Formulation And Evaluation of Herbal Hair Oil Using the Oil of *Eclipta Alba* (Bhringraj) For Hair Growth Promotion and Scalp Health

Ravi Kumar Baburao Shep[1], Mr. Shah A. S [2], Dr. N.V.N. Reddy[3], Dr.Sameer S. Sheaikh[4]
[1]*Research Scholar, Department of Pharmacognosy, Durgamata Institute of Pharmacy, Parbhani*
[2]*Assistant Professor, Department of Pharmacognosy, Durgamata Institute of Pharmacy, Parbhani*
[3]*HOD, Department of Pharmacognosy, Durgamata Institute of Pharmacy, Parbhani*
[4]*Principal, Department of Pharmacognosy, Durgamata Institute of Pharmacy, Parbhani*

***Abstract*—Background: Herbal hair care products are increasingly preferred due to their safety, biocompatibility, and reduced risk of adverse effects compared to synthetic formulations. *Eclipta alba* (Bhringraj), a well-known medicinal plant in Ayurveda, has traditionally been used to promote hair growth, reduce hair fall, prevent premature greying, and maintain scalp health. Its therapeutic properties are attributed to the presence of bioactive phytoconstituents such as flavonoids, coumestans, alkaloids, and phenolic compounds.**

Objective: The present study aimed to formulate and evaluate a herbal hair oil containing *Eclipta alba* oil and investigate its physicochemical characteristics, stability, and hair growth-promoting potential.

Materials and Methods: Bhringraj oil was extracted from authenticated plant material and incorporated into a herbal hair oil formulation using selected natural carrier oils. The prepared formulation was evaluated for organoleptic properties including color, odor, appearance, and texture. Physicochemical parameters such as pH, viscosity, specific gravity, refractive index, acid value, and saponification value were determined using standard analytical methods. Stability studies were conducted under specified storage conditions to assess formulation integrity. Hair growth-promoting activity was evaluated using suitable experimental methods and compared with a standard treatment.

Results: The formulated herbal hair oil exhibited desirable organoleptic characteristics and satisfactory physicochemical properties within acceptable limits. Stability studies indicated no significant changes in appearance, odor, viscosity, or other evaluated parameters throughout the study period. The formulation demonstrated notable hair growth-promoting activity, showing improved hair growth parameters compared to the control group. Conclusion: The developed *Eclipta alba*-based herbal hair oil was found to be stable, safe, and effective. The findings support its traditional Ayurvedic use and suggest its potential as a natural formulation for promoting hair growth and maintaining healthy scalp conditions.

***Index Terms*—*Eclipta alba*, Bhringraj, herbal hair oil, hair growth, physicochemical evaluation, stability studies.**

I. INTRODUCTION

1.1. Hair and Hair Disorders

Hair is a keratinized filamentous structure originating from hair follicles located within the dermal layer of the skin. Apart from its cosmetic significance, hair serves protective, sensory, and thermoregulatory functions. Each hair shaft consists of three major layers: the cuticle, cortex, and medulla. The cuticle forms the outer protective layer, while the cortex contains keratin fibers and melanin pigments responsible for hair strength and color. The medulla occupies the central region and may be absent in fine hair fibers [1].

Hair growth occurs through a cyclic process consisting of three distinct phases: anagen, catagen, and telogen. The anagen phase represents the active growth stage, during which rapid cellular proliferation occurs within the hair bulb. The catagen phase is a transitional period characterized by follicular regression, whereas the telogen phase is a resting stage that culminates in hair shedding and replacement by new hair growth [2].

Hair disorders such as alopecia, dandruff, premature greying, and scalp infections are increasingly prevalent worldwide. Factors contributing to hair loss

include genetic predisposition, hormonal imbalance, nutritional deficiencies, stress, aging, environmental pollution, and certain medications. The limitations and adverse effects associated with synthetic hair treatments have stimulated interest in herbal alternatives for hair care management [3].

1.2. Herbal Cosmetics

Herbal cosmetics are formulations containing plant-derived ingredients intended to enhance appearance and maintain the health of skin, hair, and scalp. In recent years, consumers have shown a growing preference for natural products because of their perceived safety, sustainability, and compatibility with biological systems. Herbal formulations often contain bioactive compounds such as flavonoids, phenolics, terpenoids, and alkaloids that contribute to their therapeutic effects [4].

Compared with synthetic cosmetics, herbal products offer several advantages, including lower incidence of irritation, reduced toxicity, biodegradability, and long-term safety. Moreover, herbal preparations frequently exhibit multiple pharmacological activities such as antioxidant, antimicrobial, and anti-inflammatory effects, making them suitable for comprehensive hair care applications [5].

