Current Clinical and Medical Education

Received 23 Jul 2025 | Accepted 23 Aug 2025 | Published Online 28 Sep 2025

vision publisher
Medical Journals

Published By: Vision Publisher

ISSN 2942-0792

CCME 3 (9), 12-25

Development of a multi-extract polyherbal shampoo targeting Malassezia-associated dandruff: formulation optimization, antifungal screening and performance evaluation

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Abstract:

Dandruff is a chronic scalp disorder primarily associated with the proliferation of *Malassezia* species, leading to itching, flaking, and inflammation. The present study aimed to develop and standardize a polyherbal anti-dandruff shampoo with effective antifungal activity using selected medicinal plants traditionally known for scalp and hair care. Extracts of *Punicagranatum*, *Azadirachtaindica*, *Murrayakoenigii*, *Hibiscus rosa-sinensis*, *Sapindusmukorossi*, *Acacia concinna*, *Glycyrrhiza glabra*, and *Citrus sinensis* were prepared using suitable aqueous and hydroalcoholic extraction methods.

The antifungal activity of individual extracts and formulated shampoos was evaluated against dandruff-associated fungal strains using the disc diffusion method. Based on base optimization studies, an optimized formulation was developed incorporating triethanol lauryl sulphate as a surfactant and methyl paraben as a preservative. The prepared polyherbal shampoo was evaluated for physicochemical properties including pH, viscosity, foam stability, wetting ability, cleaning efficiency, and ocular safety.

Preliminary phytochemical screening confirmed the presence of bioactive constituents such as flavonoids, saponins, tannins, glycosides, and phenolic compounds. FT-IR analysis demonstrated the presence of characteristic functional groups and confirmed the compatibility between herbal extracts and formulation excipients. HPLC analysis was performed for standardization using gallic acid, quercetin, and glycyrrhizin as marker compounds, showing well-resolved peaks and good linearity. Quantitative estimation revealed significant amounts of marker compounds, supporting the antifungal and anti-inflammatory potential of the formulation.

Keywords: Polyherbal shampoo; Anti-dandruff formulation; *Malassezia*; Antifungal activity; FT-IR analysis; HPLC standardization; Herbal extracts; Natural surfactants.

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INTRODUCTION

Cosmeceuticals represent one of the fastest-growing segments of the personal care and cosmetic industry. The term *cosmeceuticals*was first introduced by Dr. Albert Kligman to describe products that lie at the interface between cosmetics and pharmaceuticals, offering cosmetic benefits along with certain biological or pharmacological effects. Although these products are primarily intended for aesthetic purposes, they may contribute to maintaining skin and hair health without necessarily exerting a direct therapeutic action. At present, the cosmeceutical sector involves a large number of manufacturers worldwide, with multinational companies accounting for a significant share of the global market.

The use of plants for health and personal care dates back to early human civilization. Beyond fulfilling basic needs, plants have been extensively utilized for therapeutic and cosmetic purposes due to their diverse bioactive constituents. It is estimated that approximately 13,000 plant species worldwide possess medicinal value and are used directly or indirectly as therapeutic agents. In recent years, herbal extracts and plant-derived materials have gained considerable attention in cosmetic formulations owing to their perceived safety, multifunctional benefits, and consumer preference for natural products. Herbal cosmetics are commonly employed for skin protection, enhancement of complexion, and promotion of hair health.

India possesses rich biodiversity and a long-standing tradition of using medicinal plants in healthcare systems. Nearly 7,000–7,500 plant species are reported to be used by indigenous and traditional communities for medicinal purposes. Plant-derived constituents have also played a crucial role in the development of several modern synthetic drugs. Consequently, plant-based materials continue to remain an integral part of phytomedicine as well as herbocosmeceutical formulations for both therapeutic and cosmetic applications.

Herbal cosmetics offer several advantages over synthetic products. Being of natural origin, they are generally free from harsh chemicals and contain naturally occurring nutrients that help maintain healthy skin and hair. These products are considered safer for routine use, with a lower incidence of adverse effects such as irritation or hypersensitivity reactions. Herbal formulations are also compatible with different skin and hair types and are often more cost-effective than synthetic cosmetic products. According to estimates by the World Health Organization, a large proportion of the global population relies on natural products for primary healthcare due to their affordability and favorable safety profile.

