



Development and Characterization of Hair oil for Control Hair fall and Stimulating Hair Growth

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Abstract

The goal of the current study was to create, describe, and assess a topical therapeutic system for the treatment of alopecia using extracts from *Trigonella foenumgreacum* seeds, *Hibiscus rosa sinensis* Linn flowers, *Eclipta alba* (L.) leaves, and *Allium cepa* L. bulbs. Formulations for polyherbal oils were created and refined. Alopecia, or hair loss, is a common patient complaint and a source of significant psychological and physical distress. Apart from a variety of other factors, androgens are thought to be one of the most important causes of alopecia. Minoxidil, a drug of scientific origin, was scientifically proven for the treatment of alopecia; however, the side effects associated with this drug have limited its pharmacological benefits, so the drug of plant origin is necessary to replace the synthetic one. As alopecia is a dermatological disorder, the number of men and women who suffer from hair loss and/or hair thinning is increasing recently.

Key words: Hair loss, hair oil, herbal medicine.

Introduction

Hair and hair Loss:

Hair, one of the vital parts of the body derived from ectoderm of skin, is protective appendages on the body and considered accessory structure of the integument along with sebaceous glands, sweat glands and nails. Hair is an important of the overall appeal of the human body. Throughout history and in most of civilizations, scalp hair has been associated with positive signals such as beauty and power. Baldness or hair loss on the other hand has a negative attribute. Each hair grows in three cyclicphases viz., anagen (growth), catagen (involution) and telogen (rest). The anagen phase can be as short as 2-6 years. In the catagen phase, the growth activity increases and hair moves to the next phase, catagen phase is between 2-3 weeks. The telogen phase is a state at which the hairs

move into resting state. This phase lasts for 2-3 months.

Dandruff:

Dandruff is a common scalp disorder affecting almost half of the post pubertal population of any ethnicity and both genders. The exact nature and etiology of dandruff has always been controversial since the time of the Greeks, through Sabouraud's era in late nineteenth century till to-date. Dandruff represents 25 % of all scalp disorders. It is present in an estimated 15-20 % of the total population and more than 50 % of adult population.

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Alopecia:

Alopecia is dermatological disorder that has been recognized for more than 2000 years and a common problem in cosmetics as well as primary health care practice. It is common throughout the world and has been estimated to affect between 0.2 % and 2 % of the world population. Androgens are considered to be one of the most important causes for alopecia apart from a variety of other factors. 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.

Importance of herbal therapies:

Even though varieties of synthetic hair care products are available in the market, the consumer requirement is ecofriendly, natural and environmentally safe cosmeceuticals without producing any side effects. Herbal plants are the richest sources of antioxidants like vitamin A, vitamin C, vitamin E and other components like gallic acid, saponins, amino acids, elemental sulphur, enzymes, mucilages, flavanoids, tannins, essential oils and polysaccharides. In traditional knowledge itself number of plant parts was used in skin and hair care preparation like cucumber, burdock, marigold, watercress, daisy flower, witch hazel, hops, birch, gentian, fir, Indian cress, rosemary, sage, horsetail and thyme. Natural products in the form of herbal formulations are available on the market and are used as hair tonic, hair growth

Material & Methods

Collection of plant material:

The seeds of *Trigonella foenum greacum* and *Allium cepa* L were obtained from local market while *Hibiscus rosa sinensis*, Linn and *Eclipta alba* (L.) were collected from natural habitat and authenticated by Dr. S. N. Dwivedi, Prof. & Head, Department of Botany, Janata PG College, APS, University, Rewa, M.P. and Voucher specimen No. JC/Bot/2019/SX-526 was deposited in our department.

Preparation of plant powder:

The seeds of *Trigonella foenum greacum* were pulverized, sieved through 40 mesh to obtain a coarse powder. While flower of *Hibiscus rosa sinensis* Linn, leave of *Eclipta alba* (L.) were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve No. 40 and stored in an airtight container for further use.

promoter, hair conditioner, hair- cleansing agent, antidandruff agents, as well as for the treatment of alopecia and lice infection.

