconut oil in the mixture with three parts of other suitable base oils will be ideal base oil.\textsuperscript{14–15}

**Components of the formulation**

On the basis of literature survey, there can possible of formulating a poly herbal hair oil having antidandruff with hair growth stimulant activity. Eucalyptus oil is obtained by steam distillation and rectification from the fresh leaves and terminal branches. It is colour less or pale yellow, it has aromatic and camphoraceous odour.\textsuperscript{16, 17–22} The essential oil which is obtained from fresh leaves of Ocimum gratissimum by hydro distillation, the yield is about 0.69\%.\textsuperscript{23, 24} Hibiscus possesses antifungal property.\textsuperscript{25} It is used in management of dandruff\textsuperscript{20} Leaves and flowers are good for healing ulcers and for promoting growth and colour of hair.\textsuperscript{27} Amla helps in good growth of hairs that’s the region most of the marketed herbal hair oils contain amla as chief

<table>
<thead>
<tr>
<th>Name</th>
<th>Botanical name</th>
<th>parts used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus oil</td>
<td>Eucalyptus globules</td>
<td>Fresh leaf</td>
</tr>
<tr>
<td>Ocimum oil</td>
<td>Ocimum gratissimumonum</td>
<td>Fresh leaf</td>
</tr>
<tr>
<td>Hibiscus</td>
<td>Hibiscus rosa sinensis</td>
<td>Leaf</td>
</tr>
<tr>
<td>Amla</td>
<td>Phyllanthus emblica</td>
<td>Fruit</td>
</tr>
<tr>
<td>Tridax</td>
<td>Tridax procumbens</td>
<td>Entire aerial part</td>
</tr>
<tr>
<td>Mixture of base oils</td>
<td>Cocos nucifera and sesamum indicum</td>
<td>Vegetable oils</td>
</tr>
</tbody>
</table>
Development and Evaluation of Polyherbal Antidandruff Hair Oil

EXPERIMENTAL I

Screening for Antifungal activity

Required strain of Malassezia furfur MTCC1374 tested in the growth medium like modified Emmons’s Saboraudud’s agar medium. Fluconazole was used as standard drug for the comparison of antifungal activity. Stock solutions of each 10 ml were prepared in sterile dimethyl sulfoxide (DMSO) at a concentration of 1mg/ml. Both solid and liquid Emmons’s modifications of Saboraudud’s medium were used for the study. Activation of culture, inoculums and media has preparered. [34]

Cup-plate/ Cylinder-plate method

The cup plate methods [35] for each Extract were done in duplicate methods and the average diameter (zone of inhibition) value has shown (fig. 2 and table 2).

Disc diffusion methods for Volatile oils

It was found that the antifungal assay of volatile oils O. gratissimum and E. globulus couldn’t complete successfully in disc-plate methods as volatile oils didn’t diffuse in the agar media. The antifungal screening of these volatile oils was done by disc diffusion methods.[9-36]

The average diameter (zone of inhibition) value (fig. 3 and table 3) of volatile oil was described. Fig. 4 shows the chat of zone of inhibition in mm of different extracts and volatile oils. Minimum inhibitory concentration (MIC) value of the extracts and volatile oils are also calculated (table 4 and fig. 5).

Hair growth activity of extraction on mice

Twenty four mice (25-30g body weight) were used in this study. Animals were procured from Institutional animal house (Regd. no. 621/02/ac/CPCSEA) of Birla Institute of Technology, Mesra. All animals were kept in polycrylic cages and maintained under standard housing conditions (room temperature 24-27°C and humidity of 60-65% with 12:12; light: dark cycles). Food was provided in the form of dry pellets and water ad libitum. The animals were allowed to get acclimatized to the laboratory conditions for 7 days before the commencement of the experiment. All experiments involving animals complies with the ethical standards of animal handling and approved by Institutional Animal Ethics Committee.

