

Formulation and *In Vitro* Evaluation of a Polyherbal Hair Tonic Containing *Clitoria ternatea*, *Mimusops elengi*, and *Oroxylum indicum* Extracts for the Management of Hair Fall

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ABSTRACT

Hair fall is one of the most prevalent cosmetic and dermatological concerns affecting individuals of various age groups worldwide. Conventional treatments such as minoxidil and finasteride are often associated with adverse effects, limited patient compliance, and high treatment costs. Consequently, there has been increasing interest in herbal formulations as safer alternatives for promoting hair growth and reducing hair fall. The present study aimed to formulate and evaluate a polyherbal hair tonic containing extracts of *Clitoria ternatea*, *Mimusops elengi*, and *Oroxylum indicum*. The selected plants are rich in flavonoids, phenolic compounds, tannins, and antioxidants that may contribute to scalp nourishment and hair follicle protection. Hydroalcoholic extracts of the plant materials were prepared and incorporated into a hair tonic formulation. The prepared formulations were evaluated for physicochemical properties including appearance, pH, viscosity, specific gravity, spreadability, and stability. In vitro studies including antioxidant activity, protein protection assay, and antimicrobial activity against scalp-associated microorganisms were performed. The optimized formulation exhibited acceptable physicochemical characteristics, good stability, significant antioxidant potential, and effective antimicrobial activity. The findings suggested that the developed polyherbal hair tonic may serve as a promising natural formulation for hair fall management and scalp health maintenance. Further investigations involving preclinical and clinical studies are warranted to establish its therapeutic efficacy and safety.

Keywords: Polyherbal Hair Tonic; *Clitoria ternatea*; *Mimusops elengi*; *Oroxylum indicum*; Hair Fall Management

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1. INTRODUCTION

Hair plays a significant role in personal appearance and psychological well-being. Hair fall is a common condition that may arise due to genetic predisposition, nutritional deficiencies, hormonal imbalance, stress, scalp infections, environmental pollution, and aging. Excessive hair loss often results in reduced self-confidence and diminished quality of life. Conventional therapeutic agents such as minoxidil and finasteride have shown effectiveness in managing hair loss; however, their prolonged use

may lead to adverse reactions and limited patient compliance [1].

Medicinal plants have long been utilized in traditional systems of medicine for maintaining hair health and promoting hair growth [2]. Herbal products are generally considered safer and more acceptable due to their natural origin and reduced side effects [3]. *Clitoria ternatea* possesses antioxidant, anti-inflammatory, and antimicrobial properties attributed to its rich flavonoid and

anthocyanin content [4]. *Mimusops elengi* contains tannins, triterpenoids, and phenolic compounds that exhibit antioxidant and protective effects on biological tissues [5]. *Oroxylum indicum* is recognized for its abundant flavonoids and bioactive constituents that demonstrate antimicrobial and free-radical scavenging activities [6].

Oxidative stress has been implicated in hair follicle damage and premature hair loss, highlighting the importance of antioxidants in hair care formulations [7]. Plant-derived phenolics and flavonoids can help neutralize reactive oxygen species and protect follicular structures [8]. Herbal extracts may also contribute to scalp hygiene by inhibiting microbial growth associated with dandruff and follicular disorders [9].

Polyherbal formulations often provide synergistic effects through the combination of multiple bioactive constituents [10]. Previous studies have demonstrated that botanical preparations enriched with antioxidants and antimicrobial compounds can support scalp health and reduce factors contributing to hair fall. Therefore, the development of a polyherbal hair tonic incorporating *Clitoria ternatea*, *Mimusops elengi*, and *Oroxylum indicum* was undertaken to explore its potential as a natural alternative for hair fall management.

2. MATERIALS AND METHODS

2.1 Materials

Fresh flowers of *Clitoria ternatea* were collected from the medicinal plant garden of the institute. Bark of *Mimusops elengi* and stem bark of *Oroxylum indicum* were procured from a certified herbal supplier and authenticated by a qualified taxonomist.

