

FORMULATION AND CHARACTERIZATION OF HERBAL-BASED ANTIDANDRUFF SHAMPOO AND ASSESSMENT OF ITS ANTIFUNGAL ACTIVITY

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Abstract

*Dandruff is a widespread scalp issue affecting over 50% of the global population. The rising demand for natural and herbal products in the cosmetic industry has prompted the exploration of plant-based ingredients for hair care formulations. The purpose of this study is to formulate antidandruff shampoo using a combination of traditional herbs known for their therapeutic, antimicrobial, and scalp-nourishing properties. The extraction and incorporation of these herbal components were optimized to ensure the effectiveness of the shampoo in addressing dandruff-related concerns. The bioactive components from neem, fenugreek seed, aloe vera, and clove were extracted using different solvents. Additionally, the presence of active compounds in the herbal extracts was confirmed through phytochemical screenings. Fourier transform infrared spectroscopy (FTIR) detected the presence of bioactive chemicals and functional groups, such as nitro compounds and amide groups, in the plant extracts. Two different combinations of anti-dandruff shampoos were formulated. The formulated shampoo was characterized physically and chemically. The shampoo's antibacterial activity was tested against *Staphylococcus aureus* and *Staphylococcus epidermidis* and for antifungal activity, it was tested against *Malassezia furfur*, a fungus associated with dandruff using the agar well diffusion method. Parameters such as pH, foaming agents, solid content, and organoleptic properties were analyzed to ensure product stability and user acceptance. The findings of this study contribute to the development of herbal-based hair care products with a specific focus on addressing dandruff issues. The characterization results offer insights into the formulation's stability and quality, while the antifungal activity assessment provides valuable information on its potential therapeutic benefits. The herbal antidandruff shampoo presented in this study represents a promising natural alternative for individuals seeking effective and plant-derived solutions for scalp health.*

Keywords: Herbs, Anti-Dandruff, Therapeutic Properties, Formulation.

INTRODUCTION

Our environment is filled with microbes which are both useful and harmful to humans. 50% of people are affected by these microbes in day-to-day life. One of the main problems people face is dandruff which is caused by yeast-like fungus *Malassezia furfur* and other bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Dandruff is not harmful to humans but they cause irritation, itching and so on. Dandruff is differentiated into powdery and flaky types. People follow chemical based products, which stop the growth of the microbes for a particular period but that does not cure the problem completely. Also, chemicals can cause side effects, which are unpredictable. Instead of using chemicals to cure the problem, natural herbs can be used which have more effect on killing microbes naturally. These herbs are easily available in our day-to-day life and it also has no side effects. Plant extracts are the ones which were used by our ancestors to cure various diseases (Meena *et al.*, 2019).

One of the major challenges of hair is to treat dandruff. But it cannot be treated completely by chemicals (Pal *et al.*, 2020). People with *Seborrheic dermatitis* are more susceptible to dandruff and it is flaking off the skin on the scalp (Rathi *et al.*, 2019). *Malassezia* is a lipophilic fungus that causes dandruff, white flakes are formed from skin and fall off from the scalp. People with this problem have more sebaceous glands which produce oily scalp, for this condition there is no complete cure (Vijayakumar *et al.*, 2006).

The dandruff absorbs and forms an enzyme lipase which helps in breakdown of sebum to oleic acid. So, this fatty acid penetrates into the scalp surface and causes inflammation and other problems (Sahraie-Rad *et al.*, 2015). *Malassezia furfur* not only causes dandruff but also other infections like *Pityriasis versicolor*, *Malassezia folliculitis* and so on (Kulkarni *et al.*, 2020). Shampoos are the most commonly used cosmetic product in our day-to-day life. Herbal shampoos, derived from traditional formulation works same as the conventional shampoo, and they are safe to use as well as environmentally friendly. Herbal shampoos are a combination of plant-based product and traditional herbs. Nowadays, people prefer traditional based product compared to chemical formulation (Maurya *et al.*, 2021).