Treatment protocols

Group 1: Control rats received no treatment.
Group 2: This group was treated with 2% extract of Hibiscus rosa- sinensis in base oils
Group 3: This group was treated with 2% extract of Tridax procumbens in base oils
Group 4: This group was treated with only placebo (Base oil without any drugs)

Base oils (Sesame oil and Coconut oil in ratio of 7:3) are taken for the study. 2gm. of each extract Hibiscus rosa sinensis and Tridax procumbens was dissolved in 100

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Table 2: Zone of inhibition of extracts in compare to fluconazole

<table>
<thead>
<tr>
<th>Zone of inhibition (Diameter in mm)</th>
<th>Concentration in mcg/ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EX 2000 1000 500 250 125 62.5 31.2 15.6</td>
</tr>
<tr>
<td></td>
<td>PE 11 10 8 6 -- -- -- --</td>
</tr>
<tr>
<td></td>
<td>TP 9 8 6 -- -- -- -- --</td>
</tr>
<tr>
<td></td>
<td>HR 7 6 -- -- -- -- -- --</td>
</tr>
<tr>
<td></td>
<td>F NA 35 28 20 18 14 12 7</td>
</tr>
</tbody>
</table>

ml base oil to produce 2% active compound and it was further used for the evaluation of potential hair growth effects in vivo. 1 ml of the prepared oils of each extract was applied topically to the denuded area of each mice once a day.

Six animals in each group were taken, and shaved 2 cm² area of the hair from dorsal portions off all the rats was shaved off and wiped out with surgical sprits. One ml of the prepared oils and the placebo were applied to the denuded area of the respective groups once a day and control group received no treatment, and this treatment was continued for 30 days.²⁷

Hair was plucked randomly from the shaved area of all mice, of each group on 15th and 30th day of treatment (fig. 6 and 7). The length of 25 hairs was measured and the average length of 25 hairs was determined. The result was expressed as the mean length ± S.D. of 25 hairs.³⁷

**Results and Discussion**

Hair growth was observed from the denuded area at the end of 1st week and the length of hair began to increase until the end of the treatment course. In comparison to the control, for all the groups the whole denuded area was covered with hair during the 4th week. Moreover, the hair growth was sparse in all the groups except the leaf extract-treated group and there was no considerable change in hair texture. It was found that the extract of *Hibiscus rosa sinensis* produce a significant growth with respect to the control and placebo. The groups those were treated with the extract of *Tridax procumbens* had also significant hair growth in comparison to the control and placebo group however lower than Hibiscus treated groups. The hair growth activity of the extracts was compared with the control and the placebo groups. Results were significantly

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**Table 3: Zone of inhibition by volatile oil in compare to fluconazole**

<table>
<thead>
<tr>
<th>Concentration in mcg/ml.</th>
<th>VO 2000</th>
<th>1000</th>
<th>500</th>
<th>250</th>
<th>125</th>
<th>62.5</th>
<th>31.2</th>
<th>15.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>EG</td>
<td>12</td>
<td>10</td>
<td>8</td>
<td>6</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>OG</td>
<td>11</td>
<td>7</td>
<td>6</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>F</td>
<td>NA</td>
<td>35</td>
<td>28</td>
<td>20</td>
<td>18</td>
<td>14</td>
<td>12</td>
<td>7</td>
</tr>
</tbody>
</table>


**Table 4: Minimum inhibitory concentration (MIC) value of the extracts and volatile oils**

<table>
<thead>
<tr>
<th>Extract and Volatile oils</th>
<th>MIC Values (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E.globulus</em></td>
<td>&lt; 250</td>
</tr>
<tr>
<td><em>O. gratissimum</em></td>
<td>&lt; 500</td>
</tr>
<tr>
<td><em>P.embelica</em></td>
<td>&lt; 250</td>
</tr>
<tr>
<td><em>Hibiscus rosa sinensis</em></td>
<td>&lt; 1000</td>
</tr>
<tr>
<td><em>Tridax.Procumbens</em></td>
<td>&lt; 500</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>&lt; 15.6</td>
</tr>
</tbody>
</table>

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**Figure 3.** Zone of Inhibition (ZOI) of Volatile Oil: A and B: *Eucalyptus globules*; C and D: *Ocimum gratissimum*.

**Figure 4.** Chart of zone of inhibition in mm of different extracts and volatile oils.

**Figure 5.** Chart of MIC value of the extracts and volatile oils.
prepared with the corresponding control values and p values were calculated by Dunnett test.

**Preparation of polyherbal formulation**

From the above experiments it was found that the extracts and the volatile oils were effective against the *Malassezia furfur* which is the main cause of dandruff. The 2% extracts of *Hibiscus rosa sinensis* and *Tridax procumbens* had showed good hair growth activity. So on the base of the MIC values of extracts, volatile oils and the extract concentration that showed hair growth activity, the hair oil was developed.

