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ISSN: 0973-4945; CODEN ECJHAO
E-Journal of Chemistry
Vol. 5, No. 1, pp. 34-38, January 2008

Development and Evaluation of Herbal Formulations for Hair Growth

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Received 13 January 2007; Accepted 2 March 2007

Abstract: Hair formulation of *Emblica officinalis* (Euphorbiaceae), *Bacopa monnieri* (Scrophulariaceae), *Trigonella foenumgraecum* (Leguminosae), *Murraya koenigii* (Rutaceae) in various concentrations in the form of herbal oil were studied for their 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 *Murraya koenigii*, leaf (Rutaceae), *Bacopa monnieri*, leaf (Scrophulariaceae), *Trigonella foenumgraecum* (Leguminosae), *Murraya koenigii* (Rutaceae) were prepared in varying concentration in the form of herbal hair oil by three different oils preparation techniques and were tested for hair growth activity. The result revealed that the hair growth activity of each drug was found proportional to the concentration range tested. Similarly higher concentrations of drug in the formulation were found to have higher hair growth activities. 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 cloth pouch decoction method of oils preparation technique.

Keywords: Hair formulation, Herbal oil, Euphorbiaceae, Leguminosae, Scrophulariaceae, Rutaceae

Introduction

Hair suffers aggression; there can be some ailments to normal health of hair and cause trouble. The main problems associated with hair such as pigmentation problems (Fading), dandruff and falling of hair (Shedding)¹. 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 years². Long-term treatment with local side effects may be a problem with continuing use of minoxidil lotion^{3,4}. 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. The Plant *Embllica officinalis* belongs to family Euphorbiaceae. Amla is rich source of vitamin C and contains appreciable amount of pectin rich in mineral matters like phosphorous, iron, and calcium⁵. *Bacopa monnieri* belongs to the family Scrophulariaceae, is known for its action on brains so that it is called as nervine tonic. It has been reported that bacosides A & B enhance protein kinase activity and new protein synthesis in cells. So that it is also used for treatment of dementia⁶. *Trigonella foenum-graecum* belongs to family Leguminosae used as high protein fodder and for its cleansing and softening activity. It promotes scalp health and prevents hair falling⁷. Leaves of *Murraya koenigii* belongs to family Rutaceaceae is used for its antiseptic properties⁸. *Cocos nucifera* from the family Palmae is said to promote the growth of hair.

Experimental

Collection and identification of plant

The fruits of *Embllica officinalis*; leaves of *Bacopa monnieri*; leaves of *Murraya koenigi*; seeds of *Trigonella foenumgraecum* were purchased from local market and identified by comparing with standard herbarium specimens available in AICRP on Medicinal & Aromatic plants, J.N. Krishi Vishwa Vidyalaya, College of Agriculture, Indore. 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.

Preparation of hair formulation

The hair formulations of 1, 2 and 3% of *Embllica officinalis*, *Bacopa monnieri*, *Murraya koenigi*, *Trigonella foenumgraecum* of each drugs were separately prepared by cloth pouch method and similarly the 4, 5, 6, 7, 8, 9 and 10% formulation of each drugs were prepared. The method used for carrying out these formulations was holding the individual drug in cloth pouch and boiling continuously with stirring arrangement.

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 cm² 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 meetha neem 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 the preliminary growth initiation with individual drug oil the all drugs are selected and mixed in three concentration as 2.5, 5, & 7.5% for maximum⁹ activity (Table 1).

Table 1. Selection of Concentration of Oil for Hair Growth Activity.

%	Amount of drugs/100mL of oil, g			
	Amla	Brahmi	Methi	Meetha Neem
2.5 (OD1)	2.5	2.5	2.5	2.5
5.0 (OD2)	5.0	5.0	5.0	5.0
7.5 (OD3)	7.5	7.5	7.5	5.0

Primary skin irritation test

The institute has been cleared for the said biological evaluation by Animal Ethics Committee (Letter No.465/01/AB/CPCSEA). Three healthy male rats, weighed 200-250g 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 1cm² was shaved on the side of the spine to expose sufficiently large test areas, which could accommodate three test sites were cleaned with surgical spirit. 1mL quantity (5% w/w) of the formulations OD1, OD2 and OD3 were applied over the respective test sites of one side of the spine. The test sites were observed for erythema and edema for 48h after application¹².

Chemical evaluation

The prepared formulations were evaluated using standard methods of general characterization, physical and chemical evaluation including specific gravity, pH, refractive index, acid value and saponification value.