Common types of hair loss:

Androgenetic alopecia:

This is generally recognized as the most common hair loss cause and may be responsible for over 95 % of pattern hair loss for both men and women. It is usually associated with ageing and develops in predictable stages over varying periods of time.

Alopecia areata:

This is an immune system disorder which causes follicles to stop producing hairs usually in patches on the head. In some cases alopecia areata can advance to the stage where all hair on the head is lost (alopecia totalis) or there is a complete absence of body hair (alopecia universalis). There are three types of Alopecia areata which are named according to their severity.

Telogen effluvium:

This hair loss cause is characterized by a slowing of new hair growth following a major stress inducing incident or event. This is followed by the delayed shedding of hair with the result that more follicles than normal enter the resting stage causing excessive numbers of those follicles to eventually shed hair at the same time.

Anagen effluvium:

Hair loss is due to chemicals or radiation.

Physico-Chemical Analysis:

The powdered plants material of was subjected to standard procedure for the determination of various physicochemical parameters.

Determination of ash values:

The determination of ash values is meant for detecting low-grade products, exhausted drugs and sandy or earthy matter. It can also be utilized as a mean of detecting the chemical constituents by making use of water-soluble ash and acid insoluble ash.

Total ash value:

Accurately about 3 gms of air dried powder was weighed in a tared silica crucible and incinerated at a temperature not exceeding 450⁰C until free from carbon, cooled and weighed and then the percentage of total ash with reference to the air dried powdered drug was calculated. The percentage of

total ash with reference to the air-dried drug was calculated.

Acid insoluble ash:

The ash obtained in the above method was boiled for 5 minutes with 25ml of dilute HCl. The residue was collected on ash less filter paper and washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

Water soluble ash:

The ash obtained in total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weight at a low temperature. The weight of insoluble matter was subtracted from the weight of the ash. The difference in weights represents the water-soluble ash. The percentage of water-soluble ash with reference to the air-dried drug was calculated.

Determination of moisture content (Loss on drying):

About 10 g of drug (without preliminary drying) after accurately weighing was placed in a tared evaporating dish and kept in oven at 105⁰ C for 5 hours and weigh. The percentage loss on drying with reference to the air-dried drug was calculated.

Extracts:

The commonly employed technique for separation of active substance from crude drug is called as 'Extraction' which involves the use of different solvents. The plant material used for extraction should be properly authenticated or identified. The choice of the plant material for extraction depends upon its nature and the components required being isolated. The dried powdered plant material is commonly used for extraction. The solvent used for extraction is called menstrum and the residue is known as marc.¹¹³⁻¹¹⁵

Methods for plant extraction:

There are various methods of extraction. Some of them are described below:

Maceration:

The word maceration means softening. It is the simplest method of crude drug extraction and was official in I.P.1966. The process consists of keeping the crude drug in intimate contact with whole menstrum in a closed vessel with occasional shaking for seven days, straining, pressing the marc, mixing the liquids and finally clarifying by subsidence or filtration. The process may take up to 14 days in

some cases for complete extraction. The drug: menstrum ratio should be 1: 10.

Infusion:

Infusions are usually prepared from vegetable drugs containing water soluble and easily extractable principles. The process consisted of moistening the drug with water, macerating it with boiling water, straining and making up the volume.

Digestion:

This is a modified maceration process in which extraction is accomplished at a higher temperature at which the active ingredients are not adversely affected. Use of higher temperature provides for enhanced solvent action of menstrum and constant mechanical agitation of the system accelerates establishment of equilibrium in a short time.

Decoction:

Decoction is also employed for extracting vegetable drugs containing water-soluble and heat-soluble constituents. The process consisted of boiling the drug with water, cooling, expressing, straining liquid and finally make up the volume.

