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Devlopment and Evaluation of Herbal Hair Oil Formulation of *Helianthus annuus* and *Martynia annua* for Hair Growth Potential

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Abstract: The differentiation of hair matrix cells on the surface of the scalp during migration is caused by strong proliferation. Anagen, catagens, telogens, exogens, and kenogens are the stages of the hair development cycle. The primary aim of the present study was to investigate the potential hair growth benefits of a herbal oil formulation containing petroleum ether extract from *Helianthus annuus* and *Martynia annua* seeds. Using conventional finasteride, *in vitro* screening was conducted to determine the 5α- reductase inhibitory from herbal hair oil formulation. To measure the telogen to anagen phase transition in albino mice, applied prepared herbal formulation oil to the rear skin surface. The quantitative and qualitative analysis was performed and observed. The outcomes of the research revealed that herbal hair oil formulation FO1, FO2 and FO3 exhibited prominent activity. Formulation of herbal oils compared to normal finasteride, FO1, FO2, and FO3 showed greater 5α-reduction inhibitory effect. A different herbal formulation ratio in per cent of petroleum ether extract of both plants was given topically to FO1, FO2, and FO3 on the shaved skin of the albino mice treatment groups, and the results were monitored for 30 days. The findings indicated that FO1, FO2, and FO3 had considerably more potential for hair development in distinct ratios, especially on albino mice formulation of herbal hair oil FO3. The *in vitro* screening of 5α-reductase inhibition ability yielded a result of 27.88 ± 1.76. At different growth stages, the herbal formulation FO3 treated albino mice group needed less time than the control (positive) group and the 2% minoxidil (Standard) group. The hair length of albino mice treated with herbal formulation FO3 showed remarkable increase in length in 30 days (22.260±0.25 mm) and the hair thickness of albino mice were 0.020±0.005 mm with respect to 2% minoxidil (0.021±0.008). These results suggested that *Martynia annua* and *Helianthus annuus* seeds may promote hair development.

Keywords: *Helianthus annuus*, *Martynia annua*, 5α-Reductase inhibitor, Finasteride, Minoxidil, Hair growth

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Introduction

One of the oldest, wealthiest, and most varied cultural traditions in India is linked to the usage of medicinal plants, as the country is naturally endowed with a wide diversity of herbs. While it is

true that we are exposed to a variety of persistent chemicals in our environment that negatively impact our bodies, taking herbs helps our bodies begin the process of cleansing and purification. Rather from being a band-aid remedy, herbal therapies are long-term fixes that balance physiological systems. The natural components of herbs give the body extra essential nutrients without posing any harm. The most noticeable feature of a person's appearance, hair is also the most vital organ and should be taken very seriously.

The most dependable and trustworthy hair care products available are hair oils. Natural hair oils can be enhanced with a wide variety of herbs, the health benefits of which can aid in replenishing the natural nutrients in hair. Ayurveda is thought to have its roots in India 3,000 years ago. Whether or not modern medicine is involved, it has been found to be the most dependable medical treatment since that time. The Indian Ancient Sanskrit term "sneha" means "to oil" as well as "to love," which eloquently demonstrates the relationship between hair oil and hair care (Sharma, 2021)

Different areas of the human body often exhibit varying rates of hair growth. The male and female bodies grow at different rates as well. The following observations are made on the pace of hair development at various body parts (Saitoh *et al*., 1970): Growth rate-- 0.44 mm/day on Scalp; 0.44 mm/day on Chest; 0.39 mm/day on temples; and 0.27 mm/day on beard. Average hair growth is affected slightly by the age of human being, but overall average hair growth rate per day is 0.37mm (Dominique, 2003).

Numerous studies documented other herbal formulations for hair growth potential, quality, and quantity from a wide range of plants, herbs, and herbal formulations (Vaidya *et al.,* 2007). Plant extracts and their chemical constituents have been traditionally and scientifically stated to be useful when used in the current generation of medications to treat alopecia and hair loss.

