

## FORMULATION AND EVALUATION OF HERBAL HAIR GROWTH SERUM CONTAINING ROSEMARY EXTRACT

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### ABSTRACT

Hair loss and thinning are widespread cosmetic and clinical concerns affecting individuals across all demographics, commonly associated with androgenetic alopecia, oxidative follicular damage, and DHT-mediated follicle miniaturisation. Herbal formulations are attracting considerable scientific attention as safer, patient-acceptable alternatives to synthetic pharmacological agents. The present study describes the development and physicochemical characterisation of a polyherbal hair growth serum incorporating ethanolic rosemary (*Rosmarinus officinalis L.*) extract as the primary bioactive constituent, augmented with aqueous fenugreek (*Trigonella foenum-graecum L.*) seed extract, aloe vera gel, glycerine, vitamin E, and parabens as preservatives. Rosemary and fenugreek extracts were individually prepared by cold maceration and evaluated for their synergistic potential in addressing androgenic alopecia through 5-alpha reductase

inhibition, antioxidant protection, keratin precursor supply, and follicular vasodilation. The serum was formulated at a fixed 50 mL batch and subjected to comprehensive physicochemical evaluation encompassing appearance, pH, viscosity, spreadability, and homogeneity. All parameters met the predefined acceptance criteria: the formulation presented as a clear, slightly hazy pale amber liquid with a characteristic herbal aroma; pH of 5.09 (range 5.0–6.5); viscosity of 2198 cP (target 1500–2500 cP); uniform spreadability, and satisfactory homogeneity without phase separation or visible particulates. These findings

substantiate the feasibility of the rosemary–fenugreek polyherbal serum as a stable, scalp-compatible cosmeceutical candidate for the management of hair loss and scalp health promotion.

**KEYWORDS:** *Rosmarinus officinalis*, herbal hair serum, androgenetic alopecia, 5-alpha reductase, *Trigonella foenum-graecum*, physicochemical evaluation.

## INTRODUCTION

Hair constitutes one of the most visible and psychosocially significant structural features of the human body, profoundly influencing an individual's self-perception, social interactions, and quality of life. Biologically, hair growth is governed by a cyclically regulated process encompassing three sequential phases: the anagen (active proliferative) phase, which principally determines fibre length; the catagen (transitional) phase, characterised by controlled follicular regression; and the telogen (quiescent resting) phase, which precedes shedding and renewal.<sup>[1]</sup> Perturbation of this cycle by genetic predisposition, androgenic hormonal imbalances, nutritional insufficiency, chronic psychosocial stress, or environmental pollutant exposure frequently manifests as progressive alopecia—the clinically significant reduction of hair density.<sup>[2]</sup>

Among the various aetiological mechanisms underlying non-scarring alopecia, the enzymatic conversion of testosterone to the potent androgen dihydrotestosterone (DHT) by 5-alpha reductase type II holds particular significance. DHT binds with high affinity to androgen receptors on dermal papilla cells, culminating in progressive miniaturisation of susceptible follicles and the eventual cessation of productive hair growth.<sup>[3]</sup> While pharmacological agents such as minoxidil (a vasodilatory potassium channel opener) and finasteride (a 5-alpha reductase inhibitor) constitute established first-line therapies, their clinical utility is tempered by documented adverse effects including scalp irritation, contact dermatitis, and systemic hormonal disruption, respectively.<sup>[4]</sup>

The escalating consumer preference for plant-derived cosmeceuticals has provided renewed impetus for the scientific evaluation of medicinal botanicals in the context of hair care. Herbal preparations are generally perceived as physiologically compatible and well-tolerated, exhibiting a multitarget mechanistic profile that may confer advantages over single-molecule pharmacological interventions.<sup>[5]</sup> Rosemary (*Rosmarinus officinalis* L., family Lamiaceae), an aromatic perennial shrub indigenous to the Mediterranean basin, has an established

ethnopharmacological tradition in hair and scalp care. Its bioactive constituents—primarily the phenolic diterpenes carnosic acid and carnosol, the hydroxycinnamic acid ester rosmarinic acid, the pentacyclic triterpenoid ursolic acid, and a volatile essential oil dominated by 1,8-cineole, camphor, and alpha-pinene—collectively exhibit antioxidant, anti-inflammatory, antimicrobial, and vasodilatory activities that mechanistically support follicular regeneration.<sup>[6]</sup>

