
ORIGINAL ARTICLE

Formulation and Stability Studies on Poly Herbal Anti Dandruff Shampoo

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ABSTRACT

A novel polyherbal shampoo was formulated using extracts from Neem (*Azadirachta indica*), Tulasi (*Ocimum sanctum* L.), Bhringraj (*Eclipta prostrata*), curry leaves (*Murraya koenigii*), soapnut (*Sapindus mukoossi*), aloe vera (*Aloe barbadensis* Miller), and hibiscus (*Hibiscus rosa sinensis*). This shampoo aimed to harness the synergetic effects of these botanical ingredients to manage scalp oil levels, eliminate excess sebum, and eradicate dandruff. Comprehensive physicochemical evaluations, including pH balance, wetting time, solid content percentage, foaming capacity and retention, surface tension, detergency, and dirt dispersion were conducted to characterize the formulation's performance attributes. The product exhibited significant foam stability with small bubble size, low surface tension, and excellent cleaning properties five minutes post-application. Antifungal efficacy was demonstrated against *Candida albicans*, evidenced by the inhibition zone assay. The findings suggest that this polyherbal formulation could be effectively commercialized, offering consumers a natural and efficacious alternative to synthetic anti-dandruff shampoos.

Keywords: Polyherbal Shampoo, Antifungal Activity, Physicochemical Properties, *Candida albicans*, Natural Extracts.

Received 26.10.2025

Revised 11.01.2026

Accepted 23.02.2026

How to cite this article:

Sanjeeva Kumar A, Likhitha Reddy K, Lokeswari M, Bhakti T, Anil Kumar N, Mohitha P and Niranjan Babu M. Formulation and Stability Studies on Poly Herbal Anti Dandruff Shampoo. Adv. Biores. Vol 17 [2] February 2029. 332-339

INTRODUCTION

Herbs are multifunctional plants valued for their aroma, flavor, and wide range of therapeutic properties. Unlike spices, which are derived from seeds, roots, or bark, herbs are primarily obtained from the leafy parts of plants. Commonly used herbs such as Neem, Tulasi (Holy Basil), Bhringraj, Curry Leaves, Soapnut (Reetha), Aloe vera, and Hibiscus have long been recognized in traditional medicine for their medicinal and cosmetic benefits [1-3].

Neem (*Azadirachta indica*) is well known for its strong antimicrobial and antioxidant properties. It inhibits microbial growth by disrupting cellular structures, while compounds such as azadirachtin interfere with insect feeding and survival. Neem extracts, particularly ethanolic extracts, have demonstrated significant activity against pathogens like *Staphylococcus aureus* (including resistant strains) and fungi such as *Aspergillus* and *Rhizopus*. Similarly, Tulasi (*Ocimum sanctum*) exhibits potent antifungal activity against organisms like *Aspergillus*, *Rhizopus*, *Penicillium*, and *Fusarium*, largely due to bioactive constituents such as methyl chavicol and linalool [4,5].

Bhringraj (*Eclipta alba*), often referred to as "Kesharaj" or the "king of hair," is rich in proteins, vitamins, and antioxidants that support hair health, promote growth, and delay premature greying. Its phytoconstituents, including wedelolactone and flavonoids, contribute to its effectiveness as a natural hair tonic. Curry leaves (*Murraya koenigii*) are also valued for their antimicrobial and anti-inflammatory properties and have been traditionally used to manage digestive disorders and infections [6].

Soapnut (*Sapindus mukorossi*), commonly known as Reetha, contains natural saponins that provide cleansing and antifungal effects, particularly against organisms such as *Candida albicans* and

Trichophyton rubrum. Aloe vera is widely appreciated for its soothing, moisturizing, and healing properties, making it a key ingredient in dermatological and cosmetic applications. Hibiscus, rich in vitamin C, amino acids, and antioxidants, supports hair growth, strengthens follicles, and soothes the scalp due to its anti-inflammatory nature [7-12].

Building upon both traditional knowledge and modern scientific evidence, these herbs collectively offer antimicrobial, nourishing, and protective effects. The present study focuses on formulating a poly-herbal shampoo incorporating these natural ingredients to promote scalp health, enhance hair quality, and provide a safer, plant-based alternative to conventional synthetic formulations.

MATERIAL AND METHODS

Materials:

Table 1: Crude drugs required for the poly herbal anti dandruff shampoo

S. No	Crude Drugs	Collection
1.	Bhringraj	Collected from nearby villages of Tirupati
2.	Neem	Collected from nearby villages of Tirupati
3.	Hibiscus	Collected from nearby villages of Tirupati
4.	Aloe vera	Collected from nearby villages of Tirupati
5.	Curry leaves	Collected from nearby villages of Tirupati
6.	Rice water	Collected from home
7.	Tulasi	Collected from nearby villages of Tirupati
8.	Soapnut	Collected from Local Market.

