



PHARMACEUTICO- ANALYTICAL AND IN VITRO ANTI-FUNGAL EVALUATION OF POLYHERBAL SHAMPOO IN RELATION TO DANDRUFF

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Article Received on 15 Dec. 2025,
Article Revised on 05 Jan. 2026,
Article Published on 15 Jan. 2026,
<https://doi.org/10.5281/zenodo.18220367>

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How to cite this Article: Dr. Drishya M. V.*¹,
Dr. Surekha S. Medikeri². (2026).
Pharmaceutico- Analytical And In Vitro Anti-
Fungal Evaluation Of Polyherbal Shampoo In
Relation To Dandruff. World Journal of
Pharmacy and Pharmaceutical Sciences, 15(1),
1307–1323.

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ABSTRACT

Shampoo is a cosmetic preparation intended for cleansing the scalp and hair. However, frequent use of chemical-based formulations may lead to adverse effects such as hair fall, premature greying, and microbial resistance. To overcome these drawbacks, a polyherbal shampoo was formulated using hydro alcoholic extracts of *Nimba*, *Karanja*, *Methika* and infusion of *Sathala*, *Japa*, and *Arishtaka* for dandruff management. The study aimed to prepare, analyse, and evaluate the formulated polyherbal shampoo for its physicochemical, analytical, and antifungal efficacy. Phytochemical screening, FTIR, and HPTLC confirmed the presence of flavonoids, phenolics, saponins, and phytosterols. SEM analysis revealed smooth, intact hair cuticles after shampoo treatment, suggesting protective and conditioning effects. *In vitro* antifungal studies showed inhibition zones of 13 mm against *Malassezia furfur* and 15 mm against *M. globosa*. Thus, the formulated

polyherbal shampoo demonstrated effective antifungal properties, supporting its use as a safe, natural alternative for dandruff management.

KEYWORDS: Polyherbal shampoo, Antifungal, Hydro ethanolic extract, Infusion.

INTRODUCTION

Scalp care is essential since it determines the health and condition of hair and prevents the associated diseases. Scalp is characterized by thick skin layer with high follicular density and abundant sebaceous glands. The presence of these glands along with the dark and warm environment make the scalp more prone to mycotic infections like dandruff.^[1]

According to *Vagbata*^[2] and *Sharangadhara*^[3] *Darunaka* is *Kapala roga* but *Susrutha*^[4] and other *acharyas* explained *darunaka* under *kshudraroga*. It is caused by the aggravation of *vata* and *kapha* causing *kandu*, *keshachyuthi*, *swapa*, *rukshatha* and *twak sphutana*.^[2]

Darunaka can be correlated with dandruff which is characterized by the presence of Corneocytes that form clusters due to their high cohesive power in the form of flaky white to yellowish scales accompanied by itching.^[1] Dandruff affects approximately 50% of general adult population worldwide.^[5] Its causes vary among individuals depending upon the susceptibility, but the leading cause is considered to be a fungus named *Malassezia globosa*. The treatment of dandruff primarily includes anti-dandruff agents like Zinc Pyrithione, Selenium sulphide etc.^[1]

Shampoo may be described as a cosmetic preparation meant for the washing of hair and scalp, packed in a form convenient for use. Its primary function is cleansing the hair of accumulated sebum, scalp debris and residues of hair-grooming preparations. The added functions of shampoo include lubrication, conditioning and prevention of static charge build up. Many available shampoos are loaded with chemicals that are hazardous to skin and health which includes premature greying of hair and even hair fall. In a study it has been mentioned that shampoos with Sodium lauryl sulphate could retard healing of wounds in surface of cornea and can cause cataract in adults. It builds up in heart, liver, lungs and brain can cause major problem in these areas. It also causes flaking of skin leading to substantial roughness and corrodes the hair follicle leading to impairment of its ability to grow hair.^[6] Frequent use of these products may also lead to the development of resistance against dandruff causing organisms. Thus, there is a clear need to develop better strategies, which would be safe and free of untoward side effects.^[7]

Hence in the present study an attempt is made to formulate shampoo with extract of *Nimba*, *Karanja*, *Sathala*, *Methika*, *Japa* and *Arishtaka* so as to limit the exposure to synthetic chemicals and to study its anti-fungal activity to evaluate its probable anti-dandruff action.

MATERIALS AND METHODS

Collection of raw drugs and associated excipients

Raw drugs like *Nimba*, *Karanja*, *Methika*, *Sathala*, *Japa*, and *Arishtaka* were procured from Amrithkesari Depot, Mamulpet, Bengaluru.