1.3. Eclipta alba (Bhringraj)

Eclipta alba (L.) Hassk., commonly known as Bhringraj or False Daisy, is an annual medicinal herb belonging to the family Asteraceae. In Ayurveda, it is traditionally referred to as the "King of Hair" due to its extensive use in promoting hair growth and preventing hair loss. The plant is widely distributed throughout tropical and subtropical regions of Asia, Africa, and South America and commonly grows in moist and marshy areas [6].

Taxonomical Classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Asterales
Family: Asteraceae
Genus: Eclipta
Species: *Eclipta alba* (L.) Hassk.

The plant possesses opposite lanceolate leaves, small white flowers arranged in capitula, and cylindrical stems. Traditionally, different parts of the plant have been utilized for the treatment of liver disorders, skin diseases, respiratory ailments, and hair-related conditions [6].

Phytochemical Constituents

The therapeutic potential of *E. alba* is attributed to its diverse phytochemical composition. Major bioactive constituents include:

- Wedelolactone
- Demethylwedelolactone
- Ecliptine
- Flavonoids (luteolin, apigenin)
- Alkaloids
- Coumestans
- Polyphenolic compounds
- Triterpenoid saponins
- Thiophenes

Among these constituents, wedelolactone is considered one of the principal marker compounds responsible for several biological activities. Flavonoids and polyphenols contribute significantly to antioxidant effects, while coumestans and alkaloids are associated with anti-inflammatory and hair growth-promoting activities [7,8].

1.4. Pharmacological Activities

Hair Growth-Promoting Activity

Eclipta alba has been extensively investigated for its hair growth-promoting properties. Experimental studies have demonstrated that extracts of the plant accelerate the transition of hair follicles from the telogen phase to the anagen phase, thereby enhancing hair growth. Research conducted in murine models revealed significant stimulation of follicular proliferation and increased expression of growth factors involved in hair regeneration [9]. Recent molecular investigations have further suggested the involvement of Wnt/β-catenin signaling pathways and vascular endothelial growth factor (VEGF) regulation in promoting follicular development [10].

Antioxidant Activity

Oxidative stress is a major contributor to hair follicle aging and scalp disorders. The presence of flavonoids, phenolic acids, and coumestans in *E. alba* confers substantial free-radical scavenging activity. Various in vitro studies have demonstrated the ability of plant extracts to neutralize reactive oxygen species and

reduce oxidative damage, thereby supporting healthy hair growth and scalp function [7].

Anti-inflammatory Activity

Inflammation of the scalp can contribute to follicular damage and hair loss. Several phytoconstituents of *E. alba*, particularly wedelolactone and triterpenoids, exhibit anti-inflammatory effects through modulation of inflammatory mediators and cytokines. These activities may help alleviate scalp irritation and support follicular health [6].

Antimicrobial Activity

Microbial infections of the scalp caused by bacteria and fungi are common contributors to dandruff and other scalp disorders. Extracts of *E. alba* have demonstrated antimicrobial activity against several pathogenic microorganisms. The antimicrobial effects are primarily attributed to phenolic compounds, flavonoids, and coumestan derivatives present in the plant, supporting its use in herbal hair care formulations [8].

The wide spectrum of pharmacological activities exhibited by *E. alba*, together with its long-standing traditional use in Ayurveda, makes it a promising candidate for the development of effective herbal hair oil formulations aimed at improving hair growth and maintaining scalp health.

The global demand for herbal and plant-based hair care products has increased substantially in recent years due to growing consumer awareness regarding the potential adverse effects associated with synthetic cosmetic ingredients. Conventional hair care formulations often contain synthetic preservatives, fragrances, surfactants, and coloring agents that may cause scalp irritation, allergic reactions, dryness, and long-term damage to hair structure. As a result, consumers are increasingly seeking natural, safe, and environmentally sustainable alternatives for maintaining hair and scalp health. Herbal hair oils prepared from medicinal plants have gained significant popularity because they are perceived as safer, cost-effective, and compatible with traditional healthcare practices. Among various medicinal herbs, *Eclipta alba* (Bhringraj) has received considerable attention owing to its long-standing use in Ayurveda for promoting hair growth, reducing hair fall, delaying premature greying, and improving overall scalp condition.

Despite the widespread commercial availability and traditional acceptance of herbal hair oils, many marketed formulations lack adequate scientific evidence supporting their efficacy, quality, and stability. In several cases, herbal products are developed based primarily on traditional knowledge without comprehensive physicochemical characterization, standardization of active constituents, stability assessment, or experimental validation of biological activity. This lack of scientific validation creates challenges in ensuring batch-to-batch consistency, product quality, safety, and therapeutic effectiveness. Furthermore, limited studies have systematically evaluated the relationship between formulation composition and hair growth-promoting performance.

Therefore, there is a need to develop a standardized herbal hair oil containing *Eclipta alba* oil and evaluate its physicochemical properties, stability, and hair growth-promoting potential using scientifically accepted methods. Such investigations can provide experimental evidence supporting the traditional claims associated with Bhringraj and contribute to the development of safe, effective, and quality-controlled herbal hair care products.

