Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 25s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Formulation and Characterization of Herbal Hair Oil for Prevention of Grey Hair

Sapana P Ahirrao[1]*, Suchita Y Shirsath[2], Sarika V. Patil[3], Devyani P. Chavan[4],Anagha V.Baviskar[5], Sarika V. Algat[6], Vaidehi V. Gaidhani[7], Preetam L. Nikam[8]

[*1]Pharmaceutics Department, MET's Institute of Pharmacy, Nashik-422003, Maharashtra, India.
[2]Pharmaceutics Department, MET's Institute of Pharmacy, Nashik-422003, Maharashtra, India.
[3]Pharmaceutics Department, MET's Institute of Pharmacy, Nashik-422003, Maharashtra, India.
[4]Pharmaceutics Department, MET's Institute of Pharmacy, Nashik-422003, Maharashtra, India.
[5]Pharmaceutics Department, MET's Institute of Pharmacy, Nashik-422003, Maharashtra, India.
[6]Vishwabharti Academyis College of Pharmacy, Sarola, Baddi, Ahmednagar-414201, India.
[7]Pharmaceutics Department, MET's Institute of Pharmacy, Nashik-422003, Maharashtra, India.
[8]Pharmaceutics Department, RG Sapkal Institute of Pharmacy, Nashik-422009, Maharashtra, India.

Corresponding author:
Sapana P Ahirrao;
Email ID: Sapana.58ahirrao@gmail.com

Cite this paper as: Sapana P Ahirrao, Suchita Y Shirsath, Sarika V. Patil, Devyani P. Chavan, Anagha V.Baviskar, Sarika V. Algat, Vaidehi V. Gaidhani, Preetam L. Nikam, (2025) Formulation and Characterization of Herbal Hair Oil for Prevention of Grey Hair. *Journal of Neonatal Surgery*, 14 (25s), 947-955.

ABSTRACT

Oxidative stress is one of the major reasons of production of free radicals is discovered to be the most significant of these reasons for grey hair. Oxidative stress also accelerates the apoptosis of melanocytes found in the hair bulb, which results in a loss of regeneration ability as well as the conversion of amelanogenic melanocytes into melanogenic ones.So, the main focus of this study to find out natural alternative which is rich in minerals like iron, folic acid, and vitamins like A, C, and E with antioxidant property which is used to prevent the grey hairs. The plants used for this study is *Murraya koenigii* (Curry leaf, family rutaceae), T*rigonellafoenum-graecum* (fenugreek, family: Fabaceae) *Indigoferatinctoria* (nilli. Family: Fabaceae). Hydroalcoholic extract of M. Koenigii and powder sample of fenugreek and indigo showed the presence of alkaloid, flavonoid. tannins in their phytochemical screening. Oil based herbal formulation was prepared using finely powdered samples of curry leaves, fenugreek and nilli using pure coconut oil. Resulting formulation was evaluated by various parameters like pH, organoleptic properties (Color, Odor), acid value, saponification value. All these plants consist of essential nutrients such as flavonoid, tannins, vitamin, terpenoids and many essential oils helps to maintain normal function hair and prevention of premature hair growth.

Keywords: *Grey hair, Antioxidant, fenugreek, Curry leaves.*

1. INTRODUCTION

Hair is one of the few physical aspects of the human body that may be altered to reflect fashion trends, culture, or social ideals. (Da Gama, et al, 2017)Hair color and style have a big impact on how someone looks physically, which has a big impact on how they feel about their bodies. Premature greying of the hair (PGH) can have a negative impact on a person's self-esteem because it is thought regarded as a symptom of ageing. Normal ageing results in hair greying, also known as canities or achromotrichia. (Kumar A.B. et al, 2018) Canities (Sheeb), also known as premature greying of scalp hair, is a hair disease characterized by early greying of scalp hair.

1. Causes of grey hair

Greying of hair occurs due to various reasons, all of these come from intrinsic or extrinsic (e.g., ultraviolet radiation factors **(Tobin D.J, 2010)**. Few studies have also reported that smoking, drugs, deficiencies of trace elements, and nutritional deficiencies also play a role in PHG **(Chakrabarty, S., 2016).**

Genetic disorders: Premature grey hair is noticed in persons having the TERT mutation (human gene encoding protein component of telomerase). Many rare genetic syndromes, such as Waardenburg syndrome, Vogt Koyanagi Harada syndrome, Werner syndrome, and others, are associated with hair pigmentation loss. (Singh, R. et al, 2021)

- **Low or No Melanin Production**- Melanocytes in hair follicles create two pigments: pheomelanin and eumelanin. These pigment molecules combine to give humans natural hair colour, but with time, these are gradually diminished by creation from Melanosomes on a cell's surface—which means that as one ages, their look might change to grey or white depending on whether there is more of one type than another.