The annual dicotyledonous herbal plant *Helianthus annuus*, which is a member of the Asteraceae family, is extensively distributed over North America, Eastern Europe, Northern China, and both North and East India. Phenols, flavonoids, and alkaloids were found in *Helianthus annuus* roots, stems, leaves, and seeds. The seeds themselves contain 36–42% oil and are naturally high in linoleic acid (56–70%) and low in oleic acid (21–25%). Linoleic acid and oleic acid are two distinct fatty acids that are beneficial to our hair (Hwang *et al.*, 2021).

Martynia annua Linn is an annual plant with glandular hair that is a member of the Martyniaceae family. The plant becomes erect and branching. Their leaves are opposite, with a cordate base, a pointed apex, and repand-dentate edges. They range in size from approximately oblong to deltoid. The enormous, foxglove-shaped flowers are produced in racemes of eleven to twenty flowers and are pink and dark-purple with yellow blotches inside. The fruits are firm, the seeds are rectangular and woody, with a two-sharp recurved hook. Alkaloids, flavonoids, and phenolic material are present. Linoleic acid regulates hair water loss, maintains a healthy scalp, and promotes hair development. Because oleic acid, some flavonoids, and phenolic substances prevent hair from losing water and keep it hydrated over time, hair appears shinier and more silky (Kenwat *et al.*, 2013).

The primary aim of the current study was to evaluate the potential for hair development in some herbs, such as *Martynia annua* and *Hellianthus annuus* seeds, and to formulate an appropriate herbal oil that can help hair growth naturally.

Materials and Methods

Collection of plant seeds:

Helianthus annuus and *Martynia annua* seeds were gathered in October at the RKDF University Botanical Garden in Bhopal, MP, India. Verification of the seeds was done by Dr. Z.U. Haque, Professor of Botany Department, Madhya Pradesh's SF

Table 1: Ingredients for hair oil formulation

S. No.	Name of ingredients	Used as a	Formulation quantity (% w/w)		
			FO1	FO2	FO3
1	*Helianthus annuus* oil extract	Hair growth	5	10	7.5
2	*Martynia annua* oil extract	Hair growth	10	5	7.5
3	*Eucalyptus globulus* oil	Penetration enhancer	5	5	5
4	Cinnamon oil	Anti-microbial	5	5	5
5	Alovera pulp	Anti- oxidant	5	5	5
6	Jasmine flower extract	Fragrance	2	2	2
7	*Cocus nucifera* oil	Base oil	Up to 100	Up to 100	Up to 100

College of Sciences, Bhopal. Voucher specimens were submitted on October 22, 2021, with numbers 675/Bot/Safia/2021 and 676/Bot/ Safia/ 2021.

Helianthus annuus and *Martynia annua* seed extraction:

The seeds were dried following the removal of impurities from the seeds of *Helianthus annuus* and *Martynia annua*. Next, the seeds were ground using a commercial blender before being subjected to the Soxhlet extraction process. The well-powdered seeds were then stored in a paper thimble. Oil was extracted by immersing a water bath in a petroleum ether solvent for 10 h. Afterwards, during the extraction procedure, petroleum ether was distilled out in rotary evaporators under vacuum at 45°C. Next, the yield that was removed was weighed and noted. After that, the extracted yield was put in a vial and refrigerated for additional analysis. In cold pressing, press 29.4 to 49.0 MPa for 20 min without using any chemicals (Nadeem *et al.*, 2015; Kaushik *et al*., 2021).