In India, cosmetic products are regulated under the Drugs and Cosmetics Act, 1940, which defines cosmetics as substances intended for application to the human body for cleansing, beautifying, or altering appearance. Manufacturers are required to comply with regulatory guidelines to ensure product quality, safety, and consistency.

Hair plays an important protective and aesthetic role in humans. The hair follicle is a specialized mini-organ within the skin that undergoes cyclic phases of growth, regression, and rest. Hair growth and regeneration are regulated by complex interactions between epithelial and mesenchymal cells, along with hormonal and cytokine signaling. Disruption of normal scalp physiology can lead to various hair and scalp disorders, among which dandruff is one of the most common.

Dandruff is a prevalent scalp condition characterized by flaking, itching, and mild inflammation. It commonly appears after puberty and is reported more frequently in males. The condition is closely associated with the overgrowth of lipophilic yeast belonging to the genus *Malassezia* (formerly *Pityrosporumovale*), which is a normal inhabitant of the scalp. Under favorable conditions such as excessive sebum production, climatic changes, or stress, *Malassezia* species proliferate and produce lipase enzymes that hydrolyze sebum triglycerides into free fatty acids, particularly oleic acid. These metabolites penetrate the stratum corneum, causing irritation and increased epidermal cell turnover, leading to visible dandruff flakes.

Shampoos are widely used cosmetic products designed to cleanse the hair and scalp by removing dirt, excess sebum, and environmental contaminants. In addition to cleansing, shampoos are formulated to improve hair manageability, softness, and scalp health. Anti-dandruff shampoos are specialized formulations that contain active agents intended to control fungal growth and restore normal scalp function. These shampoos may be synthetic or herbal in nature.

Herbal anti-dandruff shampoos utilize plant-based ingredients possessing antifungal, anti-inflammatory, cleansing, and conditioning properties. Numerous medicinal plants have traditionally been used for the management of dandruff and scalp disorders. Polyherbal formulations, which combine multiple plant extracts, are believed to offer enhanced efficacy through synergistic action while minimizing adverse effects.

In view of the increasing demand for safe and effective herbal cosmeceuticals, the present study focuses on the development of a multi-extract polyherbal shampoo targeting *Malassezia*-associated dandruff. The formulation aims to combine antifungal efficacy with desirable cosmetic performance through formulation optimization, antifungal screening, and comprehensive performance evaluation.

2. Materials and Methods

2.1 Materials

Fresh plant materials including curry leaves (*Murrayakoenigii*), neem leaves (*Azadirachtaindica*), and hibiscus flowers (*Hibiscus rosa-sinensis*) were collected from local gardens in Mandsaur, Madhya Pradesh, India. Dried herbal raw materials such as pomegranate fruits (*Punicagranatum*), reetha (*Sapindusmukorossi*), shikakai (*Acacia concinna*), orange peels (*Citrus sinensis*), and licorice roots (*Glycyrrhiza glabra*) were procured from authenticated local herbal markets.

All plant materials were taxonomically authenticated by Dr. S. N. Mishra, Head, AINP on M & AP, KNK College of Horticulture. The collected samples were washed thoroughly with distilled water, shade-dried at room temperature to preserve phytoconstituents, coarsely powdered using a mechanical grinder, sieved to obtain uniform particle size, and stored in airtight containers until further use.

2.2 Preparation of Plant Extracts

2.2.1 Curry Leaf Extract

Shade-dried curry leaves were powdered and 15 g of the powder was macerated with 50 mL of distilled water for 24 h with intermittent shaking. The extract was filtered and the process was repeated thrice. The combined filtrates were concentrated to a final volume of 50 mL and stored at 4°C.

2.2.2 Pomegranate Extract

Fresh pomegranate fruits were freeze-dried and powdered. Ten grams of powder were extracted with 250 mL of 80% methanol at 25°C for 24 h using a shaking water bath. The extract was filtered through a 0.45 µm nylon membrane. Aqueous extraction was carried out similarly using distilled water. Extracts were stored under refrigeration until analysis.

2.2.3 Orange Peel Extract

Orange peels were washed, air-dried in a hot air oven at 40°C for 48 h, and powdered. Fifteen grams of powder were soaked in 200 mL distilled water for 24 h with intermittent shaking. The filtrate was concentrated to dryness on a water bath at 70°C.

2.2.4 Shikakai Extract

Powdered shikakai pods were soaked in distilled water for 48 h and boiled for 1 h. The solution was filtered and the marc was re-extracted twice. Combined filtrates were concentrated using a water bath.

