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Formulation and evaluation of hair oil for hair loss disorders

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Abstract
Hair plays a vital role in the personality of human and for their cure we use lots of cosmetic products. The fading (pigmentation problem), dandruff, alopecia (loss of hair) is the major problem associated with hairs. Ayurvedic system is the traditional system of medicine having major treatment across globe. The aim of study is to develop a hair oil formulation using *Azadirachta indica* (leaves), *Semecarpus anacardium* (fruits), *Trigonella foenum graecum* (seeds), *Cocos nucifera* (oil) for better growth of hair and diminution in loss of hair (alopecia). The oil was prepared according to Ayurvedic Formulary of India and was standardized according to Protocol for Testing Ayurvedic, Siddha & Unani Medicines, Government of India. The *Semecarpus anacardium* (fruits) are semi poisonous in nature hence purification of fruits was performed according to Ayurvedic Formulary of India. The pharmacological evaluation was performed for qualitative, quantitative, mean hair length studies and its effect on alopecia.

Keywords: Ayurvedic system, *Semecarpus anacardium*, *Azadirachta indica*, *Trigonella foenum graecum*, *Cocos nucifera*, Hair Oil, Hair loss (alopecia)

1. Introduction
Hair is one of the imperative parts of the body derived from ectoderm of the skin, it is ornament structure along with sebaceous gland. Hair is a dead part with no nerve connections. The hair follicle has the unique ability to regenerate itself [1-3]. The basic part of hair is bulb (a swelling at the base which originates from the dermis), root (which is the hair lying beneath the skin surface), shaft (which is the hair above the skin surface) [4].
The growth of hair is cyclic phase divided into following- anagen (growth), catagen (involution) and telogen (rest) [5]. Pigmentation problems (Fading), dandruff and falling of hair (Shedding) are associated problems with hair [6]. The loss of hair is not life threatening, but has profound impact on social interactions [7]. There are no concord views on hair loss, it is quite controversial issue [8, 9].
Major causes of hair loss are dihydrotestosterone (derivative of testosterone, a male hormone), poor blood flow, sebum emotional strains, stresses and nervous disorders, aging, infections, hormonal imbalance, polluted environment, toxic substances, injury and impairment, radiation [10].

Types of hair loss
Androgenetic or androgenic alopecia (baldness)
It is the most common cause of hair loss in men also known as hereditary baldness.[11] In androgenetic alopecia hair follicle size is reduced and duration of anagen is diminished while an increase in the percentage of hair follicles in telogen [7].

Alopecia areata
In alopecia areata the hair is lost from the scalp (alopecia areata totalis) or from the whole body (alopecia areata universalis) [12].

Telogen effluvium
Telogen effluvium is characterized by the early entrance of a large no of hairs in to telogen phase at one time [13].

Chemotherapy-induced alopecia
This type of hair loss is occurred due to the side-effects of cancer therapy [14].

The *Semecarpus anacardium,* [15] and *Trigonella foenum graecum* [6, 15, 21] was reported as a useful remedy for the treatment of hair loss and fresh leaf paste of *Azadirachta indica* [16] reported as topical antidote of dermatitis caused by *Semecarpus anacardium*, hence the purpose of study was to prepare an effective formulation for hair loss disorders using the above crude drugs and to evaluate quantitatively and qualitatively along with standardization of formulation.

2. Materials and Methods

Collection, identification and authentication of plants

The fruits of *Semecarpus anacardium*, seeds of *Trigonella foenum graecum* were purchased from local market of Ujjain, leaves of *Azadirachta indica* was collected from botanical garden of MIPS, Ujjain and were identified and authenticated from Department of Botany, Vikram University, Ujjain (MP).

Purification of *Semecarpus anacardium* fruit

Bhallataka (*Semecarpus anacardium* Linn.) is reported a semi poisonous drug in upavistha dravya, in classical Ayurvedic Pharmacopoeias. The purification of fruits was carried out according to the Ayurvedic Formulary of India (Figure 1) [17, 18].

