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Treatment for hair fall and premature hair greying by poly herbal formulation

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Abstract
Hair plays a vital role in making you look younger or old and also plays an important role in the personality of human. The pigmentation problem, dandruff, loss of hair is the major problem associated with hairs. Ayurvedic system is the traditional system of medicine having major treatment across the world. The aim of study was to develop a hair oil formulation using *Aloe vera* (leaves), *Indigofera tinctoria* (Whole plant), *Trigonella foenum-graecum* (seeds), *Nigella sativa* (seeds), *Cocos nucifera* (oil) are with purported claims of better growth of hair and diminution in loss of hair. The oil was prepared according to Ayurvedic Formulary of India and was standardized according to Protocol for Testing Ayurvedic, Siddha and Unani Medicines, Government of India. Purification of fruits and leaves was performed according to Ayurvedic Formulary of India. Organoleptic evaluation and physicochemical evaluation was performed.

Keywords: ayurvedic system, *aloe vera*, *indigofera tinctoria*, *trigonella foenum-graecum*, *nigella sativa*, *cocos nucifera*, hair loss

1. Introduction
Hair is one of the important parts of our body and it influences the overall appearance of the person and is derived from ectoderm of the skin, it is an ornament structure along with sebaceous gland. Hair is a dead part with no nerve connections and has the unique ability to regenerate itself [1, 3]. Alopecia is a dermatological disorder that has been recognized for more than 2000 years. It is common throughout the world [4]. Now-a-days, in the whole world there is turn to return towards the use of herbal products and to adopt more natural way of life. People prefer natural food, herbal medicines and natural curing practices for healthy life. Allopathic system alone is proving insufficient and there is need to supplement it with herbal drugs. The most appropriate way is to utilize modern as well as traditional system to look after the health of the people. Herbs and herbal drugs are clinically proved for hair growth. Hair loss problem is of great concern; the main problems associated with hair loss are hair fading, dandruff and falling of hair. Various synthetic medicines are available for hair loss which does not treat permanently and also shows severe side effects. This problem could be solved by the use of herbal medicines. Turning to Ayurveda is the safer and better option in the long run. The side-effect profile of these products is 'nil' as compared to products with a host of chemicals. Herbal hair oil helps to prevent premature graying, hair fall, dandruff and promotes hair growth. It works within the root of the hair and gives natural black colour to the hair [5].

1.1 Types of hair loss
1.1.1 Androgenetic or androgenic alopecia (baldness)
It is the most common cause of hair loss in men also known as hereditary baldness. 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 [6].

1.2 Alopecia areata
In alopecia areata the hair is lost from the scalp (alopecia areatatotalis) or from the whole body (alopecia areatauniversalis) [7].

1.3 Telogen effluvium
Telogen effluvium is characterized by the early entrance of a large number of hairs in to telogen phase at one time [8].

1.4 Chemotherapy-induced alopecia
This type of hair loss is occurred due to the side-effects of cancer therapy [9].
Aloe vera (leaves), *Indigofera tinctoria* (Whole plant), *Trigonella foenum-graecum* (seeds), *Nigella sativa* (seeds), *Cocos nucifera* (oil) was reported as a useful remedy to prevent graying, hair loss, dandruff and promotes the growth of hair. And the formulation also gives natural black colour to the hair. The study was designed to treat hair loss disorders by preparing an effective formulation using the above crude drugs [10].

2. Materials and methods
2.1 Collection, identification and authentication of plants
Aloe vera (leaves), *Indigofera tinctoria* (Whole plant), *Trigonella foenum-graecum* (seeds), *Nigella sativa* (seeds), *Cocos nucifera* (oil) were purchased from local market of Calicut, identified and authenticated from Department of Botany, University of Calicut, Kerala.

2.2 Drug materials
The various ingredients used in hair oil are collected, purified and weighed separately [11, 12]. The quantity of each ingredient

is tabulated in Table No. 1.

Table 1: Quantity of each of the ingredients and the parts used

Sl. No	Ingredients	Parts Used	Quantity (g)
1.	*Aloe vera*	Leaves	250
2.	*Indigofera tinctoria*	Whole plant	250
3.	*Trigonella foenum-graecum*	Seeds	75
4.	*Nigella sativa*	Seeds	75
5.	*Cocos nucifera*	Fixed oil from the endosperm of fruit	QS

The fresh plant drug materials except seeds of *Nigella sativa* and *Trigonella foenum-graecum* were collected and ground coarsely with mechanical grinder and filtered using muslin cloth and fresh juices were prepared. The seeds of *Nigella sativa* and *Trigonella foenum-graecum* were previously dried in shade and were stored in air tight containers.