Preparation of Herbal Hair Oil Formulation:

In order to prepare the herbal hair oil, both plant oil extracts used in the present study, were prepared in combination of both plant ratios in per cent with three different formulation FO1, FO2 and FO3. FO1 is the 5% petroleum ether extracts of *Helianthus annuus* seeds and 10% petroleum ether extracts of *Martynia annua* seeds, FO2 is the 10% petroleum ether extracts of *Helianthus annuus* seeds and 5% petroleum ether extracts of *Martynia annua* seeds and FO3 is 7.5% petroleum ether extracts of *Helianthus annuus* seeds and 7.5% petroleum ether extracts of *Martynia snnua* seeds. Other ingredient for preparation was used as penetration enhancer suh as *Eucalyptus globulus* oil 5%, used as anti-microbial agent cinnamon oil 5%, used as a anti- oxidant agent Alovera pulp 5%, used as a fragrance jasmine flower extract 2% and *Cocus nucifera* oil was used as the base oil up to make up 100% for preparation of the hair oil formulation. All of the ingredients were made with easy methods and were introduced straight into the *Cocus nucifera* oil together with FO1, FO2, and FO3. The mixture was heated and stirred continuously until the medication was fully incorporated into the oil base. These hair oils were prepared using techniques like the direct boiling method and the pillow pouch method. According to the Ayurvedic Pharmacopoeia book, a confirmatory test of produced hair oils was conducted (Kuber *et al*., 2019; Tiwari and Tiwari, 2021) (Table 1).

Evaluation of Herbal Oil Formulation:

Following preparation, the oils underwent physiochemical and biological analysis. Standard techniques for physical and chemical evaluation, including specific gravity, pH, viscosity, acid value, peroxide value, and saponification value were used to assess the generated formulations (Gautam *et al*., 2012, Sabu *et al*., 2021).

Animal:

The experiment was performed according to

animal's experimental protocols approved by RKDF University, Bhopal by the Committee -- CPCSEA (Reg. No.1693/PO/a/13/CPCSEA). All experimental procedures were reviewed and verified for the approval by the Institutional Animal Ethics Committee, RKDF University, Bhopal, MP (Reg. No. IAEC/VCP/2021/004/9).

Test for skin allergic reaction:

For the study, thirty healthy, six-week-old albino mice weighing between 150 and 180 g were chosen. Throughout the test period, food and water were provided to each confined mice at their discretion. The mice were shaved on their back 24 h before the test in order to reveal sizable test areas that could hold three centimeter-sized test sites on each side of the spine. Surgical spirit was used to clean the test locations. On the other side of the spine, the measured quantity of FO1, FO2, and FO3 were applied. After application, the test group animals were monitored for 48 h to check for erythema and edema (Lulekala *et al.*, 2019).

Hair growth promoting activity:

Determination of 5α- reductase inhibitory activity:

Microsomal suspension from albino mice was made following the procedure outlined by Kumar *et al*. (2011). In short, mice livers that had been removed were diced and sliced using scissors before being homogenized in a solution of 0.32 M sucrose and 1 mM dithiothreitol in 0.02 M phosphate buffer at pH 6.5. The liver homogenate underwent two more centrifugations, each at 4500 × g for 30 mins at 0 °C. Before being employed as an enzyme source, all of the supernatants were collected and stored at -50 °C. As described by Kumar *et al*. (2012), the 5α-reductase assay was carried out. Finasteride is an example of a standard positive group enzyme inhibitor (units of mg finasteride equivalent per 1 g extract) or finasteride equivalent 5α reductase inhibitory activity (FEA) value, was used to express all data.

In vivo Hair Growth screening of prepared formulations:

The investigation involved screening albino mice, each weighing approximately 150–180 g, for activity that promotes hair growth. Thirty healthy albino mice were chosen, and five groups of six mice each were formed. The mice's dorsal region was shaved.

The first group received *Cocus nucifera* oil treatment as a control, while the second group received conventional care, which involved applying 2% minoxidil once a day to the shaved region. The produced oil formulations FO1, FO2, and FO3 were administered to the remaining three groups of albino mice.

For thirty days, all five groups received the same course of treatment. Throughout the length of treatment, the hair growth pattern was visually monitored, and the differences between the formulation treated group, the minoxidil (Standard positive group), and the control groups were noted qualitatively (Roy *et al*., 2007; Roy *et al*., 2008).