- **Disease conditions**: Hair greying is associated with various autoimmune disorders including vitiligo and several rare premature aging syndromes including the Hutchinson Gilford and Werner'syndrome. (Jo, S.K. et al, 2018)

- **Smoking**: Tobacco smoking is a well-known exogenous source of free radical production and oxidative stress, both of which promote premature ageing changes in hair follicles. (Singh, R. et al, 2021)

- **Nutritional deficiency**: Because different minerals operate as cofactors in different stages of melanogenesis, their shortages may prematurely activate the canities. Tyrosinase, a copper-dependent enzyme that also requires calcium for phosphorylation and activation, catalyzes the conversion of L-tyrosine to levodopa. In addition to calcium and copper shortage, PGH has been linked to ferritin and zinc deficiencies. Vitamin B12 insufficiency has been discovered to have the strongest connection with PGH of any vitamin. Vitamin B7 and folic acid deficiencies have also been found in PGH patients, although more research is needed. (Singh, R. et al, 2021)

- **Stress/ ROS/ oxidation:** Oxidative stress can also be a result of ultraviolet (UV) rays, pollution, emotional factors, or inflammatory causes which shows major effect on grey hair.

Most research has focused on how reactive oxygen species (ROS) affect hair ageing. UV radiation, pollution, mental problems, or inflammatory reasons can potentially induce oxidative stress.Graying hair may result from oxidative damage caused by UV exposure to hair follicles.

2. MATERIALS AND METHODS

2.1 Materials

The leaves of *Murraya Koeinigii* were collected from the farms located at RajapurYeola, Nashik, Maharashtra, and were properly authenticated. The powder of *Trigonella foenum-graecum*, and *Indigofera tinctoria* was collected from the local market.

Methods

Formulation of extract

2.2.1 Preparation of *murraya koenigii* extract with different technique and characterization of extraction:

1) Extraction

a) Soxhlet Extraction

The thimble containing 10 g of plant material packed in a filter paper was placed between condenser and round-bottom flask containing 200 mL of 50% (v/v) methanol-water solvent. After boiling the solvent, the extraction chamber progressively furnishes with new solvent from the round bottom flask. When meeting the maximum level in the extraction chamber, the condensed solvent from the extraction chamber was flushed back into the round bottom flask by a siphon. This condensed solvent carries the solutes into the solvent. The extraction was performed for 7 h (Patil S.S. et al, 2021)

2) Solvent assisted extraction

Solvent assisted extraction was conducted using 50% methanol using a modified. Approximately 10 g of the finely ground plant material was added with 100 mL of 50% methanol and left to stand for 1 hr. The solvent was then removed leaving the residue for the second extraction. The solvent was filtered using Whatman No. 4 filter paper using a vacuum pump. (Parithy, M.T. et al, 2021)

3) Ultrasonic assisted extraction

Ultrasonic assisted extraction was conducted using a modified method. A total of 10 g powdered plant material were mixed with 50%methanol. The mixture was submerged in an ultrasonic cleaner bath and extracted for 60 min. The extracted samples

were then filtered using whatmann filter paper. Methanol was then removed from the extract using a rotary evaporator (Parithy M.T. et al, 2021)

Table no.1 Extraction of *Murraya koenigii* powder with different technique

Sr. no.	Technique used for Extraction	Amount of *Murrayakoeinigii* Powder	Solvent system	Total volume of solvent
1	Soxhlet Extraction.	10 gm	Hydro-alcoholic (Methanol: Water) 50:50	100 ml
2	Solvent assisted extraction	10 gm	Hydro-alcoholic (Methanol: Water) 50:50	100 ml
3	Ultrasonic assisted extraction	10 gm	Hydro-alcoholic (Methanol: Water) 50:50	100 ml

