Preparation, evaluation and hair growth stimulating activity of herbal hair oil

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Abstract

Herbal formulations always have attracted considerable attention because of their good activity and comparatively lesser or nil side effects with synthetic drugs. The objective of present study involves preparation of herbal hair oil using amla, hibiscus, brahmi, methi and its evaluation for increase in hair growth activity. Each drug was tested for their hair growth activity in a concentration range for 1-10% separately. Based on these results mixture of crude drugs fruits of *Embelica officinalis*, flowers of *Hibiscus rosa sinensis*, leaves of *Bacopa monnieri* and seeds of *Trigonella foenumgraecum* were prepared in varying concentration in the form of herbal hair oil by three different oils preparation techniques (direct boiling, paste and cloth method) and were tested for hair growth activity. The oil of different concentrations were characterized for proximate analysis including moisture content, total ash, acid insoluble ash, water soluble ash, water insoluble ash, sulphated ash. The formulations were also subjected to chromatographic determination and chemical tests to determine the presence of active constituents in the drugs. But looking towards the formulation viscosity the maximum concentration of combined drug was found to be 30% at their maximum level. The formulation containing 7.5% of each drug used for the study and showed excellent hair growth activity with standard (2% minoxidil ethanolic solution) by an enlargement of follicular size and prolongation of the anagen phase. It holds the promise of potent herbal alternative for minoxidil. Excellent results of hair growth were seen in formulation prepared by boiling method of oils preparation technique.

Keywords : Herbal preparation, Hair oil, Hibiscus, Amla, Methi, Brahmi

Introduction

Hair oils are the hair care preparations used for the prevention and treatment of baldness or other ailments, aggression of hair. They also promote the luxurious growth of hairs. Hair oil containing herbal drugs are used as hair tonic. Hair care products are categorized into two main category, hair tonics and hair grooming aids. These are basically the extracts of

medicinal plants in an oil base. A plethora of herbs have been employed for hair treatments. A few of these herbs are amla, henna, neem, methi, lemon, tulsi, brahmi, shikakai, reetha, liquorice root, musk root, mahabhringraj, jantamasi, chitraka, marigold, hibiscus, nutmeg, parsley, rosemary, thyme[1-2].

Synthetic drug, minoxidil is a potent vasodilator appears safe for long-term treatment. After five years use of 2 and 3% topical minoxidil, the improvement has been shown to peak at one year with a slow decline in regrowth over subsequent years[3]. Long-term treatment with local side effects may be a problem with continuing used of minoxidil lotion[4-5]. On the basis of market survey carried out on crude drugs used presently for herbal hair oils gives us clue for selection of drugs for hair oil. Hence the present study was aimed to evaluate the hair growth activity of herbal formulations, which includes oil extract of all mentioned drugs in various concentrations. In order to justify the traditional claims now a days multi ingredient hair oils are prepared and tested for their hair growth activity.

Amla is rich in vitamin C, tannins and minerals such as phosphorus, iron and calcium which provides nutrition to hair and also causes darkening of hair[6]. Hibiscus consists of calcium, phosphorus, iron, vitamin B_1, riboflavin, niacin and vitamin C, used to stimulate thicker hair growth and prevents premature graying of hair[7]. Brahmi contains alkaloids which enhance protein kinase activity[8]. Methi contains high protein fodder which supply required protein nutrition to hair[9].

There are various methods available for the preparation of hair oils direct boiling method, paste method and cloth method[10]. After preparation second main step is evaluation of preparation. The next final step is determination of its therapeutic efficacy.

Experimental Section

Plant Material

The fruits of *Embelica officinalis*, flowers of *Hibiscus Rosa sinensis*, leaves of *Bacopa monnieri* and seeds of *Trigonella foenugraecum* were procured from local market and identified by comparing with standard herbarium specimens available in AICRP on medicinal and aromatic plants, J.N.K.V.V, College of Agriculture, Jabalpur. The various parts of plant drugs are crushed in mixer and passed through the sieve number 80. The various powder drugs were subjected to pharmacognostic studies for confirmation.

