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Preparation and *In vivo* Evaluation of Hair Growth in Male Rabbits Using Poly Herbal Hair Oil

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Abstract

The study's primary goal is to create a multi-herbal hair oil formulation that can be used to stop hair loss, dandruff, balding, and dry hair. Evaluation of the developed formulations was done. It also includes physical metrics like pH, viscosity, specific gravity, refractive index, acid value, and saponification value as well as the evaluation of phytochemicals and organoleptic properties. *Chrysopogon zizanioides* roots, dried seeds of *Psoralea corylifolia*, dried fruits of *Phyllanthus embolic*, dried fruits of *Zingiber officinale*, dried seed of *Trigonella forenum-graecum* L., dried leaves of *Cyperus scariosus*, and dried flowers of *Rosa rubiginosa* are among the various herbal ingredients used in the formulation at hand. The herbal hair oil formulations were made by steeping the ingredients in coconut oil for 15 minutes at a temperature of 80°C. The F1 hair oil formulation exhibits the most colour intensity of the developed F1, F2, and F3 formulations, and the same intensity is maintained even after three shampooings. According to reports, the polyherbal hair oil formulations contain qualities including those that promote hair development, are anti-dandruff, moisturizing, and have a calming impact on the skin. Along with phytochemical, organoleptic, and physical characteristics, hair shine is also evaluated.

Keywords: Herbal • Oil • Extract • Hair • Loss • Growth

Introduction

Hair oils are a type of hair care product used to cure and prevent hair damage, baldness, and other conditions. Additionally, they encourage the luscious development of hair. As a hair tonic, hair oil containing herbal medications is utilized. Hair tonics and hair grooming aids are the two main categories for hair care products. In essence, these are herbal plant extracts with an oil base. Multi-ingredient hair oils are now made and tested for their ability to promote hair growth.

Vetiver: The scientific name for vetiver is *Chrysopogon zizanioides*. Vetiver, also known as khus, is a perennial plant in the Poaceae family that contains an oil used in perfumes. The vetiver plant is indigenous to tropical Asia, although it has been brought into both hemispheres' tropics. In certain areas, it has eluded cultivation and turned into a weed. The plant mixture was used as a sedative, nervine tonic, antidepressant, and stimulant of sexual drive. Tribal students, who depend on neighboring regions for their livelihood, make up the majority of the Indian population [1].

Babchi: The Leguminosae family includes Babchi, whose scientific name is *Psoralea coryalifolia*. This is a well-known traditional medicinal herb that has been utilized for treating a variety of illnesses since ancient times. It is widely used and plays a significant role in Ayurveda and Chinese medicine therapies. In the traditional system of medicine or in ethnomedical practices, native plants are employed as treatments for a variety of diseases. The plant has enormous biological significance and has been used extensively for centuries to treat a variety of skin conditions, including psoriasis, leukoderma, and leprosy [2].

Amla: The scientific name for amla is *Phyllanthus emblica*, and its family is Phyllanthaceae. Amla berries improve calcium absorption, resulting in better bones, teeth, nails, and hair. It strengthens the hair follicles, preserves hair colour, and delays premature greying. Amla possesses antibacterial and antioxidant characteristics that may aid in fostering the development of thick, healthy hair. The scalp and hair will be strengthened, premature hair pigment loss will be reduced, hair growth will be stimulated, and dandruff and dry scalp will be prevented or treated [3].

Ginger: *Zingiber officinale* sometimes known as ginger, belongs to the Zingiberaceae family and has a scientific name of *Hedychium*. In India, the use of ginger has a long history. The rhizomes are used to make abir, a fragrant colour used in religious ceremonies and during

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the holi celebration. They are white and starchy inside. It has been prized in the traditional medical system for having a wide range of medicinal properties, including carminative, expectorant, tranquillizer, stomachic, antiseptic, vasodilator, analgesics, anti-inflammatory, antibacterial, antifungal, antioxidant, pediculicidal, and cytotoxic activity [4].