Table 2: Chemicals required for the polyherbal anti dandruff shampoo

S. No	Chemicals	Manufacturer
1.	Sodium Lauryl Sulphate	NICECHEMICAL(P)LTD.Manimala Road, Edappally, Kochi-682024, Kerala, India.
2.	Sodium Chloride	Finar Chemicals Limited.,Ahmedabad-382110 Gujarat India.
3.	Gelatin	Finar Chemicals Limited.,Ahmedabad-380006 India.
4.	Citric acid	MOLYCHEM 78/80 Babu Genu Road, Mumbai 400002. India
5.	Tween 80	NICE CHEMICAL (P)LTD. Manimala Road, Edappally, Kochi-682024,Kerala, India.
6.	EDTA	SiscoResearch Laboratories Pvt.Ltd. PlantSite2:H-4,MIDC,Taloja-410208,Maharastra, India.

Methods:

Collection:

The botanical ingredients for the multi-herbal anti-dandruff shampoo are sourced from the adjacent locale of Venkataramapuram. The collection of these herbs is ideally carried out in the morning once the dew has evaporated, and it is performed with the use of sterilized, sharp scissors to ensure cleanliness and precision.

Drying:

To maintain the integrity of herbs' healing qualities, it's essential to dry them completely. This is done by suspending the herb clusters upside down in an area that's cool, shaded, and has good air flow. While the duration required for drying can fluctuate, it typically ranges from one to two weeks. It's crucial to ensure the herbs are entirely devoid of moisture to inhibit any mold formation.

Size Reduction:

Once the herbs are thoroughly dried, separate the leaves, stems, or flowers from the primary plant structure, being sure to eliminate any parts that show signs of mold or damage. Then, to prepare the herbs for inclusion in shampoo, employ tools like a mortar and pestle, grinder, or scissors to process them into the required consistency, which could range from a rough cut to a fine powder, tailored to the specific formula of the shampoo being produced.

Extraction:

For each ingredient, measure out 10 grams of the prepared powder and mix it with 5 milliliters of ethanol. Then, add distilled water until the total volume reaches 100 milliliters. Securely cover the beaker with aluminum foil to protect the contents and set it aside for a period of 72 hours. Remember to agitate the beaker gently every two hours to ensure the mixture infuses properly [13].



Figure 1: Extractions of formulations

Filtration:

After the extract has steeped for 72 hours, proceed by filtering it through filter paper or a cheesecloth to remove any particulate matter. Capture the filtered extract, which contains all the active compounds. Then, transfer this extract to a porcelain dish and apply gentle heat using a heating mantle. Set the temperature to 120 degrees and maintain the heat for 30 minutes. After this process, set the dish aside to cool or for further use

Table 3: Formulation table

S. No	Ingredients	Functions	F1	F2	F3	F4	F5	F6	F7	F8
1	Bhringraj	Slow down hair graying	2%	3%	1%	4%	3%	2%	1%	3%
2	Neem	Antibacterial	4%	3%	2%	3%	1%	4%	2%	1%
3	Hibiscus	Remove dandruff and itchininess	1%	3%	2%	4%	1%	2%	3%	2%
4	Tulasi	Antifungal	3%	2%	4%	1%	2%	3%	1%	3%
5	Aloevera	Remove dead cells	2%	1%	3%	2%	1%	2%	3%	2%
6	Lemongrass oil	Fragrance	1%	2%	1%	3%	2%	1%	2%	1%
7	Curry leaves	Moisture the scalp	3%	1%	2%	2%	3%	2%	1%	3%
8	Rice water	Prevent hair fall	2%	3%	1%	3%	1%	1%	3%	2%
9	Soapnut	Cleanser	1%	2%	3%	1%	2%	3%	1%	1%
10	Sodium lauryl Sulphate	Detergent	10 %	20 %	30%	-	-	-	15%	15%
11	Sodium chloride	Thickening agent	3%	3%	3%	-	-	-	1%	-
12	Gelatin	Natural gum	-	-	-	10 %	20%	30%	-	-
13	Citric acid	Ph adjuster & Preservative	-	-	-	-	-	-	0.3%	0.3%
14	Tween 80	Solubilizing agent	-	-	-	-	-	1%	1%	-
15	EDTA	Chelating agent	-	-	-	-	-	1%	1%	-

Anti-microbial activity assay:

