INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

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Research Article

Formulation and Physicochemical Evaluation of a Polyherbal Anti-Dandruff Shampoo

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ARTICLE INFO

Published: 29 Dec 2025
Keywords:
Herbal shampoo, anti-dandruff, plant extracts, Amla, Shikakai, formulation, evaluation, physicochemical analysis
DOI:
10.5281/zenodo.18085917

ABSTRACT

Background: Dandruff is a prevalent scalp disorder affecting quality of life. While synthetic anti-dandruff shampoos show efficacy, they often cause adverse effects including hair loss, scaling, and scalp irritation. Herbal-based formulations offer a safer alternative by leveraging traditional anti-fungal, anti-bacterial, and conditioning properties of plant extracts. Aim: To formulate and evaluate a herbal-based anti-dandruff shampoo using a polyherbal combination of traditionally used plant extracts, and to assess its physicochemical and performance characteristics. Methodology: A polyherbal shampoo formulation was developed using eleven plant extracts (Amla, Shikakai, Hibiscus, Aloe vera, Rose oil, Lemon juice, Fenugreek, Neem oil, Reetha, Curry leaves, and Onion juice). The prepared formulation was evaluated using organoleptic assessment, physicochemical analysis (pH, viscosity, surface tension, wetting time, foam stability), cleaning action tests, solids content determination, and 4-week stability study at 25-30°C. Results: The formulated shampoo exhibited optimal physicochemical properties: pH 6.2 (safe for scalp), viscosity 950 cP (good consumer acceptability), surface tension 35 dyn/cm (effective cleansing), foam height ≥100 mm (satisfactory), wetting time ≤25 seconds, and cleaning action efficiency 78% (greasy soil removal). The formulation remained physically and chemically stable during 4-week storage with no phase separation or discoloration. Conclusion: The developed herbal-based shampoo demonstrates safe, stable, and effective anti-dandruff and cleansing properties, making it a promising alternative to synthetic formulations. The polyherbal combination provides complementary antifungal, antibacterial, and conditioning benefits without reported adverse effects.

INTRODUCTION

1.1 Shampoo: Definition and Importance

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Shampoos are among the most widely used cosmetic preparations designed for cleansing hair and scalp of oils, environmental pollutants, dirt, and cosmetic residues. Shampoos are formulated to serve multiple functions: effective cleansing, improved hair appearance, and maintaining scalp health. The growing consumer preference for natural personal care products has driven the development of herbal shampoos as safer alternatives to synthetic formulations.[1]

1.2 Herbal Shampoos and Their Advantages

Herbal shampoos utilize plant-derived active ingredients and are formulated according to traditional Ayurvedic and modern pharmaceutical principles. They offer several advantages over conventional synthetic shampoos:

- Safety: Free from harsh synthetic surfactants, sulfates, and potentially harmful chemical additives
- Efficacy: Multiple plant extracts provide complementary therapeutic actions (antifungal, antibacterial, conditioning)
- Biodegradability: Environmentally friendly with reduced ecological impact
- Tolerability: Lower incidence of scalp and eye irritation compared to chemical formulations
- Cost-effectiveness: Affordable and increasingly accessible in commercial markets[2]

1.3 Dandruff: Clinical Significance and Current Management

Dandruff is a common, chronic scalp condition characterized by excessive shedding of dead skin cells from the scalp. It affects approximately 50% of the population worldwide and is most prevalent in young adults after puberty. The condition manifests as white flakes on the scalp and shoulders, accompanied by itching and occasionally visible erythema.

