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Polyherbal oil formulation for hair disorders

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Abstract
Hairs play a vital role in the personality of human. But, so many peoples have different hair problems like hair loss, fading, dandruff, premature greying, etc. To cure all such problem several cosmetic preparations are used. Alopecia is a medical term for hair loss or thinning of hair can be a sign of serious diseases especially if the hair loses rapidly. It is common throughout the world. Causes of hair loss are as follows: Emotional strains, stresses and nervous disorders Aging, Infections, Hormonal imbalance, Polluted environment, Toxic substances, Injury and impairment, Radiation, etc. This study aims to develop the polyherbal formulation for hairs that enhances the growth of hairs. The polyherbal formulation was developed by incorporating herbal extracts into oil base and evaluated by various parameters. The *in vivo* studies of formulation was done on rats, it showed good hair vitaliser activity.

Keywords: hair, alopecia, hair vitaliser, causes of hair loss, polyherbal formulation, etc.

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]. At the present time hair loss is common problem in men and women due to excessive exposure of chemicals in daily routine on scalp. Alopecia is a medical term for hair loss or thinning of hair can be a sign of serious diseases especially if the hair loses rapidly. Alopecia is dermatological disorder [2]. Synthetic drug, minoxidil is a potent vasodilator was scientifically proved for the treatment of alopecia. Though the use of drugs for its side effect is not advisable, the drug of plant origin is necessary to replace the synthetic one [3].
Hair has two separate structures:
1. The follicle in the skin and
2. The hair shaft, which is visible on the body surface

The hair shaft consists of a cortex and cuticle cells, and in some cases, a medulla in the central region. The medulla is the central part of the hair, whereas the cortex, which represents the majority of the hair fiber composition and plays an important role in the physical and mechanical properties of hair, the hair shaft cuticle covers the hair from the root tip of the epidermis and is formed by flat overlapping cells. The follicle is the essential growth structure of hair.
The hair bulb is the portion of the follicle which actively produces the hair. The hair bulb can be divided into two regions: a lower region of undifferentiated cells and an upper region in which the cells became differentiated [4].

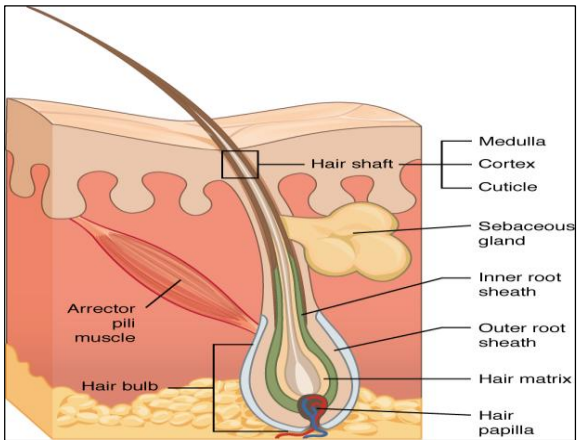


Fig 1: Structure of Hair

Natural products are fancy in cosmetics and about thousand kinds of plant extracts have been examined with respect to hair growth activity. In traditional Indian system of medicine many plants and herbal formulations are reported for hair growth promotion as well as improvement of quality of hairs [5].

2. Materials and Methods

A. Collection and procurement of crude drugs

Crude drugs were purchased from M/s Wagh Brothers Nagpur. Fresh drugs were collected from the local area.

B. Formulation of Oil

Table 1: Formulation of medicated herbal hair oil

S. No	Ingredients	HF1 (%)	HF2 (%)	HF3 (%)
1	*Ficus bengalensis*	2	2	2
2	*Cyperus rotundus*	1	1	1
3	*Citrus aurantium*	1	1	1
4	*Annona squamosal*	0.5	0.5	0.5
5	*Mangifera indica*	0.5	0.5	0.5
6	*Lawsonia alba*	2	2	2
7	*Hibiscus rosasinesis*	10	-	10
8	*Emblica officinalis*	10	-	10
9	*Eclipta alba*	-	10	10
10	*Trigonella sativum*	5	-	5
11	*Aloe vera*	-	5	5
12	*Cocus nucifera*	q.s	q.s	q.s
13	*Sesamum indicum*	q.s	q.s	q.s

C. Preparation of medicated herbal oil in laboratory

All the ingredients were procured and collected. The fresh ingredients were cleaned and weighed. All dry ingredients were powdered, weighed and mixed together. Make the decoction of all dry ingredients with water by heating. Cool it and filtered through the muslin cloth. The fresh ingredients were cleaned and weighed.

