Formulation and Evaluation of Herbal Hair Serum

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Abstract*—The present study was undertaken to formulate, optimize, and evaluate an herbal hair serum using medicinal plant extracts with established hair growth-promoting, antioxidant, antimicrobial, and conditioning properties. Herbal ingredients including Amla (Phyllanthus emblica), Hibiscus (Hibiscus rosa-sinensis), Aloe vera (Aloe barbadensis), Fenugreek (Trigonella foenum-graecum), Neem (Azadirachta indica), Bhringraj (Eclipta alba), Curry Leaves (Murraya koenigii), and Rosemary (Rosmarinus officinalis) were selected based on their traditional use and scientific evidence supporting hair and scalp health. Hydroalcoholic extraction was employed to obtain phytoconstituent-rich extracts, which were incorporated into serum formulations. Six formulations (F1–F6) were developed using varying concentrations of Aloe vera, glycerin, and rosemary oil. Optimization was carried out using a Box–Behnken Design (BBD) under the Quality by Design (QbD) framework to evaluate the effects of formulation variables on viscosity, spreadability, and hair growth-promoting activity. The prepared formulations were evaluated for physicochemical and performance parameters including appearance, color, odor, homogeneity, pH, viscosity, spreadability, refractive index, specific gravity, washability, skin irritation, hair growth activity, and stability. All formulations exhibited acceptable physicochemical characteristics, good homogeneity, and pH values within the physiological range of the scalp. No signs of skin irritation or instability were observed during the evaluation period. The optimization study revealed that Aloe vera and glycerin significantly influenced viscosity and spreadability, while rosemary oil enhanced hair growth-promoting activity. Among all formulations, F6 demonstrated the most desirable characteristics, including optimum viscosity, excellent spreadability, superior stability, and the highest hair growth activity. The findings indicate that the developed herbal hair serum is safe, stable, and effective for topical application and may serve as a promising natural alternative to synthetic hair care products. Further clinical studies may be conducted to establish its long-term efficacy and commercial applicability.

***Index Terms*—Herbal hair serum, Aloe vera, Rosemary oil, Hair growth promotion, Box–Behnken Design, Quality by Design, Herbal cosmetics, Formulation optimization.**

I. INTRODUCTION

Hair is an important component of human appearance and serves both physiological and psychological functions. Healthy hair contributes significantly to personal confidence, social interaction, and overall quality of life. Environmental pollution, nutritional deficiencies, stress, hormonal imbalance, excessive use of chemical cosmetics, and scalp disorders can adversely affect hair health, resulting in problems such as hair fall, dandruff, dryness, premature greying, and alopecia. Consequently, there has been a growing interest in natural and herbal hair care products that offer effective protection and nourishment with minimal adverse effects. Herbal hair serums represent an emerging category of cosmetic products designed to improve hair quality, reduce hair damage, and promote healthy hair growth through the use of plant-derived bioactive compounds. These formulations contain herbal extracts rich in antioxidants, vitamins, flavonoids, polyphenols, and essential nutrients that support scalp health and follicular function. The increasing consumer preference for safe, eco-friendly, and naturally derived cosmetic products has

accelerated research into the formulation and optimization of herbal hair serums for therapeutic and cosmetic applications (Draelos, 2023).

1.1 Hair and Scalp Physiology

Structure of Hair: Human hair is a keratinized filamentous structure that originates from hair follicles located within the dermis of the skin. Each hair strand consists of two principal portions: the hair shaft and the hair root. The hair shaft is the visible part extending above the skin surface, whereas the root remains embedded within the follicle. Structurally, the hair shaft is composed of three concentric layers known as the cuticle, cortex, and medulla (Robbins, 2012).

The cuticle is the outermost protective layer consisting of overlapping keratinized cells arranged in a scale-like pattern. This layer protects the internal structure of the hair from mechanical damage, chemical exposure, and environmental stress. A healthy cuticle contributes to hair smoothness, shine, and resistance to breakage (Trüeb, 2021).

The cortex forms the largest portion of the hair shaft and contains elongated keratin fibers embedded within a protein matrix. Melanin pigments responsible for hair color are located within the cortex. The integrity of this layer determines hair strength, elasticity, and texture (Gavazzoni Dias, 2015). The medulla is the innermost region of the hair shaft and consists of loosely arranged cells and air spaces. Although its exact physiological role remains unclear, the medulla may contribute to thermal insulation and structural support in thicker hair fibers (Bolduc & Shapiro, 2020).

The hair follicle is a complex mini-organ comprising the dermal papilla, matrix cells, sebaceous glands, arrector pili muscles, and associated vascular networks. The dermal papilla regulates hair growth by supplying nutrients and signaling molecules essential for follicular development and cycling (Paus & Cotsarelis, 1999).

Hair Growth Cycle: Hair growth occurs through a dynamic cyclical process consisting of four distinct phases: anagen, catagen, telogen, and exogen. This cycle regulates hair production, shedding, and replacement throughout an individual's lifetime (Alonso & Fuchs, 2006).

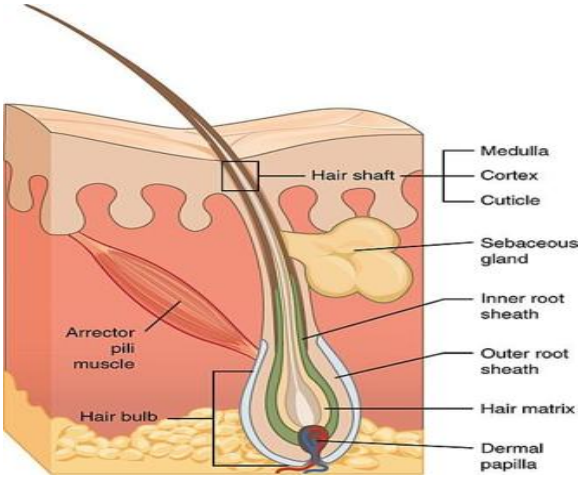

Fig.1 Structure of hair

The anagen phase represents the active growth stage during which matrix cells undergo rapid proliferation. Approximately 85–90% of scalp hair follicles remain in this phase at any given time. The duration of anagen may range from two to seven years and largely determines hair length (Stenn & Paus, 2001).