Fenugreek: The family name of fenugreek is Fabaceae, and its scientific name is *Trigonella foenum-graecum* L. Due to its culinary and therapeutic qualities as a natural cure, this plant has a long history of use dating back more than 2500 years. In essence, it is said to have originated in West Africa and is currently widely cultivated in Asia and Latin America as well. This plant's seeds and leaves are widely used in traditional Chinese medicine as anti-diabetic, anti-microbial, anti-inflammation, anti-cancer, and antioxidant agents. Because of their potential health advantages and benign side effects, the use of essential fatty acids and oils as prospective natural antioxidants is gaining popularity in culinary, cosmetic, and pharmaceutical applications [5].

Nut grass: *Cyperus scarious* is the scientific name for nut grass, and the Cyperaceae family includes it. Use of topical *Cyperus scarious* oil to inhibit hair growth is efficient and secure. On androgenic hair, the oils' flavonoids have an anti-androgenic effect. This plant is frequently used as an astringent, diuretic, anti-inflammatory, antibacterial, and central nervous system stimulant. Rhizomes are used as a snake bite remedy and for cleaning hair. Hair tonic can also be made from oil [6].

Sweet briar: *Rosa rubiginosa* and the Rosaceae family are the scientific names for sweet briar. If the blooms from this plant are consumed, it will have a cleansing effect on the intestines and urine. It also cleans up blood. The bark can be applied on the spleen externally. The uses here seem somewhat constrained and lack the subtlety of Arabic or Renaissance medicine, even if application to the chest is notable, reflecting certain discords in the spitting of blood and resurfacing in Culpeper's later usage for tuberculosis [7].

Materials and Methods

The preparation of vetiver extract

The vetiver leeches used to make the extract were gathered from the banks of the Gundlakamma River close to the Gundlakamma Project in the Mallavaram neighbourhood of Gundlapalli. The vetiver was either kept in a highly concentrated salt solution or in contact with sodium chloride crystals. The remains were then washed in distilled water to remove any excess salt that might have affected the pH of the extract that was going to be made. Leech bodies that had been cleaned were now chopped into little bits and put through a mixer to create a smooth paste. Sand was used to grind the pulp, and six times as much distilled water as pulp was used in the extraction process. The fluid from the supernatant was collected after centrifugation and filtered through coarse paper [8].

The preparation of babchi extract

Babchi seeds were gathered from a botanical garden in Mogalrajapuram, Siddhartha Nagar, Benz Circle, Vijayawada, Andhra Pradesh, GJ3X+QH4. For a few days, the plant's dry seeds were shadowed on the ground and dried at a temperature of 350 to 450 Celsius. After that, the seeds were pulverized. Boiling 20 g of babchi powder in 1 litres of distilled water for 5 minutes produced the aqueous extract of the seeds. The extract was now stirred to ensure a consistent extraction and covered until it warmed to room temperature. The extract was then concentrated in a rotary evaporator for an hour after the extraction residue was collected by filtering (1 mm pore size). The extract was now kept at -20° Celsius for additional time.

The preparation of amla extract

The Pharmacologist of the Department of Pharmacy at Hindu College of Pharmacy, Guntur, recognized the amla after it was taken from the herbal garden there. Fresh amla was rinsed with distilled water to create the cold water extract. Amla was dried in a lab oven at a steady temperature of 500 degrees Celsius to remove any remaining moisture. Now a mortar and pestle were used to grind the dried amla. About 12 grammes of powder were dissolved in 100 milliliters of distilled water. The extract was now allowed to soak for two days before being filtered using 1 mm filter paper [9].

The preparation of ginger extract

The Pharmacologist of the Department of Pharmacy, QISCP, Ongole, recognized the ginger after it was taken from the herbal garden there. By rinsing the ginger with distilled water, fresh ginger was turned into a cold water extract. Drying ginger inside a lab oven set at a steady 500 degrees Celsius removed none of the moisture from it. Using a mortar and pestle, the dried ginger was now crushed. 10 gms of powder were dissolved in 100 milliliters of distilled water. The extract was now held for 2 days to soak before being filtered using 1 mm filter paper.