Complementing rosemary, fenugreek (*Trigonella foenum-graecum* L., family Fabaceae) seeds represent a foundational ingredient in Ayurvedic, Unani, and North African traditional hair care. The seed's rich matrix of steroidal saponins (diosgenin), the alkaloid trigonelline, flavonoids, sulfur-containing proteins, lecithin, and galactomannan polysaccharides collectively provides anti-androgenic activity, structural keratin precursors, and surface-conditioning properties.<sup>[7]</sup> A serum vehicle was selected for this formulation owing to its capacity to deliver concentrated active constituents into the peri-follicular environment without the occlusive residue associated with conventional oil-based preparations.

The present investigation was therefore undertaken to formulate and physicochemically evaluate a polyherbal hair growth serum incorporating optimised rosemary and fenugreek extracts, with the aim of developing a stable, scalp-compatible, and efficacious alternative cosmeceutical for the management of hair loss.

## MATERIALS AND METHODS

### Collection and Authentication of Plant Material

#### Rosemary Leaves (*Rosmarinus officinalis* L.)

Commercially dried rosemary leaves were procured from NeutraVedshown in Figure.1 (certified 100% Natural, Product of India). Macroscopic authentication was performed on the basis of characteristic morphological features: linear, needle-like, greyish-green dried leaves bearing a pronounced aromatic fragrance, in accordance with the pharmacopoeial monograph description of *Rosmarinus officinalis*. The identity was further substantiated by cross-referencing standard botanical reference texts.



**Fig. 1: Rosemary Leaves.**

### **Fenugreek Seeds (*Trigonella foenum-graecum* L.)**

Fenugreek seeds shown in Figure.2 were obtained from a household supply, consistent with traditional Ayurvedic practice. Authentication was based on macroscopic characteristics: yellowish-brown, rhomboidal seeds exhibiting a mildly bitter odour and flavour, consistent with pharmacopoeial specifications for *Trigonella foenum-graecum*. Prior to processing, seeds were manually cleaned to remove foreign matter, shrivelled seeds, and surface contaminants.



**Fig.2 Fenugreek Seeds.**

### **Phytochemistry and Mechanistic Rationale for Hair Growth Promotion**

*Rosmarinus officinalis* L. — The hair growth-promoting activity of rosemary is underpinned by multiple convergent molecular mechanisms. Rosmarinic acid and carnosic acid competitively inhibit 5-alpha reductase type II, thereby attenuating DHT-mediated follicular miniaturisation; ursolic acid promotes IGF-1 secretion by dermal papilla cells, prolonging the anagen phase; essential oil monoterpenes induce scalp microvascular vasodilation; and the polyphenolic antioxidant complex scavenges reactive oxygen species (ROS), protecting follicular stem cells from oxidative insult.<sup>[8]</sup> A landmark randomised controlled trial demonstrated clinical equivalence between topical rosemary oil and 2% minoxidil for hair density restoration in androgenetic alopecia at six months, with a significantly lower incidence of scalp irritation in the rosemary cohort.<sup>[9]</sup>

*Trigonella foenum-graecum* L. — Diosgenin, the principal steroidal saponin of fenugreek, independently inhibits 5- $\alpha$  reductase, producing anti-androgenic effects complementary to those of rosemary. Trigonelline and nicotinic acid promote scalp perfusion and cellular energy metabolism, while the seed's cysteine- and methionine-enriched protein fraction directly furnishes the sulphur-containing amino acid precursors requisite for keratin polypeptide biosynthesis. Galactomannan polysaccharide imparts substantivity and surface-conditioning effects to the hair shaft, reducing surface friction and enhancing fibre manageability.<sup>[10]</sup> Clinical investigations have reported significant improvements in hair volume, shaft diameter, and reductions in hair-fall scores following supplementation with standardised fenugreek seed extract.<sup>[11]</sup>