The components such as Cloves, fenugreek seeds, aloe vera, and neem leaf prevent hair loss, premature greying, also promotes the growth of thick, voluminous hair and softly restores the original black Indian hair colour.

Fenugreek seeds help in elimination of dandruff and also promotes hair growth. Neem and aloe vera have a property of curing hair related problems, phytochemicals present in these plants play the major role. Clove has anti-fungal, anti-inflammatory property, help in preventing dandruff (Srivastav *et al.*, 2024).

MATERIALS AND METHODS

Sample collection:

With the help of a clean comb, scalp dandruff samples were taken and transferred to sterile containers. Cloves, fenugreek seeds, aloe vera, and neem leaves were bought from a local herbal shop. Muller Hinton agar, Luria Bertani broth, distilled water, additional chemicals, and skin pathogenic bacteria (*Staphylococcus aureus* and *Staphylococcus epidermidis*) were sourced from DGVC College, Chennai.

Isolation of *Malassezia furfur*:

After the collection of the sample, it was streaked on the Emmons modification of Sabourauds agar medium overlaid with corn oil and incubated at 30° C for 7 days (Sibi *et al.*, 2023). A loopful of the culture was added to a clean, dry, and grease free glass slide, and the mixture was thoroughly homogenized with the lactophenol cotton blue stain. A clean cover slip was then carefully placed over the specimen-reagent mixture, making sure there were no air bubbles. The slide was then examined at a 40x magnification using an optical microscope

Biochemical tests:

A biochemical test for the identification of *Malassezia furfur* from other *Malassezia spp.* based on the catalase test, tween assimilation test, and esculin hydrolysis test.

Catalase test:

3% hydrogen peroxide was added to a clean glass slide, then a chosen colony from the culture was combined with the hydrogen peroxide. The instantaneous formation of gas bubbles confirms positive results (Gueho *et al.*, 1996).

Tween assimilation tests:

The Tween assimilation test was used to determine the ability of *Malassezia spp.* To use distinct Tween compounds as a unique lipid supplement. Yeast cells were suspended in 1 ml of sterile distilled water and placed on a plate of Sabouraud Dextrose Agar (SDA) with 0.05 gm/L chloramphenicol. The plate was then chilled to around 50°C. The inoculum was evenly distributed across the plate, and following solidification, three wells were punched with a 2 mm diameter. Following that, 5 µl of Tween 20, 40, 60, and 80 were added to each well. After that, the plates were incubated at 30°C for a week. Tween usage was assessed using the zone of inhibition seen surrounding individual wells (Gueho *et al.*, 1996).

Esculin hydrolysis test:

An esculin agar tube was used to measure glucosidase activity. A sterile inoculation loop was used to deeply inoculate the agar with the yeast inoculum, which was then incubated for 3 days at 30°C to see if the colour changed. Slant darkening is an indication of esculin hydrolysis (Midgley, 2000).

Preparation of plant extracts:

Neem leaves were washed, air-dried, and powdered, while fenugreek seeds were cleaned and ground into powder. Aloe vera gel was extracted, dried, and powdered. Using maceration, 5 g of each ingredient was mixed with 50 ml of solvents (distilled water, ethanol, methanol, and chloroform), and the flasks were sealed with aluminium foil. The mixtures were kept undisturbed at room temperature for 3 days. After incubation, the extracts were filtered using Whatman filter paper, and the filtrates were evaporated to remove solvents before being stored for further analysis (Kumari *et al.*, 2022).

Extraction of essential oil from clove buds:

The maceration procedure was used to extract essential oils. Dried cloves were ground to a coarse powder and placed in a conical flask containing ethanol. The flask was wrapped in aluminium foil and left to macerate for one week. The extract was then filtered through Whatman filter paper, the ethanol was evaporated, and the clove oil was stored in an appropriate container away from light and heat to prevent degradation (Putra *et al.*, 2023).