2.2.5 Hibiscus Extract

Dried hibiscus flowers (10 g) were boiled with 50 mL of distilled water for 30 min and allowed to stand for 24 h. The extract was filtered and concentrated by evaporation.

2.2.6 Licorice Extract

Licorice powder (1 g) was extracted separately with water and ethanol (50 mL) for 240 min at room temperature. Based on extraction efficiency, aqueous extract (glycyrrhizin-rich) and ethanolic extract (glabridin-rich) were selected for further studies.

2.2.7 Neem Extract

Neem leaf powder (50 g) was macerated with 250 mL ethanol for 24 h with periodic shaking. The extraction was repeated three times and pooled extracts were concentrated on a water bath.

2.2.8 Reetha Extract

Powdered *Sapindusmukorossi* fruits were extracted using water, boiling water, ethanol, and 50% ethanol at solid-to-liquid ratios of 1:10 and 1:20. The mixtures were stirred for 6 h, filtered, and concentrated.

2.3 Microorganisms

Fungal strains associated with dandruff were obtained from MTCC, Chandigarh, India. Cultures were maintained on Dixon agar medium at 28°C and sub-cultured periodically.

2.4 Antifungal Activity

2.4.1 Disc Diffusion Method

Antifungal activity was evaluated using the disc diffusion technique. Test solutions (1000 µg/mL) were prepared by dissolving extracts in distilled water. Sterile Whatman filter paper discs (7 mm) were impregnated with test solutions and standard ketoconazole.

Prepared Dixon agar plates were seeded with fungal cultures and discs were aseptically placed on the surface. Plates were incubated at 37°C for 24–48 h. Zones of inhibition were measured in millimeters and percentage inhibition was calculated.

2.5 Formulation of Polyherbal Shampoo

Based on base optimization studies, polyherbal shampoos were formulated by incorporating various herbal extracts in defined proportions. Herbal extracts were first dispersed in distilled water, followed by gradual addition of triethanol lauryl sulphate under continuous stirring. Methyl paraben was added as a preservative.

The pH was adjusted to 5.5–6.0 using 1.5% citric acid solution. Rose oil was added for fragrance, and the final volume was adjusted to 50 mL with distilled water.

2.6 Evaluation of Polyherbal Shampoo

The formulated shampoos were evaluated for physical appearance, pH, viscosity, foam formation and stability, cleaning efficiency, wetting ability, and eye irritation potential using standard procedures.

2.7 Phytochemical Screening

Preliminary phytochemical analysis of extracts was performed to detect alkaloids, carbohydrates, glycosides, reducing sugars, saponins, proteins, phenolics, and tannins using standard qualitative tests.

2.8 FT-IR Analysis

FT-IR spectroscopy was used to identify functional groups and assess compatibility between herbal extracts and formulation excipients. Spectra were recorded in the range of 4000–400 cm^{-1}. The optimized formulation showed no significant peak shifts, indicating absence of chemical interaction.

2.9 HPLC Analysis

2.9.1 Chromatographic Conditions

HPLC analysis was carried out using a C18 reverse-phase column with acetonitrile:water (0.1% orthophosphoric acid) as the mobile phase (60:40 v/v). The flow rate was maintained at 1.0 mL/min and detection was performed at 254 nm.

2.9.2 Quantification of Marker Compounds

Gallic acid, quercetin, and glycyrrhizin were selected as marker compounds. Calibration curves showed good linearity and were used to quantify marker compounds in the formulation, confirming standardization and batch consistency.

2.10 Statistical Analysis

All experiments were performed in triplicate and results were expressed as mean ± standard deviation.

3. RESULTS & DISCUSSION

Table 5: Phytochemical Screening Test

Chemical Tests	Pomegra-nate	Neem	Curry Leaves	Hibiscus	Reetha	Shikakai	Liquorice	Orange Peel
Alkaloids	+	+	-	+	-	+	+	+
Carbohydrates	-	+	-	-	+	-	+	-
Glycosides	-	-	+	+	-	-	+	+
Reducing Sugar	-	+	+	-	-	+	-	-
Sapponins	+	+	+	+	+	+	+	+
Test for Protiens	-	-	-	-	-	-	+	-
Tests for phenolic compounds & Tannins	+	+	+	+	-	-	+	+