Etiology: Dandruff results from a combination of factors:

- Immune response to *Malassezia globosa* and related yeast species
- Excessive sebum production or dry scalp conditions
- Poor scalp hygiene and inadequate shampooing frequency
- Sensitivity to hair care products (contact dermatitis)
- Psychological stress and seasonal variations (more severe in cold, dry seasons)

Current Therapeutic Approaches: Conventional anti-dandruff shampoos contain synthetic active agents such as zinc pyrithione, selenium sulfide, ketoconazole, and coal tar. While effective, these formulations are associated with adverse effects including increased scaling, hair loss, scalp irritation, discomfort, and occasional systemic absorption.[3-4]

1.4 Rationale for Herbal Anti-Dandruff Formulation

Traditional and Ayurvedic systems of medicine have utilized plant-based preparations for centuries to address hair and scalp disorders. Recent phytochemical research has validated many traditional uses, identifying bioactive compounds in herbal extracts with documented antifungal, antibacterial, antioxidant, and anti-inflammatory properties. The present work aims to combine multiple traditionally used herbs in a single formulation to achieve synergistic anti-dandruff and conditioning effects without adverse effects associated with synthetic agents.[5]

1.5 Plant Extracts Used: Pharmacological Basis

Amla (Phyllanthus emblica L.; Euphorbiaceae)

Rich in vitamin C and tannins; exhibits strong antifungal and antibacterial activity against scalp pathogens; promotes hair strength and reduces hair loss.

Shikakai (Acacia concinna DC.; Mimosaceae)

Contains natural saponins with surfactant properties; cleanses effectively without harshness; has antimicrobial activity against *Malassezia* species.

Hibiscus (Hibiscus sabdariffa L.; Malvaceae)

Mucilage and amino acid content provide conditioning; inhibits fungal growth and prevents premature graying.

Aloe vera (Aloe barbadensis Mill.; Xanthorrhoeaceae)

Polysaccharides and active compounds calm itchy scalps; promote scalp hydration and hair growth; antimicrobial properties aid in infection prevention.

Neem (Azadirachta indica A. Juss.; Meliaceae)

Lipophilic bioactives (azadirachtin, nimbin) show potent antifungal and antibacterial activity; proven efficacy against dandruff-causing organisms.

Fenugreek (Trigonella foenum-graecum L.; Fabaceae)

Traditional Ayurvedic remedy for dandruff and scalp conditions; saponins and mucilage provide both cleansing and conditioning properties.

Additional ingredients (Lemon juice, Curry leaves, Reetha, Rose oil, Onion juice) provide antimicrobial, antioxidant, and sensory benefits.[6]

1.6 Objectives

Primary Objective:

To formulate a herbal-based anti-dandruff shampoo utilizing a polyherbal extract system and evaluate its physicochemical, safety, and performance characteristics.

Secondary Objectives:

- To optimize formulation parameters for stability and consumer acceptability
- To establish quality control standards for herbal shampoo evaluation
- To compare physicochemical profile with conventional anti-dandruff shampoos
- To assess safety through preliminary skin and eye irritation assessments[7]

2. MATERIALS AND METHODS

2.1 Plant Materials and Collection

Plant materials were collected from authenticated, verified sources:

Plant (Common/ Botanical Name)	Part Used	Botanical Family	Source
Amla (*Phyllanthus emblica* L.)	Fruit (dried, powdered)	Euphorbiaceae	Local market, Nagpur
Shikakai (*Acacia concinna* DC.)	Pods (dried, powdered)	Mimosaceae	Local market
Hibiscus (*Hibiscus sabdariffa* L.)	Flowers (dried, powdered)	Malvaceae	Local nursery
Aloe vera (*Aloe barbadensis* Mill.)	Leaves (fresh gel)	Xanthorrhoeaceae	Institutional nursery
Reetha (*Sapindus mukorossi* Gaertn.)	Dried fruits (powdered)	Sapindaceae	Local market
Curry leaves (*Murraya koenigii* Spreng.)	Leaves (fresh)	Rutaceae	Institutional nursery

Fenugreek (*Trigonella foenum-graecum* L.)	Seeds (powdered)	Fabaceae	Local market
Neem (*Azadirachta indica* A. Juss.)	Oil (extracted)	Meliaceae	Botanical supplier
Lemon (*Citrus limon* L.)	Fruit juice (fresh)	Rutaceae	Local market
Onion (*Allium cepa* L.)	Bulb juice (fresh)	Amaryllidaceae	Local market
Rose oil	Essential oil	Rosaceae	Pharmaceutical supplier

Plant Authentication: All plant materials were verified by macroscopic and microscopic examination. For herbal materials sourced from the market, identity was confirmed against standard botanical references.[8]

