Mediterranean Journal of Pharmacy & Pharmaceutical Sciences
ISSN: 2789-1895 online
www.medjpps.com
doi: 10.5281/zenodo.20757860
ISSN: 2958-3101 print

SHORT COMMUNICATION article

# Formulation and evaluation of an SLS-free polyherbal anti-dandruff shampoo containing *Sapindus mukorossi*, *Azadirachta indica*, *Acacia concinna*, *Aloe vera*, and *Phyllanthus emblica*

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**Received:** 24-03-2026, **Accepted**: 16-06-2026, **Published online:** 20-06-2026



**HOW TO CITE THIS**
Mohiuddin SA, et al. Formulation and evaluation of an SLS-free polyherbal anti-dandruff shampoo containing *Sapindus mukorossi*, *Azadirachta indica*, *Acacia concinna*, *Aloe vera*, and *Phyllanthus emblica*.
Mediterr J Pharm Pharm Sci. 2026; 6(2): 64-73. [Article number: 254]. https://doi.org/10.5281/zenodo.20757860

***Keywords:*** Anti-dandruff shampoo, *Sapindus mukorossi* biosurfactant, Malassezia furfur inhibition, natural scalp care cosmetics, SLS-free herbal formulation

**Abstract:** Heightened awareness among consumers regarding the adverse effects of synthetic chemicals in hair care products has driven growing interest in plant-based cosmetic formulations. Conventional anti-dandruff shampoos typically incorporate harsh anionic surfactants - most notably sodium lauryl sulfate (SLS) and sodium Lauretha sulfate - which, when used repeatedly over time, are known to cause scalp irritation, excessive dryness, allergic sensitization, and disruption of the scalp's natural acidic mantle. The present study was undertaken to develop a fully SLS-free, plant-derived anti-dandruff shampoo that delivers both clinical effectiveness and gentle tolerability. The formulation incorporated five well-established medicinal botanical ingredients: *Sapindus mukorossi* (Reetha) as a naturally derived foaming agent, *Azadirachta indica* (Neem) for its potent antifungal and antimicrobial properties, *Acacia concinna* (Shikakai) serving as a mild cleanser and hair conditioner, Aloe vera for scalp hydration and soothing activity, and *Phyllanthus emblica* (Amla) as an antioxidant-enriched hair tonic. Three experimental variants (F1, F2, and F3) were prepared with systematically varied concentrations of Reetha extract and Xanthan gum to identify the most suitable viscosity and performance profile. All three variants maintained a scalp-compatible pH in the range of 5.0 - 5.2. Formulation F2 exhibited the most favorable combination of properties, including optimal viscosity (2430 ± 18 cP), the greatest cleansing efficiency (82.6%), acceptable foam retention (75.0%), and superior cosmetic appeal. In antifungal testing against *Malassezia furfur*, a zone of inhibition of 17.0 ± 0.3 mm was recorded, confirming the therapeutic effectiveness of the formulation. Accelerated stability testing conducted at 40 °C / 75.0% RH over 30 days revealed no significant alterations in appearance, pH, viscosity, or microbial safety. Collectively, these findings support the developed shampoo as a viable, biodegradable, and consumer-friendly substitute for synthetic anti-dandruff products.

## Introduction

Hair constitutes an essential element of human physical appearance and personal identity, playing a considerable role in self-confidence and aesthetic appeal. The scalp represents a distinctive microenvironment defined by

elevated sebum output, high follicular density, and a resident microbial community. Among all cosmetic preparations available globally, shampoos are the most widely consumed, formulated to eliminate sebum, environmental dirt, shed corneocytes, and microbial contaminants from the scalp surface and hair shaft [1]. The majority of commercially available shampoos rely on synthetic anionic surfactants - primarily sodium lauryl sulfate (SLS) and sodium Lauretha sulfate - to achieve their cleansing effects. Although these compounds produce strong detergency, extensive research has linked chronic exposure to a range of scalp and hair problems, including dryness, irritation, pruritus, disruption of the hair cuticle structure, protein denaturation at the hair shaft, progressive weakening of follicular roots, and impairment of the scalp's natural lipid barrier. Their low environmental degradability further raises ecological concerns [2-4].