Antimicrobial Assessment of Extracted Compound:

Antibacterial activity:

The agar well diffusion method was used to determine antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermis*. Sterile MHA plates were swabbed with 24-hour test cultures using sterile cotton swabs. Wells (6 mm diameter) were made, and different solvent extracts mixed with Dimethyl Sulfoxide (DMSO) were added at a concentration of 10 mg. Plates were incubated at 37°C for 24 hours, and zones of inhibition were observed and measured (Rajalakshmi *et al.*, 2024).

Antifungal activity:

Antifungal activity was tested using the agar well diffusion method against *Malassezia furfur*. Using sterile cotton swabs, 48-hour test cultures were swabbed onto Emmons-modified Sabouraud agar plates. Wells (6 mm diameter) were made, and different solvent extracts mixed with Dimethyl Sulfoxide (DMSO) were added at a concentration of 10 mg. Plates were incubated at 30°C for 48 hours, and zones of inhibition were observed and measured (Selvakumar *et al.*, 2012).

Phytochemical analysis of plant extracts and clove oil: (Selvaraj *et al.*, 2014)

- 1) **Alkaloids:** 1 mL of extract was combined with 2 mL of concentrated HCl and Mayer's reagent. Green colour or white precipitate indicated alkaloids.
- 2) **Flavonoids:** 2–3 drops of ferric chloride were added to 1 ml of extract. Blackish-red colour indicated flavonoids.
- 3) **Tannins:** A few drops of 0.1% ferric chloride were added to 1 ml of extract. Tannins with an identified colour of blue-black or brownish green.
- 4) **Saponins:** 1 ml of extract was added to 5 ml of distilled water, the mixture was agitated for 15 minutes. A 1 cm foam layer indicated saponins.
- 5) **Terpenoids:** 2 ml of extract was mixed with chloroform and concentrated sulphuric acid. Reddish-brown at the interface confirmed terpenoids.
- 6) **Glycosides:** 2 ml of extract was mixed with glacial acetic acid, and 1 ml of concentrated sulphuric acid, and few drops of ferric chloride. Brown colour indicated glycosides.
- 7) **Quinones:** 1 ml of extract was mixed with a potassium hydroxide alcoholic solution. Red precipitate indicated quinones.

Characterization of extracted compound:

Fourier Transform Infra-red (FTIR) Spectroscopy

Equal volumes of plant extracts and clove oil were mixed. FTIR spectroscopy analysis for plant extracts was done at a wavelength range of 500-4500 cm⁻¹ using the Bruker model FT-IR spectrometer. Based on the vicinity of the infrared radiation, the FTIR analysis was performed to determine the functional groups of the bioactive chemicals included in the extract (Hemalatha *et al.*, 2016).

Formulation of anti-dandruff shampoo using extracted compound:

The shampoo was formulated in two different combinations. Herbal extracts such as neem, aloe vera, and fenugreek seed were mixed along with reetha extract. Clove essential oil and vitamin E capsules were then added and thoroughly mixed. Lastly, lemon juice was added to the solution to change its pH. The manufactured shampoo was scented with a few drops of rose essential oil, and the final volume was adjusted to 100 ml using distilled water (Table 1) (Katkale *et al.*, 2024).

Table 1: Formulation composition

INGREDIENTS	FORMULATION – 1	FORMULATION - 2
Neem extract	10ml	5ml
Fenugreek extract	10ml	15ml
Aloe vera extract	10ml	15ml
Clove oil	2ml	2ml
Reetha extract	10ml	15ml
Vitamin E capsule	2 capsules	2 capsules
Lemon juice	3ml	3ml
Rose oil	5ml	5ml
Distilled water	50 ml	40 ml
Xanthum gum	0.5g	0.5g
Total ml	100	100

Assessment of antimicrobial activity of formulated shampoo:

Antimicrobial activity was tested using the agar well diffusion method against *Malassezia furfur*, *Staphylococcus aureus*, and *Staphylococcus epidermis*. Sterile Emmons-modified Sabouraud agar and MHA plates were prepared and swabbed with test cultures using sterile cotton swabs. Wells (6 mm diameter) were made, and formulated shampoos 1, 2, and a control antibiotic were added. Plates were incubated at 30°C for 48 hours (*Malassezia furfur*) and 37°C for 24 hours (*Staphylococcus spp.*). Inhibition zones were noted and quantified (Selvakumar *et al.*, 2012).