2.2 Chemicals and Reagents

- Sodium Lauryl Sulfate (SLS), USP grade, 7.5% solution
- Sodium Chloride (NaCl), 0.1 M solution
- Acacia gum extract
- Glycerine, pharmaceutical grade
- Vitamin E capsule (400 IU)
- Activated charcoal, USP grade
- India ink (for dirt dispersion assay)
- All other chemicals of analytical or pharmaceutical grade.[9]

2.3 Extraction and Powder Preparation

Step 1: Drying and Size Reduction- Fresh plant materials were washed under running water to remove surface contamination and dried in sunlight for 7-10 days until moisture content reduced to <10%. Dried materials were subjected to size reduction using a mechanical grinder to achieve uniform particle size.

Step 2: Preparation of Plant Decoctions- Two separate decoctions were prepared:

Decoction I: Hibiscus flowers (15 g), Curry leaves (10 g), and Amla powder (10 g) were boiled in 100 ml distilled water at 100°C for 15 minutes. Volume was reduced to 25 ml by continued boiling, then cooled and filtered through muslin cloth.

Decoction II: Shikakai pods (12 g), Reetha fruits (8 g), and Fenugreek seeds (5 g) were boiled in 100 ml distilled water at 100°C for 15 minutes. Volume was reduced to 25 ml by boiling, cooled, and filtered through muslin cloth.

Storage: Both filtrates were stored at 4°C until use (within 24 hours).[10-11]

2.4 Shampoo Formulation and Preparation

The herbal-based shampoo was prepared according to the following procedure:

Step 1: Surfactant Solution Preparation- SLS (7.5%) solution (20 ml) was prepared in 0.1 M NaCl (20 ml).

Step 2: Integration of Herbal Components- Decoction I (20 ml) was added to the SLS-NaCl solution with constant gentle mixing to prevent excessive foam formation.

Step 3: Addition of Excipients- The following were added sequentially with thorough mixing:

- Aloe vera gel: 10 ml (moisturizing and conditioning agent)
- Neem oil: 2 ml (thickening agent, antimicrobial)
- Onion juice: 5 ml (antimicrobial preservative)
- Acacia gum extract: 10 ml (thickening and suspending agent)

- Glycerine: 2 ml (humectant)

Step 4: pH Adjustment and Final Additions- pH was adjusted to 6.0-6.5 using citric acid dilute solution, if necessary. Finally added:

- Vitamin E capsule (1 capsule, opened and mixed)
- Activated charcoal: 0.5 g (for color)
- Lemon juice: 2 ml (preservative, antimicrobial)
- Rose oil: q.s. (fragrance enhancer)

Step 5: Volume Adjustment and Packaging- Final volume was made up to 100 ml with distilled water. The formulation was mixed thoroughly for 10 minutes, allowed to stand for 24 hours for equilibration, then packaged in opaque, amber-colored glass bottles with airtight closures.[12-13]

2.5 Formulation Composition Summary

Sr. No.	Ingredient	Quantity (per 100 ml)	Functional Role	Concentration (%)
1	Amla extract	2.5 ml	Antidandruff, conditioning	2.5
2	Shikakai extract	2.5 ml	Natural detergent, cleansing	2.5
3	Hibiscus decoction	2 ml	Conditioning, antifungal	2.0
4	Reetha extract	1.5 ml	Foaming agent, cleansing	1.5
5	Aloe vera gel	10 ml	Moisturizing, soothing	10.0
6	Neem oil	2 ml	Antifungal, thickening	2.0
7	Curry leaves extract	1 ml	Antidandruff, antioxidant	1.0
8	Fenugreek decoction	1 ml	Conditioning, antifungal	1.0
9	Onion juice	5 ml	Antimicrobial preservative	5.0
10	Lemon juice	2 ml	Antimicrobial, pH buffer	2.0
11	Rose oil	q.s. (0.5 ml)	Fragrance	q.s.
12	SLS solution (7.5%)	20 ml	Surfactant	1.5
13	Sodium chloride (0.1 M)	20 ml	Electrolyte	2.0
14	Acacia gum extract	10 ml	Thickening agent	10.0
15	Glycerine	2 ml	Humectant	2.0
16	Vitamin E	1 capsule	Antioxidant, conditioning	0.4
17	Activated charcoal	0.5 g	Coloring agent	0.5
18	Distilled water	q.s.	Vehicle	-
-	Total Volume	100 ml	-	~100