4) Ascorbic acid estimation

500 mg of each sample was precisely weighed and put to a 25 ml conical flask. The sample was then treated with 10 ml of oxalic acid (0.05 M)-EDTA (0.02 M) solution for 24 hours to give the needed reaction time. The samples were filtered through 0.45 m filter paper after 24 hours. After that, 2.5 ml of each sample was transferred to a separate 25 ml volumetric brown flask, followed by 2.5 ml of the oxalic acid (0.05 M)-EDTA (0.02 M) solution. Following that, in each volumetric brown flask, meta phosphoric acid was mixed individually with acetic acid (0.5 ml), sulphuric acid (5% v/v) solution (1 ml), and ammonium molybdate solution (2 ml), and the volume was built up to 25 ml with distilled water. A UV/visible spectrophotometer were used to detect the absorbance at 760 nm. (Hussain, I. et al, 2011)

Formula

Concentration = (Absorbance of Test / Absorbance of std) x (Dilution factor of Std / dilution factor of test) x (Wt. of Test / Wt. of Std)

5) Total flavonoids content

100µg/ml of extract was mixed with 4 mL of distilled water.0.3 mL of sodium nitrite (5%) was added and kept for 5 min followed by addition of 0.3 mL of aluminium chloride (10%) and again kept for another 6 min for incubation. Then, 2 mL of sodium hydroxide was added followed by addition of 2.4 mL of distilled water and the absorbance was read at 510 nm using a UV spectrophotometer. (Sablania, V., et al, 2019)The standard curve for total flavonoid was made using quarcetin standard solution (0-100 mg/ml)

2.2.2 Formulation and development of herbal hair oil

Table no. 2 Formulaof herbal hair oil

Sr. no.	Ingredient	Quantity
1	*Murraya koenigii*	10%
2	*Trigonella foenum-graecum,*	10%
3	*Indigofera tinctoria*	10%
4	*Cocos nucifera oil*	50 ml

1) Procedure of formulation of herbal oil

Precisely all the dried herbs such as *Murraya koenigii*, *Trigonella foenum-graecum, Indigofera tinctoria* were weighed, grinded and mixed in Cocos nucifera oil**.** The above content was boiled for 60 min, cooled and filtered through a muslin cloth.Finer particles filtered by using whatman filter paper.

3. EVALUATION OF HERBAL HAIR OIL

3.1. Organoleptic properties of herbal hair oil

The physical characteristics like colour, odour and appearance were evaluated by visual inspection (Gautam, S.,et al, 2012)

3.2 pH

The pH of herbal oil was measured using a digital pH meter at 25 ±1 ºC. The pH meter was calibrated before use by phosphate buffer pH 4.0 and 7.0. The pH values of all formulations were determined in triplicate. (CVS Subrahmanyam. 2019) (Gautam, S.,et al, 2012)

3.3 Homogenicity Test

A clean and dry object glass was smeared with the hair oil, and a cover glass was sealed. The appearance under the light of some coarse particle/homogeneity was investigated. Herbal hair oil was tested by visual examination for homogeneity and tested for some lumps, flocculates, or aggregates

3.4. Grittiness:

Herbal oil rubbed to the skin and observed

3.5 Specific gravity:

Specific gravity bottle was rinsed with distilled water, dried in hot air oven for 15 minutes, cooled, capped, weighed and was noted as (a). Now the same specific gravity bottle was filled with the sample, capped and again weighed (b). Weight of the sample per mililiter was determined by subtracting the weights (b-a) (Kamal, A., 2009)

Specific Gravity at 30°C = A-B / C-B

Where, A = weight of specific gravity bottle with oil at 30°C (g);

B = weight of specific gravity bottle at 30°C (g);

C = weight of specific gravity bottle with water at 30°C (g).

3.6 Viscosity

Viscosity was measured with an Ostwald viscometer (Tiwari, G. et al, 2021)

Wash the relative density bottle with distilled water and dry. Take the weight of the empty bottle and filled the given liquid. Clean and rinse the viscometer properly with distilled water. Fix the viscometer vertically in the stand and filled the specific amount of given unknown liquid in the viscometer. Time of flow recorded when the liquid starts to flow from the marks C and D above and below the bulb a. the experiment was repeated 3-4 times to get the viscosity of the given unknown liquid.

3.7 Acid value:

Preparation of 0.1M KOH solution: Weigh 0.56 g KOH pellets and dissolve in 100 ml of distilled water and stir continuously. Prepared 0.1M KOH solution was filled in the burette.

Preparation of sample : Measure 10 g of oil sample and dissolve in 25 ml ethanol & 25 ml ether mixture and shake. Add 1 ml of phenolphthalein as an indicator. And titrate with 0.1M KOH solution. (Kamal, A., 2009)

Acid value = 5.61V*N/ W

Where, V = Volume of potassium hydroxide used (ml).