All the excipients used in the study was procured from pharmaceutical department of Acharya and B M Reddy college of Pharmacy, Bengaluru.

Methods

The pharmaceutical study was carried out in four stages

1. Preparation of coarse powder of individual drugs
2. Preparation of hydro alcoholic extract from the coarse powder of *Nimba*, *Methika* and *Karanja*
3. Preparation of extract of *Japa*, *Arishtaka* and *Sathala* using infusion method
4. Preparation of Polyherbal shampoo using hydro ethanolic extract and infusion.

1. Preparation of coarse powder of individual drugs

Cleaned and dried raw drugs were separately taken in a *Khalwa yantra* and pounded into small pieces. Then the raw drugs were pulverised separately using 40-number mesh. The coarse powder was collected and stored in air tight container.

Table no. 1: Showing the ingredients of polyherbal shampoo.

Drug	Botanical name	Family name	Parts used	Phytoconstituents	Activity
<i>Nimba</i>	<i>Azadirachta indica</i>	Meliaceae	Leaves	Azadirachtin, salannin, Nimbolinnin	Anti-dandruff
<i>Karanja</i>	<i>Pongamia pinnata</i>	Papilionaceae	Seed	Kaempferol	Fungicidal action
<i>Japa</i>	<i>Hibiscus rosa</i>	Malvaceae	Flower	Mucilage	Conditioning action Hair growth promoter
<i>Methika</i>	<i>Trigonella foenumgraecum</i>	Fabaceae	Seed	Nicotinic acid	Anti-dandruff
<i>Sathala</i>	<i>Acacia concinna</i>	Mimosaceae	Pod	Saponin	Cleansing action
<i>Arishtaka</i>	<i>Sapindus mukorossi</i>	Sapindaceae	Fruit	Saponin	Cleansing action

2. Preparation of hydro alcoholic extract from the coarse powder of *Nimba*, *Methika* and *Karanja*

15g each of dried coarsely powdered *Nimba*, *Methika* and *Karanja* are placed inside a muslin cloth and tightly closed with thread. The extraction solvent, hydro ethanolic solution (Absolute ethanol AR grade 99.9 %-160 ml and distilled water-40 ml) in the ratio 80:20 is poured into the round bottom flask. This one is connected with the extraction chamber in which the thimble is placed. On the top of extraction chamber, a condenser is connected to cold water inlet and outlet tubing to allow continuous condensation of the solvent vapours. The solvent is then heated from the round bottom flask. The temperature was maintained between 85 to 90 °C. The evaporated vapours were passed through the condenser where it condenses and flow down to the extraction chamber and extracts the drug by coming in contact. Consequently, when the level of solvent in the extraction chamber reaches the top of the siphon, the solvent and the extracted plant material flow back to the flask. The entire process continued repeatedly until the drug is completely extracted, a point when a solvent flowing from extraction chamber does not leave any residue behind and become transparent. The mixture of extract and solvent thus obtained in the round bottom flask is taken out and solvent recovery was done using a rotary vacuum evaporator at 78 °C and 273 rotations per minute. The extract obtained is transferred into a glass container and kept in hot air oven at 100°C till all the solvent part got evaporated. The final extract obtained is stored in glass container at 4°C.

After 13 minutes of heating the colour of solution became light green. As the heating progressed the colour changed to blackish green and towards the end it became transparent. The final product obtained was liquid in nature.



Figure No:1 showing Soxhlet extraction of coarse powder of *Nimba*, *Karanja* and *Methika* and concentration of extract.

3. Preparation of extract of *Japa*, *Arishtaka* and *Sathala* using infusion method

150 ml of distilled water is heated at 60 °C. The vessel is taken out and 10 g each of coarse powder of *Japa*, *Arishtaka* and *Sathala* is added to this water and kept undisturbed for 20 minutes. After that it is filtered well using double folded cloth and measured. It is stored in an air tight glass container.

4. Preparation of Polyherbal shampoo using hydro ethanolic extract and infusion

Three 100 ml batches of shampoos are prepared as below.

Table no. 2: showing three trial batches of polyherbal shampoo.