Dandruff - clinically referred to as seborrheic dermatitis capitis - is among the most prevalent chronic scalp conditions worldwide, with estimates suggesting it affects nearly half of the global adult population. Its underlying pathophysiology is closely tied to the overgrowth of *Malassezia furfur* (formerly *Pityrosporum ovale*), a lipophilic yeast that colonizes the human scalp. *Malassezia* species break down scalp lipids into irritant-free fatty acids, which breach the skin barrier and provoke inflammatory cascades, ultimately accelerating epidermal cell turnover and producing the characteristic flaking [5, 6]. Consumer preference for herbal and naturally sourced personal care products has shown marked growth in recent years. Herbal shampoo formulations offer multifunctional benefits - encompassing cleansing, conditioning, antioxidant protection, antimicrobial activity, anti-inflammatory action, and hair nourishment - without the safety concerns that accompany synthetic actives such as ketoconazole, zinc pyrithione, or selenium sulfide [3, 7, 8]. Dandruff is a non-inflammatory, chronic disorder of the scalp that represents the milder end of the seborrheic dermatitis clinical spectrum. Under physiologically normal conditions, scalp keratinocytes undergo desquamation across a well-regulated cycle of approximately 28 days. In individuals with dandruff, this renewal cycle is compressed to roughly seven days, generating visible clusters of shed corneocytes. The etiological involvement of *Malassezia furfur* is firmly established: Antifungal interventions that reduce *Malassezia* burden on the scalp consistently yield clinical improvement. Oleic acid liberated by the organism penetrates the stratum corneum, disrupts the organization of lamellar bilayers, and engages innate immune signaling through Toll-like receptor pathways, ultimately driving keratinocyte hyperproliferation and epidermal barrier breakdown [5, 6]. Surfactants are amphiphilic molecules that, upon exceeding the critical micelle concentration (CMC), self-organize into micellar structures capable of solubilizing sebum within their hydrophobic interior. Biosurfactants derived from plant saponins offer several distinct advantages: their CMC values and hydrophile-lipophile balance (HLB) characteristics allow effective cleansing without aggressively stripping scalp lipids, and they undergo complete biodegradation. The pericarp of *Sapindus mukorossi* yields 10.0 - 15.0% w/w saponins - chiefly hederagenin-based bidesmosidic glycosides - that assemble into stable micelles with strong surface-active properties [4, 9]. *Azadirachta indica* is a rich source of limonoid terpenoids - including azadirachtin, nimbidin, nimbidinin, and gedunin - that exhibit multifaceted antifungal mechanisms: nimbidin disrupts fungal cell membrane integrity through ergosterol interaction; azadirachtin suppresses chitin biosynthesis; and gedunin exerts direct permeabilizing effects on the fungal membrane. In assays specifically targeting *Malassezia furfur*, Neem extracts have yielded minimum inhibitory concentrations comparable to those of first-generation azole antifungals [5, 6].

Xanthan gum, an anionic heteropolysaccharide, imparts pseudoplastic (shear-thinning) flow behavior to the formulation - exhibiting high viscosity during storage (ensuring physical stability) that progressively decreases upon mechanical agitation during application (facilitating uniform distribution). Glycerin is a polyol humectant that retains moisture within the stratum corneum by forming hydrogen bonds with water molecules, thereby reducing transepidermal water loss. Methyl paraben is used as a broad-spectrum antimicrobial preservative; its