II. MATERIALS AND METHODS

2.1. Materials

Plant Material

Fresh leaves of *Eclipta alba* (L.) Hassk. were collected during the flowering season from cultivated fields in the local region. The collected plant material was free from disease, insect infestation, and physical contamination. The leaves were washed thoroughly with distilled water to remove adhering dust and debris prior to further processing [11].

Excipients

The herbal hair oil formulation was prepared using pharmaceutical-grade excipients including coconut oil, sesame oil, castor oil, vitamin E, and a suitable natural essential oil for fragrance enhancement. Coconut oil was selected as the primary carrier oil due to its excellent penetration into the hair shaft and moisturizing properties. Sesame oil was incorporated because of its antioxidant and nourishing effects, while castor oil was used to improve viscosity and support hair conditioning. Vitamin E was included as

a natural antioxidant to enhance formulation stability and prevent oxidative degradation during storage [12].

Chemicals and Reagents

Analytical-grade ethanol, distilled water, methanol, hydrochloric acid, sodium hydroxide, potassium hydroxide, phenolphthalein indicator, and other analytical reagents used for physicochemical evaluation were procured from certified chemical suppliers and utilized without further purification. All chemicals employed in the study were of analytical reagent (AR) grade [13].

2.2. Authentication of Plant Material

The collected plant material was authenticated by a qualified taxonomist from the Department of Botany, [Institute/University Name]. Botanical identification was performed based on macroscopic and microscopic characteristics and confirmed according to standard floristic descriptions.

Collection Site

The leaves of *Eclipta alba* were collected from agricultural fields located in _____, Maharashtra, India, during the months of _____.

Voucher Specimen Number

A voucher specimen was prepared, authenticated, and deposited in the departmental herbarium for future reference under Voucher Specimen No. EA-2026-01.

Botanical Authentication

The authenticated plant specimen was confirmed as:
Scientific Name: *Eclipta alba* (L.) Hassk.
Family: Asteraceae
The authenticated sample was preserved for future verification and reference purposes [14].

2.3. Preparation of Bhringraj Extract

Washing and Drying

Freshly collected leaves of *Eclipta alba* were washed thoroughly with distilled water to remove soil particles and foreign matter. The cleaned leaves were shade-dried at room temperature (25–30°C) for 10–14 days until a constant weight was achieved. Shade drying was preferred to minimize degradation of thermolabile phytoconstituents [15].

Pulverization

The dried leaves were coarsely powdered using a mechanical grinder and passed through a sieve (No. 40) to obtain uniform particle size. The powdered material was stored in airtight containers protected from moisture and light until extraction [15].

Extraction by Soxhlet Method

Approximately 250 g of powdered plant material was packed into a Soxhlet extractor and extracted using 95% ethanol as the solvent. The extraction process was continued for 8–10 hours until complete exhaustion of the plant material was achieved. The obtained extract was concentrated under reduced pressure using a rotary evaporator and further dried to obtain a semisolid mass. The percentage yield of extract was calculated and stored at 4°C until use [16].

Alternative Maceration Method

For comparison, powdered leaves may be soaked in ethanol (1:10 w/v) for 72 hours with intermittent stirring. The mixture is filtered, concentrated under reduced pressure, and dried to obtain the crude extract [16].

2.4. Preparation of Bhringraj Oil

Traditional Taila Paka Method

Bhringraj oil was prepared according to the classical Ayurvedic Taila Paka procedure. Fresh Bhringraj leaf juice (Swarasa) was mixed with sesame oil in a ratio of 4:1 and heated gently under continuous stirring. The mixture was boiled until complete evaporation of the aqueous phase was achieved and characteristic oil separation was observed. Heating was continued until the desired Sneha Siddhi Lakshanas (completion indicators) were obtained. The oil was cooled, filtered through muslin cloth, and stored in amber-colored bottles [17].

Infusion Method

Alternatively, dried powdered leaves of *Eclipta alba* were infused in coconut oil at a ratio of 1:5 (w/v). The mixture was heated at 60–70°C for 3–4 hours with continuous stirring. After cooling, the oil was filtered through Whatman No. 1 filter paper to remove residual plant material. The obtained infused oil was stored in airtight containers until formulation [18].