Male wistar albino rats, 200-250g, from Veterinary College, Mhow were used for hair growth studies. They were placed in cages and kept in (23 °C ± 10, 60% ± 10 RH) standard environmental conditions, fed with (Hindustan Liver) standard diet and allowed free access to drinking water for two days. The prepared formulations were assessed for the standard test.

Hair growth activity test

Quantitative model developed by Uno¹² for the study of hair growth was followed with slight modification. The rats were divided into 8 groups of 5 rats each 2cm² area of dorsal portion of all the rats shaved area to remove all the hair. Group 1 was kept as control, where there was no drug treatment. Group 2 was treated as standard, where 1mL of (2% Minoxidil ethanolic solution) was applied over the shaved area, once a day. The animals of remaining group were given application of 1g (5% w/w) of formulation OD1, OD2 and OD3 respectively and 1mL (5% w/v) of OD1, OD2 and OD3 respectively, once a day. This treatment was continued for 30 days. During the course the hair growth pattern was observed qualitatively and recorded^{13,14}.

Results and Discussion

The results of general characteristics, physical and chemical evaluation are summarized in Table 2 & 3. 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 non-irritant on skin of rabbits. In hair growth initiation test the activity of various concentration of hair oil were recorded in terms of qualitative changes various biological parameters formed during treatment of oil with respect to normal untreated animals. This parameter is recorded for finding minimum effective concentration of drug. A7 & A8 (7% & 8% of Amla Powder) showed a significant increase of hair follicles with in 15 d. Both of this concentration showed

growth initiation for hair follicle within 9 and 8. Similarly B7 & B8 (7% & 8% of Brahmi powder) showed a significant increase in number of hair follicles up to 12 with in 6 d. Along with this f7 and f8 (7% and 8% concentration of fenugreek) showed a significant increase in hair follicle 8 and 9 respectively in 9 d. The oil containing M7 and M8 (7% and 8% concentration of Meetha neem) showed early initiation of hair growth follicle in 6 d with 11 numbers.

Table 2. Evaluation of general characteristics.

Concentration in	2.5 %	5 %	7.5 %
Colour	Green	Green	Greenish black
Odour	-	-	Characteristic

Table 3. Evaluation of physical parameters.

Parameters	Concentration		
	2.5%	5%	7.5%
Specific Gravity	0.928	0.9384	0.9432
pH	9.1	8.4	7.5
Refractive index	1.505	1.472	1.435
Acid Value	2.49	2.18	1.558
Saponification value	256	256.9	257

From the significant quantitative changes shown by various hair oil were further subjected for hair growth activity and results are shown in Table 4 and 5 respectively.

Table 4. Qualitative observation of hair growth.

Formulation	Number of Rats	Time taken to initiate the growth in d	Time taken for complete growth in d
Control (untreated)	5	8	24
Minoxidil (standard)	5	7	19
OD1	5	10	25
OD2	5	9	23
OD3	5	8	18

Table 5. The rate of hair growth.

Formulation %	Mean length, mm±50				Population, %			
	Anagen		Catagen	Telogen	Anagen		Catagen	Telogen
	A3	A5			A3	A5		
Control	-	0.62±0.13	0.11±0.02	0.22±0.06	-	47	4	49
Standard	0.46±0.04	0.73±0.11	0.1	0.23±0.06	18	49	1	32
2.5	0.42±0.04	0.65±0.11	-	0.20±0.04	22	46	-	19
5.0	0.40±0.05	0.70±0.11	0.1	0.22±0.05	21	59	2	29
7.5	0.45±0.06	0.73±0.14	-	0.25±0.05	23	62	1	17

The time taken for complete hair growth was 18d in OD3 and 19 d in minoxidil. Table 4 shows the hair growth response within 21d. Thus on comparison OD3 and minoxidil it has been observed that patch with 7.5% hair oil formulation application shows better growth than the patch with minoxidil. On comparison with OD3 and minoxidil the complete growth of standard and OD2 was found to be 24d and 23d respectively.

The quantitative study revealed that formulation OD3 considerable increase in number of hair follicle in anagen phase of hair growth cycle, when compare to standard and control. In standard group percentage of population of anagen follicle was 67%, where as in formulation OD3 it was 83% and OD2 81%. Similarly the formulation OD1 and OD2 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 25d in OD1 and 23d in OD2 indicating late initiation and completion of hair growth. The result shows that formulation OD3 have contributed in most significant hair growth activity. Similarly, the way of method of preparation of OD3 (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.

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