To evaluate the antifungal efficacy, we utilized the Zone Inhibition Technique, also known as the Kirby-Bauer method. Sabouraud Dextrose Agar (SDA) plates were first spread with 100 microliters of the *C. albicans* fungal culture, standardized to a 0.5 McFarland standard which approximates a cellular density of 1.5×10^8 colony-forming units per milliliter. Discs impregnated with 10 microliters of varying concentrations, ranging from 0 to 100 milligrams per milliliter, were then applied to the plates. Additionally, a disc containing only the solvent served as the vehicle control, and a disc with 50 micrograms of Amphotericin B was used as a benchmark positive control. Following incubation at 37 degrees Celsius for 24 hours in a Basil Scientific Corp. incubator, the diameter of the inhibition zones around each disc was measured and documented to assess antifungal action [14].

Evaluation:

Visual Inspection:

Produced formulations were visually inspected for Colour.

Foaming ability and Foam stability:

The foam-producing capacity of the shampoo was assessed by the cylinder shake technique. Initially, 3 milliliters of shampoo were mixed with 10 milliliters of water. This mixture was vigorously shaken for less than a second, done twice in succession, and then left undisturbed for a quarter of an hour. Post-shaking, the volume of the resulting foam was measured, focusing solely on the foam's volume for calculations. This measurement was taken four times at one-minute intervals immediately after the

shaking. The foam's volume remained consistent for approximately five minutes, indicating the shampoo's ability to generate a stable and abundant lather, likely owing to the inclusion of soap nut in its formulation.

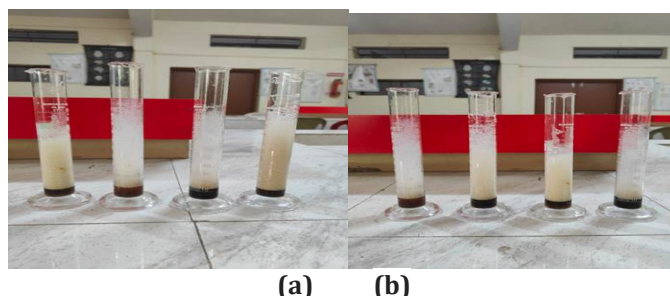


Figure 2 (a, b): Foam test of formulation

Determination of pH:

The pH of 10%v/v shampoo solution in distilled water was measured by using calibrated pH meter [15].

Percentage of Solid content:

First, take a clean and dry China dish and record its weight. Next, dispense 4 milliliters of shampoo into the dish and weigh it again to determine the exact weight of the shampoo added. Place the dish with the shampoo on a hot plate and heat it until all the liquid has evaporated. Once dry, reweigh the dish to ascertain the weight of the non-volatile components of the shampoo [16].

Wetting time:

The wetting time was assessed by measuring how long it took for a canvas paper to absorb water completely. For this test, a 1-inch circular sample, weighing 0.44 grams, was cut from canvas paper. This disk was then carefully placed atop the surface of a 1% (v/v) shampoo solution. Using a stopwatch, the duration from the paper disk's placement to the moment it submerged was recorded, providing the wetting time [17].

Stability study:

The formulation's stability was monitored over four weeks while maintaining a temperature range between 25° and 30°C. It was observed that the shampoo remained uncontaminated by microbes and retained its stability when stored at ambient temperature [18].

Surface tension:

Select a clean stalag meter fixed formally in vertical position and suck the water through the rubber tube to a level higher than the upper mark. Leave the rubber tube and allow water to flow down. Count no number of water drops when it just passes the upper mark into the clean vessel note the weight of N number of drops of reference standard that is water and then carate. The mean weight of drops has W2. Repeat the process with the sample liquid whose surface tension has to determine and note the mean weight of drop has W1. Surface tension can be calculated by using following formula [19].

$$\frac{\sigma_1}{\sigma_2} = \frac{W_1}{W_2}$$

W1= weight of sample drops, W2= weight of water drops, σ_1 =surface tension of sample, σ_2 =Surface tension of water

Ink Dispersion:

After shaking, the dispersion of the ink within the foam was observed and estimated. The amount of ink in the foam was categorized as 'none to mild', 'moderate', or 'heavy'. This subjective assessment provided an indication of the hand wash's ability to lift and disperse dirt particles, as the foam's capacity to hold ink particles could be correlated with its potential to remove and contain dirt from the skin during the washing process [20].