Percolation:

Percolation is extraction process in which granulated or powdered drug is deprived of its contents by the descent of a suitable menstrum through it. In Greek, the word 'percolate' means 'to pass through'. The process implies a slow passage of menstrum under the influence of gravity through a column of the drug. During this movement, the menstrum goes on extracting the drug particle layer wise, it being replaced by other layers above as it moves downwards.

Ultrasonic extraction:

The speed of drug extraction is enhanced by application of ultrasonic vibrations. The mixture of the drug and the menstrum is subjected to ultrasonic waves of 20 to 450 kilocycles/second followed by extraction in a soxhlet extractor. The treatment with ultrasonic vibrations provides rapid and superior extraction.

Successive solvent extraction:

Soxhlet extractor:

A Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz Von Soxhlet. It was originally designed for the extraction of a lipid from a solid material. However, a Soxhlet extractor is not limited to the extraction of lipids. Typically, a Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent.

If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance.

Principle and working of soxhlet apparatus:

Normally a solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser. The solvent is heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid.

Table 1: Polyherbal Oil Formulations containing Plants extract

Plant extracts	Combination of Extract E1	Herbal Oil Formulation F
Seed extract of Trigonella foenum greacum (% w/w)	2.5	2.5
flower extract of Hibiscus rosa sinensis Linn(% w/w)	2.5	2.5
leave extract of Eclipta alba (L.) (% w/w)	5	5
Bulb extract of Allium cepa L. (% w/w)	0.5	0.5
Coconut oil (ml)	-	q.s. to 100ml

Pharmacological Screening of Optimized Formulation

Herbal oil formulation containing plants extract selected for in-vivo hair growth study. Both qualitative hair growth and quantitative hair growth activity performed. Qualitative hair growth analysis was undertaken by visual observation of two parameters: hair growth initiation time and hair growth completion time. While hair length and histological study performed in quantitative hair growth analysis. Animal studies were approved by Institutional Animal Ethics Committee (IAEC) of R.K.D.F college of Pharmacy, Bhopal, M.P. and carried out in accordance with the Guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)

Formulation of suitable topical therapeutic system

Preparation of Hair oil Containing Extract:

The hair formulations of polyherbal hair oil for medicinal plants were separately prepared by cloth pouch method. Weight all the ingredient and extract. The method used for carrying out these formulations was holding the individual plant extract into one cloth pouch mixing and boiling continuously in the oil at a temperature of 60-80°C for 30 minutes until light brown coloured solution is obtained then cool it and filter the oil.

Animals:

Healthy Wistar rats (200-250g) were used for the study. Rats were housed in small cages in environmentally controlled (25 ± 20C, 12h light and dark cycle, with free access to food and water ad libitum). Rats were fed with the standard laboratory chow diet during the period of study.

Treatment for hair growth activity in-vivo study:

Six wistar rats are taken in that study. Hair from 3 cm² area at the dorsal portion of all the rat were shaved using electrical shavers and applied with marketed hair remover to completely remove hair. The polyherbal formulation FH was applied to the denuded area of the all six wistar rats two times in a day. This treatment was continued for 30 days during which qualitative and quantitative parameter of hair growth was observed and recorded. The data was compared with previous hair growth study of polyherbal plants extract.

Qualitative Studies on Hair Growth study

Qualitative hair growth analysis was undertaken by visual observation of two parameters: hair growth initiation time (i.e., minimum time to initiate hair growth on denuded skin region) and hair growth completion time (i.e., minimum time taken to complete cover the denuded skin region with new hair).

Quantitative hair growth study:

Hair length determination: Hairs were plucked randomly using sterile forceps from the shaved area of selected rats, from each group on 15th, 20th and 30th day of the treatment. The average length of 25 hairs was randomly selected and measured in millimeter and the results were expressed in mean ± SEM.

Histological studies: On the 10th, 20th and 30th day of treatment one rat from each group was authenticated and skin biopsies were taken from the shaved area and fixed in 10% formalin buffer. Sections of tissue were embedded in paraffin wax and sectioned in to uniform thickness of 10µm. The sectioned tissues

were stained with haematoxylin and eosin. From the stained tissue the number of hair follicles per millimeter of the skin and the percentage ratio of different cyclic phases were examined using microscope fitted with an ocular micrometer facility.