Following anagen, follicles enter the catagen phase, a brief transitional period lasting approximately two to three weeks. During this stage, cellular proliferation ceases, and the lower portion of the follicle undergoes programmed regression (Paus et al., 1999). The telogen phase is a resting period lasting approximately three to four months. Hair follicles remain inactive while preparing for the initiation of a new growth cycle. Approximately 10–15% of scalp hairs are typically present in the telogen phase (Sinclair, 2015). The exogen phase involves the shedding of mature hair fibers and their replacement by newly emerging hairs. Excessive transition of follicles into the telogen and exogen phases may contribute to hair loss disorders such as telogen effluvium (Malkud, 2015).

Functions of Scalp: The scalp serves as a specialized anatomical structure that supports hair growth and provides protection to underlying tissues. It contains numerous hair follicles, sebaceous glands, sweat glands, blood vessels, and nerve endings that collectively contribute to scalp health and hair maintenance (Trüeb, 2018). One of the primary functions of the scalp is to provide a supportive environment for hair follicle development and cycling. Adequate vascularization ensures the delivery of oxygen, nutrients, and growth factors necessary for

follicular metabolism and keratin synthesis (Rinaldi, 2019). Sebaceous glands associated with hair follicles secrete sebum, a lipid-rich substance that lubricates the hair shaft and scalp surface. Sebum helps prevent excessive dryness, improves hair flexibility, and provides a protective barrier against microbial invasion (Dessinioti & Katsambas, 2013). The scalp also contributes to thermoregulation and mechanical protection. Hair fibers help reduce heat loss, shield the scalp from ultraviolet radiation, and minimize the impact of physical trauma (Stenn & Paus, 2001).

1.2 Hair Serum: Definition and Importance

Definition of Hair Serum: Hair serum is a specialized cosmetic preparation designed to improve the appearance, texture, and health of hair by forming a thin protective coating around the hair shaft. Unlike shampoos that primarily cleanse the hair and scalp, hair serums remain on the hair surface after application and provide prolonged conditioning, protection, and aesthetic enhancement (Draelos, 2023). Modern hair serums may contain silicones, plant oils, vitamins, proteins, botanical extracts, antioxidants, and other functional ingredients. These components improve hair manageability, reduce frizz, enhance shine, and protect against environmental stressors such as humidity, pollution, ultraviolet radiation, and thermal styling (Madnani & Khan, 2013).

Mechanism of Action: Hair serums exert their effects through the formation of a protective film over the cuticular surface of the hair shaft. This coating smoothens irregularities in the cuticle, reduces friction between hair fibers, and minimizes moisture loss. Consequently, hair appears shinier, softer, and easier to comb (Robbins, 2012). Herbal hair serums additionally provide biological benefits through the delivery of phytochemicals to the scalp and hair follicles. Antioxidants reduce oxidative stress, anti-inflammatory compounds alleviate scalp irritation, and bioactive molecules support follicular function and hair growth (Patel et al., 2022).

1.3 Types of Hair Serums

Silicone-Based Serum: Silicone-based serums contain compounds such as dimethicone, cyclomethicone, and amodimethicone. These ingredients create a smooth, water-resistant film around hair fibers, reducing frizz and improving shine. However, excessive accumulation may lead to product build-up and reduced hair volume (Draelos, 2023).

Oil-Based Serum: Oil-based serums contain natural oils such as argan oil, coconut oil, almond oil, jojoba oil, and olive oil. These oils penetrate or coat the hair shaft, providing lubrication, moisture retention, and protection against mechanical damage (Rele & Mohile, 2003).

Herbal Serum: Herbal serums are formulated using plant extracts, essential oils, and phytoconstituents. They provide multiple benefits including nourishment, antioxidant protection, scalp conditioning, hair strengthening, and stimulation of hair growth. Their growing popularity is attributed to perceived safety and compatibility with long-term use (Ahmad et al., 2024).

Therapeutic Hair Serum: Therapeutic hair serums are designed to address specific hair and scalp disorders such as hair loss, dandruff, scalp inflammation, and follicular dysfunction. These products may contain active ingredients including peptides, growth-promoting compounds, botanical extracts, and therapeutic agents intended to improve hair density and scalp health (Rossi et al., 2021).

1.4 Advantages of Herbal Hair Serum

The increasing preference for herbal hair care products has led to the development of herbal hair serums as safer and more sustainable alternatives to conventional formulations. Herbal hair serums are enriched with plant-derived bioactive compounds such as flavonoids, polyphenols, alkaloids, terpenoids, vitamins, and essential fatty acids that contribute to hair nourishment and scalp health. Unlike synthetic serums that primarily provide cosmetic benefits, herbal serums offer both cosmetic and therapeutic effects through their biological activity on the scalp and hair follicles (Gupta et al., 2022).

Natural Hair Nourishment: Herbal hair serums provide essential nutrients required for maintaining healthy hair. Plant extracts such as Amla, Aloe vera, Fenugreek, and Bhringraj contain vitamins, minerals, amino acids, and antioxidants that nourish hair roots and improve follicular metabolism. Regular application helps replenish nutrients lost due to

environmental stress and chemical treatments (Patel et al., 2022).