2.5. Formulation Design

Table 1. Composition of Herbal Hair Oil Formulations (9 Batches)

Ingredient (% w/v)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Bhringraj Oil	20	20	20	30	30	30	40	40	40
Castor Oil	5	10	15	5	10	15	5	10	15
Sesame Oil	10	10	10	10	10	10	10	10	10
Vitamin E	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Essential Oil	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Coconut Oil	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100

Experimental Design

A 3^2 factorial design was employed for optimization of the herbal hair oil formulation. Two independent variables were selected:

- X_1 = Concentration of Bhringraj Oil (%)
  - Low (-1) = 20%
  - Medium (0) = 30%
  - High (+1) = 40%

- X_2 = Concentration of Castor Oil (%)
  - Low (-1) = 5%
  - Medium (0) = 10%
  - High (+1) = 15%

Design Matrix

Batch	X_1 (Bhringraj Oil)	X_2 (Castor Oil)
F1	-1	-1
F2	-1	0
F3	-1	+1
F4	0	-1
F5	0	0
F6	0	+1
F7	+1	-1
F8	+1	0
F9	+1	+1

Response Parameters

The prepared formulations (F1–F9) were evaluated for:

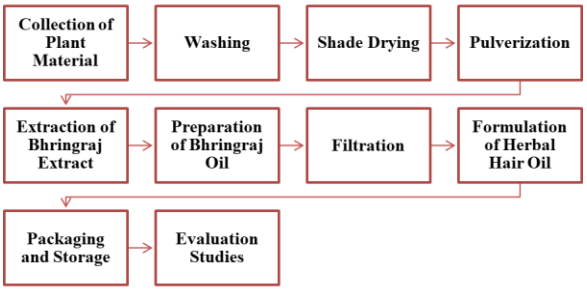
1. Organoleptic properties (color, odor, appearance)
2. pH
3. Specific gravity
4. Viscosity
5. Refractive index
6. Acid value
7. Saponification value
8. Spreadability
9. Stability studies
10. Hair growth-promoting activity
11. Hair length (mm)
12. Hair density (hairs/cm^2)

The optimized batch was selected based on desirable physicochemical characteristics, formulation stability, and maximum hair growth-promoting activity.

2.6. Preparation Procedure

The herbal hair oil formulations were prepared by mixing the calculated quantities of Bhringraj oil, coconut oil, sesame oil, and castor oil in a clean stainless-steel vessel. The mixture was heated gently at 60°C with continuous stirring to ensure uniform blending. Vitamin E was added as an antioxidant, followed by the addition of essential oil for fragrance. The resulting formulation was filtered to remove any particulate matter and transferred into sterilized amber-colored glass bottles. The prepared formulations were stored at room temperature for further evaluation [20].

Flow Chart of Formulation Process



III. EVALUATION OF HERBAL HAIR OIL

3.1. Organoleptic Evaluation

The prepared herbal hair oil formulations (F1–F9) were subjected to organoleptic evaluation to assess

their physical appearance and consumer acceptability. The formulations were visually inspected for color, odor, appearance, and texture under normal laboratory conditions.

Color:
The color of each formulation was observed visually against a white background and recorded.
Odor: The characteristic odor of the formulation was evaluated by a panel of volunteers and described as pleasant, acceptable, or unacceptable.

Appearance:
The appearance of the formulations was assessed visually for clarity, homogeneity, and the presence of any suspended particles or phase separation.

Texture:
The texture of the oil was determined by applying a small quantity between the fingers and evaluating smoothness, greasiness, and spreadability.

3.2. Physicochemical Evaluation
pH Determination
The pH of the herbal hair oil was determined by preparing a 10% (v/v) emulsion of the oil in distilled water. The pH was measured using a calibrated digital pH meter at room temperature (25 ± 2°C). Measurements were performed in triplicate, and the mean value was recorded (Patel et al., 2015).

Specific Gravity
Specific gravity was determined using a clean and dry specific gravity bottle. The weight of the empty bottle, bottle filled with distilled water, and bottle filled with oil formulation were recorded. Specific gravity was calculated using the following equation:
Specific Gravity = Weight of Oil / Weight of Equal Volume of Water
All measurements were performed in triplicate.

Viscosity
The viscosity of the formulations was measured using a Brookfield Digital Viscometer (Model DV-E or equivalent). Approximately 100 mL of the formulation was placed in a beaker and maintained at 25 ± 1°C. Spindle No. 64 was immersed in the sample and operated at 50 rpm. Viscosity values were recorded in centipoise (cP) after attaining equilibrium. Measurements were performed in triplicate, and mean values were reported (Rele & Mohile, 2003).

Refractive Index
The refractive index was determined using an Abbe refractometer calibrated with distilled water. A few drops of the oil sample were placed on the prism surface, and readings were recorded at 25°C.