The following chemicals and reagents were used:

- Ethanol (95%)
- Methanol
- Distilled water
- Propylene glycol
- Glycerin
- Tween 80
- Methyl paraben
- Propyl paraben
- Citric acid
- Sodium citrate
- DPPH reagent
- Folin–Ciocalteu reagent

- Gallic acid
- Nutrient agar
- Mueller-Hinton agar
- Potassium phosphate buffer

All reagents used were of analytical grade.

Table 1. Materials Used in the Preparation of Polyherbal Hair Tonic

S. No.	Material	Quantity
1	<i>Clitoria ternatea</i> extract	2.0 g
2	<i>Mimusops elengi</i> extract	2.0 g
3	<i>Oroxylum indicum</i> extract	2.0 g
4	Propylene glycol	10 mL
5	Glycerin	5 mL
6	Tween 80	2 mL
7	Methyl paraben	0.18 g
8	Propyl paraben	0.02 g
9	Distilled water	q.s. to 100 mL

2.2 Authentication of Plant Materials

The collected plant materials were washed thoroughly using running tap water followed by rinsing with distilled water. Foreign matter, damaged portions, and adhering contaminants were removed manually. Plant samples were authenticated based on macroscopic and microscopic characteristics by a botanist. Voucher specimens were deposited in the departmental herbarium for future reference.

2.3 Drying and Pulverization

The plant materials were shade dried at room temperature ($25 \pm 2^\circ\text{C}$) for twenty-one days. Dried materials were periodically inspected to prevent fungal contamination. Complete drying was confirmed by achieving constant weight.

The dried materials were separately pulverized using a mechanical grinder. The resulting powders were passed through sieve No. 60 and stored in airtight amber-colored containers until extraction.

2.4 Preparation of Hydroalcoholic Extracts

Extraction of *Clitoria ternatea*

Approximately 500 g of powdered flower material was transferred into a Soxhlet extraction apparatus. Extraction was performed using 70% ethanol.

The extraction process continued for 24 hours until complete exhaustion of plant material was achieved. The extract obtained was filtered and concentrated under reduced pressure using a rotary vacuum evaporator at 45°C.

The concentrated extract was dried in a vacuum desiccator and stored at 4°C.

Extraction of *Mimusops elengi*

Five hundred grams of powdered bark was extracted using 70% ethanol following the same procedure. The extract was concentrated and dried.

Extraction of *Oroxylum indicum*

Five hundred grams of powdered stem bark was subjected to Soxhlet extraction using 70% ethanol. The concentrated extract was dried and stored under refrigeration.

Table 2. Percentage Yield of Plant Extracts

Plant Material	Powder Taken (g)	Extract Obtained (g)	Yield (%)
<i>Clitoria ternatea</i>	500	68.5	13.70
<i>Mimusops elengi</i>	500	57.0	11.40
<i>Oroxylum indicum</i>	500	62.5	12.50

2.5 Preliminary Phytochemical Screening

Preliminary phytochemical investigations were performed to identify major secondary metabolites present in the extracts.

Test for Alkaloids

One milliliter of extract solution was treated with Dragendorff's reagent. Formation of orange precipitate indicated the presence of alkaloids.

Test for Flavonoids

The extract was treated with magnesium turnings and concentrated hydrochloric acid. Development of pink coloration confirmed flavonoids.

Test for Phenolics

Ferric chloride solution was added to the extract. Blue-black coloration indicated phenolic compounds.

Test for Tannins

A few drops of ferric chloride solution were added to the extract solution. Greenish-black coloration confirmed tannins.

Test for Saponins

The extract solution was vigorously shaken with distilled water. Persistent foam formation indicated saponins.

Test for Glycosides

The extract was subjected to Keller–Killiani reaction for glycoside detection.