Skin Irritation Study

In-vivo skin irritation study was conducted by 12 albino rats of either sex weighing between (200-250g) was used. Animals were divided into 3 groups of 4 animals on each. Animal studies were approved by Institutional Animal Ethics Committee (IAEC) of RGPV college of Pharmacy, Bhopal, M.P. and carried out in accordance with the Guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Hairs were depleted from the back of rats with the help of depilatories and area 2 cm² was marked on both the sides. One side served as control while the other as test and animals were used after 24 hrs. After hair depletion herbal formulation oil F was applied on test side and oil base was applied on control side once a day for 7 days in one group and site was covered with cotton bandage and observed for any sensitivity and the reaction if any was graded as under²⁰⁴:-

A – No reaction, B – Slight patchy erythema, C – Slight but confluent or moderate but patchy erythema, D – Moderate erythema, E – Severe erythema with or without edema.

Accelerated Stability Studies

The optimized formulations were subjected to a stability testing for six months as per ICH norms at a temperature and RH of 40°C ± 2°C/75% RH ± 5% RH respectively. The selected formulations were analyzed for the change in appearance, pH and drug content.

Results and Discussion

Physico-Chemical Analysis

The dried parts of plants were subjected to standard procedure for the determination of various physicochemical parameters- ash values (total ash, acid insoluble ash and water-soluble ash) and Loss on drying were determined (Table.6.1)

Extraction

The dried powder of plants was extracted with 70% v/v hydro alcoholic solution. The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator to obtain the extract. The percentage yields of various extract were presented in Table 7.1.

Phytochemical Screening

The various extracts obtained were subjected to preliminary phytochemical screening. The extraction was carried out with hydro alcoholic solution the extract was screened for the presence of various medicinally active constituents.

The results of the phytochemical screening of seed extract of *Trigonella foenum graecum*, flower extract of *Hibiscus rosa sinensis* Linn, leave extract of *Eclipta alba* (L.) and bulb of *Allium cepa* L were present in Table-7.2. Preliminary phytochemical screening was useful in prediction of nature of drugs and also useful for the detection of several constituents present in different polarity solvent. Different types of secondary metabolites such as alkaloids, tannins, terpenoids, carbohydrates, glycosides, saponins, protein and mucilage & gum were presented in extract.

Table 2: Physico-chemical analysis of Selected Plant.

S.No.	Physicochemical constants	values (% w/w) of fenugreek seed	<i>Hibiscus rosa sinensis</i> Linn	<i>Eclipta alba</i> (L.)
1	Percentage of loss on Drying	1.89% w/w	6.8 % w/w	7.8 % w/w
2	Percentage of ash content	4.25 % w/w	19.76 % w/w	15.21 % w/w
3	Percentage of acid insoluble ash	0.42 % w/w	4.0 % w/w	8.52 % w/w
4	Percentage of watersoluble ash	3.82% w/w	12 % w/w	15.66 % w/w
5	Percentage of hydroalcohol soluble extractive value	16.13 % w/wethanol	13.50 % w/v methanol	11.5 % w/v

Table 3: Phytochemical Screening of Plant Extracts

S.No	Test	Trigonella foenum greacum	Allium cepa L	Hibiscusrosa sinensis,Linn	Eclipta alba(L.)
1.	Alkaloids	+ ve	+ve	- ve	+ve
2.	Flavonoids	+ ve	+ ve	-ve	+ ve
3.	Steroids	- ve	+ ve	- ve	+ ve
4.	Tannins	+ ve	+ ve	+ ve	+ ve
5.	Saponins	+ ve	+ ve	+ ve	+ ve
6.	Carbohydrate	+ve	+ve	+ve	+ ve
7.	proteins	+ ve	+ve	+ve	- ve
8.	Glycoside	+ ve	+ ve	+ve	+ ve

Formulation Characterization and Evaluation of topical therapeutic system

Evaluation of oil Formulation:

The result showed that the developed herbal oil was brownish in color, viscous liquid in texture and showed good appearance . Formulation had good

values of viscosity, pH, drug content and during the accelerated stability studies the appearance was same as previous and no significant variation in viscosity, pH and drug content was observed. Hence formulated herbal oil physiochemical study was found to be good.