safety at concentrations up to 0.4% w/v in rinse-off products has been confirmed by regulatory authorities, including the Scientific Committee on Consumer Safety [10, 11]. The principal distinction of this investigation lies in the deliberate construction of a fully SLS-free anti-dandruff shampoo anchored on a saponin-based biosurfactant platform derived from *Sapindus mukorossi*, completely supplanting conventional synthetic anionic surfactants. While individual herbal constituents such as Neem and Shikakai have been studied separately in the literature, the purposeful integration of five pharmacologically complementary plant extracts - Reetha, Neem, Shikakai, Amla, and Aloe vera - within a single, optimized formulation represents a meaningful contribution to herbal cosmetic science. The use of Xanthan gum as a biopolymeric thickener in place of synthetic carbomers, and the incorporation of lemon juice as a natural pH-modifying agent, further enhances the environmentally conscious profile of the formulation [1, 3, 12]. The primary aim of this study was to formulate and evaluate a fully SLS-free polyherbal anti-dandruff shampoo utilizing scientifically chosen medicinal plant extracts capable of delivering effective cleansing, dandruff control, scalp protection, and hair nourishment simultaneously. The specific objectives were: (i) to replace synthetic surfactants with plant-derived biosurfactants derived from saponins; (ii) to comprehensively characterize the physicochemical properties of the prepared formulations; (iii) to assess the antifungal potential of the optimized formulation against *Malassezia furfur*; and (iv) to establish formulation stability under accelerated conditions in accordance with ICH Q1A(R2) guidelines.

## Materials and methods

All raw materials and chemicals used in this study were of pharmaceutical or analytical grade. The following were employed: Reetha extract (*Sapindus mukorossi*), Neem extract (*Azadirachta indica*), Shikakai extract (*Acacia concinna*), Aloe vera gel, Amla extract (*Phyllanthus emblica*), xanthan gum, glycerin, lemon juice, methyl paraben, and distilled water. Sabouraud dextrose agar and the fungal test strain (*Malassezia furfur*, ATCC 14521) were sourced from a certified microbiological supplier. **Table 1** summarizes the principal phyto-constituents and functional roles of the key ingredients incorporated into the formulation.

**Table 1:** Phytoconstituents and functional roles of key formulation ingredients

| Plant/ingredient | Phytoconstituents | Primary actions | Refs. |
|---|---|---|---|
| *Azadirachta indica* (Neem) | Azadirachtin, nimbidin, gedunin | Antifungal, antibacterial, anti-inflammatory | 2, 6 |
| *Sapindus mukorossi* (Reetha) | Saponins (hederagenin glycosides) | Surfactant, foam generation, antimicrobial | 1, 9 |
| *Acacia concinna* (Shikakai) | Saponins, flavonoids, tannins | Cleansing, conditioning, scalp pH regulation | 14, 17 |
| *Phyllanthus emblica* (Amla) | Ascorbic acid, tannins, emblicanin A and B | Antioxidant, hair strengthening, trichogenic | 7, 17 |
| Aloe vera | Acemannan, aloenin, vitamins C and E | Moisturizing, wound healing, and anti-inflammatory | 7, 16 |
| Xanthan gum | Heteropolysaccharide (glucose, mannose, glucuronate) | Viscosity enhancement, suspension stability | 10 |
| Glycerin | Polyol humectant | Moisture retention, plasticizing effect | 11 |

*Azadirachta indica (Neem)*, **Figure 1**, (family: Meliaceae), ranks among the most thoroughly studied medicinal plants in the traditional pharmacopoeia of India. Extracts obtained from the leaves and seeds are particularly

abundant in limonoid terpenoids, which underpin the plant's well-documented antifungal, antibacterial, and anti-inflammatory activities, including specific action against *Malassezia* species [5, 6].

*Sapindus mukorossi (Reetha)*, **Figure 2**, (family: Sapindaceae) is a deciduous tree indigenous to the sub-Himalayan foothills. Its air-dried pericarp is notably rich in saponins (predominantly hederagenin glycosides at 10.0 -15.0% concentration), which confer strong natural surface-active and foam-producing properties, along with documented antimicrobial activity against common scalp pathogens [1, 9, 13].