The preparation of fenugreek extract

The fenugreek was collected from the herbal garden of QIS College of Pharmacy, Ongole and identified by the pharmacologist of Department Pharmacy, QISCP, and Ongole. The cold water extract of fresh fenugreek was produced by rinsing the fenugreek with distilled water. Not the moisture on ginger was removed by drying then inside an oven in the lab at a constant temperature of 500 Celsius. Now the dried fenugreek was ground using a mortar and pestle. An approximately 10 gm weight of powder was dissolved in 100 ml of distilled water. Now the extract was kept for 2 days for soaking and then filtration was done using 1 mm filter paper.

The preparation of nut grass extract

Nut grass was taken from the QIS College of Pharmacy's herbal garden in Ongole. For a few days, the plant's nut grass was dried in the

shade on the ground at a temperature of 350 to 450 Celsius. After that, the nut grass was powdered. 20 g of the nut grass powder were boiled for 5 minutes in 1 liter of distilled water to create the aqueous extract of the nut grass. The extract was now stirred to ensure a consistent extraction and covered until it warmed to room temperature. The extract was then concentrated in a rotary evaporator for an hour after the extraction residue was collected by filtering (1 mm pore size). For later usage, the extract was now kept in storage at -20° Celsius.

The preparation of sweet briar extract

The QIS College of Pharmacy, Ongole's herbal garden is where the sweet briar was found. The plant's fragrant briars were shadowed on the ground and dried for a short period of time at a temperature of 350 to 450 Celsius. Following that, the sweet briar was ground and powdered. 20 g of the sweet briar powder were boiled for 5 minutes in 1 liter of distilled water to create the aqueous extract of the sweet briar. The extract was now stirred to ensure a consistent extraction and covered until it warmed to room temperature. The extract was then concentrated in a rotary evaporator for an hour after the extraction residue was collected by filtering (1 mm pore size). The extract was now kept at -20° Celsius for additional time.

Preparation of oil

Each extract was combined with the appropriate amount of coconut oil in an equal ratio. To emulsify the entire recipe, emulsifier was introduced in an approximate quantity. Now, the entire mixture was blended in a homogenizer to create a homogenous solution of oil and herbal extracts, and oil was created as illustrated in Figure 1.

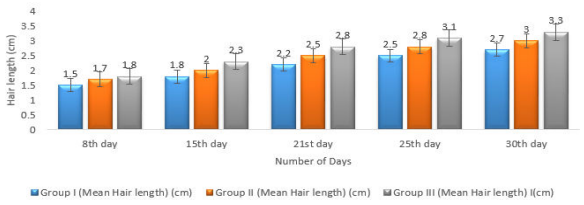

Figure 1. Preparation of the poly herbal-hair oil

Standardization of oil

To ensure the prepared oil's efficacy, stability, quality, and purity, it was assessed for a number of factors, including organoleptic and physical standards.

Physical-chemical analysis

Determining colour and smell: Organoleptic properties for different sensory qualities, such as colour and smell, were meticulously recorded down. It was discovered that the oil sample's colour and smell were characteristic of its components.

Determination of specific gravity: Bottle of specific gravity was cleaned with acetone, then it was shaken with ether. Drying the bottle and recording the weight were done. The oil was added to the bottle with specific gravity, and the weight was recorded. Repeating the process with distilled water in place of the oil. Oil can undergo changes due to heat influences. The following relationship was used to calculate the oil's specific gravity.

$$\text{Specific gravity} = (A-B)/(C-D) \quad (1)$$

Where, A=Weight of specific gravity bottle with oil at 30°C (g); B=Weight of specific gravity bottle at 30°C (g); C=Weight of specific gravity bottle with water at 30°C (g).

pH determination: The digital pH meter was used for the pH determination.