D. Evaluation of Oils

I. Physico-chemical Evaluation-[6, 7]

Prepared herbal oil formulation were subjected for various physico-chemical evaluations like colour, odour, solubility, viscosity, density, pH, refractive index, saponification value, acid value and iodine value.

II. Quantitative Estimation-[8, 9]

DPPH radical scavenging activity of each plant extract was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH according to the method of Blois. Briefly, 3 ml of extract was added to 1 ml of DPPH (2, 2-diphenyl-1-picrylhydrazyl) solution (0.2 mM in methanol) as the free radical source. The mixture was shaken and kept for 30minutes at room temperature. The decrease of solution absorbance due to proton donating activity of components of each extract was determined at 517nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH radical scavenging activity was calculated using the following formula:

$$\text{Radical Scavenging (\%)} = \frac{\text{Absorbance of Control-Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

III. Stability studies

Stabilities studies of all formulations were performed for colour, odour, solubility, viscosity, density, pH, Specific gravity, refractive index, saponification value, acid value, iodine value for initially then after 15 days, one month, two month and three month. (Temp: 25±2 °C and % RH: 60±5)

IV. Chromatographic Evaluation [11, 12]

Chromatographic evaluation was done by HPTLC. The chromatography was performed for extracts as per method reported earlier from in our lab. In brief, the samples were spotted in the form of spots with a Camag micro litre syringe on percolated silica gel aluminium plate 60 F254 (5cm×10cm with 0.2 mm thickness; E. Merck, Darmstad, Germany) using a Camag Linomat V (CAMAG, Muttenz, Switzerland). Space between two spots was 10 mm. The slit dimension was kept at 4 mm × 0.1 mm, and 20 mm/s scanning speed was employed. These parameters were kept constant throughout the analysis of samples. The mobile phase consisted Dichloromethane. Plates were developed in ascending order with a CAMAG twin trough glass tank which was pre-saturated with the mobile phase for 15 min; the length of each run was 8 cm. The TLC runs were performed under laboratory conditions of (Temp: 25±2 °C and % RH: 60±5). The plates were then dried in air. Densitometric analysis was performed at 254, 366 and 540nm with a Camag TLC scanner 4 operated by Win CATS software (Version 1.4.9).

V. Pharmacological Evaluation [1, 3, 12, 13]

Primary skin irritation test

The back side skin of rats was denuded with the help of 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.

Hair Vitalizer Activity

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 ºC-20 ºC), normal day light condition (06:00 hrs to 18:00 hrs). Six groups of rats were created for study, having 6 rats in each group. Group I served as-Control, Group II-vehicle, Group III-VH (Vrundayush Hair Oil). Group IV, V and VI-HF1, HF2 & HF3 respectively. (Kashyap *et al*. 2016)

A) Qualitative hair growth studies

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.

B) 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.

3. Results and Discussions

All the prepared formulations subjected for the various evaluation parameter. The results and discussions for the oil formulations are as follows:

I. Physico chemical evaluation

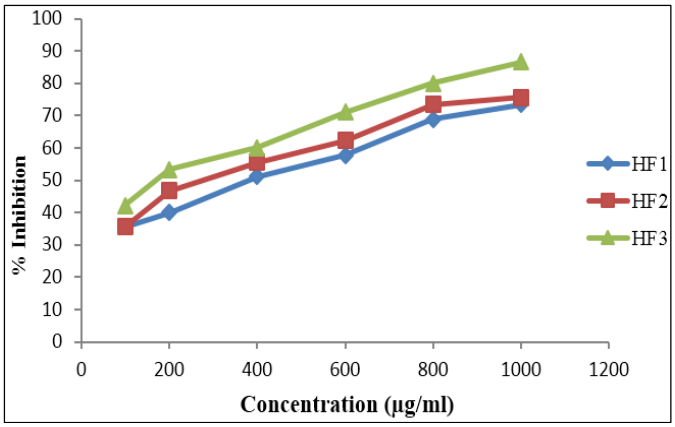
Results of physico chemical evaluation like colour, odour, solubility, viscosity, density, pH, Refractive Index, Saponification value, Acid Value and Iodine Value are shown in Table No. 2.