*Acacia concinna (Shikakai),* **Figure 3**, (family: Fabaceae) pod extracts contain a combination of saponins, flavonoids, and tannins that together deliver gentle cleansing, conditioning, and scalp pH-regulating effects. These properties help protect the natural lipid coating of the hair shaft and reduce post-wash dryness and brittleness [14, 15].

Aloe vera, **Figure 4**, (family: Asphodelaceae) gel is a complex matrix of acemannan, aloenin, vitamins C and E, and enzymatic antioxidants, including superoxide dismutase, collectively conferring moisturizing, wound-healing, and anti-inflammatory properties that are particularly beneficial for the scalp microenvironment [7, 16].

*Phyllanthus emblica (Amla)*, **Figure 5**, (family: Phyllanthaceae) is one of nature's richest sources of vitamin C, further enriched by gallic acid, ellagic acid, and distinctive hydrolyzable tannins (emblicanin A and B), endowing it with potent antioxidant activity, trichogenic stimulation, and protective effects on the scalp surface [5, 17].

**Figure 1:** Neem plant (*Azadirachta indica*)

**Figure 2:** Reetha (*Sapindus mukorossi*)

**Figure 3:** Shikakai (*Acacia concinna)*

**Figure 4:** Aloe vera

**Figure 5:** Amla (*Phyllanthus emblica*)

*Methods of extraction:* Herbal extracts were obtained via the aqueous decoction method, which selectively extracts water-soluble phytoconstituents, including saponins, flavonoids, tannins, glycosides, and polyphenolic compounds. This approach was intentionally selected to maintain the aqueous nature of the formulation and ensure compatibility with the shampoo base [18, 19]. Crude plant materials were carefully sorted, washed, and

shade-dried at ambient temperature to preserve heat-sensitive constituents - particularly the ascorbic acid content of Amla. The dried materials were reduced to a coarse powder using a mechanical grinder. Accurately weighed quantities of each powdered drug were dispersed in distilled water at a ratio of 1: 8 to 1: 10 w/v and heated to a rolling boil under continuous stirring for 30 - 60 min. The resulting decoctions were concentrated to approximately one-third of the initial volume, then cooled and filtered sequentially through muslin cloth and Whatman No. 1 filter paper. Filtered extracts were transferred into amber-glass airtight containers, refrigerated at 2 - 8 °C, and consumed within seven days of preparation. **Table 2** presents the optimized master formula for the polyherbal anti-dandruff shampoo per 100 mL of final preparation. Three formulations (F1, F2, and F3) were prepared with varying concentrations of Reetha extract and Xanthan gum, as shown in **Table 3** below, with all other components held constant.

**Table 2:** Master formula for polyherbal anti-dandruff shampoo (per 100 mL)

| Ingredient | Functional category | Quantity (per 100 mL) |
|---|---|---|
| *Azadirachta indica* (Neem extract) | Antifungal/antimicrobial | 5 mL |
| *Sapindus mukorossi* (Reetha extract) | Natural biosurfactant | 15 mL |
| *Acacia concinna* (Shikakai extract) | Natural Cleanser/conditioner | 5 mL |
| *Phyllanthus emblica* (Amla extract) | Antioxidant Hair Tonic | 5 mL |
| Aloe Vera gel | Moisturizing/soothing agent | 10 mL |
| Xanthan gum | Viscosity-enhancing agent | 1.5 g |
| Glycerin | Humectant | 2 mL |
| Lemon juice | pH adjuster | q.s. |
| Methyl paraben | Preservative | 0.1% w/v |
| Distilled water | Vehicle | Up to 100 mL |