SLS Justification: While claiming "herbal-based" formulation, minimal SLS (1.5% final concentration) was retained to ensure adequate surfactant properties and consumer satisfaction with lather. This represents a significant reduction from conventional anti-dandruff shampoos (typically 8-12% SLS) while maintaining commercial viability. Future work will explore fully herbal surfactant systems.[14-15]

2.6 Evaluation Parameters and Methods

2.6.1 Organoleptic Evaluation

Sensory characteristics were assessed visually and tactilely:

- Appearance: Visual inspection for color, clarity, and homogeneity
- Texture: Consistency assessment by manual touch and flow properties
- Odor: Subjective assessment of fragrance and any off-odors
- Foam Production: Visual observation of foam height and stability upon shaking

2.6.2 Physicochemical Evaluation

pH Determination: 1 gram of shampoo was dissolved in 9 ml of distilled water. pH was measured at 25°C using a calibrated digital pH meter (Eutech Instruments, Singapore). Measurements were performed in triplicate.

Viscosity Measurement: Viscosity was determined using a Brookfield viscometer (Model DV-II+, USA) with spindle No. 3, rotating at 100 rpm, at 25°C. 10 ml of shampoo was placed in a beaker, and the viscometer spindle was immersed for 5 minutes before recording. Values are expressed in centipoise (cP).

Surface Tension Measurement: 10% w/v shampoo solution was prepared in distilled water. Surface tension was measured at 25°C using a stalagmometer (drop count method). The number of drops of shampoo solution dispensed from a 1 ml stalagmometer was counted and compared with standard water (which typically gives 76 drops). Surface tension was calculated using the formula:

Surface Tension =

$$\frac{\text{Surface Tension of Water} \times \text{Drops of Water}}{\text{Drops of Shampoo} \times \text{Density Factor}}$$

Surface Tension=

$$\frac{\text{Drops of Shampoo} \times \text{Surface Tension of Water}}{\text{Drops of Water} \times \text{Density Factor}}$$

Values are expressed in dyn/cm.

Wetting Time: A canvas paper disc (diameter 1 inch, weight 0.44 g) was placed on the surface of 1% v/v shampoo solution (100 ml). Time required for the paper to sink completely was noted using a stopwatch. The test was performed in triplicate.

Dirt Dispersion: Two drops of shampoo were added to a test tube containing 10 ml of distilled water. One drop of India ink was added, the tube was stoppered, and shaken vigorously 10 times. The amount of ink dispersed in the foam was visually rated as: None (0), Light (1), Moderate (2), or Heavy (3).

Foam Height and Stability: 50 ml of 1% shampoo solution was placed in a 250 ml graduated cylinder. The cylinder was sealed with the palm and shaken vertically for 10 times. The total foam volume was recorded immediately after shaking (foam height), and again after 1 minute. Foam stability (%) was calculated as:

Foam Stability (%) =

$$\frac{\text{Foam Volume at 1 min}}{\text{Initial Foam Volume} \times 100}$$

Foam Stability (%) =

$$\frac{\text{Initial Foam Volume}}{\text{Foam Volume at 1 min} \times 100}$$

Solids Content Determination: A clean, dry evaporating dish was weighed, and exactly 4 grams of shampoo was added. The dish with shampoo was reweighed (total weight recorded). The dish was placed on a hot plate at 105°C until the liquid portion completely evaporated and a constant weight was achieved. The residual solid weight (dried powder) was calculated:

Solids Content (%) =

$$\frac{\text{Weight of Dried Solids}}{\text{Weight of Original Sample} \times 100}$$

Solids Content (%) =

$$\frac{\text{Weight of Original Sample}}{\text{Weight of Dried Solids} \times 100}$$

2.6.3 Cleaning Action Assessment

Cleaning efficiency was evaluated using the standard wool yarn method adapted from pharmaceutical formulation studies.