Table 2: Physico-chemical evaluation

Parameter	HF1	HF2	HF3
Colour	Dark Brown	Dark Brown	Dark Brown
Odour	Aromatic	Aromatic	Aromatic
Solubility	Non-Polar	Non-Polar	Non-Polar
Viscosity	31±1.63	29.66±1.24	35.3±1.69
Density	0.922±0.006	0.919±0.002	0.929±0.005
pH	6.66±0.04	6.72±0.03	6.73±0.06
Refractive Index	1.440±0.004	1.438±0.003	1.444±0.004
Specific Gravity	0.972±0.002	0.978±0.006	0.987±0.002
Saponification value	249.25±0.51	249.57±0.53	252.74±0.11
Acid Value	2.9±0.16	2.83±0.20	3.26±0.24
Iodine Value	7.16±0.33	7.5±0.24	8.3±0.37

II. Free radical scavenging activity

DPPH radical scavenging activity has widely used to assess the *in vitro* antioxidant activity of drugs. The free radical scavenging activity of various formulations was found to be HF1-370, HF2-270, HF3-170µg/ml. (Graph No. 1)



Graph 1: Free scavenging radical activity

III. Stability studies

Stability studies of formulated oil batches were done for three months in laboratory. Analysed data of stability studied does not shown any more changes in evaluation parameters and shows no degradation of the products. Table No. 3 shows stability studies data of hair oil.

Table 3: Stability Studied

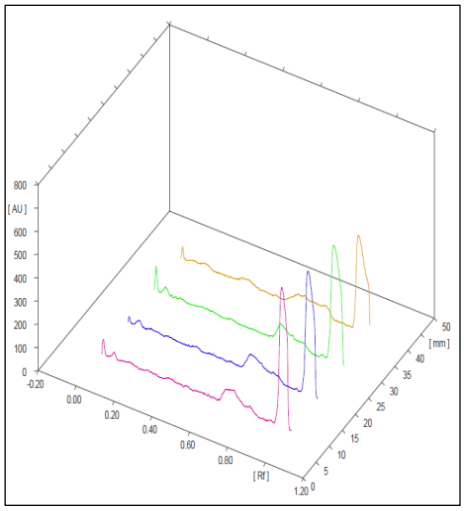
Parameters/Months	Initial			After One month			After Three month		
	HF1	HF2	HF3	HF1	HF2	HF3	HF1	HF2	HF3
Colour	Brown	Brown	Brown	Brown	Brown	Brown	Brown	Brown	Brown
Odour	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic
Solubility	NP	NP	NP	NP	NP	NP	NP	NP	NP
Viscosity	31±1.63	29.66±1.24	35.33±1.69	30.95±1.28	29.59±1.05	35.28±1.53	29.12±1.29	28.26±1.07	34.98±1.01
Density	0.922±0.006	0.919±0.002	0.929±0.005	0.920±0.005	0.918±0.003	0.928±0.004	0.917±0.001	0.914±0.008	0.926±0.001
pH	6.66±0.04	6.72±0.03	6.73±0.06	6.65±0.08	6.68±0.04	6.70±0.02	6.54±0.07	6.61±0.02	6.68±0.04
Specific Gravity	0.972±0.002	0.978±0.006	0.987±0.002	0.971±0.004	0.976±0.002	0.987±0.001	0.968±0.005	0.968±0.002	0.985±0.003
Refractive Index	1.440±0.004	1.438±0.003	1.444±0.004	1.439±0.004	1.436±0.002	1.443±0.006	1.431±0.001	1.425±0.005	1.438±0.006
Saponification Value	249.25±0.51	249.57±0.53	252.74±0.11	249.19±0.21	249.50±0.61	252.62±0.58	248.99±0.55	249.02±0.41	252.12±0.21
Acid Value	2.9±0.16	2.83±0.20	2.26±0.24	2.84±0.05	2.78±0.25	2.24±0.15	2.79±0.22	2.67±0.12	2.19±0.19
Iodine Value	7.16±0.33	7.5±0.24	8.3±0.37	7.15±0.21	7.46±0.18	8.28±0.28	7.07±0.11	7.37±0.26	8.19±0.22
Degradation of product	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Note-NP stands for Non-Polar