**Table 3:** Composition of trial formulations F1, F2, and F3

| Ingredient | F1 | F2 | F3 |
|---|---|---|---|
| *Azadirachta indica* (Neem extract) | 5 mL | 5 mL | 5 mL |
| *Sapindus mukorossi* (Reetha extract) | 10 mL | 15 mL | 20 mL |
| *Acacia concinna* (Shikakai extract) | 5 mL | 5 mL | 5 mL |
| *Phyllanthus emblica* (Amla extract) | 5 mL | 5 mL | 5 mL |
| Aloe vera gel | 10 mL | 10 mL | 10 mL |
| Xanthan gum | 1.0 g | 1.5 g | 2.0 g |
| Glycerin | 2 mL | 2 mL | 2 mL |
| Lemon juice | q.s. | q.s. | q.s. |
| Methyl paraben | 0.1% w/v | 0.1% w/v | 0.1% w/v |
| Distilled water | Up to 100 mL | Up to 100 mL | Up to 100 mL |

*Preparation procedure*: Xanthan gum was dispersed incrementally into warm distilled water (60 °C) under continuous magnetic stirring until a smooth, homogeneous, lump-free gel base was obtained. The gel was then cooled to ambient temperature prior to the addition of herbal components. Reetha and Shikakai extracts were

introduced slowly under gentle agitation to minimize excessive foam generation during incorporation. Neem extract, Amla extract, and Aloe vera gel were then added sequentially, with thorough mixing after each addition to ensure uniform dispersion. Glycerin was blended in as the humectant component. Methyl paraben (0.1% w/v), pre-dissolved in warm propylene glycol, was added to provide microbial preservation. Freshly squeezed lemon juice was introduced dropwise, while pH was continuously monitored with a calibrated digital pH meter, until the target pH of 5.0 - 5.2 was achieved. Distilled water was then incorporated to bring the final volume to 100 mL. Completed formulations were transferred to clean, properly labeled containers and allowed to rest for 24 hrs to allow entrapped air bubbles to dissipate before evaluation [2, 14].

All three formulations (F1, F2, and F3) underwent a thorough evaluation encompassing organoleptic assessment, physicochemical characterization, functional performance testing, antifungal activity evaluation, and accelerated stability studies. Each formulation was assessed subjectively for color, odor, physical appearance, consistency, texture, and transparency. Samples were also visually examined for homogeneity, evidence of phase separation, and the presence of grittiness [14]. The pH of each formulation was measured at 25.0 ± 2.0 °C using a calibrated digital pH meter (two-point calibration performed using pH 4.0 and 7.0 buffer solutions) on a 1.0% w/v aqueous dilution. A pH value between 5.0 and 6.5 was designated as the acceptable range, consistent with scalp acid mantle compatibility [14]. Viscosity was determined at 25°C using a Brookfield RV-DV-II Viscometer equipped with Spindle No. 4 operated at 20 rpm. Results are reported in centipoise (cP) as mean ± SD calculated from three independent determinations [14]. 50 ml of a 1.0% w/v shampoo solution was transferred to a 250 mL graduated cylinder and agitated uniformly for 10 strokes. Foam height was recorded immediately (H0) and again after standing undisturbed for five minutes (H5). Foam stability (%) was calculated as: (H5 ÷ H0) × 100 [2]. A Whatman No. 1 filter paper disc (about 0.44 g) was carefully placed on the surface of a 1.0% w/v shampoo solution, and the time for the disc to sink completely was recorded. A shorter wetting time indicates superior wetting and detergent activity [2]. 4.0 g of each formulation was transferred to a pre-weighed evaporating dish and dried to a constant mass at 105 °C. Percentage solid content was derived from the formula: [(mass of residue ÷ mass of sample) × 100] [14].

Cleansing performance was evaluated using wool yarn pre-contaminated with a standardized artificial sebum mixture. Known weights of contaminated yarn were immersed in a 1.0% w/v shampoo solution for five minutes with gentle mechanical agitation, then thoroughly rinsed and dried. Cleansing efficiency (%) was calculated gravimetrically from the mass of grease removed [14]. A single drop of India ink was added to 10 mL of a 1.0% w/v shampoo solution in a graduated cylinder, which was then shaken 10 times. The extent to which ink particles migrated into the foam phase relative to their retention in the aqueous phase was visually graded as low, moderate, or high. A low level of dirt dispersion into foam is preferable, as it minimizes the risk of contaminant redeposition on the hair [2].