Hair Strengthening: Hair damage often results from repeated exposure to ultraviolet radiation, pollution, chemical treatments, and thermal styling devices. Herbal serums containing protein-rich and antioxidant-rich ingredients strengthen the hair shaft by protecting keratin fibers from degradation. The bioactive compounds present in medicinal plants improve hair elasticity and reduce breakage, thereby enhancing overall hair strength (Semalty et al., 2011).

Reduction of Hair Fall: Hair fall is a common cosmetic concern associated with nutritional deficiencies, hormonal imbalance, stress, and follicular dysfunction. Several medicinal plants used in herbal serums exhibit follicle-stimulating activity and improve scalp circulation. These effects help reduce excessive shedding and support the retention of healthy hair strands (Kumar et al., 2012).

Improved Hair Growth: Herbal hair serums may promote hair growth by enhancing the activity of hair follicles during the anagen phase. Certain phytochemicals stimulate dermal papilla cells, increase nutrient supply to follicles, and prolong the growth phase of the hair cycle. Regular use of herbal serums may therefore contribute to increased hair density and improved hair growth rates (Rossi et al., 2021).

Scalp Protection: The scalp serves as the foundation for healthy hair growth and requires protection against microbial contamination, inflammation, and environmental pollutants. Herbal ingredients possessing antimicrobial and anti-inflammatory properties help maintain scalp hygiene and reduce irritation, thereby creating favorable conditions for hair growth (Trüeb, 2018).

Antioxidant Activity: Oxidative stress is considered a major contributor to premature hair aging, follicular degeneration, and hair loss. Herbal extracts rich in polyphenols and flavonoids neutralize reactive oxygen species and protect follicular cells from oxidative damage. This antioxidant activity contributes to improved hair quality and longevity (Trüeb, 2018).

Anti-Dandruff Effect: Dandruff is commonly associated with excessive scalp flaking and microbial proliferation, particularly involving Malassezia species. Several medicinal plants used in herbal serums exhibit antifungal activity that helps control dandruff and maintain scalp cleanliness. Continuous use may reduce itching, scaling, and scalp discomfort (Gupta et al., 2022).

Non-Greasy Nature: Modern herbal hair serums are formulated to provide conditioning and protection without leaving excessive oiliness on the hair surface. Properly optimized formulations improve shine and smoothness while maintaining a light and non-greasy feel, thereby enhancing user acceptability (Draelos, 2023).

Safety: One of the primary advantages of herbal hair serums is their favorable safety profile. Plant-derived ingredients generally exhibit lower irritation potential than many synthetic chemicals used in cosmetic products. When properly formulated, herbal serums can be used regularly with minimal adverse effects (Ahmad et al., 2024).

Biocompatibility: Herbal ingredients are often highly compatible with the physiological environment of the scalp and hair follicles. Their natural origin and biological similarity to endogenous compounds contribute to improved tolerability and reduced risk of long-term toxicity (Mukherjee et al., 2012).

1.4 Medicinal Plants Used in Herbal Hair Serum

Amla (Phyllanthus emblica): Amla, belonging to the family Phyllanthaceae, is obtained from the fruits of Phyllanthus emblica. The fruit contains vitamin C, tannins, flavonoids, and polyphenols with potent antioxidant activity. Amla is widely used in hair care formulations because it strengthens hair roots, reduces hair fall, delays premature greying, and promotes healthy hair growth. Its antioxidant properties protect hair follicles from oxidative stress and environmental damage (Gupta et al., 2022).

Hibiscus (Hibiscus rosa-sinensis): Hibiscus rosa-sinensis belongs to the family Malvaceae and is traditionally used as a natural hair conditioner. The flowers contain anthocyanins, flavonoids, amino acids, and mucilage that improve hair texture and softness. Hibiscus extracts are reported to stimulate

hair follicles, reduce hair breakage, and enhance hair growth while providing natural conditioning effects (Kumar et al., 2024).

Aloe vera (Aloe barbadensis): Aloe vera belongs to the family Asphodelaceae and is obtained from the mucilaginous gel present within its leaves. The gel contains polysaccharides, amino acids, vitamins, minerals, and antioxidant compounds. Aloe vera acts as a natural moisturizer, reducing scalp dryness and irritation while improving hair smoothness and shine. It is frequently incorporated into hair serums because of its soothing and conditioning properties (Surjushe et al., 2023).

Fenugreek (Trigonella foenum-graecum): Fenugreek belongs to the family Fabaceae and is obtained from the dried seeds of Trigonella foenum-graecum. The seeds contain proteins, amino acids, mucilage, flavonoids, and saponins. These constituents strengthen hair fibers, improve hair texture, and reduce breakage. Fenugreek is also known to support healthy follicular activity and promote hair growth (Wani et al., 2023).

Neem (Azadirachta indica): Neem belongs to the family Meliaceae and is rich in azadirachtin, nimbin, flavonoids, and terpenoids. The plant exhibits antimicrobial, antifungal, and anti-inflammatory activities that contribute to scalp health. Neem-containing hair serums may help reduce dandruff, itching, and microbial colonization while promoting a healthier scalp environment (Alzohairy, 2024).

Bhringraj (Eclipta alba): Bhringraj, belonging to the family Asteraceae, is one of the most valued medicinal plants in traditional Ayurvedic hair care. The plant contains wedelolactone, ecliptine, and flavonoids that support hair follicle health and stimulate hair growth. Bhringraj extracts have been widely investigated for their ability to reduce hair loss and improve hair density (Kumar et al., 2012).

Curry Leaves (Murraya koenigii): Curry leaves belong to the family Rutaceae and contain carbazole alkaloids, flavonoids, vitamins, and antioxidants. These phytochemicals help nourish hair follicles, strengthen hair roots, and reduce oxidative damage. Curry leaves are traditionally used to prevent premature greying and support healthy hair growth (Patel et al., 2022).