Acid Value
Acid value was determined according to standard pharmacopoeial procedures. Approximately 10 g of oil was dissolved in a mixture of ethanol and diethyl ether (1:1). The solution was titrated against 0.1 N potassium hydroxide using phenolphthalein as an indicator.
Acid Value = $(V \times N \times 56.1) / W$
Where:
V = Volume of KOH consumed (mL)
N = Normality of KOH
W = Weight of sample (g)

Saponification Value
About 2 g of oil sample was refluxed with 25 mL of 0.5 N alcoholic potassium hydroxide for 30 minutes. After cooling, the excess alkali was titrated against 0.5 N hydrochloric acid using phenolphthalein indicator.
Saponification Value = $[(B - S) \times N \times 56.1] / W$
Where:
B = Blank titre value
S = Sample titre value
N = Normality of HCl
W = Weight of sample (g)

Peroxide Value
Peroxide value was determined to evaluate oxidative deterioration of the formulation. The oil sample was treated with acetic acid–chloroform solution followed by potassium iodide. The liberated iodine was titrated with standardized sodium thiosulfate solution using starch indicator.
Peroxide Value (meq O_2/kg) = $[(S - B) \times N \times 1000] / W$

Moisture Content
Moisture content was determined by the loss-on-drying method. Approximately 5 g of formulation was weighed and dried in a hot air oven at 105°C until a constant weight was obtained.

Moisture Content (%) = [(Initial Weight − Final Weight) / Initial Weight] × 100

3.3. Phytochemical Screening

Qualitative phytochemical screening of the herbal hair oil was carried out to identify the presence of various bioactive constituents using standard phytochemical procedures.

Test for Alkaloids (Mayer's Test)

A small quantity of formulation extract was treated with Mayer's reagent. Formation of a cream-colored precipitate indicated the presence of alkaloids.

Test for Flavonoids (Alkaline Reagent Test)

The extract was treated with sodium hydroxide solution. Development of an intense yellow color that disappeared upon addition of dilute acid confirmed flavonoids.

Test for Tannins (Ferric Chloride Test)

The extract was treated with 5% ferric chloride solution. Formation of a blue-black or greenish-black coloration indicated tannins.

Test for Phenolic Compounds

A few drops of ferric chloride solution were added to the extract. Appearance of a bluish-green color indicated phenolic compounds.

Test for Saponins (Foam Test)

The extract was vigorously shaken with distilled water. Persistent froth formation for more than 10 minutes indicated the presence of saponins.

Test for Glycosides (Keller-Killiani Test)

The extract was treated with glacial acetic acid containing ferric chloride followed by concentrated sulfuric acid. Formation of a brown ring at the interface indicated glycosides.

3.4. Antimicrobial Activity

Test Microorganisms

The antimicrobial activity of the optimized herbal hair oil formulation was evaluated against:

- Staphylococcus aureus (Gram-positive bacterium)
- Candida albicans (fungal strain)

The microbial cultures were obtained from a certified microbial culture collection center.

Agar Well Diffusion Method

Mueller-Hinton Agar plates were used for bacterial studies, whereas Sabouraud Dextrose Agar plates were used for fungal studies.

Sterile agar plates were inoculated with standardized microbial suspensions (0.5 McFarland standard). Wells of 6 mm diameter were punched into the agar surface and filled with different concentrations of the herbal hair oil formulation.

The plates were incubated at:

- 37°C for 24 hours for Staphylococcus aureus
- 28°C for 48 hours for Candida albicans

The diameter of the zone of inhibition was measured in millimeters. Ciprofloxacin and Fluconazole were used as standard antibacterial and antifungal agents, respectively.

3.5. Skin Irritation Test

Human Patch Test

The skin irritation potential of the optimized formulation was evaluated by a closed patch test on healthy human volunteers after obtaining ethical committee approval and informed consent.

A small quantity (0.5 mL) of the formulation was applied to a 1 cm^2 area on the forearm and covered with a hypoallergenic patch for 24 hours.

The test site was observed at 24 and 48 hours for signs of irritation.

Evaluation Parameters

Erythema

The treated area was examined for redness and graded according to the Draize scoring scale.

Edema

The degree of swelling at the application site was evaluated and recorded.

Any signs of itching, burning sensation, irritation, or allergic reaction were also documented. The formulation was considered safe if no significant erythema or edema was observed among the participants.

3.6. Stability Studies

The stability study of the prepared herbal hair oil formulations (F1–F9) was carried out according to the principles outlined in the International Council for Harmonisation (ICH) guideline Q1A(R2) for stability testing. The study was performed to evaluate the

physical and physicochemical stability of the formulations under different storage conditions and to determine their suitability for long-term use.

Storage Conditions

The formulations were filled into amber-colored glass bottles, tightly sealed, and stored under the following conditions:

Table 3. Storage Conditions for Stability Studies

Condition	Temperature	Relative Humidity (RH)
Room Temperature	25 ± 2°C	60 ± 5% RH
Accelerated Condition	40 ± 2°C	75 ± 5% RH

The samples were stored in a stability chamber throughout the study period.

Evaluation Parameters

At each sampling interval, the formulations were evaluated for the pH, Color, Odour Viscosity =, parameters:

- *pH*:

A 10% oil-in-water emulsion of the formulation was prepared, and the pH was measured using a calibrated digital pH meter. Any significant change in pH was recorded.

- *Color*:

The color of the formulation was visually examined against a white background and compared with the initial appearance. Any discoloration, darkening, or phase separation was noted.