Animals

Male Wister albino rats, weighing 150-200 gm and rabbits were obtained from Veterinary College, Jabalpur. The protocol of the experiment was approved by the Institutional animal ethical committee as per the guidance of the committee for the purpose of control and supervision of experiments on animals (Reg. No-1196/a/08/CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Characterization and Identification of Powdered Raw Material

Individual drugs were subjected to Proximate analysis including Moisture content, Total ash, Acid insoluble ash, Water soluble ash, Water insoluble ash, Sulphated ash. The extracts obtained from successive solvent extractions were then subjected to qualitative chemical analysis for identification of various plants constituents using different methods. Thin layer chromatography and paper chromatography with different mixtures of solvent systems were performed to confirm the presence of constituents detected in qualitative chemical test[6, 11].

Preparation of Herbal Hair Formulation

The herbs used in the present study for making herbal oil were dried, crushed and passed through 80 mesh stainless steel sieves and olive oil was used as base. The hair oil was prepared utilizing three different methodologies.

First is the direct boiling method in which the crude drugs were powdered, weighed and directly boiled in olive oil with continuous stirring and heating until the drug had completely extracted in the oil base. 1, 2, 3% of drugs containing oils were prepared.

Secondly, paste method was used where fresh fruit or pulp or the desired part of the plants were converted into paste with very little amount of water and kept overnight. After this the wetted drug was mixed in olive oil base and boiled with continuous stirring at a constant temperature, until the water droplets in oil stop knocking and the drug has completely extracted in the oil. The oil was then filtered through a muslin cloth. Three different concentrations 4%, 5%, 6% were prepared containing 4, 5, 6 g of drug per 100 ml of oil respectively.

Last method is cloth method, in which the dried drug was weighed and tied in a muslin cloth. This cloth was then hanged in olive oil base, with continuous boiling, stirring and finally the oil was filtered. Four different concentrations 7%, 8%, 9%, 10% were prepared containing 7, 8, 9, 10 g of drug per 100 ml of oil respectively.

Hair growth initiation test

Quantitative modified model for the study of hair growth initiation was followed. The rabbits were divided into four groups of one rabbit named as group A, B, C and D respectively 2 cm^2 area of surface of each rabbits shaved area to remove all the hairs. Approximately 11 patches on each rabbit with a distance of 5cm were developed. Group A was treated with amla oil with respective concentration of 1mL each of 1-10% on each patch (A1 – A10) keeping first patch as control. Group B was treated with brahmi oil with respective concentration of 1mL each of 1- 10% on each patch (B1-B10), keeping first patch as control. Group C was treated with methi oil with respective concentration of 1mL each of 1-10 % on each patch (F1-F10), keeping first patch as control. Group D was treated with hibiscus oil with respective concentration of 1mL each of 1-10% on each patch keeping first patch as control. This treatment was continued for 15 days during the course the hair growth initiation pattern was observed and reported.

Preparation of combined drug herbal hair formulation of different concentration

After confirming growth initiation from individual oils and selection of method for preparation, multi ingredient oils of effective concentrations based on the preliminary physical and biological screening were prepared.

The method selected was direct boiling method and three different formulations having concentrations 2.5%, 5%, 7.5% of drugs were prepared for maximum activity.

Evaluation of Herbal Oil Preparation

The prepared oils were then subjected to physical and biological evaluation.

Physical evaluation

In physical evaluation, parameters like specific gravity, pH, refractive index, viscosity and acid value were determined and the formulations were subjected to biological evaluation.