Procedure:

- 5 grams of wool yarn was immersed in purified mineral oil for 2 hours
- The greased yarn was placed in 200 ml of distilled water containing 1 gram of prepared shampoo
- The solution was maintained at 35°C and agitated at 50 cycles per minute for 4 minutes in a mechanical shaker
- The yarn was removed, excess solution was drained, and the yarn was dried in an oven at 105°C to constant weight

Calculation:

Cleaning Efficacy (%)=[Weight of Grease Removed Initial Weight of Grease]×100

Cleaning Efficacy (%)=[Initial Weight of Grease Weight of Grease Removed]×100

Where: Weight of grease removed = (Weight of greased dry yarn) − (Weight of cleaned dry yarn).[16-17]

2.6.4 Skin Sensitization Test

Procedure:

The prepared shampoo (0.5 ml) was applied to a 2 cm × 2 cm area of skin (inner forearm) of healthy volunteers (n = 5) and left for 5 minutes. The area was then thoroughly rinsed with water. The skin was observed at 0, 1, 4, and 24 hours for any signs of erythema, edema, or other irritation. Each volunteer completed a sensory questionnaire assessing any sensations of itching, burning, or discomfort.

Ethical Consideration: This preliminary test was conducted with informed consent on healthy volunteers with no known dermatological conditions or sensitivities to herbal products. Institutional approval for human volunteer studies was obtained.[18-19]

2.6.5 Stability Study

The formulated shampoo was stored in amber glass bottles with airtight closures at room temperature (25-30°C) for 4 weeks. Physical and chemical parameters were assessed at 0, 1, 2, and 4 weeks:

- Physical Stability: Observation for phase separation, color change, precipitation, odor changes, or clarification
- Chemical Stability: pH measurement and organoleptic re-assessment
- Parameter Changes: Viscosity, surface tension, and foam stability were re-measured at week 4.[20-21]

3. RESULTS

3.1 Organoleptic Characteristics

The prepared shampoo exhibited the following sensory properties:

Parameter	Observation
Color	Light brownish-green (due to herbal extracts and activated charcoal)
Clarity	Translucent to slightly opaque homogeneous dispersion
Texture	Semi-liquid, viscous, creamy consistency
Odor	Pleasant herbal fragrance with rose oil note; no off-odors
Foam Production	Abundant, thick, creamy foam on shaking; appears rich and luxurious

Flow Properties	Good pourability and spreadability on skin/ scalp

Consumer Acceptability Assessment: The formulation demonstrated favorable sensory attributes suitable for commercial cosmetic product.

3.2 Physicochemical Evaluation Results

The following table presents the physicochemical evaluation parameters and observed values:

Parameter	Test Method	Acceptance Criteria	Observed Value	Result
pH	pH meter, distilled water	6.0-6.5	6.2 ± 0.1	✓ Pass
Viscosity	Brookfield viscometer (cP)	800-1200 cP	950 ± 25 cP	✓ Pass
Surface Tension	Stalagmometer (dyn/cm)	30-40 dyn/cm	35 ± 2 dyn/cm	✓ Pass
Wetting Time	Canvas disc method (sec)	≤ 30 seconds	24 ± 2 seconds	✓ Pass
Foam Height	Cylinder shake method (mm)	≥ 100 mm	125 ± 5 mm	✓ Pass
Foam Stability	After 1 minute (%)	≥ 60%	78 ± 3%	✓ Pass
Dirt Dispersion	India ink method (0-3 scale)	≥ 2 (Moderate)	3 (Heavy)	✓ Pass
Solids Content	Evaporation method (%)	12-15%	13.2 ± 0.5%	✓ Pass
Cleaning Action	Wool yarn method (%)	≥ 70%	78 ± 2%	✓ Pass

Statistical Analysis: All values represent mean ± standard deviation (SD) of triplicate measurements. Results demonstrate consistency and reliability of the formulation.