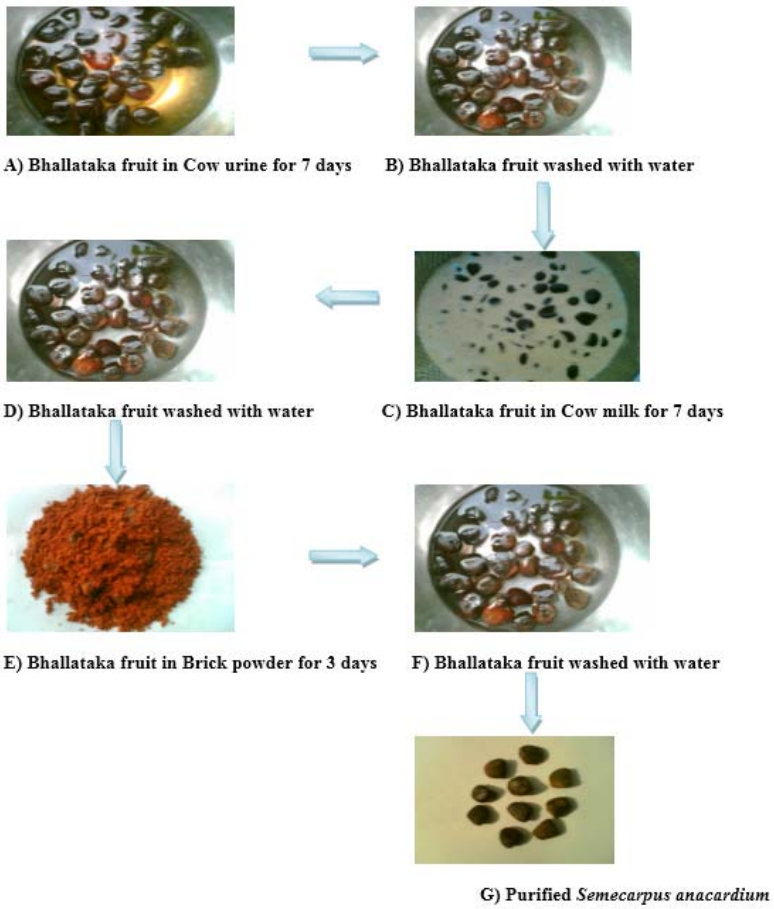

Fig 1: Purification of *Semecarpus anacardium*

Preparation of oil

The kalka of *Semecarpus anacardium* (Purified fruits), *Trigonella foenum graecum* (seeds) and *Azadirachta indica* (leaves) were prepared individually and boiled with the coconut oil and water, with continuous stirring at a constant temperature, until the water droplets in oil stopped crackling sound and the drugs were completely extracted in the oil. The oil was then filtered through a muslin cloth and stored (Table 1) [19].

Table 1: Formulation Composition Kalka: Sneha: water (1/4:1:4)

S. No.	Name of Ingredients	Part Used	Formulation compositions (%w/w)		
			F1	F2	F3
1	*Semecarpus anacardium* Linn. (Purified)	Fruit	8.3	2.5	1
2	*Trigonella foenum graecum* Linn.	Seed	8.3	11.25	12
3	*Azadirachta indica* Linn.	Leaf	8.3	11.25	12
4	*Cocos nucifera* Linn.	Oil	100	100	100
5	water		400	400	400

Physicochemical evaluation of oil

The prepared formulations were evaluated using standard methods of general characterisation, physical and chemical evaluation including colour, weight per ml, specific gravity, acid value and saponification value according to Protocol for Testing Ayurvedic, Siddha & Unani Medicines, Government of India (Table 2) [20].

Table 2: Evaluation of oils

S. No.	Parameters	Obtained Values		
		F1	F2	F3
1	Colour	Dark brown	Chocolate brown	Yellowish green
2	Weight per ml	0.926g/ml ± 0.001	0.923g/ml ± 0.004	0.918g/ml ± 0.002
3	Specific gravity	0.927	0.924	0.919
4	pH	6.89 ± 0.02	6.72 ± 0.01	6.64 ± 0.03
5	Acid value	3.2 ± 0.1	2.5 ± 0.15	2.12 ± 0.04
6	Saponification value	242.31 ± 0.6	259.4 ± 0.58	265.24 ± 0.42

All values are Mean ± SD

Pharmacological evaluation

Wistar albino rats were used for study (weight 150-200g). Animals were kept in standard environmental conditions with standard diet and free access to drinking water. Standard environmental conditions mean room temperature (24 ± 2^0C), normal day light condition (06:00 hrs to 18:00 hrs). Five groups of rats were created for study, having 6 rats in each group.

Primary skin irritation test

The back side skin of rats was denuded with the help of electric shaver followed by hair remover cream. The denuded area was kept under visual observation for any irritation or erythema for next 24 hours, and same observation was performed after applying test samples on denuded area, except time which was extended up to 48 hours (Table 3) [2, 21, 22].

Table 3: Primary Skin Irritation Test

S. No.	Formulation	Skin Irritation Result
1	F1	-
2	F2	-
3	F3	-

'+' = present '-' = absent

Application of test formulation

Equal quantities of prepared test oils, standard oil and control oil were applied to the denude skin of rats once in a day for 30 days [2, 22, 23].

Qualitative studies on hair growth

In Qualitative hair analysis hair growth initiation time and hair growth completion time were observed. The minimum time required for growth of hair from denuded skin is the hair growth initiation time, and the time taken to completely cover the denude skin is the hair growth completion time (Table 4) [2, 21-23].