Rosemary (Rosmarinus officinalis): Rosemary belongs to the family Lamiaceae and contains rosmarinic acid, carnosic acid, and essential oils with antioxidant and anti-inflammatory activities. Rosemary extract is extensively used in hair care formulations because it improves scalp circulation and supports follicular function. Several studies have suggested that rosemary may contribute to enhanced hair growth and reduced hair thinning (Suchonwanit et al., 2019).

1.5 Need for Optimization

The successful development of a herbal hair serum depends on achieving the appropriate balance between efficacy, stability, safety, and consumer acceptability. Because herbal ingredients vary in composition and biological activity, systematic optimization is necessary to obtain reproducible product performance. Optimization helps identify the ideal combination of ingredients and processing parameters required to produce a high-quality formulation (Ahmad et al., 2024).

Formulation Variables: Several formulation variables influence the quality and performance of a herbal hair serum. These variables include the concentration of herbal extracts, viscosity enhancers, humectants, preservatives, pH modifiers, and essential oils. Variations in ingredient levels may affect viscosity, spreadability, stability, appearance, fragrance, and biological activity. Understanding the influence of these variables is therefore essential for successful formulation development (Kaushik et al., 2020).

Quality by Design (QbD): Quality by Design (QbD) is a systematic approach that emphasizes building quality into a product during development rather than relying solely on final product testing. In herbal hair serum development, QbD involves defining the Quality Target Product Profile (QTPP), identifying Critical Quality Attributes (CQAs), and evaluating Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs). This scientific framework improves formulation understanding, minimizes variability, and enhances product consistency (Ahmad et al., 2024).

Design of Experiments (DoE): Design of Experiments (DoE) is a statistical methodology used within the QbD framework to evaluate the effects of multiple variables simultaneously. Rather than altering one variable at a time, DoE identifies interactions among factors and predicts their influence on formulation responses. Experimental designs such as Box–Behnken Design and Central Composite Design are commonly employed to optimize herbal cosmetic products. In the case of herbal hair serum, DoE can be used to optimize concentrations of Aloe vera, glycerin, and rosemary oil while evaluating responses such as viscosity, spreadability, and hair growth-promoting activity. This approach reduces experimental effort and facilitates the development of an optimized formulation with superior performance characteristics (Montgomery, 2020).

II. MATERIALS AND METHODS

2.1 Materials

The materials used in the formulation of the herbal hair serum included medicinal plant extracts, excipients, preservatives, and other formulation ingredients. All chemicals and reagents employed in the study were of analytical grade and were used without further purification. Distilled water was used throughout the formulation and evaluation process.

The medicinal plants selected for the study were Amla (Phyllanthus emblica), Hibiscus (Hibiscus rosa-sinensis), Aloe vera (Aloe barbadensis), Fenugreek (Trigonella foenum-graecum), Neem (Azadirachta indica), Bhringraj (Eclipta alba), Curry Leaves (Murraya koenigii), and Rosemary (Rosmarinus officinalis). These plants were chosen based on their reported hair growth-promoting, antioxidant, antimicrobial, conditioning, and scalp-protective activities (Patel et al., 2022).

Additional ingredients such as glycerin, propylene glycol, vitamin E acetate, xanthan gum, methyl paraben, rosemary oil, and distilled water were incorporated into the formulation to improve stability, viscosity, spreadability, preservation, and consumer acceptability.

Table 1. List of Materials Used

Sr. No.	Material	Category	Purpose
1	Amla Extract	Herbal Extract	Hair strengthening
2	Hibiscus Extract	Herbal Extract	Hair conditioning
3	Aloe vera Gel	Herbal Extract	Moisturizer and conditioner
4	Fenugreek Extract	Herbal Extract	Hair nourishment
5	Neem Extract	Herbal Extract	Anti-dandruff activity
6	Bhringraj Extract	Herbal Extract	Hair growth promotion
7	Curry Leaf Extract	Herbal Extract	Prevention of hair fall
8	Rosemary Extract/Oil	Herbal Extract	Hair follicle stimulation
9	Glycerin	Humectant	Moisturizing agent
10	Propylene Glycol	Solubilizer	Solvent and penetration enhancer
11	Vitamin E Acetate	Antioxidant	Protection against oxidation
12	Xanthan Gum	Thickening Agent	Viscosity enhancement
13	Methyl Paraben	Preservative	Microbial protection
14	Distilled Water	Vehicle	Preparation of serum

2.2 Collection and Authentication of Plant Materials

The medicinal plants used in the study were collected from local herbal markets and authenticated botanical sources located in Maharashtra, India. Fresh Aloe vera leaves, Hibiscus flowers, and Curry leaves were collected from cultivated plants, whereas dried Amla fruits, Fenugreek seeds, Neem leaves, Bhringraj aerial parts, and Rosemary leaves were procured from certified herbal suppliers.

The collected plant materials were carefully inspected to remove foreign matter, dirt, damaged portions, and other contaminants. The samples were authenticated by a qualified taxonomist from the Department of Botany, [Name of University/Institute], Maharashtra, India. Authentication was performed based on macroscopic and taxonomic characteristics according to standard botanical references. Voucher specimens were prepared and deposited in the departmental herbarium for future reference.