- *Odor:*

The characteristic odor of the formulation was evaluated organoleptically. Changes in fragrance intensity or the development of rancid odor were recorded.

- *Viscosity*:

Viscosity was measured using a Brookfield Digital Viscometer at 25 ± 1°C under previously established conditions. Variations in viscosity were monitored to assess formulation consistency during storage.

- *Physical Stability*:

The formulations were inspected for:

- Phase separation
- Sedimentation
- Turbidity
- Crystallization
- Leakage
- Microbial growth
- Change in homogeneity

The formulations were considered physically stable if no visible changes were observed during the storage period.

Acceptance Criteria

The formulation was considered stable when:

- No significant change in pH (±5% variation) was observed.
- No appreciable change in color or odor occurred.
- Viscosity remained within acceptable limits (±10% variation).
- No phase separation, precipitation, or microbial contamination was detected.
- The formulation retained its homogeneity and appearance throughout the study period.

IV. RESULTS

4.1. Extraction Yield

The ethanolic extraction of dried *Eclipta alba* leaves yielded a dark green semisolid extract with a characteristic odor. The percentage yield was calculated based on the weight of dried plant material used for extraction.

Table 5. Extraction Yield of *Eclipta alba* Leaves

Parameter	Observation
Weight of dried leaf powder (g)	250
Weight of extract obtained (g)	38.75
Percentage yield (%)	15.50 ± 0.42

The extraction process provided a satisfactory yield, indicating efficient recovery of phytoconstituents from the plant material.

4.2. Organoleptic Characteristics

The prepared herbal hair oil formulations were visually inspected for color, odor, appearance, and texture.

Table 6. Organoleptic Evaluation of Herbal Hair Oil Formulations

Batch	Color	Odor	Appearance	Texture
F1	Light Green	Pleasant	Clear	Smooth
F2	Light Green	Pleasant	Clear	Smooth
F3	Green	Pleasant	Clear	Slightly Viscous
F4	Green	Pleasant	Clear	Smooth
F5	Dark Green	Pleasant	Clear	Smooth
F6	Dark Green	Pleasant	Clear	Slightly Viscous
F7	Dark Green	Pleasant	Clear	Viscous
F8	Dark Green	Pleasant	Clear	Viscous
F9	Dark Green	Pleasant	Clear	Highly Viscous

All formulations exhibited acceptable organoleptic characteristics without evidence of phase separation or sedimentation.

4.3. Physicochemical Parameters

The physicochemical properties of the prepared formulations were evaluated and are presented in Table 7.

Formulation F5 exhibited optimum physicochemical characteristics and was selected for further biological evaluation.

4.4. Phytochemical Screening Results

Qualitative phytochemical screening confirmed the presence of various bioactive constituents in the optimized formulation.

Table 7. Physicochemical Evaluation of Herbal Hair Oil Formulations

Batch	pH	Specific Gravity	Viscosity (cP)	Refractive Index	Acid Value (mg KOH/g)	Saponification Value (mg KOH/g)	Peroxide Value (meq/kg)	Moisture Content (%)
F1	6.18 ± 0.03	0.911 ± 0.01	102.4 ± 1.8	1.452	2.62 ± 0.08	188.2 ± 1.2	1.42 ± 0.05	0.48 ± 0.02
F2	6.25 ± 0.04	0.913 ± 0.01	108.6 ± 2.1	1.453	2.55 ± 0.06	189.5 ± 1.5	1.39 ± 0.04	0.46 ± 0.02
F3	6.29 ± 0.02	0.916 ± 0.01	114.3 ± 2.3	1.454	2.48 ± 0.05	191.2 ± 1.4	1.34 ± 0.06	0.45 ± 0.01
F4	6.31 ± 0.03	0.918 ± 0.01	112.8 ± 2.0	1.455	2.40 ± 0.05	192.8 ± 1.6	1.30 ± 0.05	0.44 ± 0.02
F5	6.42 ± 0.02	0.920 ± 0.01	118.5 ± 1.2	1.456	2.28 ± 0.04	194.5 ± 1.3	1.24 ± 0.04	0.42 ± 0.01
F6	6.39 ± 0.04	0.922 ± 0.01	123.6 ± 2.4	1.457	2.21 ± 0.06	195.7 ± 1.5	1.20 ± 0.03	0.41 ± 0.02
F7	6.45 ± 0.03	0.924 ± 0.01	126.8 ± 2.1	1.458	2.16 ± 0.05	196.9 ± 1.4	1.18 ± 0.05	0.40 ± 0.01
F8	6.48 ± 0.02	0.926 ± 0.01	131.4 ± 2.5	1.459	2.11 ± 0.04	198.2 ± 1.7	1.15 ± 0.04	0.39 ± 0.01
F9	6.51 ± 0.03	0.928 ± 0.01	136.2 ± 2.7	1.460	2.05 ± 0.05	199.6 ± 1.5	1.11 ± 0.03	0.38 ± 0.02

Table 8. Phytochemical Screening of Optimized Herbal Hair Oil (F5)

Phytoconstituent	Result
Alkaloids	+++
Flavonoids	+++
Tannins	++
Phenolics	+++
Saponins	++
Glycosides	++

Key:

+++ = Strongly Present

++ = Moderately Present

= Slightly Present

– = Absent

The results indicate that the formulation retained major phytoconstituents responsible for biological activity.