Biological evaluation

Primary skin irritation test

The prepared formulations were assessed for primary skin irritation test. Six healthy rats were selected for the study. Each rat was caged individually food and water given during the test period 24 hrs prior to the test. The hair from the back of each rat of 1cm^2 was shaved on the side of the spine to expose sufficiently large test areas, which could accommodate three test sites were cleaned with surgical sprit. Measured quantity (1ml) (5% w/w) of the formulations OD1, OD2 and OD3 were applied over the respective test sites on one side of the spine and observed for erythema and edema for 48hrs after application[12].

Hair growth initiation test

Quantitative model by Uno was used with slight modification for the study of hair growth initiation[12]. The rabbits were divided in 4 groups of 1 rabbit each and 2 cm^2 areas were shaved to remove hairs. Eleven patches were developed on each rabbit. Rabbit of group A was treated with amla oil of 1-10% concentration on individual patches keeping first patch as control. Similarly rabbits of other three groups were subjected to brahmi, methi and hibiscus oils respectively with different concentrations in same pattern. This treatment was continued for 15 days and during the course the hair growth initiation pattern was observed and reported.

Hair Growth Activity

The rats were divided into 5 groups of 5 rats each and 2 cm^2 area of the dorsal portion of each of the rats was shaved. Group 1 was kept as control, where there was no drug treatment and in Group 2 the standard 2% minoxidil ethanolic solution was applied. In the remaining groups 3, 4, 5 the three different concentrations; 2.5% (OD_1), 5% (OD_2) and 7.5% (OD_3) of the herbal oil formulations were applied once a day respectively. The treatment was continued for 30 days and during this course the hair growth pattern was observed qualitatively and recorded[2, 13].

Results and Discussion

The prepared formulation is green to reddish-black in color with pH in accordance with human skin neutral to slightly acidic. Proximate analysis and qualitative chemical test were performed to identify plants and to show presence of active constituents responsible for increasing hair growth activity.

Table 1 & 2 summarizes the results of proximate and chemical analysis respectively. Petroleum ether extract of the drugs were subjected to qualitative thin layer chromatographic screening and the results obtained are summarized in table 3. Table 4 summarizes the various values obtained in the physical evaluation of the formulations.

Primary skin irritation test was conducted to evaluate the irritation by the prepared formulations on intact skin of rabbits. All of the prepared formulations were not showed any erythema and/or edema; this indicates that the prepared formulations were nonirritant on skin of rabbits.

The results of preliminary hair growth initiation tests were encouraging. They were recorded on the basis of time taken for initiation of growth and number of hair follicles. This parameter is recorded for finding minimum effective concentration of drug. Amla oil showed significant growth at a concentration of 7% to 8% and growth was observed in 8-9 days. Hibiscus oil showed significant growth in 6-7 days at a concentration of 6% and 7%. Brahmi oil also showed growth in 7-8 days at a concentration of 7%-8%. Methi oil showed growth in 9-10 days at a concentration of 7%-8 %. Table 5 & 6 give the results obtained after the biological screening.

Table 1: Result of proximate analysis

Name of analysis	**Amla** *(Embelica officinalis)*	**Hibiscus** *(Hibiscus Rosa sinensis)*	**Brahmi** *(Bacopa monnieri)*	**Methi** *(Trigonella foenugraecum)*
Moisture content	9.25%	8.4%	9.15%	9.30%
Total ash	23.22%	20.35%	22.33%	24.45%
Acid insoluble ash	3.22%	4.9%	3.33%	4.55%
Water soluble ash	34.5%	31.5%	32.5%	32.0%
Water insoluble ash	2.5%	4.5%	2.5%	3.5%
Sulphated ash	3.12%	3.15%	3.13%	4.15%

Table 2: Result of qualitative chemical test

Constituents	**Amla** *(Embelica officinalis)*	**Hibiscus** *(Hibiscus Rosa sinensis)*	**Brahmi** *(Bacopa monnieri)*	**Methi** *(Trigonella foenugraecum)*
Alkaloids	-	-	+	+
Carbohydrates	-	+	-	-
Phytosterols	-	+	-	-
Proteins	-	+	-	+
Saponins	-	-	+	+
Pectin	+	-	-	+
Glycosides	-	+	+	+
Vitamin C	+	+	-	-

