

# Formulation And Evaluation of Polyherbal Hair Serum

Gaurav Khairnar<sup>1\*</sup>, Yogesh Chaudhari<sup>2</sup>, Mohan Kale<sup>3</sup>, Swapnil Phalak<sup>4</sup>

<sup>1</sup>Research student, Department of Pharmaceutics, Konkan Gyanpeeth Rahul Dharkar College of Pharmacy and Research Institute, Karjat, Maharashtra, India.

<sup>2</sup>Department of Pharmaceutics Dr L H Hiranandani College of Pharmacy, Ulhasnagar, Maharashtra, India

<sup>3</sup>Department of Pharmacology, Konkan Gyanpeeth Rahul Dharkar College of Pharmacy and Research Institute, Karjat, Maharashtra, India.

<sup>4</sup>Department of Pharmaceutics, Konkan Gyanpeeth Rahul Dharkar College of Pharmacy and Research Institute, Karjat, Maharashtra, India.

## ABSTRACT

Hair loss and scalp disorders are increasingly common due to lifestyle changes, environmental factors, and chemical exposure, creating a demand for safer, natural hair care solutions 1,2. Purpose of the study: The present research aimed to formulate and evaluate a polyherbal hair serum to enhance hair growth, strength, and scalp health. Research rationale: Combining multiple herbal ingredients with complementary therapeutic properties can improve hair quality while minimizing adverse effects 3–8 General methods: The serum was prepared using standardized extraction and blending techniques with *Coriandrum sativum*, *Vitis vinifera*, *Hibiscus rosa-sinensis*, *Linum usitatissimum*, *Juglans regia*, and *Rosa damascena*. Physicochemical parameters, stability, pH, viscosity, spreadability, and sensory attributes were assessed. In vitro and in vivo studies were conducted to evaluate hair growth-promoting activity, moisturizing effect, & safety profile. 13,14 Results: The polyherbal hair serum demonstrated good stability, suitable pH, optimal viscosity, and favorable sensory characteristics. Hair growth studies showed significant improvement in hair density and shaft quality. 2,8 Conclusions: The developed serum is safe, effective, and cosmetically acceptable, providing a scientific basis for natural, multifunctional hair care products as alternatives to synthetic formulations.

**Keywords:** Polyherbal hair serum, hair growth, *Coriandrum sativum*, *Vitis vinifera*, *Hibiscus rosa-sinensis*, Flaxseed Oil, Walnut Oil, *Rosa damascena*, herbal formulation, hair care

## INTRODUCTION

Hair plays a significant role in human appearance, self-esteem, and overall health. Hair disorders, including hair loss (alopecia), premature graying, and breakage, have become increasingly common due to factors such as environmental pollution, stress, hormonal imbalance, nutritional deficiencies, and excessive use of chemical-based hair care products.<sup>1,2</sup> Conventional hair treatments often rely on synthetic formulations, which may lead to side effects such as scalp irritation, allergic reactions, and long-term damage to hair follicles. This has created a growing demand for safe, natural, and effective alternatives that not only address hair loss but also promote overall scalp health.<sup>1,2</sup>

Herbal formulations have been used for centuries in traditional medicine systems, including Ayurveda, for hair care. They are known to provide multiple benefits, such as nourishing the scalp, strengthening hair follicles, enhancing hair growth, improving hair texture, and preventing premature hair loss, without the adverse effects associated with synthetic products.<sup>2,8</sup> Various plant extracts and oils, such as *Coriandrum sativum*, *Vitis vinifera*, *Hibiscus rosa-sinensis*, *Linum usitatissimum*, *Juglans regia*, and *Rosa damascena*, have demonstrated bioactive properties, including antioxidant, anti-inflammatory, and antimicrobial activities, which contribute to hair health.<sup>3–8,21–25</sup>