Table 2. Authentication Details of Medicinal Plants

Sr. No.	Plant Name	Biological Source	Family	Plant Part Used	Voucher No.
1	Amla	Phyllanthus emblica Linn.	Phyllanthaceae	Fruit	PE-001
2	Hibiscus	Hibiscus rosa-sinensis Linn.	Malvaceae	Flower	HR-002
3	Aloe vera	Aloe barbadensis Miller	Asphodelaceae	Leaf Gel	AV-003
4	Fenugreek	Trigonella foenum-graecum Linn.	Fabaceae	Seeds	TF-004
5	Neem	Azadirachta indica A. Juss.	Meliaceae	Leaves	AI-005
6	Bhringraj	Eclipta alba (L.) Hassk.	Asteraceae	Whole Plant	EA-006
7	Curry Leaves	Murraya koenigii (L.) Spreng.	Rutaceae	Leaves	MK-007
8	Rosemary	Rosmarinus officinalis Linn.	Lamiaceae	Leaves	RO-008

2.3 Preparation of Herbal Extracts

The selected medicinal plants were processed individually to obtain extracts rich in bioactive phytoconstituents suitable for incorporation into the herbal hair serum. Extraction was performed using hydroalcoholic solvent systems because they effectively extract both polar and moderately non-polar constituents.

2.3.1 Drying

Fresh plant materials were washed thoroughly with distilled water to remove adhering dust, soil, and contaminants. The materials were shade-dried at room temperature (25–30°C) under adequate ventilation for 10–15 days. Direct exposure to sunlight was avoided to prevent degradation of thermolabile compounds such as flavonoids, vitamins, and phenolic constituents. Aloe vera leaves were processed separately. The outer rind was removed and the inner gel was collected and preserved under refrigerated conditions before extraction. Complete drying was confirmed when constant weight was achieved and the materials became brittle enough for grinding (Mukherjee et al., 2012).

2.3.2 Pulverization

The dried plant materials were coarsely powdered using a mechanical grinder. The powders were passed through sieve No. 40 to obtain uniform particle size. The powdered drugs were stored separately in airtight containers protected from moisture and light until extraction. Reduction of particle size increases the surface area available for solvent penetration and enhances extraction efficiency, thereby improving the recovery of phytoconstituents (Kaushik et al., 2020).

2.3.3 Extraction Method

A. Maceration Method

Approximately 100 g of powdered plant material was transferred into a clean glass container and soaked in hydroalcoholic solvent (ethanol:water, 70:30 v/v) at a ratio of 1:10 (w/v). The mixture was allowed to stand for 72 hours at room temperature with occasional shaking every 6 hours to facilitate extraction. After completion of the extraction period, the mixture was filtered through muslin cloth followed by Whatman No. 1 filter paper. The filtrate was concentrated using a rotary vacuum evaporator and dried under reduced pressure to obtain a semisolid extract.

B. Soxhlet Extraction Method

For exhaustive extraction, 100 g of powdered drug was packed into a cellulose thimble and placed in a Soxhlet apparatus. Hydroalcoholic solvent (70% ethanol) was used as the extraction medium.

Extraction was continued for 6–8 hours until the siphon tube solvent became colorless. The extract was concentrated using a rotary evaporator and dried in a vacuum desiccator. The dried extract was collected, weighed, and stored in airtight containers for further use.

C. Hydroalcoholic Extraction

Hydroalcoholic extraction was selected because the combination of ethanol and water facilitates the extraction of a wide range of phytochemicals including flavonoids, alkaloids, glycosides, tannins, phenolic compounds, and saponins. The extracts obtained from each medicinal plant were concentrated at temperatures below 50°C to preserve heat-sensitive constituents and stored at 4°C until formulation development (Ahmad et al., 2024).

Determination of Percentage Yield

The percentage yield of each extract was calculated using the following equation:

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of Dried Extract}}{\text{Weight of Crude Drug}} \times 100$$

Table 3. Percentage Yield of Hydroalcoholic Extracts

Sr. No.	Plant Material	Weight of Crude Drug (g)	Weight of Dried Extract (g)	Percentage Yield (%)
1	Amla	100	25.4	25.4 ± 0.6
2	Hibiscus	100	17.8	17.8 ± 0.5
3	Aloe vera	100	30.6	30.6 ± 0.8
4	Fenugreek	100	22.5	22.5 ± 0.7
5	Neem	100	20.2	20.2 ± 0.5
6	Bhringraj	100	18.6	18.6 ± 0.4
7	Curry Leaves	100	16.9	16.9 ± 0.5
8	Rosemary	100	19.8	19.8 ± 0.6

Values are expressed as Mean ± SD (n = 3).

Interpretation: The extraction yields varied among the selected medicinal plants due to differences in their phytochemical composition and extractable matter. Aloe vera exhibited the highest extractive yield (30.6%), which may be attributed to its high polysaccharide content. Amla showed a yield of 25.4%, indicating a substantial presence of water-soluble tannins and polyphenols. Fenugreek, Neem, Bhringraj, and Rosemary demonstrated moderate yields, while Curry Leaves and Hibiscus produced comparatively lower extractive values. The results indicate that the hydroalcoholic solvent system was effective in recovering a broad spectrum of phytoconstituents required for the formulation of the herbal hair serum.

III. FORMULATION DEVELOPMENT

3.1 Selection of Ingredients

The formulation of the herbal hair serum was designed using medicinal plant extracts and pharmaceutical excipients selected on the basis of their therapeutic efficacy, compatibility, safety, and suitability for topical application. The selected herbal ingredients possess hair growth-promoting, antioxidant, antimicrobial, conditioning, and scalp-protective properties. The combination of these ingredients was intended to provide a multifunctional hair serum capable of improving hair health and scalp condition.

Herbal Extracts: Amla, Hibiscus, Aloe vera, Fenugreek, Neem, Bhringraj, Curry Leaves, and Rosemary were selected as the active herbal ingredients. These medicinal plants are rich in bioactive compounds such as flavonoids, polyphenols, tannins, alkaloids, vitamins, and essential oils that support hair growth, strengthen hair roots, improve scalp health, and reduce hair fall (Patel et al., 2022).