Table 3. Preliminary Phytochemical Screening of Extracts

Phytoconstituent	<i>C. ternatea</i>	<i>M. elengi</i>	<i>O. indicum</i>
Alkaloids	+	+	+
Flavonoids	+++	++	+++
Phenolics	+++	+++	+++
Tannins	++	+++	++
Saponins	+	+	+
Glycosides	++	++	++

Key: + Present; ++ Moderately Present; +++ Abundantly Present

2.6 Determination of Total Phenolic Content

The Folin–Ciocalteu method was employed.

One milliliter of extract solution was mixed with 5 mL of diluted Folin–Ciocalteu reagent. After five minutes, 4 mL of sodium carbonate solution was added.

The mixture was incubated for thirty minutes in the dark. Absorbance was measured at 765 nm using a UV–Visible spectrophotometer. Total phenolic content was calculated using gallic acid calibration standards.

2.7 Determination of Total Flavonoid Content

One milliliter of extract was mixed with aluminum chloride reagent and incubated for thirty minutes. Absorbance was recorded at 415 nm.

Flavonoid content was calculated using quercetin standard calibration.

2.8 Formulation of Polyherbal Hair Tonic

Four trial formulations were prepared.

The required quantities of extracts were weighed accurately. Methyl paraben and propyl paraben were dissolved in a small quantity of warm distilled water.

Propylene glycol and glycerin were mixed separately. The extracts were dispersed uniformly into the solvent mixture under continuous stirring.

Tween 80 was incorporated gradually to ensure homogeneous distribution of herbal extracts. The preservative solution was added slowly with constant stirring.

The volume was adjusted to 100 mL using distilled water.

The formulations were stirred for 30 minutes using a magnetic stirrer at 500 rpm until a clear and uniform tonic was obtained.

2.9 Optimization of Polyherbal Hair Tonic Formulations

Four trial formulations (F1–F4) were prepared by varying the concentration of the herbal extracts while maintaining the concentrations of other excipients constant. The formulations were evaluated for physical appearance, clarity, pH, viscosity, stability, and ease of application. Based on preliminary evaluation, the formulation exhibiting optimum physicochemical characteristics was selected as the optimized formulation for further studies.

Table 4. Composition of Trial Hair Tonic Formulations

Ingredient (g/100 mL)	F1	F2	F3	F4
<i>Clitoria ternatea</i> extract	1.0	1.5	2.0	2.5
<i>Mimusops elengi</i> extract	1.0	1.5	2.0	2.5
<i>Oroxylum indicum</i> extract	1.0	1.5	2.0	2.5
Propylene glycol	10	10	10	10
Glycerin	5	5	5	5
Tween 80	2	2	2	2
Methyl paraben	0.18	0.18	0.18	0.18
Propyl paraben	0.02	0.02	0.02	0.02
Distilled water	q.s.	q.s.	q.s.	q.s.

2.10 Evaluation of Polyherbal Hair Tonic

Organoleptic Evaluation

The prepared formulations were visually examined for color, odor, clarity, homogeneity, and phase separation.

Determination of pH

The pH of each formulation was determined using a calibrated digital pH meter. Ten milliliters of formulation were diluted with distilled water and measurements were performed in triplicate at room temperature.

Determination of Specific Gravity

Specific gravity was determined using a specific gravity bottle. The bottle was weighed empty and after filling with distilled water and formulation respectively.

Determination of Viscosity

Viscosity measurements were performed using a Brookfield viscometer at 25°C using spindle number 62 at 50 rpm.

Spreadability

One milliliter of formulation was placed between two glass slides and spreadability was evaluated by

measuring the diameter of spreading under a standardized weight.

2.11 Accelerated Stability Studies

Stability studies were conducted according to ICH guidelines.

The optimized formulation was filled into amber-colored glass bottles and stored under the following conditions:

- $4 \pm 2^\circ\text{C}$
- $25 \pm 2^\circ\text{C}$
- $40 \pm 2^\circ\text{C} / 75 \pm 5\% \text{ RH}$

The study was conducted for three months.

Samples were withdrawn at predetermined intervals of 0, 30, 60, and 90 days.