Sl no	Ingredients	S ₁	S ₂	S ₃
1	Infusion	73 ml	68.3 ml	60 ml
2	Xanthan gum	1.2 g	1.54 g	2 g
3	Sodium CMC	0.5g	0.64 g	1 g
4	Glycerin	3 ml	4.36 ml	6 ml
5	Hydro ethanolic extract	4.9 ml	4.9 ml	4.9 ml
6	Decyl glucoside	10 ml	13.3 ml	15 ml
7	Cocamidopropyl betaine (CAPB)	8 ml	10.36 ml	12 ml
8	Geogard ECT	0.8 ml	0.98 ml	1 ml
9	Sodium hydroxide	Q S	Q S	Q S
10	Rose oil	Q S	Q S	Q S

1. Hydration of xanthan gum and sodium CMC-The known quantity of xanthan gum and sodium CMC is taken and mixed uniformly. It is added with glycerine and mixed well. This is then kept for 30 minutes for proper hydration.

2. Preparation of shampoo base-The known quantity of freshly prepared infusion is taken in a China dish and added with the specified quantity of mixture of hydrated xanthan gum and sodium CMC. This mixture is kept in a water bath and heated at 45°C temperature with stirring till the gum mixes uniformly in the infusion. This mixture is allowed to cool down completely.

3. Formulation of shampoo-The previously prepared base was added with hydro ethanolic extract and mixed properly. Then Decyl glucoside and CAPB was added and gently mixed. Geogard ECT was added followed by the addition of rose oil sufficient to give the fragrance. At the end the pH was checked and adjusted using sufficient quantity of sodium hydroxide.

ANALYTICAL STUDY OF POLYHERBAL SHAMPOO

A. Physico chemical analysis

1. Organoleptic characters: The organoleptic characters like colour, odour and touch are noted.

2. Determination of pH: The pH of 10% shampoo solution in distilled water was determined at room temperature 25°C.

3. Determination of Percent of solids contents: A clean dry evaporating dish was weighed and added 4 grams of shampoo to the evaporating dish. The dish and shampoo were weighed. The evaporating dish with shampoo was put on the hot plate until the liquid portion was evaporated. The weight of the shampoo only (solids) after drying was calculated.

$$\text{Percentage of solid contents} = \frac{\text{weight of residue}}{\text{Weight of sample}} \times 100$$

4. Dirt dispersion: Two drops of shampoo were added in a test tube containing 10 ml of distilled water. 1 drop of India ink was added; the test tube was stoppered and shook it ten times. The amount of ink in the foam was estimated as None, Light, Moderate, or Heavy.

5. Cleansing action: 5g of hair was placed in oil, after that it was placed in 200 ml. of water containing 1 gram of shampoo in a conical flask. Temperature of water was maintained at 35°C. The flask was Shaken for 4 minutes at the rate of 50 times a minute. The solution was removed and sample was taken out, dried and weighed. The amount of oil removed was calculated by using the following equation.

$$\text{Soil Removed (\%)} = [(W_1 - W_2) / W_1] \times 100$$

Where: W_1 = Initial weight of soiled hair (before washing)

W_2 = Final weight after washing and drying

6. Foaming ability and foam stability: The foaming ability was evaluated using the cylinder shake method. In this procedure, 50 ml of a 1% shampoo solution was transferred into a 250 ml graduated cylinder, which was then covered with the hand and shaken vigorously 10 times. The total foam volume formed was measured immediately after shaking, and subsequent foam volumes were recorded at 1-minute intervals for up to 4 minutes. The foam height after 1 minute was used to calculate the foaming capacity, while changes over time indicated foam stability.

7. Viscosity: The viscosity of the shampoo was measured using an Anton Paar MCR 302 rotational rheometer with a P-PTD200 measuring cell and D-CP50-1 geometry. The sample was made homogeneous and free of air bubbles before loading. It was placed between the plates with a set gap, and the temperature was maintained at 25 ± 0.1 °C for equilibration. A shear rate sweep ($0\text{--}110\text{ s}^{-1}$) was performed, and viscosity was automatically calculated from torque and shear stress data. The test was repeated for reproducibility, and the mean viscosity was obtained from the instrument's regression analysis.