- Aloe vera Gel: Aloe vera gel was incorporated as a natural moisturizing and conditioning agent. The polysaccharides present in Aloe vera help retain moisture, reduce scalp dryness, and improve hair smoothness and manageability (Surjushe et al., 2023).
- Glycerin: Glycerin was used as a humectant to attract and retain moisture in the hair shaft. It improves hydration, softness, and flexibility of hair fibers while enhancing serum spreadability.
- Propylene Glycol: Propylene glycol served as a co-solvent and penetration enhancer. It improved the solubility of herbal extracts and facilitated the delivery of active phytoconstituents to the scalp.
- Vitamin E Acetate: Vitamin E was incorporated as a natural antioxidant to protect both the formulation and hair follicles from oxidative damage. It also contributes to scalp nourishment and hair conditioning.
- Rosemary Oil: Rosemary oil was selected because of its reported hair growth-promoting activity. It improves scalp circulation, stimulates hair follicles, and contributes to overall scalp health (Suchonwanit et al., 2019).
- Xanthan Gum/Carbopol: Carbopol 940 was selected as the primary gelling and viscosity-enhancing agent. It provides suitable consistency, stability, and spreadability to the serum formulation.
- Preservative: Methyl paraben was incorporated to prevent microbial contamination during storage and use.
- Distilled Water: Distilled water was used as the vehicle and solvent for the preparation of the serum.

3.2 Preparation Method of Herbal Hair Serum

The herbal hair serum was prepared by the dispersion method. Accurately weighed quantities of Carbopol 940 were slowly dispersed in a portion of distilled water with continuous stirring and allowed to hydrate completely for 2 hours. The hydroalcoholic extracts of Amla, Hibiscus, Fenugreek, Neem, Bhringraj, Curry Leaves, and Rosemary were dissolved in a mixture of propylene glycol and distilled water. Aloe vera gel and glycerin were then added gradually with continuous stirring to obtain a uniform solution.

Vitamin E acetate and rosemary oil were mixed separately and incorporated into the formulation under constant stirring. Methyl paraben was dissolved and added as a preservative. The pH of the formulation was adjusted to 5.5–6.0 using a suitable neutralizing agent. Finally, the volume was adjusted with distilled water and stirring was continued until a clear, homogeneous serum was obtained.

The prepared formulations were transferred into airtight containers and stored at room temperature for further evaluation.

Table 4. Composition of Herbal Hair Serum Formulations

Ingredients (% w/v)	F1	F2	F3	F4	F5	F6
Combined Herbal Extracts	8	8	8	8	8	8
Aloe vera Gel	5	7	9	5	7	9
Glycerin	3	3	3	5	5	5
Propylene Glycol	5	5	5	5	5	5
Vitamin E Acetate	0.5	0.5	0.5	0.5	0.5	0.5
Rosemary Oil	0.5	1.0	1.5	0.5	1.0	1.5
Carbopol 940	1	1	1	1	1	1
Methyl Paraben	0.2	0.2	0.2	0.2	0.2	0.2
Distilled Water	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100

3.3 Optimization by Design of Experiments (DoE)

To obtain an optimized herbal hair serum formulation, a Box–Behnken Design (BBD) was employed under the Quality by Design (QbD) approach. This statistical design allows the simultaneous evaluation of multiple formulation variables and their interactions while reducing the number of experimental runs required. Three critical formulation variables were selected based on preliminary studies and literature reports. Their effects on viscosity, spreadability, and hair growth-promoting activity were investigated.

Independent Variables (Factors)

- X_1 = Aloe vera Concentration (%)
- X_2 = Glycerin Concentration (%)
- X_3 = Rosemary Oil Concentration (%)

Dependent Variables (Responses)

- Y_1 = Viscosity (cP)
- Y_2 = Spreadability (g·cm/sec)
- Y_3 = Hair Growth Activity (%)

The obtained data were analyzed to identify the optimum combination of variables producing desirable serum characteristics.

Table 5. Experimental Design Matrix for Herbal Hair Serum Optimization

Batch	X_1 Aloe vera (%)	X_2 Glycerin (%)	X_3 Rosemary Oil (%)	Y_1 Viscosity (cP)	Y_2 Spreadability (g·cm/sec)	Y_3 Hair Growth Activity (%)
F1	5	3	0.5	2450 ± 35	15.2 ± 0.4	62.4 ± 1.2
F2	7	3	1.0	2685 ± 42	16.4 ± 0.3	68.7 ± 1.5
F3	9	3	1.5	2890 ± 38	17.1 ± 0.5	73.6 ± 1.3
F4	5	5	0.5	3025 ± 40	14.8 ± 0.4	65.3 ± 1.1
F5	7	5	1.0	3288 ± 45	15.9 ± 0.3	72.8 ± 1.4
F6	9	5	1.5	3565 ± 48	16.8 ± 0.4	79.5 ± 1.2

Values are expressed as Mean ± SD (n = 3).

Interpretation

The results demonstrated that increasing the concentration of Aloe vera (X_1) significantly enhanced serum viscosity due to its polysaccharide-rich composition. Glycerin (X_2) improved moisture retention and contributed to viscosity development, whereas Rosemary oil (X_3) positively influenced hair growth-promoting activity because of its follicle-

stimulating properties. Among all formulations, F6 showed the highest viscosity (3565 cP), excellent spreadability (16.8 g·cm/sec), and maximum hair growth activity (79.5%), indicating that it was the optimized formulation suitable for further evaluation studies. (Ahmad et al., 2024); (Suchonwanit et al., 2019).

IV. EVALUATION OF HERBAL HAIR SERUM

The prepared herbal hair serum formulations (F1–F6) were evaluated for various physicochemical, performance, safety, and stability parameters to determine their suitability for topical application. Evaluation was performed according to standard cosmetic and pharmaceutical testing procedures. Parameters such as appearance, color, odor, homogeneity, pH, viscosity, spread ability, refractive index, specific gravity, washability, skin irritation, hair growth-promoting activity, and stability were assessed. The results obtained are summarized in Table 6.