The following parameters were evaluated:

- Appearance
- pH
- Viscosity
- Color
- Odor
- Phase separation

2.12 Determination of Antioxidant Activity

DPPH Radical Scavenging Assay

The antioxidant activity of the extracts and optimized formulation was evaluated using DPPH radical scavenging assay.

A 0.1 mM DPPH solution was prepared in methanol.

Different concentrations of extracts (20, 40, 60, 80, and 100 $\mu\text{g/mL}$) were mixed with DPPH solution.

The reaction mixtures were incubated in darkness for 30 minutes at room temperature.

Absorbance was measured at 517 nm against a blank using a UV-Visible spectrophotometer.

The percentage radical scavenging activity was calculated using the formula:

$$\% \text{ Inhibition} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

Ascorbic acid was used as the reference standard.

2.13 Protein Protection Assay

Hair primarily consists of keratin proteins. Therefore, the protective effect of the formulation against protein denaturation was investigated.

A bovine serum albumin solution was prepared in phosphate buffer.

The optimized formulation was mixed with protein solution and incubated at 37°C for 30 minutes.

The mixtures were subsequently heated to induce denaturation.

Absorbance was recorded at 660 nm.

The percentage inhibition of protein denaturation was calculated.

2.14 Antimicrobial Activity

The antimicrobial activity of the optimized formulation was evaluated against scalp-associated microorganisms.

Test Organisms

The following microorganisms were employed:

- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*
- *Candida albicans*

Agar Well Diffusion Method

Sterile Mueller-Hinton agar plates were prepared.

Microbial suspensions equivalent to 0.5 McFarland standard were spread uniformly over agar surfaces.

Wells measuring 6 mm diameter were punched aseptically.

The optimized formulation, extract solution, and standard antimicrobial agent were introduced into respective wells.

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The plates were incubated:

- Bacteria: 37°C for 24 h
- Fungi: 28°C for 48 h

Zones of inhibition were measured using a Vernier caliper.

2.15 Determination of Hair Protein Conditioning Potential

Human hair strands collected from healthy volunteers were washed and dried.

Hair samples were immersed in:

- Distilled water
- Individual extracts
- Optimized formulation

The samples were maintained for 24 h.

The strands were evaluated visually for:

- Smoothness
- Shine
- Manageability
- Surface texture

Observations were recorded.

3. RESULTS AND DISCUSSION

The extraction process yielded satisfactory quantities of hydroalcoholic extracts from all three medicinal plants. The highest extractive yield was observed for *Clitoria ternatea* (13.70%), followed by *Oroxylum indicum* (12.50%) and *Mimusops elengi* (11.40%).

Phytochemical screening revealed the presence of alkaloids, flavonoids, phenolics, tannins, glycosides, and saponins in all extracts. The abundance of flavonoids and phenolics suggested strong antioxidant potential.

All formulated hair tonics appeared homogeneous, dark brown in color, and free from phase separation. No precipitation was observed during storage.

Among the trial formulations, F3 demonstrated optimum appearance, viscosity, pH, and spreadability

characteristics. Therefore, F3 was selected as the optimized formulation.

Table 5. Physicochemical Evaluation of Formulations

Parameter	F1	F2	F3	F4
Appearance	Clear	Clear	Clear	Slightly Turbid
pH	5.10	5.45	5.82	6.10
Specific Gravity	1.02	1.03	1.04	1.05
Viscosity (cP)	41.2	48.4	56.8	68.5
Spreadability (cm)	5.2	5.8	6.4	5.9

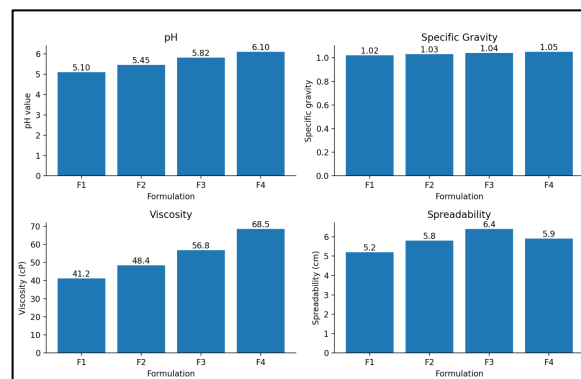


Figure 1: Physicochemical Evaluation of Formulations

The total phenolic content of the extracts ranged from 78.6 to 112.5 mg GAE/g extract, whereas flavonoid content ranged from 41.3 to 78.2 mg QE/g extract.