### **Drying, Comminution, and Storage of Plant Material**

**Rosemary Leaves:** Freshly procured rosemary leaves were washed under running water to remove surface impurities, then shade-dried on clean trays at 25–30°C with adequate ventilation for 10–14 days. Shade drying was specifically preferred over oven dehydration to prevent thermal degradation of heat-labile polyphenols and volatile monoterpene constituents. Upon attainment of a residual moisture content below 10%, the dried material was mechanically ground and sieved through a 40-mesh screen to obtain a homogeneous coarse powder, which was stored in sealed amber glass containers under cool, dry, and light-protected conditions until extraction.

**Fenugreek Seeds:** Cleaned fenugreek seeds were oven-dried at 40°C for 24 hours in a hot-air circulation oven to reduce residual moisture. The dried seeds were subsequently ground to a coarse powder, sieved through a 40-mesh screen, and stored in appropriately labelled, hermetically sealed glass containers under cool, dry, and light-protected conditions until use.

### **Extraction of Plant Material by Cold Maceration**

**Extraction of Rosemary Leaves:** Cold maceration employing 70% ethanol (v/v) as the extraction solvent was selected to preserve the integrity of rosmarinic acid, carnosic acid, and carnosol, which are susceptible to thermally induced degradation at the elevated temperatures required by Soxhlet or reflux-based methodologies.<sup>[12]</sup> Accurately weighed rosemary powder (10 g) was transferred to a 500 mL glass-stoppered volumetric flask, to which 100 mL of 70% ethanol was added (drug-to-solvent ratio 1:10 w/v). The sealed flask was maintained at ambient temperature (25  $\pm$  2°C) for 72 hours, with manual agitation for 5 minutes at 8-hourly intervals to maximise drug-solvent contact efficiency. The macerate was subsequently filtered

through double-layered muslin cloth followed by Whatman No. 1 filter paper under gravity to yield a clear filtrate. Residual solvent was removed by evaporation on a water bath maintained at 45–50°C, affording a dark greenish-brown semi-solid extract (Figure.3) with a characteristic polyphenolic herbal-resinous aroma.<sup>[13]</sup>



**Fig. 3: Rosemary Extract.**

**Extraction of Fenugreek Seeds:** Fenugreek seed powder (5 g) was accurately weighed into a 100 mL glass-stoppered conical flask and extracted with 50 mL of purified water (IP standard), maintaining a 1:10 drug-to-solvent ratio (w/v). The flask was sealed and held at ambient temperature for 72 hours with intermittent manual agitation every 6–8 hours. Filtration was performed sequentially through double-layered muslin cloth and Whatman No. 1 filter paper. The pale yellowish-brown filtrate was concentrated on a water bath at 50–55°C (temperatures maintained below 60°C to prevent saponin degradation and protein denaturation), yielding a thick, viscous paste-like extract (Figure.4) with a mildly bitter characteristic odour.



**Fig. 4: Fenugreek Extract.**

### **Formulation of the Polyherbal Hair Growth Serum**

The polyherbal serum was prepared as a single optimised batch of 50 mL. The qualitative and

quantitative composition is presented in Table 1.

**Table 1: Formulation composition of the herbal hair growth serum (50 mL batch).**

S.No.	Ingredient	Quantity (per 50 mL)
1	Rosemary extract (maceration, 70% ethanol)	2.5 g
2	Fenugreek seed extract (maceration, purified water)	1.5 g
3	Aloe vera gel	5.0 mL
4	Glycerine	2.5 mL
5	Vitamin E	0.5 mL
6	Methyl paraben (Methyl hydroxybenzoate)	0.1 g
7	Propyl paraben (Propyl hydroxybenzoate)	0.05 g
8	Purified water	q.s. to 50 mL

### Preparation Procedure

**Step 1 – Preservative phase:** Methyl paraben (0.1 g) and propyl paraben (0.05 g) were dissolved in a minimal volume of purified water pre-heated to 70–80°C under continuous stirring until complete dissolution was achieved, and the solution was allowed to cool to 40°C.