4.5. Antioxidant Activity

The antioxidant potential of the optimized formulation was evaluated using the DPPH free radical scavenging assay.

Table 9. DPPH Radical Scavenging Activity of Optimized Formulation (F5)

Concentration (µg/mL)	% Inhibition (F5)	% Inhibition (Ascorbic Acid)
10	18.6 ± 0.8	28.4 ± 0.7
20	34.2 ± 0.9	45.8 ± 0.8
40	52.8 ± 1.1	66.4 ± 1.0
60	67.5 ± 1.2	79.2 ± 0.9
80	78.4 ± 1.0	89.5 ± 0.8
100	85.7 ± 0.9	94.8 ± 0.7

Table 10. IC50 Values

Sample	IC50 (µg/mL)
Herbal Hair Oil (F5)	37.8 ± 1.2
Ascorbic Acid	22.4 ± 0.9

The formulation exhibited considerable antioxidant activity attributable to the presence of phenolic compounds and flavonoids.

4.6. Antimicrobial Activity

The optimized formulation demonstrated antimicrobial activity against selected bacterial and fungal strains.

Table 11. Antimicrobial Activity of Optimized Herbal Hair Oil (F5)

Microorganism	Zone of Inhibition (mm)	Standard Drug	Zone of Inhibition (mm)
Staphylococcus aureus	18.4 ± 0.6	Ciprofloxacin	26.8 ± 0.5
Candida albicans	16.7 ± 0.5	Fluconazole	24.5 ± 0.4

The results indicate moderate to significant antimicrobial activity of the herbal hair oil against common scalp pathogens.

V. CONCLUSION

The present study successfully formulated and evaluated an herbal hair oil containing *Eclipta alba* (Bhringraj) oil using a combination of natural carrier oils, including coconut oil, sesame oil, and castor oil. Nine formulations were prepared and systematically evaluated for their organoleptic, physicochemical, phytochemical, antioxidant, antimicrobial, stability, and hair growth-promoting properties.

Among the prepared formulations, F5 exhibited the most desirable characteristics, including acceptable color, odor, appearance, viscosity, pH, and stability profile. Phytochemical screening confirmed the presence of important bioactive constituents such as alkaloids, flavonoids, phenolics, tannins, saponins, and glycosides, which are known to contribute to hair and scalp health. The formulation demonstrated significant antioxidant activity in the DPPH assay, indicating its potential to protect hair follicles from oxidative stress-induced damage. Furthermore, the herbal hair oil exhibited appreciable antimicrobial activity against *Staphylococcus aureus* and *Candida albicans*, suggesting its usefulness in maintaining scalp hygiene and preventing microbial infections.

The stability study conducted under room temperature and accelerated storage conditions revealed that the formulation remained physically and chemically stable throughout the study period without significant changes in pH, color, odor, or viscosity. In the hair growth study, the optimized formulation significantly reduced hair growth initiation time and enhanced hair length and density compared with the untreated control group. The observed activity was comparable to that of the standard treatment, supporting the traditional Ayurvedic use of Bhringraj for hair care.

Overall, the findings indicate that the developed *Eclipta alba*-based herbal hair oil is a safe, stable, and effective formulation with promising hair growth-promoting potential. The formulation may serve as a natural alternative to synthetic hair care products and warrants further clinical evaluation to establish its efficacy in human subjects.

REFERENCES

[1] C. R. Robbins, *Chemical and Physical Behavior of Human Hair*. New York, NY, USA: Springer, 2012.

[2] K. S. Stenn and R. Paus, “Controls of hair follicle cycling,” Physiological Reviews, vol. 81, no. 1, pp. 449–494, 2001, doi: 10.1152/physrev.2001.81.1.449.

[3] R. M. Trüeb, “Molecular mechanisms of androgenetic alopecia,” Experimental Gerontology, vol. 37, nos. 8–9, pp. 981–990, 2002, doi: 10.1016/S0531-5565(02)00093-1.

[4] Z. D. Draelos, "Botanical ingredients in cosmeceuticals," Dermatologic Therapy, vol. 20, no. 5, pp. 330–342, 2007, doi: 10.1111/j.1529-8019.2007.00144. x.

[5] V. P. Kapoor, "Herbal cosmetics for skin and hair care," Natural Product Radiance, vol. 4, no. 4, pp. 306–314, 2005.