Table 6: Evaluation of Formulation for Physical Appearance and pH

Formulation	Physical appearance	pH
F1	Dark brown	5.23
F2	Dark brown	5.42
F3	Dark brown	5.22
F4	Dark brown	5.62
F5	Dark brown	5.51
F6	Dark brown	5.42

The pH of shampoos has been shown to be important for improving and enhancing the qualities of hair, minimizing irritation to the eyes and stabilizing the ecological balance of the scalp. Mild acidity prevents swelling and promotes tightening of the scales, there by inducing shine. As seen from(Table 6) all six formulations shows the shampoos were acid balanced and were ranged 5-5.6, which is near to the skin pH and all formulations shows good pH range and also good physical appearance.

Table 7: Viscosities & Solid Contents of Developed Formulations

Formulation	Viscosity (Cp, at 10rpm)	**% Solid contents**
F1	1260	21.02
F2	2478	23.05
F3	4259	24.10
F4	5789	27.02
F5	6259	28.06
F6	9058	29.01

The result of viscosity & percent of solids contents is tabulated in (Table 7), and percent of solids was found between 21-29%. As a result, they were easy to wash out. If the shampoo has too many solids it will be hard to work into the hair or too hard to wash out Viscosity of all six formulations increased with increase in concentration.F1 shows less viscosity as compared to other formulation and F6 shows highest viscosity. In order to better observe the difference between shampoos, the viscosity at a low rotational speed (10rpm) were compared. As we found the literature that the shear rate applicable to the flow from the bottle are about 5-10rpm.

Table 8: Cleansing, Surface Tension and Detergency Parameters of Developed Formulation

Formulation	Cleaning (%)	Surface tension (dynes/cm)	Detergency (%)
F1	25.50	28.23	58.54
F2	27.21	29.06	60.23
F3	27.52	31.22	61.29
F4	28.16	32.43	62.25
F5	29.23	33.21	62.89
F6	29.72	33.56	63.22

It has been mentioned that a proper shampoo should be able to decrease the surface tension of pure water to about 40 dynes/cm. The reduction in surface tension of water from 72.8 dynes/cm to 34.2 dynes/ cm by the herbal shampoos is an indication of their good detergent action. The results are shown in (Table 8). Cleaning action was tested on wool yarn in grease. As seen from the results, there is a significant difference in the amount of sebum removed by the different shampoos.F6 formulation show good cleaning action.

Table 9: Evaluation of Foam Stability of Polyherbal Shampoo Formulations

Time (min)	Foam volume					
	F1	F2	F3	F4	F5	F6
1min	173	170	172	175	171	172
2min	172	169	170	173	169	171
3min	170	167	169	172	167	168
4min	169	166	167	170	166	167
5min	167	165	166	169	164	166

All the six shampoo formulations showed similar foaming characteristics in distilled water. All six formulations showed comparable foaming properties. The foam stability of herbal shampoos is listed in (Table 9). A point to be noted here is that there does not seem to be any correlation between detergency and foaming, which only confirms the fact that a shampoo that foams well need not clean well.

Table 10: Stability Studies of Optimized Formulation (F6)

Parameters	After 1month	After 2month	After 3month
Physical appearance/visual inspection	clear	clear	clear
pH	5.20	5.23	5.26
Solids contents (%)	24.13	23.78	23.56
Surface tension measurement (dy. /cm)	31.69	32.21	32.43
viscosity	9057	7043	6352
Detergency ability (%)	66.21	64.23	62.77
Foaming ability and foam stability (ml)	170	168	166

Stability and acceptability of organoleptic properties (odor and color) of formulations during the storage period indicated that they are chemically and physically stable. The stability of herbal formulation is listed in (Table 10).which shows that the stability of F6 formulation in terms of all parameters is not more changed as compared to the prepared previous formulation.