8. Determination of surface tension: First the specific gravity of distilled water and the shampoo was determined using a pycnometer by recording the weights of the empty bottle (W_1), bottle with water (W_2), and bottle with shampoo (W_3). Specific gravity was calculated as.

$$\text{Specific Gravity} = \frac{W_3 - W_1}{W_2 - W_1}$$

For surface tension, the drop count method using a stalagmometer was employed. The instrument was cleaned and calibrated with distilled water, and the average number of drops (n_1) between two marks was recorded. The same procedure was repeated using a 10% shampoo solution to obtain the average number of drops (n_2). The surface tension of the shampoo (γ_2) was then calculated using.

$$\gamma_2 = \gamma_1 \times \frac{n_1 d_2}{n_2 d_1}$$

9. Wetting time: A canvas paper was cut into 1-inch diameter discs having an average weight of 0.449 g. The smooth surface of disc was placed on the surface of 1% shampoo solution and the stopwatch started. The time required for the disc to begin to sink was noted down. This experiment was repeated for three times and average value was calculated.

10. Stability studies: The formulated shampoo was placed in appropriate container in a stability chamber at 45°C temperature and 75% relative humidity. It's appearance and physical stability were evaluated at an interval of one month.

B. Instrumental analysis

1. High performance thin layer chromatography (HPTLC)

Table No. 3: showing materials used for HPTLC development of shampoo.

Parameter	Details
Stationary phase	Silica gel 60 F ₂₅₄ plates (Merck)
Mobile phase	Toluene: Ethyl acetate (7:3)
Sample solvent	Methanol
Detection wavelengths	254 nm (UV) and 366 nm (fluorescence)
Samples applied	Shampoo at 3 μ L, 6 μ L, 9 μ L, 12 μ L
Development distance	90 mm
Visualization	Blue-green or fluorescent bands under UV light

2. Fourier transform infrared spectrometry (FTIR): The sample was placed on the ATR crystal, and the clamp was adjusted to ensure proper contact. IR radiation ($4000\text{--}400\text{ cm}^{-1}$) from a black-body source passed through an aperture to regulate the energy reaching the sample. The beam then entered the interferometer, where spectral encoding occurred, producing an interferogram signal. This signal interacted with the sample, where specific frequencies were absorbed based on the sample's molecular characteristics. Finally, the beam reached the detector, and the digitized signal was processed by the computer using Fourier transformation to generate the IR spectrum.

3. Scanning Electron Microscopy (SEM): Surface morphology of the hairs was examined by scanning electron microscopy (Leo 430, Leo Electron Microscopy Ltd., Cambridge, England). The hair samples were mounted directly on the SEM sample stub, using double side stitching tape and coated with gold film (thickness 200nm) under reduced pressure (0.001 mm of Hg). The photomicrographs of suitable magnification were obtained for surface characterization.

C. Phytochemical screening

1) Detection of Alkaloids: Mayer's Test: 1 ml of the sample was taken, and 1–2 drops of Mayer's reagent were added along the sides of the test tube. Formation of a white precipitate indicates the presence of alkaloids. Here there was no white precipitate formed.

2) Detection of Saponins – Foam Test

50 mg of the sample was diluted with distilled water and the volume was adjusted to 20 ml in a measuring cylinder. The solution was shaken vigorously for 15 minutes. A stable foam layer

of about 2 cm indicates the presence of saponins. 6 cm foam height was observed and it was stable for 30 minutes.

3) Detection of Phytosterols – Liebermann-Burchard's Method

The sample was dissolved in 2 ml of acetic anhydride and 1–2 drops of concentrated H_2SO_4 were added along the sides of the test tube. A colour change from purple to green indicates the presence of phytosterols. An array of colour changes from purple to green was observed.

4) Detection of Phenolic Compounds

Ferric Chloride Test: The extract was dissolved in 5 ml of distilled water, followed by the addition of a few drops of neutral 5% FeCl_3 solution. A dark green colour indicates the presence of phenols which was observed during the test.

5) Detection of Flavonoids – Aluminium Chloride Method

A 0.5 ml portion of the sample was mixed with 2 ml of distilled water and 0.15 ml of 5% NaNO_2 solution. After 6 minutes, 0.15 ml of 10% AlCl_3 solution was added and the mixture was allowed to stand for another 6 minutes. Then, 2 ml of 4% NaOH solution was added, followed by dilution with distilled water. The mixture was incubated at room temperature for 15 minutes. A pink colour indicates the presence of flavonoids. Development of pink colour was observed during the test.

6) Detection of Glycosides – Borntrager's Test

50 mg of the sample was hydrolysed with concentrated hydrochloric acid for 2 hours in a water bath and then filtered. To 2 ml of the filtrate, 3 ml of chloroform were added and the mixture was shaken well. The chloroform layer was separated and treated with 10% ammonia solution. A pink colour indicates the presence of glycosides. There was no development of pink colour.