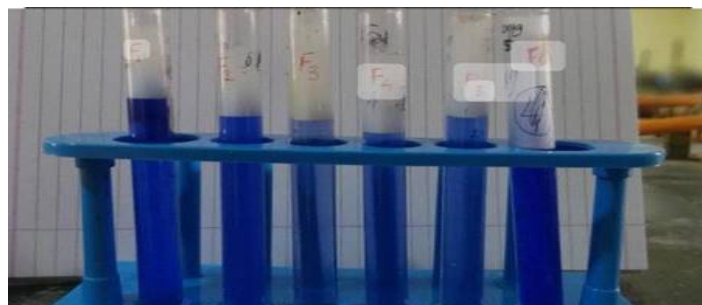


Figure 3: Ink Dispersion results

RESULT AND DISCUSSION

Table 4: Characters of poly-herbal anti-dandruff shampoo

S.NO	Name of the extract	Colour	Consistency	Percentage Yield (W/W)
1.	Neem leaves extract	Green	Semisolid	2.2
2.	Tulasi leaves extract	Green	Semisolid	2.9
3.	Bhringraj leaves extract	Green	Semisolid	2.9
4.	Curry leaves extract	Green	Semisolid	2.3
5.	Hibiscus flowers extract	Red	Semisolid	2.8
6.	Soapnut fruit extract	Brown	Semisolid	2.4

The poly-herbal anti-dandruff shampoo was formulated using extracts from six medicinal plants, each of which was assessed for percentage yield (w/w) and physical characteristics. Extracts of Neem, Tulasi, Bhringraj, and Curry leaves were green in appearance and exhibited a semisolid consistency, with yields of 2.2%, 2.9%, 2.9%, and 2.3%, respectively. The Hibiscus flower extract showed a red coloration with a semisolid texture and a yield of 2.8%, whereas the Soapnut extract appeared brown and semisolid, with a yield of 2.4%. The consistent semisolid nature observed across all extracts suggests their suitability for uniform blending and stable incorporation into the shampoo base, as indicated in Table 4.

The inclusion of these plant extracts is supported by their long-standing use in traditional medicine for managing scalp conditions. They are rich in various phytochemicals such as flavonoids, saponins, tannins, and phenolic compounds, which are known to exhibit antimicrobial and soothing properties. The green color of most extracts can be linked to chlorophyll content, while the red coloration of Hibiscus is due to anthocyanins. These natural compounds may contribute not only to the functional benefits of the formulation but also to its visual appeal [7].

Differences in extraction yield among the plant materials may be attributed to variations in their chemical composition and extraction efficiency. However, a higher yield does not necessarily guarantee greater effectiveness. The overall activity of the formulation depends more on the presence and interaction of bioactive constituents rather than quantity alone. The combination of multiple herbal extracts may result in a synergistic effect, enhancing the overall performance of the shampoo [11-3].

While the initial observations indicate encouraging properties, further studies are required to substantiate the efficacy and safety of the formulation. Detailed investigations, including pharmacological and clinical evaluations, will be necessary to validate its potential as an effective natural anti-dandruff product.

Table 5: Evaluation of prepared polyherbal shampoo formulation

Evaluation parameters	F1	F2	F3	F4	F5	F6	F7	F8
Visual Inspection	Brick red	Dark brown	Dark brown	Light brown	Dark brown	Dark brown	Brick red	Dark brown
Foaming ability & Foam stability	110ml	150ml	140ml	100ml	130ml	120ml	100ml	150ml
Determination of pH	6.54	7.74	7.67	6.32	5.86	7.57	6.25	7.43
Percentage of Solid content	25%	26%	21.7%	23.8%	24%	25.3%	22.5%	21%
Wetting Test	35 sec	40 sec	45 sec	90 sec	80 sec	85 sec	55 sec	50 sec
Stability Test	Stable	Stable	Stable	Stable	Stable	Stable	Stable	Stable
Surface tension	32.15	33.22	31.37	34.60	33.61	35.43	33.23	31.23
Dirt dispersion	Moderate	Moderate	Light	Moderate	Light	Light	Moderate	Light

The table presents various evaluation parameters for eight formulations of polyherbal shampoo. Visual inspection revealed variations in color, ranging from brick red to light and dark brown. Foaming ability and foam stability were measured, with F2 and F8 showing the highest foam volume at 150 ml, and F4 and F7 the lowest at 100 ml. The pH levels varied from slightly acidic at 5.86 (F5) to more alkaline at 7.74 (F2), while the percentage of solid content was highest in F2 at 26% and lowest in F8 at 21%. The wetting test times ranged significantly, with F4 taking the longest at 90 seconds and F1 the shortest at 35 seconds. All formulations were stable upon the stability test. Surface tension measurements ranged from 31.23 (F8) to 35.43 (F6). Dirt dispersion was noted as light in F3, F5, and F6, and moderate in the other formulations.