N = Normality of potassium hydroxide.

W= Weight of sample (g)

3.8 Saponification value:

Accurately weigh 1g of oil sample into a 250ml conical flask and 10ml of ethanol: ether mixture (2:1) was added. To this flask, 25ml of 0.5N alcoholic KOH was added. Keep the flask 30 min for reflux then cool the flask. The cooled solution was titrated against 0.5N HCL solution using phenolphthalein as an indicator (a). Similarly, blank titration was performed without taking oil sample (b). The amount of KOH in mg used was calculated. (Gautam, S.,et al, 2012)

Saponification value = 28.05 *(b-a) /W

Where, b= ml of KOH required for blank

a= ml of KOH required to neutralize the substance.

W= weight of sample (g).

3.9 Sensitivity test:

The prepared hair oil was applied on 1cm skin of hand and exposed to sunlight for 4-5 minute. (Kamal, A., 2009)

3.10 Skin Irritation Test

A skin irritation test was performed on skin of dorsal side of hand with a small amount of the produced herbal hair oil. For 3 to 4 hours, the test site was monitored for erythema and edema (Tiwari, G. et al, 2021)

3.11. Antioxidant Property of herbal oil

Different concentrations of 100 ppm solution (20,40,60,100 mg/ml) of Herbal oilwere combined with 4 ml of 0.004% methanol solution of DPPH. The mixture was mixed well and permitted for 30 minutes undisturbed at normal room temperature in the dark. The lowering of the DPPH radical was obtained by reading the transmittance at 517nm (Ahmad, A et al, 2012)

3.12 SEM Analysis

SEM analysis were done on the treated and untreated hair samples which exposed to to artificial ultraviolet radiation. (Da Gama, R.M., 2017)

Treated Sample:

Tresses of hair sample were washed and cleaned properly. Samples of each hair type were exposed to the mercury vapor lamp for 24 hours. The hair sample were exposed to UVA radiation at 365 wavelengths after the application of herbal hair oil. This procedure continue for 10 days. During the irradiation process, the hair samples were rotated at regular time intervals to ensure that both sides received similar radiation quantities.

Untreated sample

Samples of each hair type were exposed to the mercury vapor lamp for 24 hours. The hair sample were exposed to UVA radiation at 365 wavelength. This procedure continue for 10 days. During the irradiation process, the hair samples were rotated at regular time intervals to ensure that both sides received similar radiation quantities. (Nogueira, A.C.S 2004)

SEM analysis of hair

For scanning electron microscopy (SEM), hair fibers were placed in the proper stub, covered with gold in a sputtering unit and observed with a microscope. (Da Gama, R.M., 2017)

4.13 Effect of temperature and humidity (Stability study)

Effect of temperature and humidity was studied by analyzing the herbal oil kept at room temperature, 40° C and 75% $\pm$ 5% relative humidity (stability chamber) and after one month, the formulation was analysed for colour, odour, pH, appearance, sensitivity, specific gravity etc.

4. RESULTS AND DISCUSSION:

Evaluation of formulation

Table no.3 Evaluation of herbal hair oil

Sr. no.	Parameters	Observation
1	Color	Greenish
2	Odour	Pleasant
3	Appearance	Smooth
4	pH determination	6.71 $\pm$ 0.18
5	Homogeneity Test	homogeneous
6	Grittiness:	smooth
7	Specific gravity	0.93
8	Viscosity:	0.927
9	Acid Value	0.617

10	Saponification Value	120.6
11	Sensitivity Test	Non sensitive
12	Skin Irritation test	No irritation

Antioxidant Property of herbal oil

Determination of antioxidant property of herbal oil:

Table no.4 % scavenging activity of herbal hair oil.

Sr. No.	Concentration	%RSA
1	20	83.6 ± 0.15
2	40	84.77 ± 0.21
3	60	85.94 ± 0.12
4	80	87.11 ± 0.09
5	100	88.87 ± 0.05

Mean ± SD (n = 3).