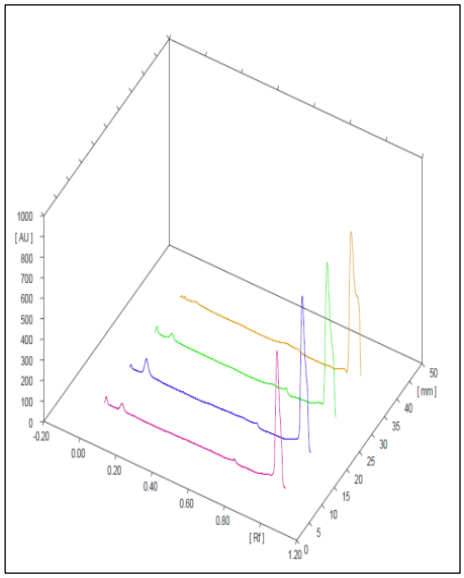
IV. Chromatographic evaluation

The numbers of different solvent systems were employed to generate fingerprint profiles for these oils. The separation of phytoconstituents in the formulated oil occurred in the mobile phase comprised of Dichloromethane, and the stationary phase is silica gel G F_{254} using CAMAG twin trough TLC chamber**.**

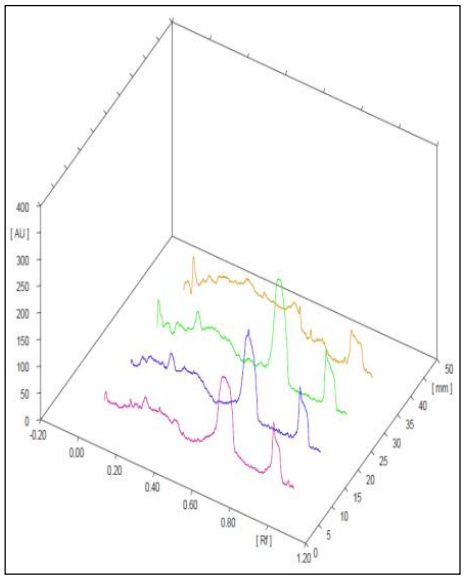
Graph No. 2, 3 and 4 shows HPTLC chromatogram of all tracks of prepared and marketed hair oil formulations at 254nm, 366nm and 540nm respectively. Track1, Track2, Track3 and Track4 represents HF1, HF2, HF3 and Vrundayush Hair oil (VH) marketed formulation.



Graph 2: At 254nm



Graph 3: At 366nm



Graph 4: At 540nm

V. Pharmacological Activity

A) Skin Irritation Test

Primary skin irritation test was performed for 48 hours for all the formulations, and there was no sign of erythema or edema found on skin. The results of skin irritation test are shown in Table No.4 and there was no sign of irritations was found.