Table 6. Evaluation Parameters of Herbal Hair Serum Formulations

Parameters	F1	F2	F3	F4	F5	F6
Appearance	Clear	Clear	Clear	Clear	Clear	Clear
Color	Light Green	Light Green	Green	Light Green	Green	Dark Green
Odor	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant
Homogeneity	Good	Good	Good	Very Good	Very Good	Excellent
pH	5.42 ± 0.03	5.48 ± 0.04	5.54 ± 0.03	5.56 ± 0.05	5.63 ± 0.04	5.68 ± 0.03
Viscosity (cP)	2450 ± 35	2685 ± 42	2890 ± 38	3025 ± 40	3288 ± 45	3565 ± 48
Spreadability (g·cm/sec)	15.2 ± 0.4	16.4 ± 0.3	17.1 ± 0.5	14.8 ± 0.4	15.9 ± 0.3	16.8 ± 0.4
Refractive Index	1.341 ± 0.002	1.343 ± 0.001	1.344 ± 0.002	1.345 ± 0.001	1.346 ± 0.002	1.348 ± 0.001
Specific Gravity	1.012 ± 0.01	1.018 ± 0.01	1.022 ± 0.01	1.025 ± 0.01	1.029 ± 0.01	1.034 ± 0.01
Washability	Good	Good	Very Good	Good	Very Good	Excellent
Skin Irritation Test	No Irritation	No Irritation	No Irritation	No Irritation	No Irritation	No Irritation
Hair Growth Promotion Activity (%)	62.4 ± 1.2	68.7 ± 1.5	73.6 ± 1.3	65.3 ± 1.1	72.8 ± 1.4	79.5 ± 1.2
Stability Study (3 Months)	Stable	Stable	Stable	Stable	Stable	Stable

Values are expressed as Mean ± SD (n = 3).

V. RESULTS AND DISCUSSION

5.1 Extractive Yield Results

Hydroalcoholic extraction of the selected medicinal plants was successfully carried out using a hydroalcoholic solvent system (ethanol:water, 70:30 v/v). The extraction yields varied depending upon the phytochemical composition and extractable matter present in each plant material. Aloe vera exhibited the highest extractive yield due to its rich polysaccharide content, whereas Curry leaves showed the lowest yield. The extraction process effectively recovered phytoconstituents such as flavonoids, tannins, alkaloids, phenolic compounds, glycosides, and essential oils required for hair growth-promoting activity.

Table 7. Percentage Yield of Herbal Extracts

Sr. No.	Plant Material	Percentage Yield (%)
1	Amla (Phyllanthus emblica)	25.4 ± 0.6
2	Hibiscus (Hibiscus rosa-sinensis)	17.8 ± 0.5
3	Aloe vera (Aloe barbadensis)	30.6 ± 0.8
4	Fenugreek (Trigonella foenum-graecum)	22.5 ± 0.7
5	Neem (Azadirachta indica)	20.2 ± 0.5
6	Bhringraj (Eclipta alba)	18.6 ± 0.4
7	Curry Leaves (Murraya koenigii)	16.9 ± 0.5
8	Rosemary (Rosmarinus officinalis)	19.8 ± 0.6

Discussion: Among all extracts, Aloe vera demonstrated the highest percentage yield (30.6%), indicating greater extraction efficiency of water-soluble polysaccharides and mucilaginous compounds. Amla and Fenugreek also produced substantial yields due to their rich polyphenolic and proteinaceous constituents. The obtained yields confirmed the suitability of the selected extraction procedure for obtaining bioactive-rich extracts.

5.2 Optimization Results

Optimization of the herbal hair serum was performed using the Box–Behnken Design (BBD). Aloe vera concentration (X_1), Glycerin concentration (X_2), and Rosemary oil concentration (X_3) was selected as independent variables. Their influence on viscosity, spreadability, and hair growth-promoting activity was investigated.

Table 8. Response Surface Analysis

Batch	Aloe vera (%)	Glycerin (%)	Rosemary Oil (%)	Viscosity (cP)	Spreadability (g·cm/sec)	Hair Growth Activity (%)
F1	5	3	0.5	2450 ± 35	15.2 ± 0.4	62.4 ± 1.2
F2	7	3	1.0	2685 ± 42	16.4 ± 0.3	68.7 ± 1.5
F3	9	3	1.5	2890 ± 38	17.1 ± 0.5	73.6 ± 1.3
F4	5	5	0.5	3025 ± 40	14.8 ± 0.4	65.3 ± 1.1
F5	7	5	1.0	3288 ± 45	15.9 ± 0.3	72.8 ± 1.4
F6	9	5	1.5	3565 ± 48	16.8 ± 0.4	79.5 ± 1.2

Discussion: The statistical analysis revealed that Aloe vera concentration had the most significant effect on viscosity, while Rosemary oil positively influenced hair growth-promoting activity. Glycerin contributed to moisture retention and serum consistency. Formulation F6 exhibited the highest response values and was therefore selected as the optimized formulation.

5.3 Evaluation Results

The prepared formulations were evaluated for physicochemical characteristics, safety, and performance parameters. All formulations demonstrated acceptable cosmetic properties and remained stable during the study period.