*Antifungal activity against malassezia furfur*: Antifungal activity was assessed using the agar well diffusion method on Sabouraud Dextrose agar supplemented with 1.0% v/v olive oil. A standardized inoculum of *Malassezia furfur* (0.5 McFarland, about 1 - 10 × 106 CFU/mL) was spread uniformly across agar plate surfaces. Wells of 8 mm diameter were bored using a sterile cork borer. One hundred microliters each of F2, Neem extract alone, ketoconazole 2.0% w/v (positive control), and distilled water (negative control) were introduced into separate wells. Plates were incubated at 32.0 ± 1.0 °C for 48 - 72 hrs, and zones of inhibition were measured in millimeters (mean ± SD, n = 3) [5]. Stability assessment was conducted in accordance with ICH Q1A(R2) guidelines. Sealed formulation samples were stored at 40°C ± 2 °C / 75% RH ± 5.0% for a total duration of 30 days. Samples were withdrawn at Days 0, 10, 15, and 30 and evaluated for physical appearance, color, odor, pH, viscosity, phase separation, and microbial contamination via standard plate count methodology [9, 21].

## Results and discussion

All three formulations displayed distinctive herbal characteristics and acceptable consistency, with no observable phase separation or grittiness at any point during evaluation. The progressively deepening brown coloration across F1 to F3 was consistent with the increasing Reetha extract concentration, as the saponin-glycoside complexes present in the extract impart a characteristic dark hue. The translucent appearance of F1 can be attributed to its lower Xanthan gum content (1.0 g), whereas F2 and F3 presented the typical opacity associated with higher concentrations of biopolymeric thickener. All three formulations-maintained pH within the physiologically acceptable range of 5.0 - 5.2, confirming their suitability for regular application on the scalp without disturbing the natural acid mantle [3, 6]. Among the three variants, F2 demonstrated the most appropriate viscosity (2430 ± 18 cP), which correlated well with satisfactory spreadability, effective foam generation, and ease of rinsing. F3, with a viscosity of 3125 ± 22 cP, was too thick to pour comfortably, while F1 at 1850 ± 15 cP showed comparatively reduced foam stability. Viscosities in the 2000-3000 cP range are generally recognized as optimal for liquid shampoo formulations [6].

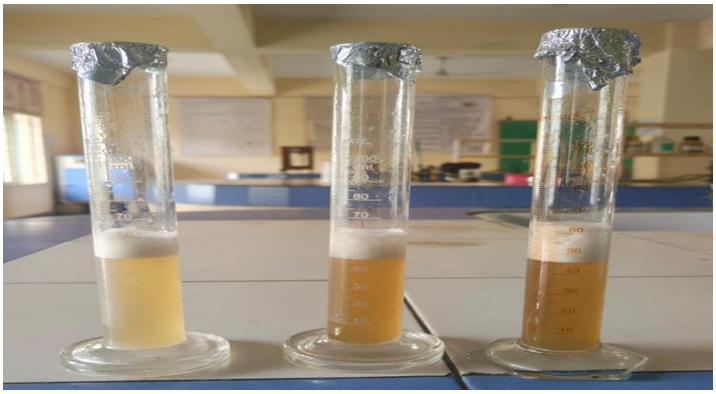
**Table 4:** Organoleptic and physical characterization of formulations F1, F2, and F3

| Parameter | F1 | F2 | F3 |
|---|---|---|---|
| Color | Light brown | Medium brown | Dark brown |
| Odor | Characteristic herbal | Pleasant lemon-herbal | Pleasant lemon-herbal |
| Appearance | Translucent | Opaque | Opaque |
| Texture | Smooth | Smooth | Slightly thick |
| pH | 5.1 ± 0.02 | 5.2 ± 0.03 | 5.0 ± 0.01 |
| Solid content (%) | 24.98 ± 0.12 | 26.14 ± 0.18 | 28.10 ± 0.15 |
| Viscosity (cP) | 1850 ± 15 | 2430 ± 18 | 3125 ± 22 |