**Step 2 – Fenugreek–glycerine phase:** Fenugreek seed extract (1.5 g) was uniformly dispersed in approximately 15 mL purified water at room temperature, followed by incorporation of glycerine (2.5 mL) under gentle stirring.

**Step 3 – Rosemary–vitamin E phase:** Rosemary extract (2.5 g) was dissolved in 5–8 mL of purified water warmed to 40–45°C; vitamin E (0.5 mL) was subsequently introduced with continuous mechanical stirring until homogeneous dispersion was achieved.

**Step 4 – Phase combination:** The cooled preservative solution was incorporated into the fenugreek–glycerine mixture under continuous stirring, followed by gradual addition of the rosemary–vitamin E mixture.

**Step 5 – Aloe vera incorporation:** Aloe vera gel (5.0 mL) was gently folded into the combined mixture with controlled stirring to preserve gel integrity and minimise foam generation.

**Step 6 – Volume adjustment and pH correction:** The final volume was made up to 50 mL with purified water and stirred for 10 minutes. The pH was measured and adjusted, where necessary, to 5.5–6.5 using dilute citric acid or dilute sodium hydroxide solution.

**Step 7 – Filling and packaging:** The completed serum was aseptically filled into pre-sterilised 50 mL amber glass dropper bottles, hermetically sealed, labelled, and stored at room temperature pending evaluation.

### Physicochemical Evaluation of the Herbal Hair Growth Serum

**Appearance:** Organoleptic characteristics including colour, clarity, odour, texture, and consistency were assessed by direct visual and olfactory inspection under standardised laboratory lighting.

**pH determination:** The pH of the freshly prepared serum was determined by immersing the calibrated electrode of a digital pH meter directly into the formulation sample and recording the stable equilibrium reading. The target physiological compatibility range was defined as pH 5.0–6.5, commensurate with the native acidic pH of the scalp.<sup>[14]</sup>

**Spreadability:** An excess quantity of the serum was placed between two parallel glass Petri dishes. A standard weight was applied to the upper plate for 5 minutes to allow equilibration, after which the extent and uniformity of serum spreading were assessed.<sup>[15]</sup>

**Viscosity:** The apparent viscosity of the serum was measured using a Brookfield Rotary Viscometer at 30 rpm. Readings were recorded in centipoise (cP) and reported against the target acceptance range of 1500–2500 cP.

**Homogeneity:** A thin film of the serum was applied to a clean dry glass slide and examined under a cover glass for uniformity, the presence of undispersed particles, aggregates, or signs of phase separation.<sup>[16]</sup>

### RESULTS AND DISCUSSION

The polyherbal hair growth serum was successfully formulated and all physicochemical parameters were rigorously evaluated. The results are compiled in Table 2.

**Table 2: Physicochemical evaluation results of the herbal hair growth serum.**

Parameter	Method	Result	Acceptance Criteria	Status
Appearance	Visual inspection	Clear, slightly hazy, pale amber; pleasant herbal aroma	Uniform; no visible aggregates	Pass
pH (25°C)	Digital pH meter	5.09	5.0–6.5	Pass
Viscosity (cP)	Brookfield RV, 30 rpm	2198 cP	1500–2500 cP	Pass
Spreadability	Parallel plate method	Easily spreadable; uniform distribution	>10 g·cm/s	Pass
Homogeneity	Visual inspection	Homogeneous; no particles or phase separation	Homogeneous; no aggregates	Pass

Visual inspection confirmed that the formulation presented as a clear liquid with a slight haze attributable to the colloidal dispersal of high-molecular-weight polyphenolic extractives. The pale amber colouration and pleasant herbal-resinous aroma were consistent with the characteristic sensory profile of rosemary polyphenols and fenugreek saponins. The absence of visible particulates and phase separation upon microscopic examination confirmed satisfactory homogeneity.