Table11: Antimicrobial Activity of Polyherbal Shampoo (50ml)

formulation	Test organism	concentrations (mg/mL) (zone of inhibition in mm)			
		50	100	150	200
F1	*Malassezia furfur.*	21	20	18	16
F2	-	22	20	18	17
F3	-	23	18	16	15
F4	-	24	20	17	16
F5	-	25	23	21	19
F6	-	28	26	24	21
Base	-	10	8	7	5
Synthetic Marketed product	-	30	28	27	25
Herbal marketed product	-	28	27	26	25

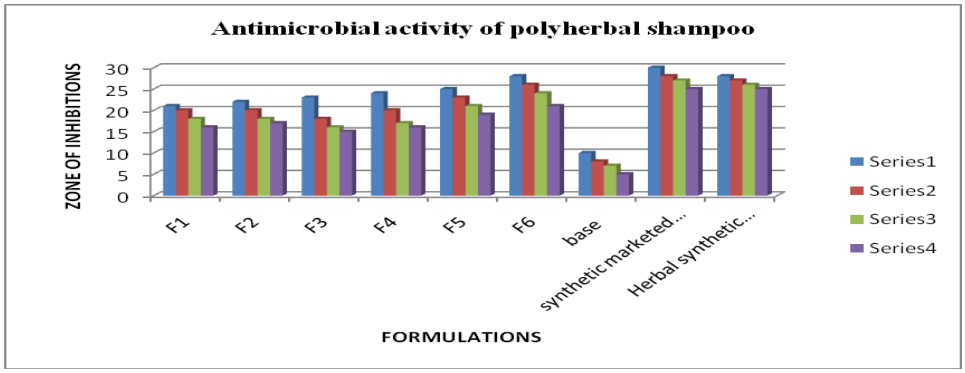


Figure 15: Antifungal activities of prepared formulations [F1-F6]