**Table 5:** Foam stability and wetting time findings

| Parameter | F1 | F2 | F3 |
|---|---|---|---|
| Initial foam height (cm) | 19.0 ± 0.3 | 16.0 ± 0.2 | 15.0 ± 0.2 |
| Foam height after 5 min (cm) | 14.0 ± 0.2 | 12.0 ± 0.2 | 12.0 ± 0.1 |
| Foam stability (%) | 73.68 | 75.00 | 80.00 |
| Wetting time (sec) | 19.16 ± 0.4 | 19.28 ± 0.3 | 25.35 ± 0.5 |

**Figure 7:** Foam test performed at laboratory scale



All three formulations generated satisfactory foam, despite the total absence of synthetic surfactants, confirming the functional adequacy of Reetha-derived saponins as effective biosurfactants [1, 11]. F1, by virtue of its lower viscosity, produced the greatest initial foam volume (19.0 cm). F3, having the highest Xanthan gum loading, achieved superior foam stability (80.0%), as the elevated bulk viscosity retarded the rate of foam drainage. F2 presented the most well-balanced foam characteristics: adequate initial foam height (16.0 cm), acceptable stability (75.0%), and excellent rinsability. Regarding surface wetting activity, F1 and F2 demonstrated markedly shorter wetting times (19.16 and 19.28 seconds, respectively) compared to F3 (25.35 seconds), indicating stronger detergency and more rapid wetting of hair surfaces [2, 8].

**Table 6:** Cleansing efficiency and dirt dispersion results

| Parameter | F1 | F2 | F3 |
|---|---|---|---|
| Cleansing efficiency (%) | 68.4 ± 0.5 | 82.6 ± 0.6 | 74.1 ± 0.4 |
| Dirt dispersion | Moderate | Low | Moderate |
| Ease of washing | Good | Excellent | Good |

F2 recorded the highest cleansing efficiency (82.6 ± 0.6%), a result attributable to the synergistic surfactant activity of Reetha (15 mL) and Shikakai (5 mL) extracts at concentrations sufficient for robust micelle formation and effective sebum solubilization, while avoiding the viscosity-related impediment to surface wetting observed in F3. The characteristically low dirt dispersion into the foam phase noted for F2 is a particularly favorable quality attribute, as it indicates that particulate contaminants remained preferentially within the aqueous phase rather than migrating into the foam - thereby greatly reducing the probability of contaminant redeposition on hair fibers during rinsing. This characteristic differentiates high-quality shampoo formulations from inferior ones [2, 6].

**Table 7:** Antifungal activity against *Malassezia furfur* (agar well diffusion)

| Sample/treatment | Zone of inhibition (mm) |
|---|---|
| Control (untreated medium) | 0 (no inhibition) |
| Neem extract alone | 12 ± 0.2 |
| Polyherbal shampoo F2 | 17 ± 0.3 |
| Standard - Ketoconazole 2% w/v | 22 ± 0.4 |

Formulation F2 generated a zone of inhibition of 17 ± 0.3 mm against *Malassezia furfur*, representing a clinically relevant antifungal response. The primary antifungal contribution is attributed to the neem-derived limonoids (nimbidin, azadirachtin, gedunin), whose multi-target mechanism - encompassing ergosterol binding, inhibition of chitin biosynthesis, and direct membrane permeabilization - is well supported in the literature. [4, 14] Although ketoconazole (positive control) produced a somewhat larger inhibition zone (22 ± 0.4 mm), the polyherbal formulation achieved approximately 77.0% of the standard drug's antifungal activity while additionally offering scalp-compatible pH, anti-inflammatory benefits, and a superior safety profile with no risk of long-term antifungal drug resistance or adverse effects [20]. The notably larger zone generated by F2 compared to Neem extract alone (12 ± 0.2 mm) strongly suggests additive or synergistic contributions from Shikakai saponins, Amla polyphenols, and Aloe vera acemannan [5, 6, 17].