Determining the length of hair:

On days 10, 20, and 30 of formulation therapy,

mice's shaved dorsal areas were randomly selected for hair collection using sterile forceps. After measuring the length of each hair, the results were expressed as the mean length ± SEM of 25 hairs (Park *et al*., 2023).

Determining the thickness of hair:

At the conclusion of the thirty-first day of treatment, hair from the shaved area was removed using sterile forceps from each of the groups: Control, Standard (minoxidil 2% solution), and the FO1, FO2, and FO3 formulation. The thickness of the hair was measured using a micrometer, whose least count was 0.01 mm. For each group, the average hair thickness for 25 hairs was calculated, observed, and documented. The thickness of the hair was found to have significantly increased (Park *et al.*, 2023).

Results and Discussion

Phytochemical Screening:

Table 2: Evaluation of physical parameters of herbal hair oils formulated

Formulations	FO1	FO2	FO3
Colour	Greenish brown	Brownish green	Slightly darker
Odour	Pleasant	Pleasant	Pleasant
Appearance	Sediment and matter free	Sediment and matter free	Sediment and matter free
pH	7.0	7.2	7.3
Specific gravity	1.100	1.178	1.203
Refractive index	1.271	1.316	1.339
Viscosity(cps)	112.1	112.3	112.9
Acid value	0.81	0.92	0.93
Peroxide value (mE/1000 g)	6.0	6.36	6.42
Saponification value	248	250	249

Table 3: observations of formulated hair oils effect on 5α – reductase inhibition ability

S. No.	Formulation code	Finasteride 5α – reductase inhibition ability (mg Finasteride/ 1 ml oil formulation
1	FO1	26.30 ± 1.64
2	FO2	27.32 ± 1.73
3	FO3	27.88 ± 1.76

Alkaloids, carbohydrates, fixed oils, flavonoids, proteins, tannins, and vitamin C were found in the petroleum ether extract of *Martynia annua* seeds, while phytochemical screening of *Helianthus annuus* seeds revealed the presence of aromatic acids, triterpenoids, reducing sugars, alkaloids, phenolic compounds, saponins, tannins, flavonoids, and phytosterols.

Assessment of the herbal hair oil formulation:

The hair oils formulation FO1, FO2 and FO3 were further studied for their physical stability parameters like specific gravity, acid value, saponification value, viscosity and pH and also showed biological activity on the test group animals were observed, none of them showed any erythema and edema for 48 h after application in albino mice. On evaluation all these parameters were found to be satisfactory and within the prescribed limit (Table 2).

In vitro study Determination of 5α- reductase inhibitory activity:

The findings are presented as mg of finasteride equivalent per 1 g formulation, or finasteride equivalent 5α-reductase inhibitory activity value. The formulations FO1, FO2, and FO3 had values of 26.30 ± 1.64, 27.32 ± 1.73, and 27.88 ± 1.76. The FO3 formulation exhibited 5α-reductase inhibitory efficacy equivalent to finasteride, as demonstrated in Table 3 (Kumar *et al*., 2012).

In vivo study in albino mice model:

Hair length determination:

In all the five groups i.e. the FO1, FO2, FO3, Control and Minoxidil treated groups, the hair looked parsley. This denotes the presence of greater number of hair follicles in the anagen phase of the hair growth cycle.