Figure 16: Nutrient media plate

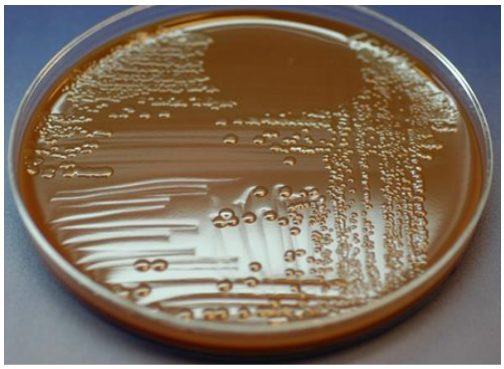


Figure 17: Plate after inoculation

Figure 18: Plate after fungus growth

Figure 19: Zone of inhibition F1&F2

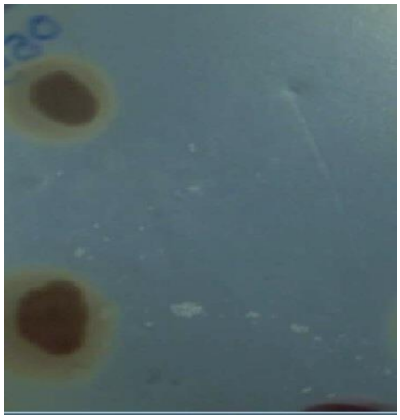


Figure 19: zone of inhibition F3 & F4

Figure 20: zone of inhibition F5&F6

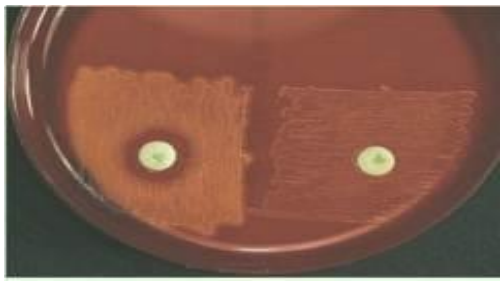
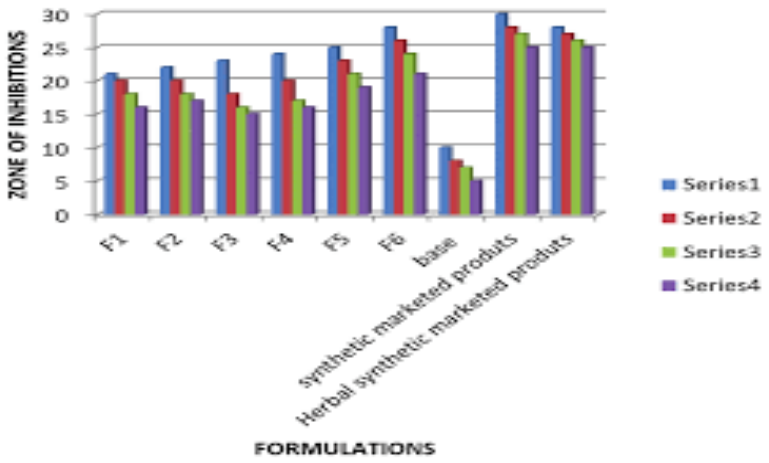
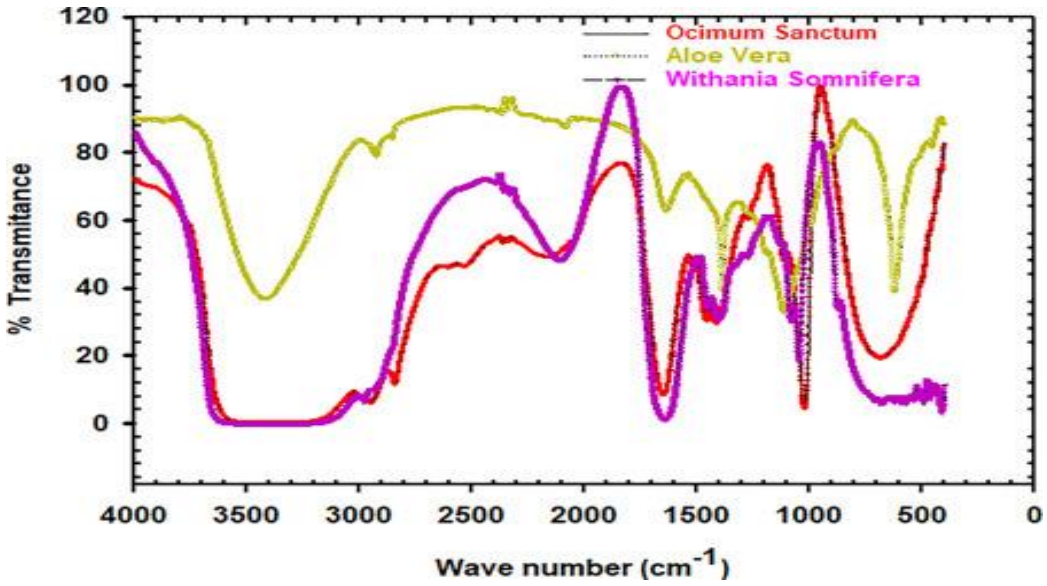


Figure 21: Zone of inhibition of marketed formulations





4. CONCLUSION

The present study was aimed at developing a completely herbal shampoo with properties comparable to, or exceeding, those of commercially available synthetic shampoos. A polyherbal shampoo was formulated using plant extracts traditionally recognized for their hair cleansing, conditioning, and antifungal properties across Asia.

The ingredients selected—Pomegranate (Punicagranatum), Reetha (Sapindusmukorossi), Shikakai (Acacia concinna), Orange peel (Citrus sinensis), Licorice (Glycyrrhiza glabra), Curry leaves (Murrayakoenigii), Neem leaves (Azadirachtaindica), and Hibiscus flower (Hibiscus rosa-sinensis)—were incorporated to provide a multifunctional hair care product. These botanical extracts offer a safer alternative to synthetic conditioners such as silicones and polyquaterniums, minimizing the risk of adverse effects often associated with chemical-based hair care formulations.

Instead of using conventional cationic conditioners, natural extracts such as Shikakai, Hibiscus, and Reetha were employed to impart conditioning, smoothness, and manageability to hair. Moreover, Pomegranate, Curry leaves, and Neem leaves were incorporated for their antifungal properties, particularly targeting Malassezia furfur, which is one of the primary organisms responsible for dandruff and seborrhoeic dermatitis. Pomegranate, a novel addition in this context, exhibited significant antifungal activity, especially when combined with other herbal extracts at optimized concentrations.

Extensive physicochemical evaluations were performed to assess critical quality parameters such as pH, foam stability, wetting ability, detergency, solubility, and eye irritation potential. The shampoo demonstrated good clarity, stability, acceptable viscosity, and effective cleansing properties. Foam formation and stability, as well as wetting time, were found to be satisfactory, ensuring a consumer-friendly experience. The antifungal activity of the polyherbal shampoo showed promising results, indicating its potential as an effective natural alternative to commercial anti-dandruff shampoos.

In conclusion, this study demonstrates that a well-formulated polyherbal shampoo can provide effective hair cleansing, conditioning, and antifungal activity, while remaining safe, natural, and cost-effective. However, further research is required to enhance the formulation's aesthetic attributes such as fragrance, foam quality, and long-term stability to ensure complete commercial viability. Additionally, clinical trials on human volunteers could provide more robust evidence of its efficacy and safety.

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