Formulation F2 retained excellent physicochemical stability throughout the entire 30-day accelerated study period. The minor variations in pH (from 5.2 to 5.1) and viscosity (from 2430 to 2395 cP) fall well within

acceptable tolerance thresholds and carry no practical clinical or functional significance. No phase separation, color alteration, or development of off-odor was detected at any of the designated sampling intervals. The complete absence of microbial contamination at all time points confirms the adequate preservative efficacy of methyl paraben at the incorporated concentration (0.1% w/v). These observations are consistent with stability data reported for comparable herbal cosmetic formulations in the published literature [21, 22].

**Table 8:** Accelerated stability study of formulation F2 (40°C / 75% RH, ICH Q1A(R2))

| Parameter | 0 day | 10 days | 15 days | 30 days |
|---|---|---|---|---|
| Physical Appearance | Acceptable | No change | No change | No change |
| pH | 5.2 | 5.2 | 5.1 | 5.1 |
| Viscosity (cP) | 2430 | 2425 | 2410 | 2395 |
| Phase Separation | Absent | Absent | Absent | Absent |
| Microbial Growth | Absent | Absent | Absent | Absent |

The current investigation was performed exclusively at laboratory scale, and the feasibility of manufacturing at an industrial scale has not been evaluated. Real-time long-term stability studies extending beyond 30 days were not conducted. Clinical assessment of scalp compatibility and therapeutic efficacy in human subjects was outside the scope of this work. Advanced instrumental analyses such as comprehensive rheological profiling, zeta potential measurement, and particle size distribution analysis were not performed. Antifungal evaluation was restricted to in vitro agar well diffusion methodology; minimum inhibitory concentration determination and in vivo experimental models were not employed.

*Conclusion:* This investigation successfully achieved the formulation and evaluation of a fully SLS-free polyherbal anti-dandruff shampoo based on a biosurfactant-driven cleansing platform, incorporating a rational combination of medicinal plant extracts selected for their synergistic cleansing, antifungal, antioxidant, conditioning, and scalp-protective properties. Of the three formulations evaluated, F2 -prepared with 15 mL Reetha extract and 1.5 g Xanthan gum - emerged as the most optimally performing formulation, demonstrating a well-balanced profile across all measured parameters: viscosity, cleansing efficiency, foam stability, antifungal activity against *Malassezia furfur*, and sustained physicochemical stability over 30 days under accelerated conditions. The developed formulation addresses dandruff through multiple synergistic antifungal and anti-inflammatory pathways while circumventing the scalp-barrier disruption, protein denaturation, and environmental persistence associated with synthetic anionic surfactants.

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**Acknowledgements**: The authors extend their sincere gratitude to the Department of Pharmaceutics, Deccan School of Pharmacy, Hyderabad, Telangana, India, for generously providing the laboratory infrastructure and facilities that made this research possible.
**Author's contribution:** SAM conceived and designed the study. MS & FM collected data. MM & MA contributed to data analysis. MS, MM & MA performed the data analysis and interpretation. SAM & MS drafted the manuscript. All authors approved the final version of the manuscript and agreed to be accountable for its contents.
**Conflict of interest:** The authors declare the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
**Ethical issues:** The authors have considered ethical issues, including plagiarism, informed consent, data fabrication or falsification, and double publication or submission.
**Data availability statement:** The raw data that support the findings of this article are available from the corresponding author upon reasonable request.
**Generative AI disclosure:** No Generative AI was used in the preparation of this manuscript.