Characterization of formulated shampoo:**Organoleptic Properties:**

The prepared anti-dandruff shampoos were inspected for colour, clarity, odour, and texture as per standard method (Maurya *et al.*, 2021).

Determination of pH:

The pH values of prepared shampoos and marketed selected shampoos were determined using a digital pH meter (Saripalla *et al.*, 2022).

Solid Contents Determination:

The percentage of solid content of the samples was assessed with the loss-on-drying technique. Petri plates that were previously weighed and cleaned were then taken; 5 g of each shampoo formulation was placed on into each dish. The dishes were dried for 1 hour at 50 °C in a convection oven, until they were completely dry. The weight after drying was noted, and solid content was found out using the under mentioned equation:

$$\text{Solid content (\%)} = \frac{W_0 - W_1}{W_0}$$

Where W_0 is the initial weight of the sample while W_1 is the weight of solid contents (Al Badi *et al.*, 2014).

Foam stability:

A 250 ml graduated cylinder was filled with 50 mL of a 10% shampoo solution, and it was shaken ten times. Using the cylinder's graduation, the total volume of foam was promptly measured after 1, 2, 3, and 4 minutes of shaking. The value was recorded after the procedure was carried out three times (Bartkute *et al.*, 2024).

Dirt Dispersion:

In a test tube, 10 ml of distilled water were combined with 2 drops of shampoo, and then 1 drop of Indian ink was added. 10 gentle shakes were given to the sealed test tube. The foam's ink content was visually evaluated as either heavy, light, moderate, or none. Shampoos with colour retention in the foam were deemed to be of poor quality (Saad *et al.*, 2011).

Wetting Time:

Wetting time was measured by adding 50 ml of 1% aqueous shampoo solution to 1 g of wool yarn in a 100 mL beaker. The time taken for the wool yarn to float and then sink in the solution was recorded using a stopwatch (Zlabiene *et al.*, 2024).

Stability studies:

Glass tubes were placed at room temperature for 3 weeks in order to conduct stability investigations. During that time, the tubes' appearance and physical stability were examined.

RESULTS

Isolation of *Malassezia furfur*:

A dandruff sample was collected by scraping and streaked on Emmons modified of SDA medium then incubated for 7 days. After incubation, colonies were observed in whitish cream suggesting *M. furfur*. Microscopic examination was done at 40x after affixing lactophenol cotton blue, budding yeast cells were noted confirming the identification of *M. furfur* (Fig 1).

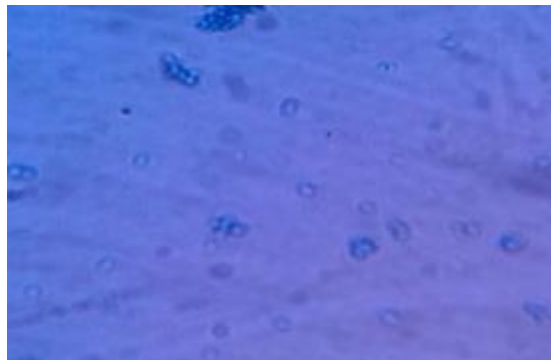


Fig 1: LPCB Staining

Biochemical tests:

The morphology of *Malassezia* was noted and the biochemical test was performed using different biochemical tests such as the Catalase test, Tween assimilation test, and Esculin hydrolysis test for confirmation of *M. furfur*.

Catalase test:

The immediate formation of gas bubbles upon the addition of 3% hydrogen peroxide to the culture was observed which indicates a positive reaction.

Tween assimilation tests:

After one week of incubation at 30°C, the growth of *M. furfur* was observed around each of the wells containing Tween 20, 40, 60, and 80, respectively. The appearance of growth is indicating a positive result for the assimilation of the Tween test (Fig. 2).