Table 4: Physical evaluation of all formulations

Color	Odour	Texture	Refractive index	Saponification value	Viscosity (cps)	Ph	Drug content (%)
Brown	Characteristic	Viscous Liquid	1.434	232	129.3	5.9	98.32

Standard curve of Plant extract

Standard calibration curve of plant extracts was determined by plotting absorbance vs concentration at 234 nm. Table no.6.4 and Fig-7.1 shows the

standard curve for herbal extract. The method obeyed Beer's law limit in the concentration range of 2-12 mcg/ml at 234 nm with a regression value of 0.998.

Table 5: Calibration curve of plant Extracts at 234 nm

S. No	Concentration	Absorbance at 234 nm
1	2	0.221
2	4	0.323

3	6	0.425
4	8	0.556
6	10	0.639
8	12	0.753

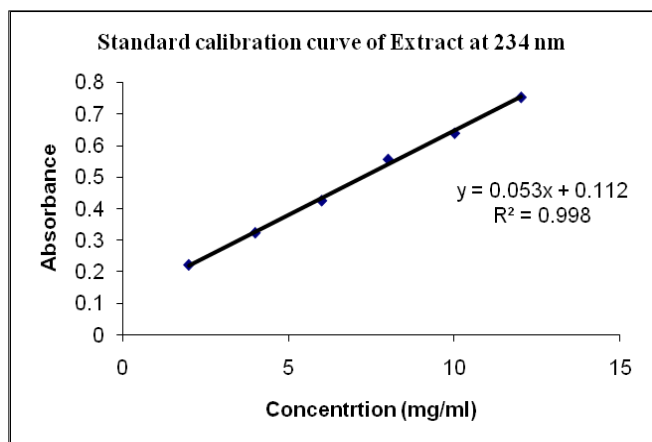


Fig 1: Calibration curve of plant Extracts at 234 nm

Pharmacological Screening of Optimized Formulation

Optimized formulation containing plants extract selected for in-vivo hair growth study. Both qualitative hair growth and quantitative hair growth activity performed. Qualitative hair growth analysis was undertaken by visual observation of two parameters: hair growth initiation time and hair growth completion time. While hair length, hair weight and histological study performed in quantitative hair growth analysis.

Qualitative Evaluation of In Vivo Hair Growth

Herbal formulation containing Combination of extract FH treated animals showed significant reduction in hair growth initiation and completion time as compared to control and minoxidil treated animals (Table 7.5). Throughout the 30 days study period, all the groups of animals were observed closely to determine the hair growth initiation and completion time. This was achieved using a magnifying lens that enabled observation of minute changes in the hair growth pattern. The point at which a tiny prickle of hair growth was observed and it was noted as the initiation time. Oil formulation containing combination of extract treated animals showed significant reduction in hair growth initiation and completion time as compared to control and minoxidil treated animals.

In control group animals, initiation of hair growth in denuded area was observed in 10 days. Hair growth initiation was noted in the first week (6 days) in mice of minoxidil treated standard group. The extract combination Oil formulation exhibited hair growth initiation on 5th day. Similarly, the time taken for complete hair growth on shaved area was promoted with minoxidil treatment as well as Oil formulation. Complete hair growth with minoxidil and control group as observed in 20 and 29 days respectively while Oil extract formulations was recorded at 19 days.