Determination of acid value: A conical flask containing 10 g of oil was used. It received 50 ml of acid-free alcohol-ether mixture (25 + 25 ml), which had been titrated against 0.1N potassium hydroxide solution and earlier neutralized by the addition of 1 ml of phenolphthalein solution. With the development of a light pink colour that lasts for 15 seconds, the end point is identified. Triglycerides are transformed into fatty acids and glycerol during oxidation, which causes the acidity to increase. Therefore, there is a direct connection between acid value and rancidity. In the current work, the oil's acid value was under 2, indicating higher quality.

$$\text{Acid value} = 5.61VN/W \quad (2)$$

Where, V=Volume of standard sodium hydroxide used (ml); N=Normality of the sodium hydroxide solution; W=Weight of the sample (g).

Determination of peroxide value:

$$\text{Peroxide value} = (10(a-b))/W \quad (3)$$

Where, a=ml of NaOH required to neutralize the substance, b=ml of NaOH required for blank, w=weight of sample in (g).

Determination of saponification value: A 250 ml round bottom flask weighed about 2 g of the oil. After adding 25 ml of the KOH alcoholic solution, a reflux condenser was connected. It was heated for one hour on a water bath while the contents of the flask were periodically turned. The flask was chilled before adding 1 ml of the phenolphthalein solution and titrating the surplus alkali with 0.5N HCl. It was indicated how many ml were needed. Because they have a comparatively smaller number of carboxylic functional groups per unit mass of the fat than short chain fatty acids, long chain fatty acids present in fats have a low saponification value.

$$\text{Saponification value} = (28.05(B-S))/W \quad (4)$$

Where, S=ml of KOH required to neutralize the substance; B=ml of KOH required for blank; and; W=Weight of the sample taken for the test (g).

Animals study

At 28.2°C, nine male rabbits weighing between 1.5 and 3 kg each were housed in three different cages with three animals each. The humidity was kept between (50 and 55%). The experimental setup is depicted in Table 1 and the animals were fed a regular pellet diet and free access to water in the CPCSEA-approved animal house of QISCP, Ongole (Institute Animal Ethics Committee number is QISCP/IAEC/02/2020-21 for Research for Education Purpose on Small Animals).

S. no.	Group number	Group name	Dosing	Number of animals
1	Group-I	Control	Coconut oil	3
2	Group-II	Standard	4% Minoxidil ethanolic solution	3
3	Group-III	Test	Laboratory herbal oil	3

Table 1. Experimental design.

Using the described method, the potential for hair development in male rabbits was assessed. Male rabbits' dorsal hair was clipped at a length of 3-6 cm, and then it was covered in hair removal cream (Veet, a commercial hair removal product). On days 8, 15, 21, 25, and 30, the length of hair in each group was measured. After 30 days of therapy, the length of hair in both groups was now compared.

Results and Discussion

Standardization of herbal oil

The formulation had a pleasant aroma because of the light green colour of the produced oil, which was also the cause. The distinctive smell is a nice one. The pH was discovered to be neutral to accommodate all the various types of scalp and hair. If the saponification value exceeds the usual range, smaller molecular saturated fatty acids are present. The likelihood of photo-oxidation and rancidity increases with increasing acid value. The characteristic odor is pleasant. pH was found to be neutral to suit all the different types of scalp as well as hair. If Saponification value is more than normal range, it indicates lower molecular saturated fatty acids. If acid value is more, then chances of photo-oxidation and rancidity are more. The obtained values of these tests were found within normal limits in prepared formulation which indicate good quality of product. In addition, no rancidity was found in finished product. The sample's specific gravity was 0.857, which is not too dense and was closer to that of unrefined coconut oil (0.9). The amount of free fatty acids present in the oil was indicated by the acid value, which was 1.4 w/v. It was discovered that the saponification value was 98 w/v. Table 2 lists all the assessment information found for the herbal oil.