Visual inspection results suggest that color variations may be due to the concentration and type of extracts used, which could influence consumer preference. The higher foaming ability of F2 and F8 might appeal to consumers who associate foam with cleaning efficacy, though it does not necessarily correlate to better cleaning. The pH values are within the range typical for shampoos, with F5 being the most acidic, potentially better for maintaining scalp health, and F2 the most alkaline, which might be harsher on the hair and scalp.

The solid content indicates the concentration of active ingredients in the shampoo; F2 had the highest, which could imply better cleaning potential or conditioning effects, though it may also impact the rinse-off characteristics. Wetting time is indicative of how quickly the shampoo will spread on the hair and scalp; shorter times, such as in F1, suggest quicker action, which can be a selling point.

Stability across all formulations is a positive result, indicating that the shampoos are likely to maintain their properties over time. Surface tension relates to the shampoo's ability to remove oil and dirt from the hair; lower values, as in F3 and F8, typically indicate better cleaning ability. Dirt dispersion capabilities also contribute to cleaning effectiveness, with light dispersion potentially being less effective than moderate dispersion.

The optimal formulation would depend on the desired shampoo characteristics, balancing between foaming ability, pH balance for scalp health, wetting time for convenience, and cleaning efficiency. Formulations F2 and F8 show promise with high foaming and lower surface tension but may require adjustments to pH for a more skin-friendly product. Further studies could look into the consumer sensory experience and clinical testing to evaluate the overall performance and acceptance of these formulations.



Amount present per disc in µg
 Dispensed Volume- 10µL
 Positive Control - 50 µg

Figure 4: Antifungal activity

Table 6: Antifungal activity of polyherbal antidandruff shampoo on *C.albicans*

Amount (µg/Disc)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	18	18	19	18.333333	0.57735	0.408248
0	0	0	0	0	0	0
50	9	9	10	9.333333	0.57735	0.408248
125	8	8	10	8.666667	1.154701	0.816497
250	10	10	8	9.333333	1.154701	0.816497
500	10	11	11	10.66667	0.57735	0.408248
1000	12	12	12	12	0	0

The antifungal activity of the polyherbal shampoo was assessed at concentrations ranging from 50 to 1000 µg/disc. The positive control demonstrated a pronounced zone of inhibition (ZOI) with an average diameter of 18.33 mm. In comparison, the shampoo exhibited no inhibitory effect at 0 µg/disc, confirming

the validity of the control conditions. At increasing concentrations, the shampoo produced measurable zones of inhibition, reaching a maximum of 12 mm at 1000 µg/disc.

A clear concentration-dependent trend was observed, with antifungal activity improving as the dose increased. This indicates that the formulation possesses dose-responsive antifungal potential against *Candida albicans*. However, at all tested concentrations, the activity of the shampoo remained lower than that of the standard antifungal agent, as shown in Table 6 and Figure 4.

Interestingly, the results at intermediate concentrations (125 µg/disc and 250 µg/disc) did not show a strictly linear increase compared to lower doses, suggesting the possibility of a threshold effect or minor experimental variation. This may indicate that beyond a certain concentration, the increase in antifungal efficacy is not proportionally significant.

The absence of inhibition at 0 µg/disc confirms that the observed antifungal effects are solely due to the active components of the formulation. Furthermore, the low standard deviation (SD) and standard error of the mean (SEM) values across all readings demonstrate good consistency and reliability of the experimental data.

Overall, the findings confirm that the polyherbal shampoo possesses moderate antifungal activity with a clear dose-dependent response. While it does not match the potency of the standard drug, its natural composition and consistent performance highlight its potential as a safer alternative for managing dandruff, warranting further investigation [18,19].

CONCLUSION

The poly-herbal anti-dandruff shampoo formulated with Neem, Tulasi, Bhringraj, Curry leaves, Hibiscus, and Soapnut demonstrates promising stability, acceptable physical characteristics, and notable therapeutic potential. Among the tested formulations, F2 and F8 showed superior foaming, while F1 exhibited optimal wetting ability. Although most formulations performed well, slight pH adjustments may be needed to ensure scalp compatibility. The presence of substantial solid content, particularly in F2, suggests good active ingredient concentration. Cleansing efficiency was supported by favorable surface tension and dirt dispersion results. Antifungal studies against *C. albicans* confirmed dose-dependent activity, though efficacy remained lower than the standard control. Overall, the formulation offers a natural, stable, and potentially safer alternative for dandruff management, despite moderate antifungal potency.

ACKNOWLEDGEMENT

The authors are extremely thankful to Seven Hills college of Pharmacy, Tirupathi, Andhra Pradesh-India.

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