Qualitative observation of hair growth

Table No. 6: Effect of Extracts on Hair Growth Initiation and Completion Time

Group	Treatment	Time taken to initiate the growth (in days)	Time taken for complete growth (in days)
Group I	Control	10.4±0.45	29.2±1.51
Group II	2% Minoxidil	6.5±0.41	20.0±0.21
Group III	E1 Extract combination	7.06±64	22.2±0.71
Group VI	Formulation	5.2±0.32	19.3±0.41

Values are mean ± SEM

Quantitative hair growth Study Hair length Determination:

The length of the hair began to increase until the end of the treatment course. The extract combination Oil formulation produced a greater effect on the length of hair when compared to other group. The Oil Formulation combination extract exhibited hair

length 1.48 mm at 30 days whereas with 2% Minoxidil marketed formulation exhibited hair length 1.38 nm at 30 days while control group exhibited hair growth 1.21 mm at 30 days. Oil Formulation treated groups produced a greater effect on the length of hair when compared to other groups. This may be due to the premature switching of

follicles from the telogen to anagen phase of hair growth cycle. Average hair length of each group at

10th day, 20th day and 30th day has been given in Table. 7.

Table No 7. Effect of different combination of Extract on Hair length

Group of	Formulation	Hair Growth in mm Mean+ S.D		
		10 days	20 days	30 days
Group I	Control	0.0	0.312±00	1.21±0.21
Group II	2% Minoxidil	0.6±0.20	1.16±0.22	1.38±0.21
Group III	E1 Extract combination	0.6±0.21	1.18±0.24	1.39±0.24
Group VI	Formulation	0.8±0.20	1.20±0.22	1.48±0.20

Values are mean ± SEM

Histological studies Development of Hair Follicles:

The hair follicle count, skin thickness and color appearance were observed. Formulation containing plant extracts showed significantly considerable results and exhibited significant increase in hair regrowth. Increase in the thickness and presence of hair follicles in the subcutis layer were taken as an

evidence for transition of follicles from telogen to anagen phase of hair growth. The photomicrographs obtained indicated that formulation treated animals had showed maximum percentage of anagenic hair follicles (71.6 %) and hair follicles density while the E1 extract (70.8 %) and minoxidil (71.4 %). (Table.8)

Table No. 8: Effect of Extracts different combination on per cent of hair follicles

Group of 20 days	Formulation	Anagen	Telogen	T/A ratio
Group I	Control	53.3±0.69	45.4±0.89	0.85
Group II	2% Minoxidil	71.4±0.65	28.2± 0.17	0.39
Group III	E1 Extract combination	70.8±0.42	25.5±0.42	0.36
Group VI	Formulation	71.6±0.41	29.1±0.21	0.40

Values are mean ± SEM

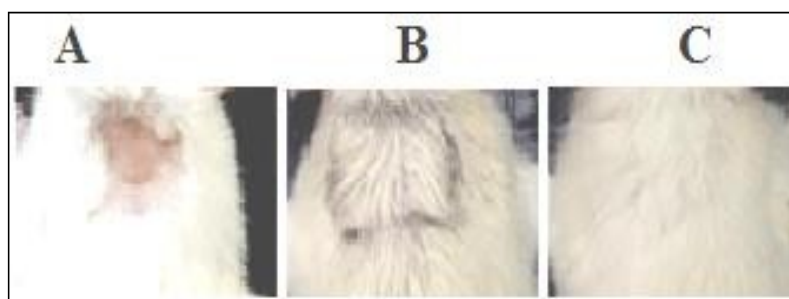


Figure 2: Formulation herbal oil treated Rat (A= 10 days, B= 20 days C = 30days)

Skin Irritation Study

The skin irritation test was conducted for a period of seven days and the results are tabulated in Table 6.9. The results indicated that the control preparation, oil

formulation and marketed products did not cause any skin reaction. It can be assured that both plants extract and the excipients did not cause any skin irritation and can be used in the Oil formulation.

Table 9: Skin irritation study

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	A	A	A	A	A	A
Formulation	A	A	A	A	A	A	A
Minoxidil Gel	A	A	A	A	A	A	A

A – No reaction, B – Slight patchy erythema, C – Slight but confluent or moderate but patchy erythema, D – Moderate erythema, E – Severe erythema with or without edema.