7) Detection of proteins-Biuret Test

Add 2 ml of the extract solution to 2 ml of Biuret reagent (a mixture of copper sulphate and sodium hydroxide). Appearance of violet or purple colour indicates the presence of proteins. Appearance of purple colour was observed during the test.

IN VITRO ANTI FUNGAL STUDY

1. Zone of inhibition-Antifungal activity was evaluated against *Malassezia furfur* (ATCC 14521) and *M. globosa* (ATCC MYA-4612) using the agar well diffusion method on Muller Hinton Agar (HiMedia, MH290-500G). Inoculum were prepared from 16–18 h cultures in Muller Hinton broth and adjusted to 1×10^6 cells/mL using the 0.5 McFarland standard. Wells (6 mm) were made on inoculated agar plates (90 mm), and 100 μ L of test samples were added: shampoo at 10%, 25%, 50%, and 100%, and itraconazole (100 μ g/well) as standard. Plates were incubated at 37 °C for 24 hours, and zones of inhibition were measured.

2. Minimal inhibitory concentration (MIC)-The MIC of the shampoo was determined against *Malassezia furfur* (ATCC 14521) and *M. globosa* (ATCC MYA-4612) using the broth microdilution method with resazurin in 96-well microtiter plates. Cultures grown in Mueller–Hinton broth for 16–24 h at 37 ± 2 °C were adjusted to 1×10^6 cells/ml. The shampoo (stock 100%, v/v) was serially diluted in sterile water to final concentrations ranging from 0.25% to 16%, while itraconazole (2 mg/ml in DMSO) served as the standard. Each well received 90 μ L of test dilution and 10 μ L inoculum, with controls containing broth, DMSO, and inoculum only. After 24 h incubation at 37 °C, 20 μ L of 0.015% resazurin was added and incubated for 2–4 hours; blue wells indicated inhibition and pink wells indicated growth. The MIC was recorded as the lowest concentration showing no colour change.

RESULTS

a) Pharmaceutical study results

The hydroethanolic extract of *Nimba*, *Karanja* and *Methika* was prepared from 45 g of coarse powder using 200 ml of solvent, yielding 26 ml of final extract. Similarly, the infusion extract of *Japa*, *Arishtaka* and *Sathala* was prepared from 30 g of coarse powder with 150 ml solvent, producing 71 ml of extract. The polyherbal shampoo was then formulated using these extracts, and the final volumes obtained from three trial batches were 83 ml (S_1), 81 ml (S_2), and 78 ml (S_3) respectively.

b) Analytical study results

Organoleptic characters-The formulation is dark brown in colour, possesses an aromatic odour, and has a smooth texture to the touch.

Table no. 4: showing the evaluation parameters of shampoo.

pH	5.1
Percentage of solid contents	20.9502
Dirt dispersion	Light
Cleansing action	72.7%
Viscosity	219.7 mPa·s
Surface tension	1.0423 dyne/cm

Table no. 5: showing foam volume and foam stability of shampoo.

Time	Foam volume(cm)
Initial	154
1 minute	154
2 minutes	152
3 minutes	152
4 minutes	152

Table no. 6: showing the wetting time of shampoo.

Repetition of experiment	Wetting time(seconds)
1	10
2	12
3	17
Average	13.3

The time required for the disc to begin to sink-13.3 seconds.

Table no.: 7 showing the stability study of shampoo.

Parameters	First month	Second month	hird month
Organoleptic characters	Brown colour and aromatic odour	Brown colour and aromatic odour	Brown colour and aromatic odour
pH	5.1	5.0	5.0
Percentage of solid contents	20.6183	21.9520	21.5249
Dirt dispersion	Light	Light	Light
Cleansing action	72.7	70.17	70.17
Viscosity	220.21 mPa·s	216.9 mPa·s	212.1 mPa·s
Surface tension	36.8792 dyne/cm	36.20 dyne/cm	36.50 dyne/cm
Foam volume	154 cm	150 cm	148 cm
Wetting action	13.6	13	12.6