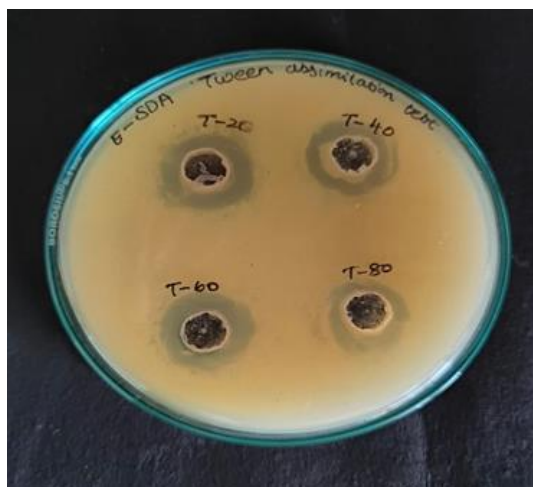


Fig 2: Tween assimilation test

Esculin hydrolysis test:

Darkening of the esculin agar tube slant at 30°C for 3 days was observed, which indicates the presence of the glucosidase enzyme in *M. furfur* (Fig. 3).

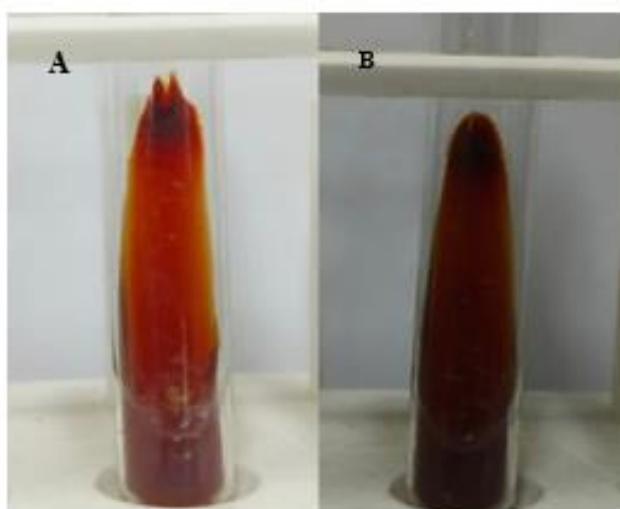


Fig 3: Esculin hydrolysis test: A- Control, B- Sample

Preparation of plant extracts:

The maceration process was used to extract bioactive components from neem, fenugreek, aloe vera, and clove. After 3 days of incubation, the mixture was filtered and concentrated by steam distillation.

Extraction of essential oil from clove buds:

The maceration method was used to extract essential oil from ground cloves with coconut oil. After 1 week, the mixture was filtered, the yield was about 10 % and stored away from light and heat.

Antimicrobial Assessment of Extracted Compounds:

Antibacterial activity:

The agar well-diffusion method was used to assess the antibacterial properties of neem, fenugreek, aloe vera, and clove oil extracts against *Staphylococcus aureus* and *Staphylococcus epidermidis*. Out

of all the test materials, the clove oil in coconut oil exhibited the greatest activity (17 mm for *S. aureus* and 18 mm for *S. epidermidis*). Ethanol extracts of neem, fenugreek, and aloe vera yielded higher inhibition zones measuring 26 mm, 12 mm, and 8-18 mm, respectively.

Methanol and chloroform extracts of neem, aloe vera, and fenugreek plants were moderately active. Clove oil and ethanolic extracts were found to be the most potent (Fig. 4) and (Table 2).

Table 2: Antibacterial activity of the herbal extract

Solvents	Zone of inhibition in mm against <i>Staphylococcus aureus</i>			
	Neem	Fenugreek seed	Aloe vera	Clove oil
Distilled water	10	0	0	17
Ethanol	26	12	8	
Methanol	25	10	1	
Chloroform	22	3	3	
	Zone of inhibition in mm against <i>Staphylococcus epidermidis</i>			
Distilled water	12	0	0	18
Ethanol	13	12	18	
Methanol	10	10	10	
Chloroform	12	3	12	

Antifungal activity:

The agar well-diffusion method was used to measure antifungal activity. Among the tested substances, clove oil inhibited the growth of *Malassezia furfur* the most, with a zone of inhibition measuring 21 mm.