3.3 Biological Safety Assessment

Skin Sensitization Test Results

Five healthy volunteers were patch-tested with the formulated shampoo. No erythema, edema, pruritis (itching), or other signs of dermatological irritation were observed at any time point (0, 1, 4, 24 hours). All volunteers rated the sensation as "comfortable" with no burning or stinging perception.

Conclusion: The formulated shampoo demonstrated excellent skin tolerability and absence of sensitization potential.

Eye Irritation Consideration

Standard Draize rabbit eye irritation test was not performed in this study due to institutional guidelines favoring alternatives to animal testing. The formulation's low SLS concentration (1.5%), neutral pH, and herbal base suggest minimal eye irritation potential compared to conventional shampoos (8-12% SLS).

3.4 Stability Study Results

The formulation was stored at room temperature (25-30°C) for 4 weeks and monitored for physical and chemical changes:

Parameter	Week 0	Week 1	Week 2	Week 4	Status
Appearance	Clear translucent	Clear translucent	Clear translucent	Clear translucent	✓ Stable
Color	Light brownish-green	Light brownish-green	Light brownish-green	Light brownish-green	✓ Stable
Odor	Pleasant herbal	Pleasant herbal	Pleasant herbal	Pleasant herbal	✓ Stable

pH	6.2	6.2	6.2	6.1	✓ Stable
Viscosity (cP)	950	945	940	935	✓ Minor change
Phase Separation	Absent	Absent	Absent	Absent	✓ Stable
Precipitation	Absent	Absent	Absent	Absent	✓ Stable
Foam Height (mm)	125	123	121	120	✓ Minimal change

Conclusion: The formulation demonstrated excellent short-term stability with only minor, non-significant changes in physical parameters over 4 weeks storage at room temperature.

4. DISCUSSION

4.1 Formulation Strategy and Rationale

The polyherbal anti-dandruff shampoo was designed based on traditional Ayurvedic principles and contemporary pharmaceutical knowledge. The combination of eleven plant-derived ingredients was selected to provide complementary therapeutic actions through different phytochemical mechanisms:

Antifungal Components: Neem oil (azadirachtin, nimbin), Aloe vera, Amla, and Fenugreek collectively target fungal pathogens, particularly *Malassezia globosa*, responsible for dandruff initiation and perpetuation. These ingredients work synergistically through multiple mechanisms—cell wall disruption, ergosterol synthesis inhibition, and immune modulation.

Natural Surfactants and Cleansing Agents: Shikakai and Reetha provide natural saponins with documented cleansing efficacy comparable to synthetic surfactants but with significantly lower irritation potential. Lemon juice's acidity and antimicrobial citric acid content aid in removing sebum and oil buildup.

Conditioning and Scalp Soothing: Aloe vera polysaccharides, Hibiscus mucilage, and Curry leaf compounds provide deep hydration and anti-inflammatory benefits. These address the dual pathophysiology of dandruff (either oily or dry scalp) by maintaining optimal sebum balance.

Preservation and Antimicrobial Functions: Onion juice (sulfur compounds) and Lemon juice (organic acids) serve as preservative agents while maintaining antimicrobial activity against bacteria and fungi. This natural preservation system avoids synthetic preservatives like methylparaben or propylparaben.

4.2 Physicochemical Properties and Functional Significance

pH Neutrality (6.2): The neutral pH achieved ensures scalp compatibility and minimizes irritation to skin and eyes compared to conventional anti-dandruff shampoos (often pH 3-4 due to high zinc pyrithione content). A pH of 6.2 maintains the scalp's natural acidic mantle while supporting natural flora balance.

Viscosity (950 cP): The measured viscosity falls within optimal range (800-1200 cP) for consumer satisfaction. It ensures adequate spreadability, good sense of feel during washing, and facilitates even distribution over scalp. The viscosity is achieved through natural gums and oils rather than synthetic thickening polymers.

Surface Tension (35 dyn/cm): The reduced surface tension relative to water (72 dyn/cm) indicates effective surfactant action provided primarily by Shikakai and Reetha saponins. This reduction facilitates penetration of the formulation into sebaceous follicles and enhanced dirt displacement from hair fibers.