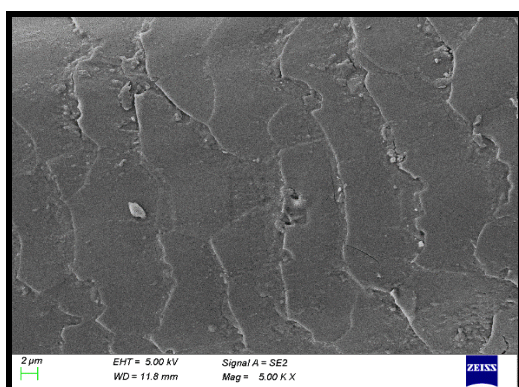
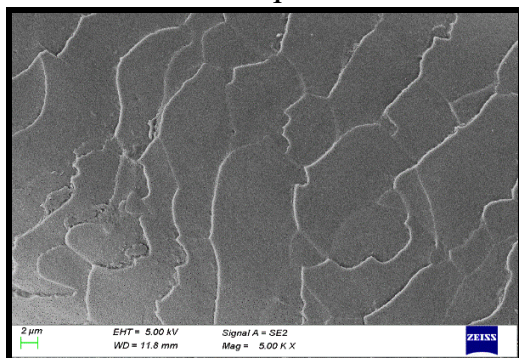
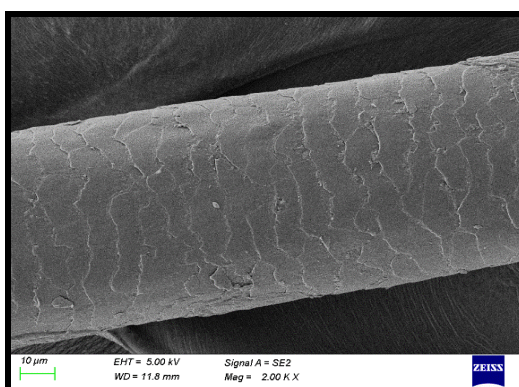
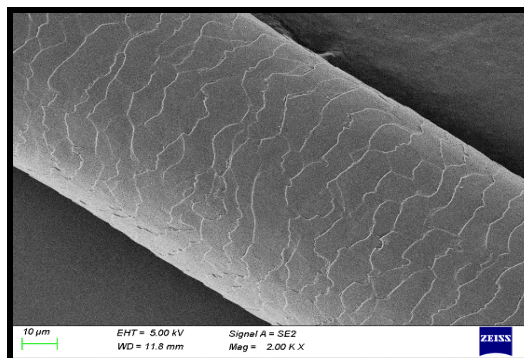
Table no. 8: showing the results of phytochemical screening of Polyherbal shampoo.

Phytochemical	Result
Alkaloids	
Mayer's test	-
Wagner's test	-
Hager's test	-
Dragendroff's test	-
Saponins	+ (6 cm)
Phytosterols	+++

<u>Phenolic compounds</u>	
FeCl ₃	+++
Gelatin	+++
FC reagent	+++
<u>Flavonoids</u>	
AlCl ₃	+++
Glycosides	-
Protein	++

Table No. 9: showing the FTIR result of Polyherbal shampoo.

Sample peak frequency cm ⁻¹	Specific type of bond	Bond	Functional group/compound
3326.83	Strong, broad	O–H stretching	Alcohol/Phenol (polyphenols from plant extracts)
2118.27	Medium	C≡C stretching or C≡N stretching	Alkyne / Nitrile group (plant alkaloids or minor constituents)
1634.44	Strong	C=O stretching (amide I) / C=C stretching	Amide (proteins/peptides) or Alkene (flavonoids, tannins)
1045.46	Strong	C–O stretching	Alcohols, Esters, Ethers, Carbohydrates (e.g., saponins)

H₁H₂Figure No.: 2 showing the morphological characterisation of hair with (H₁) and without (H₂) the treatment of polyherbal shampoo (SEM analysis).