**Formula**

Each 100 ml contains

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eucalyptus globulus</em> (Volatile oil)</td>
<td>0.11 ml</td>
</tr>
<tr>
<td><em>Ocimum gratissimum</em> (Volatile oil)</td>
<td>0.43 ml</td>
</tr>
<tr>
<td><em>Phylanthus embelica</em> (Dried water extract)</td>
<td>0.1 gm</td>
</tr>
<tr>
<td><em>Tridax procumbens</em> (Dried pet. ether extract)</td>
<td>2.0 gm</td>
</tr>
<tr>
<td><em>Hibiscus rosa sinensis</em> (Dried pet. ether extract)</td>
<td>2.0 gm</td>
</tr>
<tr>
<td>Perfume</td>
<td></td>
</tr>
<tr>
<td>Base oils (sesame oil + coconut oil)</td>
<td>Q.s</td>
</tr>
</tbody>
</table>

The base oil was taken in a glass vessel. Then it was allowed to boil, when the oil started to boil then extract of *Phyllanthus embelica* was added to it and stirred continuously so that it was not allowed to adhere to the vessels. The dry petroleum ether extracts of hibiscus and tridax were added one by one with continuously stirring. Boiling of oil for long time was avoided, and then the oil was filtered through cloth twice. The require amount volatile oils like ocimum and eucalyptus were added after cooling. The perfume was added the end and stored in a glass container.

**Evaluation of formulated hair oil**

The physical evaluation of formulated hair oil was observed in naked eyes and the colour of formulation was found to be dark green. The relative density of the formulated hair oil determined by using pycnometer was found to be 0.917 gm/ ml. Viscosity of a liquid measured by Ostwald viscometer was found to be 69.23 centipoise. In the chemical evaluations, saponification values is 228, iodine value is 83.25, peroxide value is 5 and acid value is 1.2 were determined by the same procedure which followed for base oils. All the chemical parameters observation came within the range. The oil was kept in the bottle at room temperature for stability study.

**EXPERIMENTAL II**

**Antifungal activity of formulation**

After the successful formulation of hair oil, the antifungal (*Malassezia furfur MTCC1374*) activity was studied (fig. 8 and table 6).
Table 6: Zone of inhibitions of formulation

<table>
<thead>
<tr>
<th>Screening</th>
<th>Concentration in(μg/ml)</th>
<th>Compound</th>
<th>2000</th>
<th>1000</th>
<th>500</th>
<th>250</th>
<th>125</th>
<th>62.5</th>
<th>31.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulated oil</td>
<td></td>
<td>9</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

Hair growth activity of formulation on mice

The method for hair growth activity is studied in mice (table 7, fig. 9 and 10). The method is same as the previous method followed [27,37]

Allergic test of formulated hair oil

To know about the allergic and other adverse effect of the formulated hair oil open study was non-comparative, non-randomized which was conducted among some M. Pharma students of Department of pharmaceutical sciences, B.I.T, Mesra, Ranchi. Twenty five volunteers, from the age groups 22-27 years, who were willing to give informed consent, were enrolled in the study. The formulated hair oil was applied on the lower surface of forehead and back side of ear pinna of twenty five volunteers (both male and female). The volunteers were told not to wash the applied area for 24 hrs. After 24 hrs, no irritation, no allergic and no redness cases found among all the volunteers. It was concluded that, the formulated hair oil free from allergic and other skin related topical adverse effects.

Evaluation of antidandruff activity of polyherbal Hair oil [8,9]

An open pilot clinical study was planned to evaluate the clinical efficacy and safety. This pilot study was an open, non-comparative, non-randomized which was conducted among some M. pharma students and mess workers of B.I.T, Mesra, Ranchi. A total of 10 patients were enrolled in the study, there was a significant reduction in dandruff and itching at the end of 2nd weeks, similarly there was significantly reduction of white scales. In subjective evaluation, majority of the patients experienced remarkable overall improvement.

Stability study

After the chemical analysis the formulated hair oil was kept in a glass container at room temperature. The same oil again tested after the stipulated period. Physical evaluation of 30 days stored prepared oil, the colour found to be dark green, relative density is 0.916 and viscosity found to be 68.1 centipoise. In the chemical evaluation the formulation, saponification value is 239, acid value is 2.1, iodine value is 87.29 and peroxide value is 5.8. The stability study of the formulation, physical and chemical parameters remained unchanged after one month of preparation.
ACKNOWLEDGEMENTS

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