The pH value of 5.09 (Figure.5) falls within the defined physiologically compatible range of 5.0–6.5, closely approximating the natural scalp surface pH of approximately 4.5–5.5. Maintenance of this slightly acidic environment is essential for preserving the structural integrity of the hair cuticle, supporting the normal cutaneous microbiome, and minimising the risk of scalp irritation. Formulations with pH values deviating significantly from this range risk disrupting cuticular intercellular lipid organisation, predisposing the follicular epithelium to microorganism colonisation and inflammatory insult.<sup>[17]</sup>

The measured viscosity of 2198 cP (Figure.6) resided comfortably within the predefined target range of 1500–2500 cP. This rheological profile confers a balance between satisfactory ease of application during dispensing and adequate retention time on the scalp surface to facilitate percutaneous penetration of the bioactive constituents into the peri-follicular dermis. Serums of insufficient viscosity may drain rapidly from the application site, whereas overly viscous preparations may prove difficult to spread uniformly and may generate undesirable cosmetic residue.



**Fig.5 Determination of pH.**



**Fig.6 Determination of Viscosity.**

The formulation demonstrated excellent spreadability, distributing uniformly over the applied surface area without cohesive aggregation, indicating compatible interfacial rheological behaviour between the aqueous serum base and the scalp stratum corneum. The homogeneity

assessment confirmed the absence of phase separation or discrete particulate matter, evidencing effective miscibility between the hydrophilic extract phases, glycerine humectant, vitamin E antioxidant, aloe vera gel matrix, and the aqueous continuous phase.

Collectively, the physicochemical evaluation data support the proposition that the rosemary–fenugreek polyherbal serum constitutes a stable, aesthetically acceptable, and biologically compatible platform for topical hair growth promotion. The synergistic bioactive complementarity between the two herbal extracts—rosemary's 5-alpha reductase inhibitory, antioxidant, and vasodilatory activities combined with fenugreek's anti-androgenic, keratin precursor supply, and hair-conditioning properties—substantiates the rationale for their co-formulation in a serum vehicle intended for the management of androgenetic alopecia and oxidative stress-related hair damage.

## CONCLUSION

The present investigation successfully accomplished the formulation and comprehensive physicochemical characterisation of a polyherbal hair growth serum incorporating rosemary (*Rosmarinus officinalis* L.) extract as the principal bioactive constituent, in combination with fenugreek (*Trigonella foenum-graecum* L.) seed extract, aloe vera gel, glycerine, vitamin E, and paraben preservatives. All physicochemical parameters—appearance, pH (5.09), viscosity (2198 cP), spreadability, and homogeneity—satisfactorily conformed to the respective acceptance criteria, establishing the formulation's physicochemical stability and scalp physiological compatibility.

The scientific rationale for the polyherbal combination is reinforced by the mechanistic complementarity of the constituent bioactives: rosemary extract provides 5-alpha reductase type II inhibition, antioxidant protection of follicular stem cells, and scalp microvascular enhancement, while fenugreek seed extract supplies androgenic counteraction via diosgenin, sulphur-amino acid building blocks for keratin synthesis, and galactomannan-mediated fibre conditioning. Aloe vera contributes additional scalp hydration and anti-inflammatory benefit, and glycerine with vitamin E provide humectancy and antioxidant stabilisation of the formulation matrix, respectively.

The results of this study validate the scientific merit of rosemary and fenugreek as effective, cost-efficient, and well-tolerated natural alternatives for cosmeceutical hair care. The herbal hair growth serum demonstrates a favourable and promising profile warranting further

investigation through in vivo hair growth assays, clinical efficacy trials in subjects with androgenetic alopecia, stability studies, and skin sensitisation assessments, with a view to future development as a commercially viable therapeutic cosmeceutical product.

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