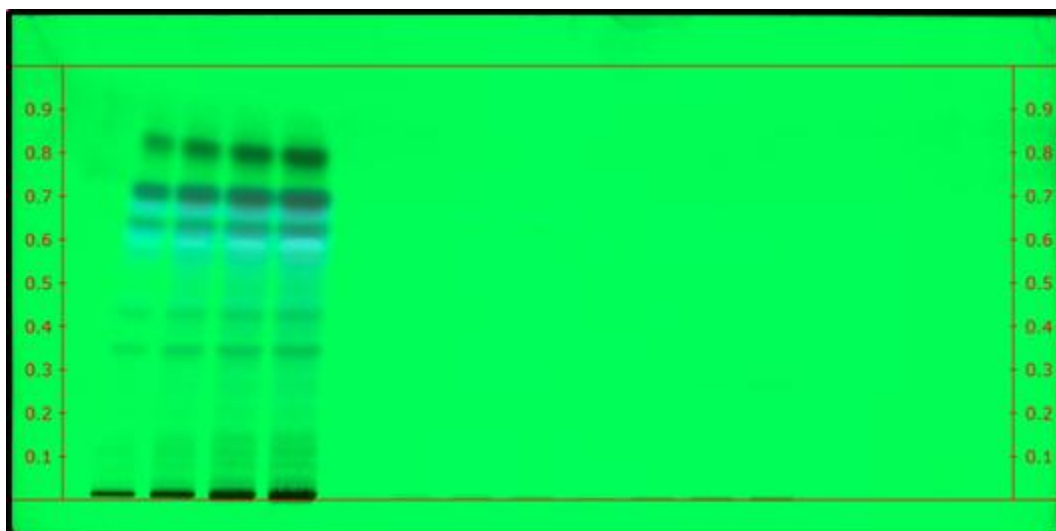


Figure No: 3 showing the HPTLC band of polyherbal shampoo at 254 nm.

Table No. 10: showing zone of inhibition against *M. furfur* and *M. globosa*.

Test sample concentration		Zone of inhibition (mm)	
		<i>M. furfur</i>	<i>M. Globosa</i>
Standard		20	22
control		-	-
Shampoo	10%	12	13
	25%	12	13
	50%	12	14
	100%	13	15

Table No. 11: showing Activity of test samples and standard against test pathogens.

well	1	2	3	4
Conc/well	Shampoo (%)	Std ($\mu\text{g/mL}$)	Shampoo (%)	Std ($\mu\text{g/mL}$)
A	Ct	Ct	Ct	Ct
B	0.25	2	0.25	2
C	0.5	4	0.5	4
D	1	8	1	8
E	2	16	2	16
F	4	32	4	32
G	8	64	8	64
H	16	128	16	128

DISCUSSION

The findings of the present study were discussed in relation to the formulation, evaluation, and biological efficacy of the prepared polyherbal shampoo to understand its potential as a natural hair care product. In order to prepare extract, coarse powder of *Nimba*, *Methika* and *Karanja* was extracted using 80:20 ethanol–water at 85–90 °C until solvent became clear. 80:20 ethanol–water chosen as it extracts both polar (saponins, glycosides, proteins) and

semi-polar compounds (flavonoids, terpenoids, sterols). Moreover, ethanol is safe, volatile, and pharmaceutically acceptable. Coarse powder (passed through sieve no. 40) preferred to avoid clogging, ensure uniform solvent penetration, and prevent co-extraction of unwanted materials. Soxhlet extraction offers continuous hot percolation, efficient solvent recycling, and maximum yield without thermal degradation. It ensures reproducible results suitable for phytochemical studies.

The infusion of *Japa*, *Sathala*, and *Arishtaka* was prepared using water at 60 °C to preserve heat-sensitive anthocyanins and saponins, maintaining the desired foaming and conditioning properties.

The polyherbal shampoo was formulated using mild and biodegradable surfactants such as Decyl glucoside and Cocamidopropyl betaine, natural thickeners like sodium CMC and xanthan gum, and Geogard ECT as a safe, eco-certified preservative. Glycerine was incorporated as a humectant to retain moisture and enhance texture. Among the three trials, the second formulation (S₂) exhibited optimum viscosity, stability, and yield.

The final shampoo showed a pH of 5.1, which is mildly acidic and suitable for scalp compatibility, ensuring cuticle smoothness and preventing irritation. The solid content of 20.95% indicated good washability, while a viscosity of 219.7 mPa·s provided satisfactory consistency and spreadability. The cleansing ability was 72.7%, and the surface tension was reduced to 36.87 dyne/cm, signifying effective detergent action. The foaming ability was excellent, with stable foam volume maintained even after five minutes, and a wetting time of 13.3 seconds suggested balanced cleansing efficiency. Stability studies conducted for three months at 45 °C and 75% relative humidity revealed no significant change in pH, viscosity, or appearance, confirming good physical and chemical stability with an estimated shelf life of about one year.

Phytochemical screening demonstrated the presence of phenolics, flavonoids, saponins, and phytosterols, all of which contribute to the antioxidant, antimicrobial, and conditioning effects of the shampoo.