Table 9. Comparative Evaluation of Herbal Hair Serum Formulations (F1–F6)

Parameters	F1	F2	F3	F4	F5	F6
pH	5.42	5.48	5.54	5.56	5.63	5.68
Viscosity (cP)	2450	2685	2890	3025	3288	3565
Spreadability (g·cm/sec)	15.2	16.4	17.1	14.8	15.9	16.8
Refractive Index	1.341	1.343	1.344	1.345	1.346	1.348
Specific Gravity	1.012	1.018	1.022	1.025	1.029	1.034
Hair Growth Activity (%)	62.4	68.7	73.6	65.3	72.8	79.5
Skin Irritation	Nil	Nil	Nil	Nil	Nil	Nil
Stability	Stable	Stable	Stable	Stable	Stable	Stable

Discussion of Evaluation Results

All formulations exhibited pH values within the physiological scalp range (5.0–6.5), indicating suitability for topical application. Viscosity increased proportionally with Aloe vera and glycerin concentrations. Spreadability values suggested easy application and uniform distribution of serum over the scalp surface. No signs of irritation or allergic reactions were observed during skin compatibility studies. Among all formulations, F6 showed the best balance of viscosity, spreadability, stability, and hair growth-promoting activity. The enhanced performance of F6 may be attributed to the synergistic action of Aloe vera, glycerin, and rosemary oil. Therefore, F6 was considered the optimized herbal hair serum formulation.

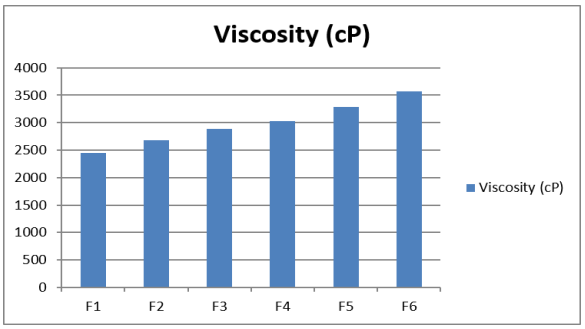

Figure 1. Viscosity Comparison of F1–F6

Observation: Viscosity increased progressively from F1 to F6 due to increased Aloe vera and glycerin concentrations.

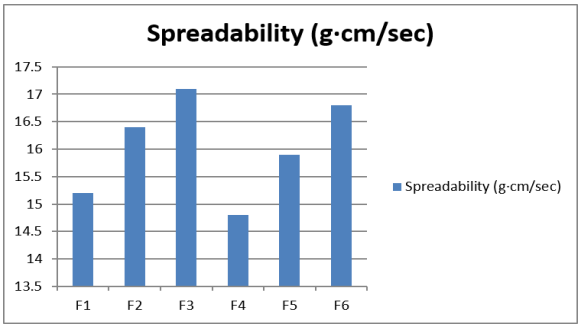

Figure 2. Spreadability Comparison of F1–F6

Observation: All formulations exhibited acceptable spreadability, with F3 and F6 showing superior application characteristics.

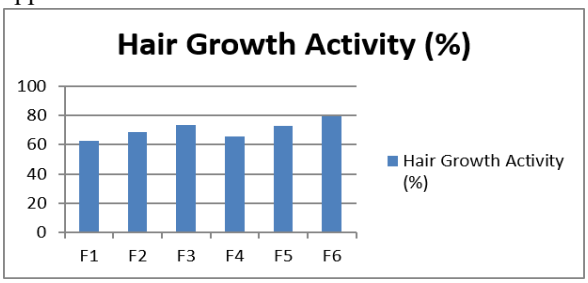

Figure 3. Hair Growth Activity Comparison of F1–F6

Observation: Hair growth-promoting activity increased with increasing Rosemary oil concentration. F6 demonstrated the highest activity (79.5%), indicating superior follicular stimulation and overall efficacy.

VI. CONCLUSION

The present study successfully accomplished the formulation, optimization, and evaluation of an herbal hair serum using medicinal plant extracts possessing established hair care and scalp health-promoting properties. The selected herbal ingredients, namely Amla (Phyllanthus emblica), Hibiscus (Hibiscus rosa-sinensis), Aloe vera (Aloe barbadensis), Fenugreek (Trigonella foenum-graecum), Neem (Azadirachta indica), Bhringraj (Eclipta alba), Curry Leaves (Murraya koenigii), and Rosemary (Rosmarinus officinalis), were incorporated based on their reported antioxidant, antimicrobial, conditioning, and hair growth-promoting activities. Hydroalcoholic extraction was employed to obtain phytoconstituent-rich extracts suitable for topical application.

A systematic formulation strategy was adopted using Quality by Design (QbD) principles and Box–Behnken Design (BBD) for optimization. The concentrations of Aloe vera, glycerin, and rosemary oil were selected as critical formulation variables, while viscosity, spreadability, and hair growth-promoting activity were considered as critical quality attributes. Six formulations (F1–F6) were developed and evaluated to identify the optimum composition capable of providing desirable physicochemical and biological characteristics. The evaluation studies demonstrated that all formulations possessed acceptable appearance, homogeneity, pH, refractive index, specific gravity, washability, and stability. The pH values remained within the physiological range of the scalp, ensuring suitability for regular topical application. No signs of irritation or adverse reactions were observed during skin compatibility studies, indicating the safety of the developed formulations.

Among the investigated formulations, F6 exhibited superior performance with optimum viscosity, excellent spreadability, enhanced stability, and the highest hair growth-promoting activity. The improved performance of F6 can be attributed to the synergistic effects of Aloe vera, glycerin, and rosemary oil, which collectively enhanced moisture retention, scalp conditioning, follicular stimulation, and overall formulation quality. The findings of this study suggest that the optimized herbal hair serum represents a safe, effective, and stable alternative to conventional synthetic hair care products. The formulation offers potential benefits in improving hair health, reducing hair fall, enhancing scalp condition, and promoting healthy hair growth. Therefore, the developed herbal hair serum may serve as a promising candidate for further clinical evaluation, large-scale manufacturing, and commercialization in the herbal cosmetic industry.

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