Table 4: Skin Irritation Test

S. No	Formulations	Skin Irritation Result
01	HF1	-
02	HF2	-
03	HF3	-
04	VH	-

'-' = No Irritations & '+' = Irritations

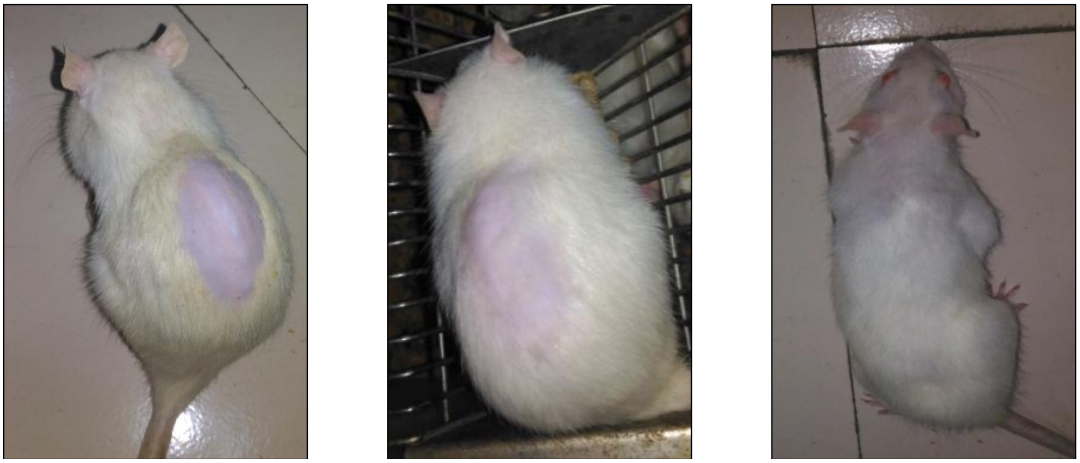
B) Hair vitaliser activity

Qualitative hair growth studies

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, vehicle and control formulations. The hair growth initiation time was found to be on 13th, 10th, 9th, 10th, 11th, and 7th Day for control, vehicle, VH, HF1, HF2, and HF3 groups respectively. The hair growth completion time was found to be 28th, 27th, 23rd, 26th, 25th, and 21st Day for control, vehicle, VH (Vrundayush hair oil), HF1, HF2, and HF3 groups respectively. (Table No. 5).

Table 5: Qualitative Hair Growth Studies

Sr. No.	Groups	No. of Rats	Time Taken for Initiation of Hairs growth (In Days)	Time Taken for complete Hair growth (In Days)
01	Control	06	13	28
02	Vehicle	06	10	27
03	VH	06	09	23
04	HF1	06	10	26
05	HF2	06	11	25
06	HF3	06	07	21

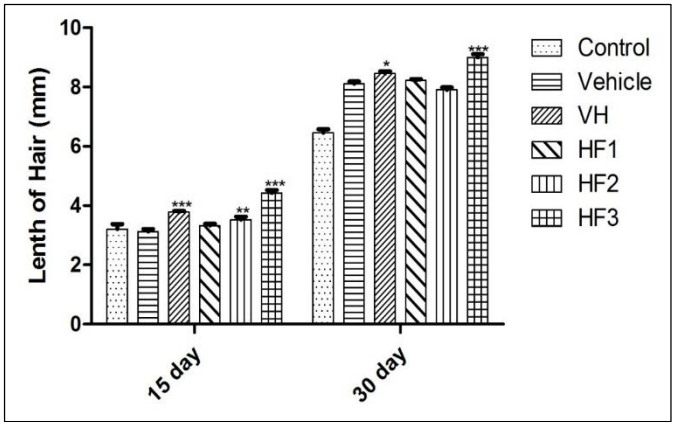


A **B** **C**

Picture showed in Fig A, B and C of animal back area nude and treated with formulation showed hair growth on the 0th Day, 15th Day and 30th Day respectively

Hair length studies

Hair length studies were performed by measuring plucked hairs was carried out on 15th and 30th day for each group and the average length (mm) was computed.



Graph 5: hair Length on 15th and 30th Day

Graph No. 5 Shows effects of various formulations of Hair oil in rat hair growth. The Values are represented as mean ± SEM (n=6) **P*<0.05, ***P*<0.01, ****P*<0.001 Considered as statistically Significant as compared with vehicle. Data was analysed by Two Way ANOVA Bonferroni Posttests

4. Summary and Conclusions

The polyherbal oil formulations were formulated and evaluated by various parameters. Physico-chemical parameters shows that formulations have brown colour, with good aromatic odour and more solubility in the non-polar solvents. Other evaluation parameters were also done, for all the batches (HF1, HF2 and HF3) shows results are under range of limits. Viscosity of HF3 showed 35.3±1.69 CP maximum as compared with other formulation. Density of HF3 showed 0.929±0.005. Specific gravity are 0.972±0.002 to 0.987±0.002 for hair oil formulations. The Saponification, acid and iodine value of HF3 batch was more as compared to other formulation batches.

Antioxidant studies using *In-vitro* DPPH method also proven

a good antioxidant activities of the prepared Ayurvedic medicine derived herbal formulations. The various Ayurvedic medicine derived herbal formulation HF1, HF2 and HF3 showed IC50 values at the concentration of 370, 270, 170 µg/ml, when compared with standard Ascorbic Acid showed IC_{50} values at the concentration 9 µg/ml. Stability studies data for 0, 1, 3 months studies found to be stable in room temperature.

Chromatographic evaluation was done by using HPTLC. The formulation were dissolved in dichloromethane in concentration of 1000µg/ml, and subjected to HPTLC, the chromatogram were recorded in 254, 366 and 540 nm after spraying with Vanillin sulphuric acid reagent.

Formulations were evaluated for hair vitaliser activity in experimental animal. Hair vitaliser activity was evaluated by hair initiation and hair lengthening measurements. The HF3 formulation takes 7 days for initiation. Similar results were obtained in case of mean hair length in mm for HF3 formulation groups.

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