FTIR analysis was done for hydro ethanolic extract, infusion and shampoo. The FTIR spectrum of the formulated shampoo showed a broad O–H peak at 3326 cm⁻¹, confirming the presence of polyphenolic compounds and flavonoids from herbal extracts. The C=O/C=C

band at 1634 cm^{-1} indicated flavonoid or protein-like compounds, signifying the retention of plant bioactives after formulation. A distinct C–O peak at 1045 cm^{-1} supported the presence of carbohydrate derivatives such as saponins likely from *Shikakai* or *Soapnut*. The overall spectral fingerprint of the shampoo was more similar to the infusion than to the hydroalcoholic extract. This is because infusions mainly contain water-soluble constituents like saponins, polysaccharides, and phenolics, which remain stable during formulation due to similar solvent polarity and mild processing. In contrast, the hydroalcoholic extract includes both polar and semi-polar compounds, and possible loss or modification of these during formulation may explain its differing FTIR pattern.

The SEM analysis of shampoo-treated hair (H_2) revealed a smooth and compact surface with intact cuticle layers, indicating a protective and conditioning effect of the formulation. In contrast, untreated hair (H_1) showed roughness, raised cuticles, and micro-cracks, reflecting structural damage. EDAX analysis supported these findings, showing a lower O:S ratio (1.64) in H_2 compared to 1.93 in H_1 , suggesting reduced oxidation and better preservation of sulphur-containing keratin residues. Overall, the SEM–EDAX results confirm that the formulated shampoo effectively protects hair integrity and minimizes oxidative degradation.

HPTLC profiling further confirmed the presence of multiple phytoconstituents corresponding to the original extracts, verifying the integrity of active compounds post-formulation.

The antifungal activity of the formulated polyherbal shampoo was evaluated against *Malassezia furfur* and *Malassezia globosa* using agar diffusion and resazurin-based MIC assays. In the agar diffusion method, the shampoo produced inhibition zones of 12 mm at 10–50% concentrations and 13 mm at 100% concentration against *M. furfur*, while the standard itraconazole showed a 20 mm zone under similar conditions. Against *M. globosa*, the zones ranged from 13–15 mm, indicating slightly higher susceptibility. This variation may be due to differences in the cell wall structure and lipid dependence of the two fungi, allowing easier penetration of the phytoconstituents in *M. globosa*. The nearly constant inhibition zone at lower concentrations with minimal increase at 100% suggests limited diffusion of active compounds through the agar medium.

In the resazurin-based MIC assay, the colour change from blue to pink confirmed fungal viability, enabling determination of inhibitory concentrations. The shampoo exhibited MIC values of 16% (v/v) for *M. furfur* and 8% (v/v) for *M. globosa*, whereas itraconazole showed

64 µg/mL and 32 µg/mL, respectively. The greater sensitivity of *M. globosa* may be attributed to its thinner cell wall and higher permeability, facilitating entry of active phytochemicals such as flavonoids, phenolics, and saponins. Compared to itraconazole, the herbal shampoo demonstrated moderate antifungal potency, which can be explained by its complex, diluted composition and slower diffusion of active molecules. Nevertheless, the formulation exhibited consistent antifungal activity against both species, supporting its potential as a safe and natural alternative for managing dandruff-associated fungal infections.

CONCLUSION

The present study successfully formulated and evaluated a polyherbal shampoo containing *Nimba*, *Karanja*, *Methika*, *Japa*, *Sathala*, and *Arishtaka* as a safer alternative to synthetic shampoos. Hydroethanolic extracts of *Nimba*, *Karanja*, and *Methika* revealed phytosterols, phenolics, and flavonoids, while aqueous infusions of *Japa*, *Sathala*, and *Arishtaka* provided saponins and proteins. FTIR and HPTLC analyses confirmed the presence and retention of these bioactive constituents in the final formulation. The shampoo formulated exhibited desirable physicochemical properties with suitable pH, viscosity, foam stability, cleansing ability, and surface tension. Stability studies over three months indicated minimal changes, confirming good formulation stability. Antifungal studies demonstrated moderate activity against *Malassezia furfur* and *M. globosa*, supporting its anti-dandruff potential. Overall, the formulation proved stable, safe, and biologically effective as a natural, eco-friendly hair care product.

ACKNOWLEDGEMENT

Authors are grateful to Dr. Venkatesh, HOD, Department of pharmaceuticals, Acharya and BM Reddy college of Pharmacy, Bengaluru, for his guidance in the pharmaceutical study of present work. Also, we extend our heartfelt thanks to Dr. Naganand G.S, Assistant Professor, CIIRC unit, Jyothy Institute of Technology, Bengaluru for his valuable advice, continuous support and patience during the study.

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