Wetting Time (24 seconds): Rapid wetting promotes quick scalp contact and efficient cleansing action. The value well below the acceptance criterion (≤30 seconds) indicates excellent detergent properties and immediate action on hair and scalp surfaces.

Foam Height and Stability: Abundant foam (125 mm) and excellent stability (78% at 1 minute) provide the psychological satisfaction associated with effective cleansing. Consumer perception of shampoo efficacy is significantly influenced by lather characteristics. The herbal formulation achieves foam production comparable to or exceeding synthetic shampoos while using lower surfactant concentrations.

Cleaning Action (78%): The cleaning efficiency of 78% in removing mineral oil from wool yarn demonstrates effective degreasing capacity. This exceeds the minimum acceptance criterion (70%) and is comparable to commercial herbal shampoos, indicating that the polyherbal combination provides efficient cleansing of sebum and oily soils without harsh synthetic surfactants.

Solids Content (13.2%): The solid content represents suspended herbal particles, active compounds, thickening agents, and other non-volatile components. This level ensures adequate delivery of active herbal ingredients without excessive settling.

4.3 Safety Profile

The formulation demonstrated excellent skin tolerability in preliminary testing (5 healthy volunteers, zero adverse reactions). The safe profile is attributed to:

1. Low SLS Concentration (1.5%): Significantly reduced from conventional shampoos (8-12%), minimizing irritation potential
2. Herbal Base: Plant-derived ingredients carry centuries of traditional use safety data
3. Physiological pH (6.2): Matches scalp pH, preventing disruption of acid mantle and natural protective barrier
4. Anti-inflammatory Components: Aloe vera and Hibiscus provide soothing properties that counteract any irritant potential

Limitations of Safety Assessment: The present study performed only preliminary skin sensitization testing on 5 volunteers. Comprehensive safety evaluation would include:

- Larger human volunteer studies ($n \geq 30$)
- Standardized irritancy tests (OECD guidelines)
- Formal ocular irritation testing per Draize protocol (with appropriate ethical approval)
- Patch testing in individuals with sensitive skin or history of dermatological conditions

These comprehensive safety studies are recommended before commercial distribution or clinical claims.

4.4 Stability Characteristics

The 4-week stability data demonstrate excellent formulation stability at ambient temperature. The minimal changes in viscosity (950→935 cP, 1.6% change) and foam height (125→120 mm, 4% change) indicate strong preservation of formulation integrity. No phase separation, precipitation, discoloration, or odor changes were

observed, confirming adequate preservation by the natural antimicrobial system (Onion juice + Lemon juice).

For commercial product shelf life determination, extended stability studies (minimum 6 months at 40°C/75% RH per ICH Q1A guidelines) are recommended.

4.5 Comparison with Existing Formulations

Versus Synthetic Anti-Dandruff Shampoos:

- Lower pH (6.2 vs. 3-4 for zinc pyrithione formulations)
- Reduced surfactant concentration (1.5% vs. 8-12% SLS)
- Natural preservatives (vs. synthetic parabens/EDTA)
- Comparable cleaning efficacy (78% vs. typically 75-85% for commercial brands)
- Multiple mechanism of action (vs. single active agent)

Versus Previous Herbal Formulations: This formulation represents an advance through:

- Polyherbal synergistic design with documented rationale
- Comprehensive physicochemical characterization
- Quantified cleaning and anti-dandruff potential
- Systematic stability evaluation

4.6 Proposed Mechanism of Anti-Dandruff Action

The formulated shampoo addresses dandruff through multiple complementary pathways:

1. Antifungal Activity: Neem azadirachtin and Aloe polysaccharides inhibit *Malassezia* growth through cell wall destabilization and anti-inflammatory mechanisms.
2. Sebum Normalization: Shikakai and Reetha saponins gently remove excess sebum without over-drying; Hibiscus and Curry leaves promote healthy sebaceous gland function.
3. Scalp Anti-inflammation: Aloe vera and Hibiscus compounds downregulate inflammatory cytokine production in response to *Malassezia*-associated antigens.
4. Immune Modulation: Multiple herbal constituents (polyphenols, tannins) enhance innate immune function against pathogenic organisms.
5. Microbiome Rebalancing: Gentle cleansing preserves beneficial scalp microbiota while selectively suppressing pathogenic *Malassezia* overgrowth.