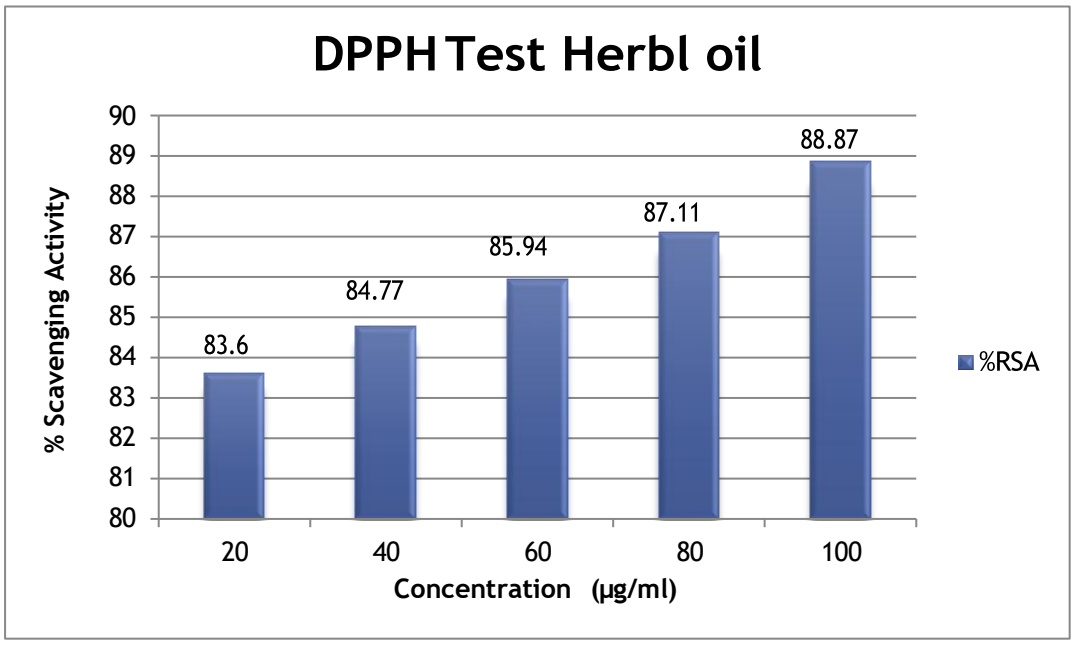

Fig. no.1 % scavenging activity of herbal oil shown by graph

Radical scavenging potential of maceration extract of *murraya koenigii* was evaluated DPPH method. The radical scavenging activity (RSA) was significantly higher at 100 ppm (88.87 ± 0.05). Measurements were done in triplicate for each sample. Results are expressed as mean ± SD (n = 3)

SEM analysis

SEM images revealed some differences in the conditions of the surface morphology of the hair samples due to the treatments. Some hair fibers were evaluated and images of untreated fibers and those treated with the herbal oil sample given in fig.7.25. Images of irradiated fibers were also obtained and the restorative capacities were compared. Irradiation of Untreated hair showed the largest differences as clearly lifted cuticles when compared with the non-irradiated hair sample (UT). The application of the formulation on hair led to a slight improvement in the hair surface without open cuticles when subjected to UV radiation. The improvement in hair surface of the formulations could be attributed to the antioxidant. These formulations help to prevent the oxidation of hair fibers subjected to UV, repairing the cuticle and protecting the hair.