2.3 Formulation of Polyherbal Hair oil

All the drug materials except *Trigonella foenum-graecum* and *Nigella sativa* seeds were made to juice and were filtered through muslin cloth. *Trigonella foenum-graecum* and *Nigella sativa* seeds were dried and finely ground. Powdered drugs and juices were mixed with coconut oil. The hair oil was prepared by direct boiling method, one of the three methods which were used traditionally. In this method the fresh plant material was weighed and mixed with powdered drug materials and directly boiled along with coconut oil taken in steel vessel, with continuous stirring and heating until the drug has completely extracted in to the oil base. The boiling process with continues stirring was carried out for 2 hour and 45 minutes. The temperature was kept between 80-100 ˚C. Then the oil was filtered through muslin cloth and stored for further use. The optimized formula was used for further preparation.

2.4 Evaluation of prepared hair oil

The prepared formulation were evaluated using standard methods of general characterization, physical and chemical evaluation including Specific gravity, pH, Refractive index, Acid value, Saponification value and Iodine value[13].

1. **Organoleptic properties:** Colour and odour, were determined manually.
2. **Specific gravity:** Specific gravity of the prepared oil was determined using pycnometer or specific gravity bottle. Determine and record weight of the empty, clean and dry pycnometer, W. Place 10g of oil sample taken in the pycnometer. Determine and record weight of the pycnometer containing water and record as WA. Dry the apparatus and place 10g of oil sample in the pycnometer. Determine and record weight of the pycnometer containing the oil, WO. Empty the pycnometer and clean it.

$$\text{Specific gravity, } G = \frac{WA - W}{WO - W} \quad (1)$$

Where

W = weight of empty pycnometer
WA = weight of pycnometer filled with water
WO = weight of pycnometer filled with oil

3. **Viscosity:** Viscosity is the property of a fluid that determines its resistance to flow. Viscosity was determined using Ostwald's viscometer. Viscosity is very sensitive to temperature, so all solutions and the viscometer must be kept at 30 °C in the water bath. Always handle the viscometer by one limb only and never squeeze the two arms together. Rinse the viscometer with water and place it in position in water bath by carefully clamping one limb. Check that it is vertical using a plumb line. Introduce exactly 20 ml of water into the bulb with a syringe or pipette. Leave for 5 minute to equilibrate, then either apply positive pressure to the wide limb or gentle suction to the other limb until the meniscus rises above the upper graduation mark. Release the pressure and measure the time for the liquid to flow between the two graduation marks. Repeat the experiment with oil until the flow times agree within 0.2S. Calculate the average flow time. Calculate the relative viscosities (t_1/t_0) using the values from the curves.
4. **Refractive index:** It was determined using Abbe's refractometer. Since this experiment requires high accuracy, it is necessary to have the spectrometer in complete adjustment. Focus the eyepiece on the cross-hair. Direct towards the light, move the eyepiece tube until the cross-hair are most distinctly observed. Light rays from the cross-hair which enter the eye are parallel rays. Level the spectrometer. Place a level on the spectrometer plate, adjust the foot screws, turn the level on the plate and adjust again till the spectrometer is absolutely horizontal. Level the spectrometer table. Place a level on the table so that it is parallel to a line through two of the leveling screws, and level by adjusting these screws. Then place the level at right to its first position, and level by adjusting the third screw. Level the telescope and collimator. Place the level on top of the barrel of the telescope, and adjust by use of the leveling screw. In a similar manner adjust the collimator. Adjust slit of the collimator. Rotate the telescope until it is directly opposite the collimator and in alignment with it. Slide the slit assembly in the collimator tube until it is in sharp focus and without parallax with respect to the cross-hair. It has been focused for parallel light. Put the prism on the prism table. The prism table should be leveled such that if the light through the hole in the Gaussian eyepiece, the two reflected images on the cross-hair from the two reflecting faces are both at the same height as the cross-hair. Measure the prism angle θ. Turn the prism table so that light can be reflected from both faces simultaneously. Record the positions at which these reflections are seen in the telescope. Be able to show that the difference between these two readings is 2θ. Rotate the prism and the telescope to observe the deviated ray. Turn the prism table until minimum deviation is observed and record the readings. Then remove e prism from the table and turn the telescope to observe straight-through or un-deviated ray. Record the scale-readings. The difference between this set of readings and the readings of the light deviation is δ m, the angle of minimum deviation.
5. **pH:** pH of the herbal oil was detected using pH meter

(Labtech). Glass and reference electrodes are connected to appropriate terminals at the rear panel. Set the temperature compensation knob to the temperature of the solution. Place the electrodes in buffer 7. Press read switch on the front panel to read. Adjust control knob to read pH 7 in the display. Bring the read switch back to standby mode. Remove the electrodes from the buffer 7. Wash them and place in buffer 4 and 9.2. Confirm the display reading same as that of the buffer solution used. Rinse the electrodes with distilled water. Place the oil sample, dip the electrodes and read the pH.