Table 6. Total Phenolic and Flavonoid Content

Extract	Total Phenolics (mg GAE/g)	Total Flavonoids (mg QE/g)
<i>Clitoria ternatea</i>	112.5	78.2
<i>Mimusops elengi</i>	98.4	56.7

Oroxylum indicum	105.6	69.5
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Figure 2: Total Phenolic and Flavonoid Content

The DPPH assay demonstrated concentration-dependent antioxidant activity for all extracts and the optimized formulation.

Table 7. DPPH Radical Scavenging Activity

Concentration (µg/mL)	Ascorbic Acid (%)	Optimized Formulation (%)
20	28.5	22.4
40	45.8	39.2
60	61.4	55.6
80	78.2	71.5
100	91.3	84.7

Figure 3: DPPH Radical Scavenging Activity

The antioxidant activity increased with concentration. The optimized formulation exhibited 84.7% inhibition at 100 µg/mL, indicating substantial free radical scavenging capacity.

The protein protection assay revealed significant inhibition of protein denaturation by the optimized formulation.

Table 8. Protein Protection Activity

Sample	Inhibition of Protein Denaturation (%)
Control	0
<i>Clitoria ternatea</i> Extract	62.4
<i>Mimusops elengi</i> Extract	58.7
<i>Oroxylum indicum</i> Extract	60.8
Optimized Formulation	76.5

Figure 4: Protein Protection Activity

The polyherbal combination exhibited greater activity than individual extracts, suggesting synergistic interactions among the phytoconstituents.

Antimicrobial activity studies demonstrated broad-spectrum activity against bacterial and fungal microorganisms commonly associated with scalp disorders.

Table 9. Antimicrobial Activity of Optimized Formulation

Microorganism	Zone of Inhibition (mm)
<i>Staphylococcus aureus</i>	18.4 ± 0.5
<i>Pseudomonas aeruginosa</i>	16.2 ± 0.4
<i>Candida albicans</i>	19.1 ± 0.6

Figure 5: Antimicrobial Activity of Optimized Formulation

The observed antimicrobial activity indicated the potential of the formulation to support scalp hygiene and reduce microbial colonization.

Stability studies conducted over ninety days revealed no significant alterations in physical characteristics.

Table 10. Stability Study of Optimized Formulation (F3)

Day	Appearance	pH	Viscosity (cP)
0	Clear	5.82	56.8
30	Clear	5.80	56.4
60	Clear	5.79	55.9
90	Clear	5.77	55.5

The formulation remained physically stable throughout the study period without evidence of precipitation, phase separation, or microbial contamination.

Hair conditioning assessment demonstrated enhanced smoothness, improved shine, and better manageability of treated hair strands compared with untreated controls. The formulation imparted a healthier appearance and reduced surface roughness, indicating favorable conditioning effects.

4. CONCLUSION

A polyherbal hair tonic containing *Clitoria ternatea*, *Mimusops elengi*, and *Oroxylum indicum* extracts was successfully formulated and evaluated. The hydroalcoholic extracts exhibited appreciable levels of phenolics and flavonoids, contributing to strong antioxidant activity. The optimized formulation demonstrated desirable physicochemical characteristics, acceptable pH, adequate spreadability, and good stability during storage. Significant antioxidant, protein protective, and antimicrobial activities were observed, suggesting its potential role in protecting hair follicles and maintaining scalp health. The synergistic combination of the three medicinal plants enhanced the biological performance of the formulation compared with individual extracts. The developed polyherbal hair tonic may represent a promising natural approach for the management of hair fall and improvement of hair quality. Further in vivo studies and clinical investigations are recommended to establish long-term efficacy and safety.

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