S. no.	Parameters	Result
1	Color	Light green
2	Odor	Pleasant
3	pH	6.8
4	Specific gravity	0.857
5	Acid value	1.4
6	Peroxide value	1.6
7	Saponification value	95

Table 2. The parameters observed and evaluated by standardization of the oil are as follows.

Evaluation of effectiveness of herbal oil

On the 8th, 15th, 21st, 25th, and 30th days, the length of the hair in Group I, Group II, and Group III was measured and compared before and after the use of oil. Male rabbits' dorsal hair development after shaving was observed, and hair length in all groups was measured and compared on dates 8, 15, 21, 25, and 30 following shaving, as shown. These parameters were used to evaluate the experiment's outcomes in Tables 3 and 4 and Figures 2 and 3.

S. no.	Days	Group-I (Mean hair length) (cm)	Group-II (Mean hair length) (cm)	Group-III (Mean hair length) (cm)
1	8th day	1.5	1.7	1.8
1	15th day	1.8	2	2.3
2	21st day	2.2	2.5	2.8
3	25th day	2.5	2.8	3.1
4	30th day	2.7	3	3.3

Table 3. The mean hair length was recorded on 8, 15, 21, 25 and 30 days in male rabbit of Group-I, Group-II and Group-III.

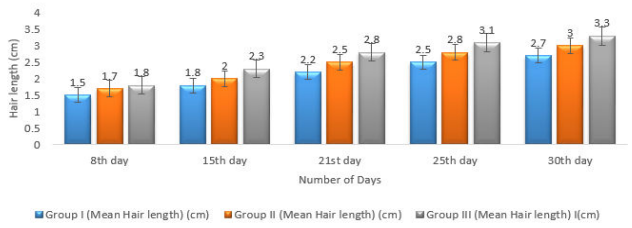

Figure 2. A comparative graph of hair length on days 8, 15, 21, 25 and 30 after hair removal. X-axis: Number of days on which hair length was recorded. Y-axis: The length of hair grown on dorsal area of male rabbits in centimeters.

S. no.	Time to initiate hair growth in days
Group-I	8
Group-II	6
Group-III	4

Table 4. The table shows initiation of hair growth in days (mean) in all groups.

S. no.	Group number	Group name	Telogen	Anagen	A/T
1	Group-I	Control group	63.2 ± 0.5320%	54.2 ± 0.5321%	0.86
2	Group-II	Standard group	43.2 ± 0.5223*** %	74.2 ± 0.5226*** %	1.72
3	Group-III	Treatment group	35.6 ± 0.8534*** %	78.2 ± 0.5352*** %	2.2

Note: Values are mean ± SEM, *p<0.05, **p<0.01, ***p< 0.0 01, significance *vs.* control

Table 5. Anagen/Telogen ratio for estimation of hair growth population of 100 hairs for all groups.

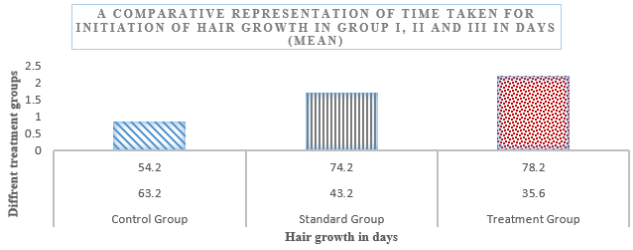

Figure 3. A comparative representation of time taken for initiation of hair growth in Group I, II and III in days (mean). X-axis: Shows the different groups on basis of dosing and treatment I, II and III. Y-axis: Shows the time taken to initiate hair growth in days

Conclusion

The constituents of a prepared formulation are confirmed by the results of its standardization. This composition, which contains natural substances, works wonders to combat the widespread issue of hair loss caused by a variety of factors. To determine the efficacy of the prepared herbal oil, the findings were tabulated, graphed, and compared. The effectiveness of the produced herbal oil was assessed using Minoxidil as a benchmark. When treating alopecia, it was discovered that the herbal oil was more effective at promoting hair growth than the regular form of Minoxidil.

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