After ten days, the average hair length in the control group was measured at 4.29±0.160 mm, whereas standard group, and the formulations FO1, FO2, and FO3 showed 12.22±0.155, 9.328±0.158 mm, 10.480±0.188 mm, and 11.680± 0.198 mm, respectively (Table 4). After twenty days, the average hair length in the control group was measured as 6.20±0.120 mm, 16.123±0.255

Table 4: Hair growth after 10, 20, and 30 days for control, standard and formulas FO1, FO2, and FO3

S. No.	Group	Hair length in mm		
		10 days	20 days	30 days
1.	Control	4.29±0.160	6.20±0.120	9.39±0.260
2.	Standard (2% Minoxidil)	12.22±0.155	16.123±0.255	23.22±0.355
3.	FO1	9.328±0.158	13.428±0.258	20.128±0.158
4.	FO2	10.480±0.188	14.280±0.288	21.220±0.188
5.	FO3	11.680±0.198	15.280±0.168	22.260±0.250

From the first day of therapy to the finish, the length of the hair was measured. When compared to the corresponding results using the Student's t-test, the mean value S.D. P <0.005 was displayed (n = 25 hair, and the result is expressed in terms of mean ± SEM).

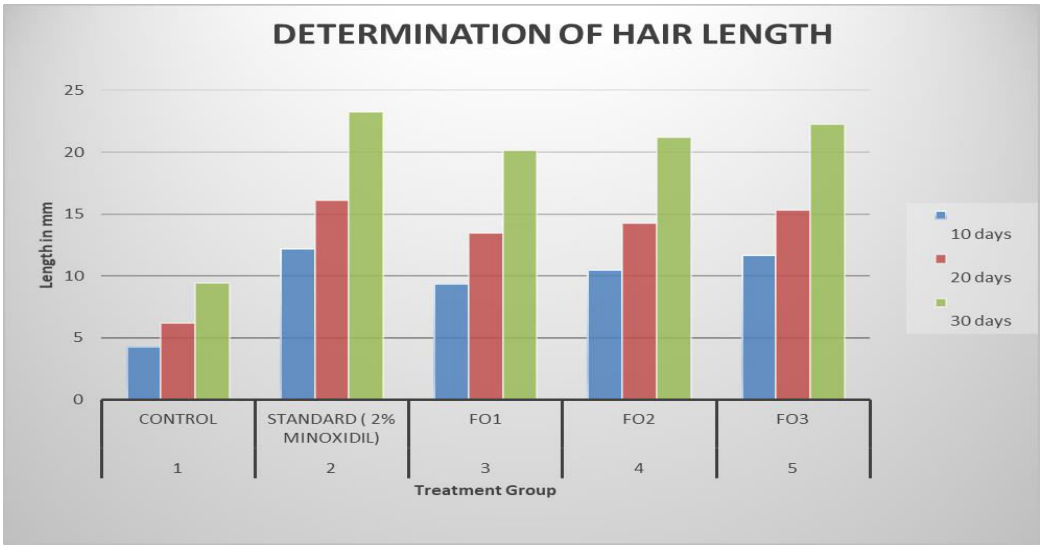

Fig. 1: Observation of hair length in a day's 10, 20, and 30 days.

Table 5: Measurement of Hair Thickness

S. No.	Treated Group	Hair thickness in mm
		30days
1.	Control	0.014±0.002
2.	Standard (2% Minoxidil)	0.021±0.008
3.	FO1	0.018±0.009
4.	FO2	0.019±0.002
5.	FO3	0.020±0.005

From the first day of therapy to the finish, the length of the hair was measured. When compared to the corresponding results using the Student's t-test, the mean value S.D. P <0.005 was displayed (n = 25 hair, and the result is expressed in terms of mean ± SEM).