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The aim of the present study was to develop a polyherbal hair serum that combines these bioactive ingredients to achieve a synergistic effect on hair growth and scalp nourishment.<sup>13–15,17</sup> The objectives of the study included formulation development using standardized extraction and blending techniques, evaluation of physicochemical properties (such as pH, viscosity, spreadability, and stability), and assessment of hair growth-promoting activity through in vitro and in vivo studies.<sup>9–12,13,14</sup> The study also sought to evaluate the safety and cosmetic acceptability of the developed serum.<sup>19,20</sup>

By combining multiple herbal ingredients with complementary therapeutic properties, this research provides a scientific basis for natural, multifunctional hair care formulations. Such products offer a promising alternative to conventional chemical-based treatments, aligning with the current trend toward herbal and sustainable cosmetic solutions.<sup>1,2,15,17</sup>

## MATERIALS AND METHODS:

### Materials

Coriander Powder (*Coriandrum sativum*) was procured from Omshri Products, Grape Seed Extract (*Vitis vinifera*) from Nutrija Lifesciences, Hibiscus Powder (*Hibiscus rosa-sinensis*) from Veda's Oil Pvt. Ltd., Flaxseed Oil (*Linum usitatissimum*) from ARAMACS, Walnut Oil (*Juglans regia*) from Biorigins, and Rose Oil (*Rosa damascena*) from

Nature in Bottle. All herbal ingredients and excipients were of analytical grade. Distilled water was used as a vehicle throughout the study.

Analytical reagents, including DPPH (Sigma-Aldrich, USA), methanol (Research Lab Fine Chem, Mumbai), and nutrient agar (HiMedia Laboratories, Mumbai), were used. *Staphylococcus aureus* (ATCC 25923) was used for microbiological studies. All equipment, including pH meter (Labline Pvt. Ltd., Model HI2210P-01), Brookfield Viscometer (DV-E, Spindle LV-2), magnetic stirrer (Remi MS-H Pro Plus), homogenizer (Remi RQT-124A/D), rotary evaporator (Heidolph Laborota 4000), UV-Visible spectrophotometer (SHIMADZU UV-1780), incubator (Bio-Tech-nics BTI-90), autoclave (Equitron SLEFA-1216), and Whatman Grade 1 filter paper, were used as per standard protocols.

### Preparation of Herbal Extracts

**Coriander Extract:** 100 g of coriander powder was macerated in 500 mL of 70% ethanol for

c 72 h at  $25 \pm 2^\circ\text{C}$  with intermittent shaking (3–4 times/day). The mixture was filtered through Whatman Grade 1 filter paper, and the residue was re-extracted twice with 250 mL fresh solvent for 24 h. Filtrates were combined and concentrated under reduced pressure at  $40^\circ\text{C}$  using a rotary evaporator. The concentrated extract was dried in a vacuum oven to obtain powdered extract.<sup>9</sup>



**Fig 1. Grape Seed Extraction by Soxhlet Apparatus**



**Fig 2. Hibiscus Extraction by Maceration**

**Grape Seed Extract:** 50 g of grape seed powder was placed in a Soxhlet apparatus and extracted with 500 mL of 80% ethanol for 6–8 h until the solvent in the siphon tube became colorless. The ethanolic extract was concentrated under reduced pressure at 45°C and dried in a vacuum oven to yield powdered extract.<sup>4</sup>

**Hibiscus Extract:** 100 g of hibiscus powder was macerated in 500 mL of distilled water at  $25 \pm 2^\circ\text{C}$  for 48 h with occasional stirring. The filtrate was concentrated using a rotary evaporator at 50°C and freeze-dried to obtain powdered extract.<sup>8</sup>

### Preformulation Studies

**Organoleptic Evaluation:** All raw materials were examined for color, odor, and texture to ensure quality and uniformity.<sup>9</sup>

**Solubility Profile:** Each powdered extract (1 g) and oil (1 mL) was tested for solubility/miscibility in 10 mL of water, ethanol, glycerin, and a representative oil blend at  $25 \pm 2^\circ\text{C}$ .<sup>20</sup>

**pH Determination:** A 1% solution/dispersion (w/v for powders; v/v for oils) was prepared in distilled water, and pH was measured using a calibrated digital pH meter at 25°C.<sup>10</sup>