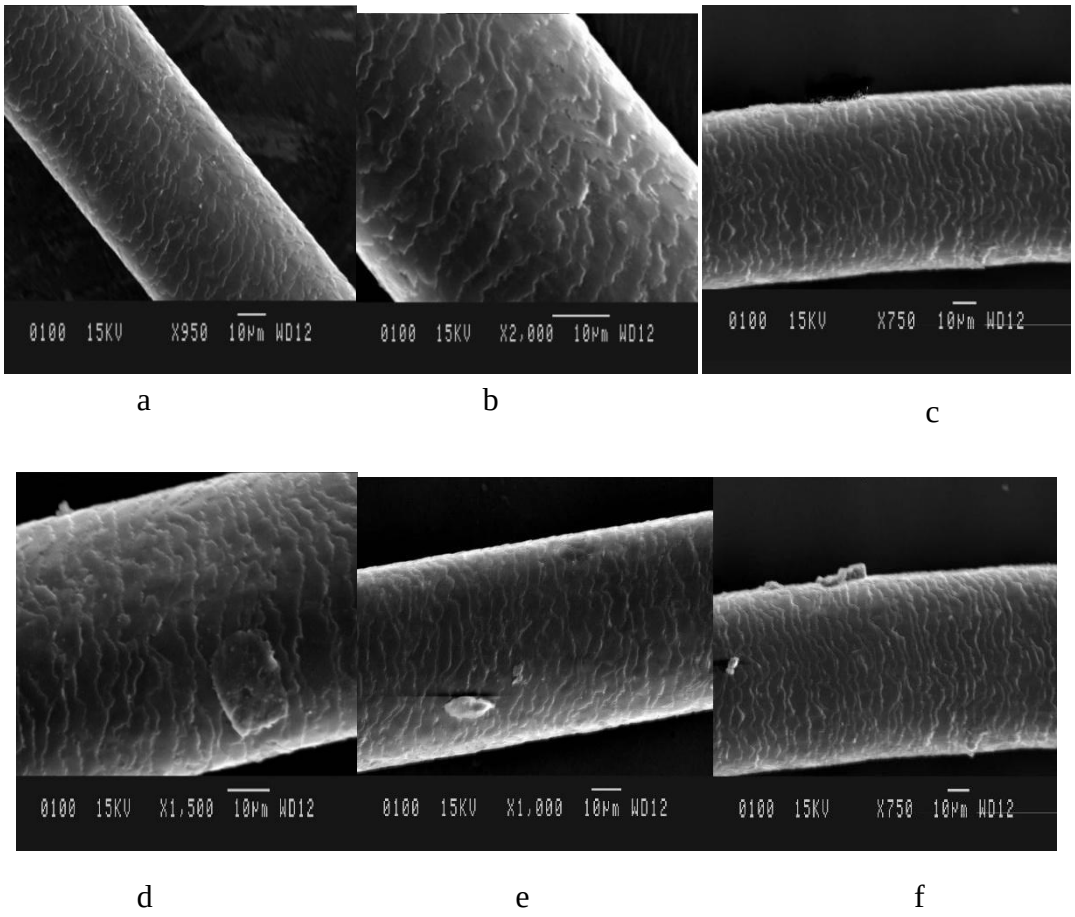

Fig.2 Scanning electron microscope (SEM) images of the surface of hair exposed to ultraviolet (UV) radiation of treated and untreated hairs and hair exposed to UV radiation. Images of the surface of hair exposed to UV radiation with application of sample ×2,000 (**a**), ×950 (**b**) ×750 (**c**); images of the surface of hair exposed to UV radiation without application of sample, ×1,500 (**c**) ×1000 (**d**); ×750

Stability

Effect of temperature and humidity on formulation was carried out by analyzing the formulation keeping at 40° C and 75% ± 5% relative humidity in stability chamber for 1 month. The formulation was analyzed for colour, odour, pH, appearance, sensitivity, specific gravity.

Sr. No.	**Parameter**	**Observation**						
		Initial	**After 1 month**		**After 2 month**		**After 3 month**	
			RT	**40 ± 2^{0}C & 70 ± 5%**	**RT**	**40 ± 2^{0}C & 70 ± 5%**	**RT**	**40 ± 2^{0}C & 70 ± 5%**
1	**Colour**	No change	No change	No change	No change	No change	No change	No change
2	**Odour**	No change	No change	No change	No change	No change	No change	No change

3	**pH**	6.69	6.66	6.60	6.65	6.61	6.66	6.59
4	**Appearance**	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny
5	**Sensitivity**	No irritation	No irritation	No irritation	No irritation	No irritation	No irritation	No irritation
6	**Specific gravity**	0.93	0.92	0.94	0.92	0.93	0.93	0.95
7	**% scavenging activity**	88.87 ± 0.05	88.83 ± 0.05	88.32 ± 0.03	88.29 ± 0.04	87.77 ± 0.07	88.11 ± 0.04	87.53 ± 0.04

5. CONCLUSION

Herbal hair oil is one of the most well recognized hair treatments. Herbal hair oil not only moisturizes scalp but also reverses dry scalp and dry hair condition. In this research herbal hair oil was prepared from various herbs and various parameters of herbal oil were evaluated for their physicochemical characteristics such as physical appearance, pH determination, Homogeneity Test, Grittiness, Specific gravity, Viscosity, Acid Value, Saponification Value, Sensitivity Test, Skin Irritation test FTIR, antioxidant property and UV protection efficiency.

Preformulation and formulation studies indicates that herbal hair oil was effective in managing oxidative stress that prevents premature hair greying

6. ACKNOWLEDGMENTS

The authors are grateful to Savitribai Phule Pune University, MET's Institute of Pharmacy, Adgaon, Nashik for providing facilities to carry out this research.

7. FUNDING/CONFLICT OF INTERESTS IF ANY

No conflict of interest.

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