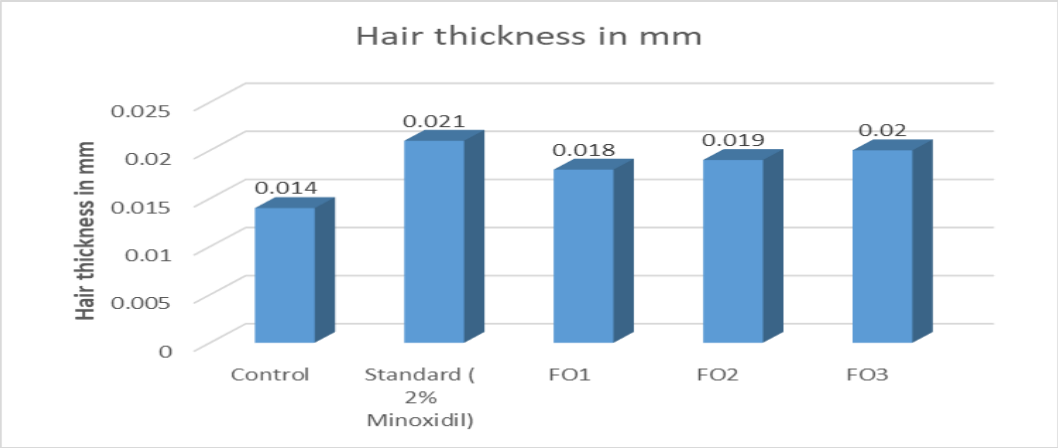

Fig. 2: Measurement of the thickness of hair on day thirty.

mm for the standard and 13.428±0.258 mm, 14.280±0.288 mm, and 15.280±0.168 mm for the formulations FO1, FO2, and FO3, respectively (Table 4). After the thirty days of treatment, a complete growth of hair length was observed and recorded, which was quite noticeable. The formulations FO1, FO2, and FO3 had the highest hair growth activity in terms of hair growth length, measuring 20.128±0.158 mm, 21.220± 0.188 mm, and 22.260±0.250 mm, respectively (Table 4), when compared to standard. These findings unequivocally show that the produced formulations of FO3 have superior activity and are just as effective as regular minoxidil and at FO3 may be a viable herbal substitute for minoxidil.

Following a 30-day treatment period, all observations were recorded. Following statistical analysis, the above hair gram graph was plotted to examine the cumulative growth of each formulation. A cumulative hair gram graph was also plotted for each formulation. An indication of the growth in hair length was obviously visible in formulas FO1, FO2, and FO3, as the peak area demonstrates. The average length of hair for each group on days 10, 20, and 30 is provided in Table 4 and Figure 1.

Hair thickness determination:

For the purpose of the data analysis, the hair thickness for each formulation was measured and noted. For each group, the average hair thickness for 25 hairs was measured, observed, and documented (Table 5; Fig. 2). Comparing the FO3 treated animals to the positive control (minoxidil 2% solution) and other group treated animals, it was found that the hair thickness in FO3 treated groups increased significantly. The data obtained from the 30-day treatment completion for the formulation FO1, FO2, and FO3 treated groups were 0.018±0.009 mm, 0.019±0.002 mm, and 0.020±0.005 mm, respectively. These values were similar to the 0.021±0.008 mm of the positive control (minoxidil 2% solution). The outcome was comparable to the test formulation, demonstrating strong hair tensile strength and a slower rate of hair loss.

Conclusion

Vigorous proliferation induces the process of differentiation during migration of hair matrix cells to the scalp's surface during hair development. Ectoderm on the skin generates hair at a rate of six inches per year, particularly if the protein is comprised of creatinine. The primary scourge of humanity is alopecia, or head hair loss, which is a dermatological condition that has long been recognized. The current study uses petroleum ether extract of *H. annuus* and *M. annua* seeds for phytochemical screening. The results showed the presence of terpenoids, linoleic acid, steroids, and flavonoids. FO1, FO2, and FO3

formulations are then used. The formulation FO3 had a considerable influence on hair growth. In order to ascertain whether the formulation FO3 has facilitated hair development the hair cycle was analyzed. In albino mice, the effect of hair development was noted in several formulations. The outcome was similar to that of the commercially available standard formulation of finasteride or 2% minoxidil. These results suggested that the inclusion of flavonoids, linoleic acid, and some steroids in formulations FO3 prepared by *Helianthus annuus* and *M. annua* seed extract may contribute to the high hair growth potential.

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