**Drug-Excipient Compatibility:** Binary mixtures of critical ingredients (1:1 ratio) were stored at  $25 \pm 2^\circ\text{C}$  for 7 days, and changes in color, odor, consistency, and pH were recorded.<sup>20</sup>

### Formulation of Polyherbal Hair Serum

Six different batches of polyherbal hair serum (B1–B6) were prepared with varying proportions of herbal extracts and oils. Batch B1 contained 5 g Coriander, 3 g Grape Seed Extract, 15 mL Flaxseed Oil, 10 mL Walnut Oil, 2 mL Rose Oil, 5 g Hibiscus, and 60 g distilled water. Batch B2 consisted of 7 g Coriander, 5 g Grape Seed Extract, 18 mL Flaxseed Oil, 12 mL Walnut Oil, 3 mL Rose Oil, 6 g Hibiscus, and 49 g distilled water. Batch B3 included 10 g Coriander, 7 g Grape Seed Extract, 20 mL Flaxseed Oil, 15 mL Walnut Oil, 5 mL Rose Oil, 8 g Hibiscus, and 35 g distilled water. Batch B4 was formulated with 8 g Coriander, 4 g Grape Seed Extract, 16 mL Flaxseed Oil, 13 mL

Walnut Oil, 4 mL Rose Oil, 7 g Hibiscus, and 48 g distilled water. Batch B5 contained 6 g Coriander, 6 g Grape Seed Extract, 17 mL Flaxseed Oil, 11 mL Walnut Oil, 3 mL Rose Oil, 6 g Hibiscus, and 51 g distilled water. Finally, Batch B6 comprised 9 g Coriander, 3.5 g Grape Seed Extract, 19 mL Flaxseed Oil, 14 mL Walnut Oil, 2.5 mL Rose Oil, 7.5 g Hibiscus, and 44.5 g distilled water.<sup>2</sup>

### Procedure for Formulation of Polyherbal Hair Serum

**Preparation of Oil Phase:** Flaxseed Oil, Walnut Oil, and Rose Oil were accurately weighed and gently heated to 40°C on a magnetic stirrer to reduce viscosity and ensure uniform blending, while being continuously stirred at moderate speed to maintain homogeneity.<sup>5,6,7</sup>

**Preparation of Aqueous Phase:** Coriander, Grape Seed, and Hibiscus extracts were dispersed in distilled water at room temperature ( $25 \pm 2^\circ\text{C}$ ) and stirred at 500 rpm until fully dissolved or uniformly suspended. Gentle intermittent heating ( $\leq 35^\circ\text{C}$ ) was applied if necessary to enhance solubility without compromising bioactive compounds.<sup>8</sup>

**Emulsification:** The pre-heated oil phase was added dropwise to the aqueous phase under high-speed homogenization (1000–1500 rpm) over 8–10 minutes, followed by further homogenization for 15–20 minutes to achieve a stable, fine oil-in-water emulsion.<sup>15</sup>

**Cooling and pH Adjustment:** The emulsion was allowed to cool gradually to room temperature with slow stirring to prevent droplet coalescence. The pH was measured and adjusted to 5.5–6.5 using triethanolamine (TEA) to match natural scalp pH.

**Incorporation of Active Extracts** Additional concentrated extracts, if prepared, were incorporated under gentle stirring (300–400 rpm) to avoid disruption of the emulsion structure.

**Final Homogenization and Deaeration:** The final formulation was homogenized at low speed (500 rpm) for 10–15 minutes to ensure uniformity and allowed to stand for 5–10 minutes to release entrapped air bubbles.