Table 4: Qualitative Studies on Hair Growth

S. No.	Groups	No. of Rats	Time taken to initiate the hair growth (in days)	Time taken to complete hair growth (in days)
1	Standard (Trichup oil)	6	7	21
2	Control (Coconut oil)	6	7	23
3	F1	6	5	19
4	F2	6	9	24
5	F3	6	10	25

Hair length studies

The 30 strands of hair from each group were plucked randomly with the help of pincer from denuded skin area and average length (mm) was computed with the help of ruler (Table 5) [21].

Table 5: Hair Length Studies

S. No.	Days	Mean hair length (mm)				
		Standard	Control	Test		
				F1	F2	F3
1	15 Day	6.9± 0.22	5.9± 0.26	7.2± 0.25	5.4± 0.21	5.2± 0.35
2	30 Day	9.6 ± 0.25	9.1± 0.26	9.9 ± 0.3	8.7 ± 0.32	8.3 ± 0.21

All values are Mean ± SD

Quantitative studies on hair growth

In quantitative hair analysis the percentage ratio of hair follicles per mm area of skin in different cyclic phases, like anagen (growth phase) and telogen (resting phase) were evaluated. For this purpose after 30 days treatment one rat from each group was euthanized and sample for skin biopsy was taken from the denuded skin area. Formalin (10%) was used as preservative for sample and samples were sent to laboratory for evaluating number of hair follicles per mm area in different cyclic phases (Table 6) [2, 21-23].

Table 6: Quantitative Studies on Hair Growth

S. No.	Groups	No. of Rats	Percentage of different hair follicles		No. of hair follicles per mm area
			Anagen	Telogen	
1	Standard (Trichup oil)	6	63	31	4-7
2	Control (Coconut oil)	6	56	39	4-6
3	F1	6	66	29	5-7

3. Result and Discussion

Formulation development was done with the optimized formula and evaluated by means of various parameters like colour, weight per ml, specific gravity, pH, acid value and saponification value.

The colour of all formulations were found to be Dark brown for F1, Chocolate brown for F2 and yellowish green for F3. The weight per ml and specific gravity of all formulations were found to be 0.926g/ml ± 0.001 and 0.927 for F1 respectively; 0.923g/ml ± 0.004 and 0.924 for F2 respectively; 0.918g/ml ± 0.002 and 0.919 for F3 respectively. The pH of all formulations was found to be 6.89 ± 0.02 for F1; 6.72 ± 0.01 for F2; 6.64 ± 0.03 for F3. The acid values of all formulations were found to be 3.2 ± 0.1 for F1; 2.5 ± 0.15 for F2; 2.12 ± 0.04 for F3. The saponification values of all formulations were found to be 242.31 ± 0.6 for F1; 259.4 ± 0.58 for F2; 265.24 ± 0.42 for F3.

Primary skin irritation test was performed for 48 hours for all formulations (F1, F2 and F3) and there was no sign of erythema or edema found on skin.

Qualitative hair analysis was performed to evaluate the hair growth initiation time and hair growth completion time for all the test formulations and for standard and control formulations. The hair growth initiation time was found to be 7th day, 7th day, 5th day, 9th day and 10th day for standard, control, F1, F2 and F3 respectively. The hair growth completion time was found to be 21 day, 23 day, 19 day, 24 day and 25 day for standard, control, F1, F2 and F3 respectively. Measurement of plucked hairs was carried out for each group and the average length (mm) was computed. The mean hair length was found to be 9.6 ± 0.25, 9.1± 0.26, 9.9 ± 0.3, 8.7 ± 0.32 and 8.3 ± 0.21 for standard, control, F1, F2 and F3 respectively.

Quantitative hair analysis was performed to evaluate the number of hair follicles per mm area of skin and percentage ratio of hair follicles in different cyclic phases, like anagen and telogen. The number of hair follicles per mm area was found to be 4-7, 4-6 and 5-7 for standard, control and F1 respectively. The percentage of hair follicles of anagen phase was found to be 63%, 56% and 66% for standard, control and F1 respectively. The percentage of hair follicles of telogen phase was found to be 31%, 39% and 29% for standard, control and F1 respectively.

Among all three formulations F1 was found to be effective in all preparation and showed promising results in qualitative, quantitative and mean hair length studies over standard oil.

4. Conclusion

The formulations were evaluated for skin irritation and for qualitative and quantitative growth of hair. The F1 formulation was found to be better. It showed promising results in hair growth, mean hair length and quantitative evaluation. The optimization of formulation showed that *Semecarpus anacardium* had more potential for hair growth and reduction in hair loss. From the results it can be concluded that *Semecarpus anacardium* showed promising effects in qualitative, quantitative and mean hair length studies over standard oil and found to be good and effective remedy for hair growth and hair loss treatment.

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