Stability Study

The formulated oil were subjected to stability studies. No color fading was observed for all

prepared oil. The pH of all formulations remained unchanged and was found to be within the range of 6.2-7.2. The viscosity of oil remained unaltered and found to be within the range. The drug content was found to be in the limit 90% -103% for formulation. (Table..10)

Table 10: Accelerated Stability study of formulated oil

Color	Odour	Texture	Viscosity(cps)	Ph	Drug content(%)
Brown	Characteristic	Viscous Liquid	134.3	6.3	98.92

Conclusions

In the present study, an attempt was made to prepare, characterize and evaluate of topical therapeutic system for management of alopecia from seed extract of *Trigonella foenumgreacum*, flower extract of *Hibiscus rosa sinensis* Linn, leave extract of *Eclipta alba* (L.) and bulb of *Allium cepa* L herbal plant. Polyherbal oil formulations were designed and optimized.

Recently, the number of men and women who suffered from hair loss and/or hair thinning is increasing. Hair loss is a dermatological disorder, and the surge for discovering natural products with hair growth promoting potential is continuous. Hair loss or alopecia is a common patient complaint and a source of significant psychological and physical distress. Androgens are considered to be one of the most important causes for alopecia apart from a variety of other factors. Minoxidil, a drug of scientific origin was scientifically proved for the treatment of alopecia. Though the side effect associated with this drug has limited its pharmacological benefits hence the drug of plant origin is necessary to replace the synthetic one. Thus it is very important to develop

new therapeutic materials to stop hair loss and to enhance hair growth. Alternative medicine is one interesting area, which is getting more popular. Although it has not yet been incorporated into the mainstream of medical care because of limited scientific evidences and lack of mechanistic understanding, alternative medicine is becoming an increasingly attractive approach all over the world. Natural products in the form of herbal formulations are available on the market and are used as hair tonic, hair growth promoter, hair conditioner, hair- cleansing agent, antidandruff agents, as well as for the treatment of alopecia and lice infection. A number of herbal products have been acclaimed with hair growth promoting activity. The traditional system of medicine in India acclaims a number of herbal drugs for hair growth promotion but lack of sound scientific backing and information limits their use.

From the present study, the following conclusion can be drawn:

- Physicochemical analysis of powder of aerial part of plant was carried out which provides referential information for the correct identification of crude drug. In this

study ash values (total ash, acid insoluble ash and water-soluble ash) and moisture content (M.C.) were determined. The physic-chemical analysis studies can be used as a diagnostic tool for the correct identification of the selected species of plants. Hence, these standardization parameters are useful in detecting the adulterants if any in this plant and will lead to efficacy and purity of the selected plant. Hence, all these findings shall be helpful in the correct identification, identity and purity of the selected endangered plant.

- The extraction of seed of *Trigonella foenum-graecum*, flower extract of *Hibiscus rosa sinensis* Linn, leave extract of *Eclipta alba* (L.) and bulb of *Allium cepa* L herbal plant were carried out with hydroalcoholic solvent by using soxhlet apparatus. The extracts were screened for the presence of various medicinally active constituents. Physicochemical studies revealed that major active constituent are present in hydroalcoholic extract like alkaloids, tannins, terpenoids, carbohydrates, glycosides, saponins, protein and flavonoids as a major component.
- The polyherbal Oil formulation of extracts combination E1 was formulated and then decreased as several problems like homogeneity, texture and viscosity were encountered.
- The result showed that the developed herbal Oil was brown in colour with viscous liquid texture. Formulation had good values of viscosity, pH, drug content and during the accelerated stability studies.
- Pharmacological Screening of Optimized Formulation study revealed that formulation F treated animals showed significant reduction in hair growth initiation and completion time. Hair growth initiation was noted on 5th day. Similarly the time taken for complete hair growth on shaved area was recorded at 19 days. The formulated Oil F combination extract exhibited hair length 1.48 mm at 30 days.

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