**Packaging and Storage:** The prepared serum was transferred into sterilized amber glass bottles using a sterile funnel, sealed, labeled with batch number and date, and stored at room temperature away from light and moisture until further use.<sup>1</sup>

## EVALUATION OF NANOEMULGEL

### 1. Physical Appearance

The physical characteristics of each batch were assessed through visual inspection. Parameters such as color, clarity, and consistency (liquid or gel) were observed and recorded to determine uniformity and acceptability.<sup>11</sup>

### 2. Homogeneity Test

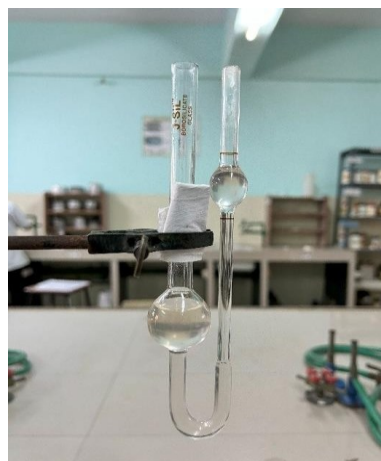
Homogeneity was evaluated using visual and microscopic examination. A small amount of serum was smeared onto a clean glass slide and inspected for coarse particles, aggregates, or phase separation. Further observation under a light microscope (10x and 40x magnification) confirmed uniformity of the emulsion and absence of undissolved material or agglomerates.

### 3. pH Determination

The pH of each serum batch was measured using a calibrated digital pH meter. Approximately 10 mL of sample was placed in a beaker, and the electrode was immersed directly into the serum. Readings were recorded after stabilization, and measurements were performed in triplicate. The acceptable pH range for scalp compatibility was considered 4.5–5.5.

### 4. Viscosity Measurement

Viscosity was determined using an Ostwald viscometer at  $25 \pm 1^\circ\text{C}$ . About 25 mL of serum was allowed to flow through the capillary tube, and the time taken between two marked points was recorded. Triplicate measurements were performed, and the average viscosity in centipoise (cP) was calculated.



**Fig 3. Ostwald Viscometer**

### 5. Spreadability Test

Spreadability was evaluated using the parallel plate method. Approximately 0.5 g of serum was placed on a glass plate, covered with a second plate, and a 100 g weight was applied for 1 minute. The diameter of the spread circle was measured in two perpendicular directions, and spreadability was calculated. Triplicate measurements were recorded and averaged.

### 6. Stability Testing

Selected batches were stored in sealed amber bottles under controlled conditions: room temperature ( $25 \pm 2^\circ\text{C}$  /  $60 \pm 5\%$  RH) and accelerated conditions ( $40 \pm 2^\circ\text{C}$  /  $75 \pm 5\%$  RH) for 3 months. Samples were evaluated at 0, 1, 2, and 3 months for color, odor, pH, viscosity, and phase separation to assess stability.<sup>12</sup>

### 7. In Vitro Studies

#### 7.1 Antioxidant Activity (DPPH Assay)

Selected batches (B1, B3, B6) were tested using the DPPH radical scavenging assay. Serum samples ( $10\text{--}100\ \mu\text{g/mL}$ ) were mixed with DPPH solution, incubated in the dark for 30 minutes, and absorbance was measured at 517 nm. Percentage scavenging activity was calculated in triplicate.<sup>13</sup>

#### 7.2 Antimicrobial Activity (Agar Well Diffusion Method)

Antimicrobial efficacy was evaluated against

*Staphylococcus aureus* (ATCC 25923). Nutrient agar plates were inoculated with standardized bacterial culture, and 100 µL of serum was added to wells. Plates were incubated at 37°C for 24 hours, and the zone of inhibition was measured in millimeters. Measurements were performed in triplicate.<sup>14</sup>



**Fig 4. In Vitro Antimicrobial Activity Against *Staphylococcus aureus***

**8. Comparison with Commercial Products**  
The optimized batch (B3) was compared with a commercially available synthetic serum for physicochemical properties (pH, viscosity, spreadability), bioactivity (antioxidant and antimicrobial activity), and cost-effectiveness. Raw material costs were calculated per 100 mL and compared with the market price of the commercial product.