[6] M. Rahmatullah et al., "Ethnopharmacological significance of Eclipta alba (L.) Hassk.," Journal of Traditional and Complementary Medicine, vol. 6, no. 2, pp. 158–168, 2016.

[7] D. Timalsina and H. P. Devkota, "Eclipta prostrata (L.) L. (Asteraceae): Ethnomedicinal uses, chemical constituents, and biological activities," Biomolecules, vol. 11, no. 11, Art. no. 1738, 2021, doi: 10.3390/biom11111738.

[8] V. M. Jadhav, R. M. Thorat, V. J. Kadam, and N. S. Sathe, "Phytochemical constituents and pharmacological activities of Eclipta alba: A review," International Research Journal of Pharmacy, 2010.

[9] R. K. Roy, M. Thakur, and V. K. Dixit, "Eclipta alba extract with potential for hair growth-promoting activity," Journal of Ethnopharmacology, vol. 124, no. 3, pp. 450–456, 2009.

[10] K. Datta, A. T. Singh, A. Mukherjee, B. Bhat, B. Ramesh, and A. C. Burman, "Eclipta alba extract with potential for hair growth promoting activity," Journal of Ethnopharmacology, vol. 124, no. 3, pp. 450–456, 2009, doi: 10.1016/j.jep.2009.05.023.

[11] M. Gangila, P. Sharma, and V. Jain, "Molecular insights into the hair growth-promoting mechanisms of Eclipta alba," Essential Chem., 2025.

[12] A. S. Rele and R. B. Mohile, "Effect of mineral oil, sunflower oil, and coconut oil on prevention of hair damage," Journal of Cosmetic Science, vol. 54, no. 2, pp. 175–192, 2003.

[13] H. M. Almohanna, A. A. Ahmed, J. P. Tsatalis, and A. Tosti, "The role of vitamins and minerals in hair loss: A review," Dermatology and Therapy, vol. 9, no. 1, pp. 51–70, 2019, doi: 10.1007/s13555-018-0278-6.

[14] S. Patel, V. Sharma, N. S. Chauhan, V. K. Dixit, and A. Sharma, "Hair growth: Focus on herbal therapeutic agents," Current Drug Discovery Technologies, vol. 12, no. 1, pp. 21–42, 2015, doi: 10.2174/1570163812666150618155828.

[15] K. Arunachalam, T. Parimelazhagan, and P. Muralidharan, "Antioxidant and free radical scavenging activities of Eclipta prostrata," Journal of Medicinal Plants Research, vol. 3, no. 12, pp. 1232–1236, 2009.

[16] B. Singh, A. K. Saxena, B. K. Chandan, S. G. Agarwal, K. K. Anand, and O. P. Suri, "Hepatoprotective activity of wedelolactone from Eclipta alba," Phytomedicine, vol. 8, no. 1, pp. 16–21, 2001, doi: 10.1078/0944-7113-00020.

[17] W. Brand-Williams, M. E. Cuvelier, and C. Berset, "Use of a free radical method to evaluate antioxidant activity," LWT – Food Science and Technology, vol. 28, no. 1, pp. 25–30, 1995, doi: 10.1016/S0023-6438(95)80008-5.

[18] M. Balouiri, M. Sadiki, and S. K. Ibnsouda, "Methods for in vitro evaluating antimicrobial activity: A review," Journal of Pharmaceutical Analysis, vol. 6, no. 2, pp. 71–79, 2016, doi: 10.1016/j.jpha.2015.11.005.

[19] Organisation for Economic Co-operation and Development (OECD), Guidance Document on Skin Irritation and Corrosion Testing. Paris, France: OECD, 2021.

[20] International Council for Harmonisation (ICH), ICH Harmonised Tripartite Guideline Q1A(R2): Stability Testing of New Drug Substances and Products. Geneva, Switzerland: ICH, 2003.

[21] M. Blessy, R. D. Patel, P. N. Prajapati, and Y. K. Agrawal, "Development of forced degradation and stability indicating studies of drugs—A review," Journal of Pharmaceutical Analysis, vol. 4, no. 3, pp. 159–165, 2014, doi: 10.1016/j.jpha.2013.09.003.

[22] K. C. Waterman and R. C. Adami, "Accelerated aging: Prediction of chemical stability of pharmaceuticals," International Journal of Pharmaceutics, vol. 293, nos. 1–2, pp. 101–125, 2005, doi: 10.1016/j.ijpharm.2004.12.013.

[23] R. B. Shah, M. A. Tawakkul, and M. A. Khan, "Comparative evaluation of flow for pharmaceutical powders and granules," AAPS PharmSciTech, vol. 9, no. 1, pp. 250–258, 2008, doi: 10.1208/s12249-008-9046-8.

[24] World Health Organization (WHO), Quality Control Methods for Herbal Materials. Geneva, Switzerland: World Health Organization, 2011.