6. **Acid value:** The acid value is the number of mg of potassium hydroxide required to neutralize the free fatty acids in 1 g of the fat. The acidity is an expression of the content (in %, m/m; percentage) of free fatty acids as content of dominant or chosen fatty acid. An increment in the amount of FFA in a sample of oil or fat indicates hydrolysis of triglycerides. Such reaction occurs by the action of lipase enzyme and it is an indicator of inadequate processing and storage conditions (i.e., high temperature and relative humidity, tissue damage). The source of the enzyme can be the tissue from which the oil or fat was extracted, or it can be a contaminant from other cells including microorganisms. Besides FFA, hydrolysis of triglycerides produces glycerol. Measure 10ml into a titration vessel. Dissolve it in 25 ml of the solvent mixture of ethanol and diethyl ether. Add 0.2 ml of phenolphthalein solution and titrate by shaking, with the solution of potassium hydroxide in ethanol to the pink colour persisting for at least 10 seconds. Carry out simultaneously a blank test.

   Acid value = 5.61n/w (2)

   Where,
   n= Number of ml of 0.1M KOH
   w= Weight of oil

7. **Saponification value:** Saponification value indicates the average molecular weight of a fat or oil. The saponification value may be defined as the number of milligrams of potassium hydroxide required to neutralize the fatty acids obtained by complete hydrolysis of one gram of oil or fat. Thus saponification value gives us information whether an oil or fat contains high proportion of lower or higher fatty acids. Coconut oil has comparatively higher saponification value. Saponification value gives us an idea about the molecular weight of fat or oil. 2g of oil was accurately weighed and transferred into a 250ml iodine flask. 25ml of 0.5M alcoholic potassium hydroxide was added and refluxed on a water bath for 30minutes. Phenolphthalein was added as indicator and titrated against 0.5M HCl (‘a’ ml). Similarly blank was performed (‘b’ ml) without the sample.

   Saponification Value = 28.05(b-a)/w (3)
   (w= Weight of oil)

3. Results and Discussion

3.1 Formulation of Poly-Herbal Hair oil

Table 2: Percentage quantity of each of the ingredients and the parts used

Sl. No	Ingredients	Parts Used	Quantity (%)
1.	*Aloe vera*	Leaves	8.25
2.	*Indigofera tinctoria*	Whole plant	8.25
3.	*Nigella sativa*	Seeds	2.47
4.	*Trigonella foenum-graecum*	Seeds	2.47
5.	*Cocos nucifera*	Fixed oil from the endosperm of fruit	12.55

The poly herbal hair oil was formulated as per standard procedure by direct boiling method and stored in air tight container for further study. The percentage quantity of each of the ingredients and the parts used are tabulated and given in Table No. 2. The percentage of leaves of *Aloe vera* and *Indigofera tinctoria* were 8.25%, seeds of *Nigella sativa* and *Trigonella foenum-graecum* were 2.5% and 12.55% of oil from *Cocos nucifera*, which is similar to standard other poly herbal hair oils.

3.2 Evaluation of prepared hair oil

3.2.1 Organoleptic evaluation

Table 3: Result showing organoleptic evaluation

Sl. No.	Parameters	Observation
1	Colour	Greenish brown
2	Odour	Characteristic
3	Texture	Greasy to touch

Organoleptic parameters were evaluated. The oil is having greenish brown colour, greasy to touch and having characteristic odour, which is similar to standard poly herbal hair oils.

3.2.2 Physicochemical evaluation

Table 4: Result showing physicochemical evaluation

Sl. No.	Parameters	Observation
1	Specific gravity	0.9831±0.0003
2	Viscosity	0.9432±0.0002 Poise
3	Refractive index	1.573±0.001
4	Acid value	2.69±0.03
5	Saponification value	259.67±0.02
6	Iodine value	15.66±0.01
7	pH	6.10±0.005

The weight per ml and specific gravity of the formulation was found to be 0.9831 g/ml±0.0003, Viscosity was found to be 0.9432±0.0002 Poise, Refractive index was found to be

1.573±0.001, Acid value was found to be 2.69±0.03, pH was found to be 6.10±0.005.

4. Conclusion

For the treatment of hair fall and premature hair greying a poly herbal hair oil containing *Aloe vera* (leaves), *Indigofera tinctoria* (Whole plant), *Trigonella foenum-graecum* (seeds), *Nigella sativa* (seeds), using coconut oil as the vehicle were formulated by direct boiling method using prescribed formula.Various physicochemical evaluations were carried out on the formulated Polyherbal hair oil. The result shows that the physicochemical parameters were within safe limit. From the results it can be concluded that this herbal formulation found to be good and effective remedy for hair growth, pigmentation problem and hair loss treatment.

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