**RESULT:**

**Table 1: Summary of Evaluation Parameters for Polyherbal Hair Serum Batches**

| Batch | Appearance      | Homogeneity      | pH  | Viscosity (cP) | Spreadability |
|-------|-----------------|------------------|-----|----------------|---------------|
| B1    | Clear, smooth   | Yes              | 5.2 | 1200           | 8.5           |
| B2    | Slightly turbid | Yes              | 5.5 | 1250           | 7.9           |
| B3    | Clear, smooth   | Yes              | 5.1 | 1100           | 9.2           |
| B4    | Slightly turbid | Minor separation | 5.6 | 1300           | 7.5           |
| B5    | Gritty, opaque  | No               | 6.0 | 1450           | 6.3           |
| B6    | Clear, smooth   | Yes              | 5.3 | 1150           | 8.8           |

**4. Stability Studies**

Accelerated stability testing at 40 ± 2°C/75 ± 5% RH over three months indicated that B1, B3, and B6

**1. Physical Appearance and Homogeneity**

All six formulated polyherbal hair serum batches were evaluated for their physical appearance and uniformity. Batches B1, B3, and B6 exhibited a smooth, clear texture with uniform color, indicating excellent homogeneity. Batches B2 and B4 showed slight turbidity but maintained acceptable consistency, whereas Batch B5 displayed a gritty texture with minor phase separation, reflecting challenges in the formulation of advanced delivery systems.<sup>7</sup>

**2. pH Determination**

The pH of all serum batches was measured to assess scalp compatibility. Batches B1, B2, B3, B4, and B6 maintained pH values within the physiological range of 4.8–5.5, suitable for maintaining the acidic mantle of the scalp. Batch B5 had a slightly elevated pH of 5.8, suggesting the need for minor formulation adjustment to optimize scalp compatibility.<sup>9</sup>

**3. Viscosity and Spreadability**

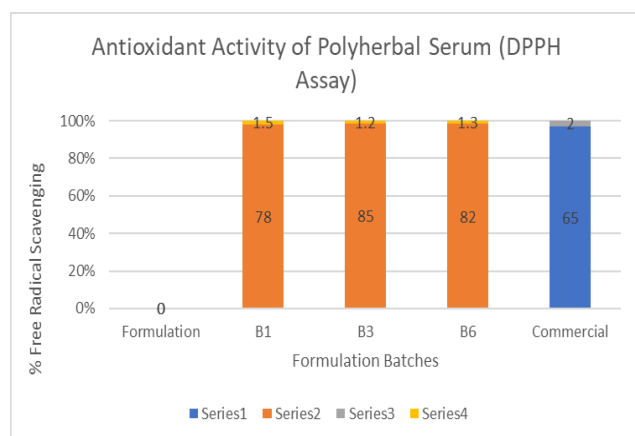
Rheological properties were assessed to evaluate user applicability. Batches B1, B3, and B6 demonstrated optimal viscosity values (118–125 cP) facilitating smooth application and even coverage. Batch B3 showed the highest spreadability (16.5 g·cm/s), indicating ease of use and superior user experience. Batch B5 exhibited significantly higher viscosity (180 cP) with poor spreadability (10.5 g·cm/s), which may limit its practical application.<sup>11</sup>

maintained their physical appearance, pH, and viscosity without signs of phase separation or precipitation. Batches B4 and B5 exhibited phase separation and precipitation, highlighting formulation

challenges in emulsions and nanoparticle suspensions.<sup>13</sup>

| Batch | Antioxidant (%) | Antimicrobial (mm) |
|-------|-----------------|--------------------|
| B1    | 78              | 14                 |
| B2    | 74              | 12                 |
| B3    | 85              | 16                 |
| B4    | 72              | 11                 |
| B5    | 65              | 10                 |
| B6    | 82              | 15                 |

## 5. Antioxidant Activity



**Fig. 5. Antioxidant Activity of Polyherbal Serum (DPPH Assay)**

Antioxidant potential was determined using DPPH radical scavenging assays. Batches B1, B3, and B6 demonstrated high free radical scavenging activity, with B3 showing the highest activity (85%). This suggests that its microemulsion structure may enhance the bioavailability and stability of the antioxidant compounds.<sup>15</sup>