4.7 Limitations of the Current Study

1. No In Vitro Antifungal Testing: Direct assessment against *Malassezia globosa* and *Malassezia furfur* would strengthen anti-dandruff claims
2. No Clinical Efficacy Study: Human subject efficacy in actual dandruff reduction requires controlled clinical trial (Comparison vs. marketed shampoo, randomized design, blinded assessment, minimum 4-week application)
3. Limited Safety Data: Only 5 volunteers in sensitization testing; comprehensive studies needed
4. Short-Term Stability: 4-week stability sufficient for project scope; long-term stability (6-12 months) recommended for commercial product
5. No Comparative Analysis: Direct head-to-head comparison with a marketed anti-dandruff shampoo would strengthen positioning

4.8 Future Directions and Recommendations

Immediate Next Steps (3-6 months):

- Conduct standardized antifungal susceptibility testing of herbal extract against pathogenic *Malassezia* species
- Perform clinical efficacy trial with 30-50 volunteers having mild-moderate dandruff
- Expand safety testing to 25-30 healthy volunteers plus subset with sensitive skin

Medium-Term Development (6-12 months):

- Develop fully herbal surfactant system to eliminate SLS entirely
- Optimize concentrations of individual herbal components through design of experiments (DOE)
- Conduct long-term stability studies (6 months at 40°C/75% RH)
- Evaluate shelf life extension through alternative packaging or preservative systems

Commercial Development:

- Pilot scale-up and GMP compliance for production
- Stability testing per ICH Q1A(R2) guidelines
- Preparation of product master file and regulatory submissions for appropriate jurisdiction (India: Drug Controller approval pathway)

5. CONCLUSION

This study successfully developed a herbal-based anti-dandruff shampoo formulation utilizing a synergistic combination of eleven plant-derived ingredients. The formulation demonstrated:

- **Optimal Physicochemical Properties:** pH 6.2 (scalp-safe), viscosity 950 cP (consumer-acceptable), superior foam characteristics (125 mm, 78% stability), effective cleansing action (78% efficiency)
- **Safe Tolerability Profile:** No dermatological sensitization observed in preliminary human volunteer testing; low synthetic surfactant concentration minimizes irritation potential
- **Excellent Formulation Stability:** No phase separation, precipitation, or significant property changes over 4-week ambient temperature storage; stable pH and minimal viscosity change
- **Evidence-Based Botanical Design:** Formulation rationale based on traditional use, contemporary phytochemical research, and documented antimicrobial/ anti-inflammatory properties

The polyherbal approach offers significant advantages over conventional synthetic anti-dandruff formulations by providing:

- Multiple mechanisms of action through complementary phytochemical interactions
- Superior safety profile through reduced irritant potential
- Compliance with consumer preferences for natural personal care products
- Sustainable sourcing of renewable botanical resources

Clinical Impact: This formulation represents a promising therapeutic alternative for consumers seeking effective, safe, and naturally-derived anti-dandruff solutions without the adverse effects associated with synthetic active agents.

Regulatory Path: The formulation may be classified as a cosmetic (under most regulatory jurisdictions) or as a Ayurvedic cosmeceutical (India), pending specific active ingredient concentration levels and efficacy claims. The formulation meets acceptance criteria for

physicochemical characterization suitable for cosmetic product quality standards.

This work contributes to the growing body of evidence supporting the scientific validation of traditional herbal remedies in modern pharmaceutical formulation development.

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HOW TO CITE: Tejaswini Mankar, Prafull Wairagadwar, Dr. Awdhut Pimpale, Pallavi Zode, Heena Mahurkar, Formulation and Physicochemical Evaluation of a Polyherbal Anti Dandruff Shampoo, Int. J. of Pharm. Sci., 2025, Vol 3, Issue 12, 3883-3896. https://doi.org/10.5281/zenodo.18085917