Table 3: TLC Screening of petroleum ether extracts of drugs

Solvent system used	**Detection reagent**	**Observation**	**Inference**	**Petroleum ether extract**			
				A	**H**	**B**	**M**
Ethylacetate:Methanol:Water 100:13.5:10	Dragendorff reagent	Orange brown (vis)	Alkaloid	-	-	+	+
	Vanillin sulphuric acid	Blue (vis)	Saponin	-	-	+	+
N,N dimethyl formamide:Benzene 7 : 93	Alcoholic NaOH	Yellow	Glycoside	-	+	+	+

A= Amla *(Embelica officinalis);* **H= Hibiscus** *(Hibiscus Rosa sinensis)*
B= Brahmi *(Bacopa monnieri);* **M= Methi** *(Trigonella foenugraecum)*

Table 4: Evaluation of physical parameters

Physical Parameters	Concentration		
	2.5%	**5%**	**7.5%**
Specific gravity	0.928	0.9384	0.9432
pH	9.1	8.4	7.5
Refractive index	1.492	1.472	1.435
Acid value	2.49	2.18	1.558

Table 5: Qualitative observations of hair growth

Formulations	Number of rats	Time taken to initiate growth (in days)	Time taken for complete growth (in days)
Control (untreated)	5	8	24
Standard (10%minoxidil)	5	7	19
OD_1	5	10	25
OD_2	5	9	23
OD_3	5	8	18

Table 6: The rate of hair growth in different phases of hair growth cycle

Formulation	Mean length (mm ± 50)				Population %			
	Anagen		Catagen	Telogen	Anagen		Catagen	Telogen
	A3	A5			A3	A5		
Control	-	0.60±0.14	0.11±0.03	0.20±0.06	-	47	4	49
Standard	0.48±0.04	0.69±0.12	0.1	0.21±0.05	19	50	1	32
2.5% (OD_1)	0.39±0.03	0.62±0.11	-	0.22±0.04	26	48	-	19
5% (OD_2)	0.42±0.06	0.70±0.11	0.1	0.21±0.04	23	64	2	29
7.5% (OD_3)	0.44±0.05	0.71±0.13	-	0.24±0.04	22	67	1	17

The significant quantitative changes shown by various hair oils prompted the hair growth activity screening of the herbal hair oil formulation. It was observed that the herbal preparation showed excellent activity at a concentration of 7.5% better than the standard drug minoxidil. While minoxidil showed complete hair growth in 19 days, OD_3 gave similar results in 18 days.

The quantitative study revealed that formulation OD_3 showed considerable increase in number of hair follicle in anagen phase of hair growth cycle when compared to control and standard. In standard group, percentage of population of anagen follicle was 67% while in OD_3 it was 89% and OD_2 it was 87%. The formulation OD_1 and OD_2 were shown time of initiation of hair growth late when compared with standard and control. It also observed that the time taken for complete hair growth the late initiation and completion of hair growth was 25days in OD_1 and 23days in OD_2 indicating late initiation and completion of hair growth.

The result shows that formulation OD_3 have contributed in most significant hair growth activity. Similarly, the way of method of preparation of OD_3 (7.5% concentration of all drugs) boiling in pouch method showed maximum extraction of active principles responsible for hair growth. The hair growth studies finally prove that formulation OD3 have significant increase in hair growth activity when compared to the standard. It holds the promise of potent herbal alternative for minoxidil. Also suggest excellent results of hair growth in formula prepared by cloth pouch boiling method.

Conclusion

The hair growth studies showed that formulation OD_3 has excellent potential to be developed as herbal alternative to minoxidil. The various constituents of the herbal extracts such as minerals and amino acids may be the cause for the significant hair growth activity. All these drugs not only show remarkable activity but are also devoid of potential side effects as compared to synthetic drugs.

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