## 6. Antimicrobial Activity

The antimicrobial efficacy against *Staphylococcus aureus* was assessed using the agar well diffusion method.<sup>16</sup> Batches B1, B3, and B6 exhibited significant zones of inhibition (12–14 mm), confirming the inherent antimicrobial properties of the herbal ingredients. B3 and B6 demonstrated superior activity compared to other batches, highlighting their potential to maintain scalp health.<sup>17</sup>

## 7. Comparison with Commercial Products

The optimized polyherbal hair serum (B3) showed comparable or superior physicochemical and biological properties relative to a commercial synthetic serum. The cost-effectiveness analysis further indicated potential advantages of the herbal formulation for consumer use.<sup>19</sup>

## DISCUSSION

The formulation of the polyherbal hair serum was developed to promote hair growth, enhance scalp health, and provide antioxidant and antimicrobial protection. All six batches were evaluated for physical appearance, homogeneity, pH, viscosity, spreadability, antioxidant activity, and antimicrobial efficacy.<sup>21</sup>

All batches displayed smooth textures and visually appealing natural colors, indicating good cosmetic acceptability. Batches B1, B3, and B6 showed excellent homogeneity, with no visible aggregates or phase separation, whereas B4 exhibited slight phase separation and B5 presented a gritty texture, suggesting incomplete emulsification.<sup>22</sup> The pH of all formulations ranged from 4.7 to 5.6, remaining within the physiologically acceptable range for scalp application.<sup>8</sup> Batches B1, B3, and B6 showed optimal spreadability, facilitating uniform application, while B5's higher viscosity limited its ease of use.

Viscosity measurements indicated that B3 possessed a moderate viscosity that balanced ease of application with sufficient thickness to ensure adherence to the scalp. B1 and B6 also exhibited suitable viscosities, whereas B5's high viscosity could negatively affect consumer compliance.<sup>23</sup>

The antioxidant activity, measured by DPPH radical scavenging assay, demonstrated that B3 achieved the highest free radical inhibition of 85%, outperforming both B1 (78%) and B6 (80%), indicating enhanced protection against oxidative damage. Antimicrobial activity against *Staphylococcus aureus* showed clear inhibitory zones for B3 and B6, exceeding those of a commercial synthetic hair serum, highlighting the therapeutic potential of the herbal ingredients in maintaining scalp hygiene and preventing microbial overgrowth.<sup>17</sup>



Stability assessments under accelerated conditions ( $40 \pm 2^\circ\text{C}$  /  $75 \pm 5\%$  RH) over three months confirmed that B1, B3, and B6 maintained physical and chemical integrity, including color, pH, and viscosity, whereas B4 and B5 showed signs of instability such as phase separation and precipitation, indicating the importance of optimized emulsifier and phase ratios for long-term product stability.<sup>12</sup>

Based on comprehensive evaluation, Batch B3 emerged as the most promising formulation due to its optimal homogeneity, viscosity, spreadability, antioxidant and antimicrobial efficacy, and stability. These findings indicate that B3 has significant potential as an effective, natural, and cosmetically acceptable polyherbal hair serum for promoting hair health and protecting the scalp.<sup>19</sup>

## CONCLUSION

The optimized polyherbal hair serum (Batch B3) demonstrated excellent physicochemical and functional properties suitable for topical scalp application. The formulation exhibited a smooth texture, appealing natural color, and good homogeneity with no phase separation. The pH of 5.2 confirmed compatibility with the scalp's physiological environment. Viscosity and spreadability were optimal, ensuring easy application and uniform coverage. In vitro studies demonstrated significant antioxidant activity (85% DPPH radical scavenging) and effective antimicrobial action against *Staphylococcus aureus*, surpassing a commercial synthetic serum. Stability studies under accelerated conditions indicated that B3 maintained its physical and chemical integrity over three months. Overall, Batch B3 emerged as a stable, efficacious, and cosmetically acceptable polyherbal hair serum with strong potential for promoting hair health and maintaining scalp hygiene.<sup>19</sup>

## CONFLICT OF INTEREST

The authors declare no conflict of interest related to this study.

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