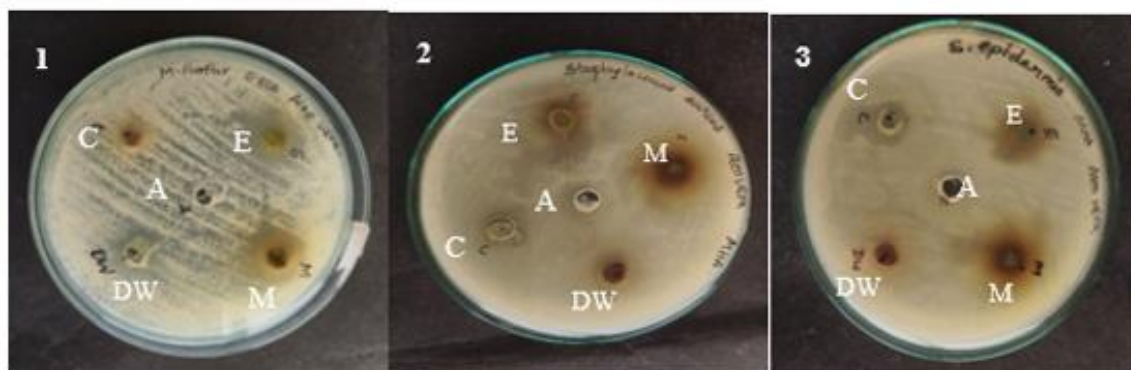
The ethanolic extracts of neem (18 mm), fenugreek (17 mm), and aloe vera (10 mm) were also quite active. Methanol and chloroform extracts demonstrated moderate inhibition, predominantly exhibited by neem and fenugreek. The ethanolic extracts, as well as the clove oil, were found to have the most potent antifungal effects (Fig 4) and (Table 3).

Table 3: Antifungal activity of the herbal extract against *Malassezia furfur*

Solvents	Zone of inhibition in mm (Plant extract)			
	Neem	Fenugreek seed	Aloe vera	Clove oil
Distilled water	0	0	0	21
Ethanol	18	17	10	
Methanol	17	13	5	
Chloroform	16	16	4	



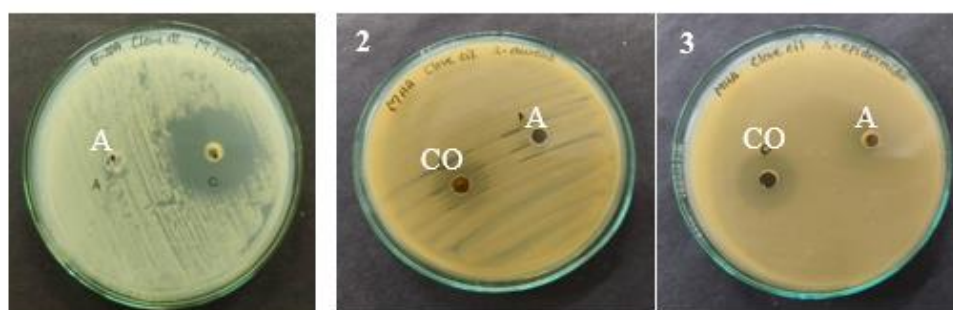
Antimicrobial activity of neem extract



Antimicrobial activity of Aloe vera extract



Antimicrobial activity of fenugreek seed extract



Antimicrobial activity of Clove oil

Fig 4: Antimicrobial activity of extracted compounds: 1- *Malassezia furfur*, 2 - *Staphylococcus aureus*, 3 – *Staphylococcus epidermidis*

*E-ethanol, M-methanol, C-chloroform, DW- distilled water, CO-clove oil, A-antibiotic

Phytochemical analysis of extracted compounds:

The phytochemical constituents were evaluated for the ethanolic plant extracts along with clove oil. Alkaloids, flavonoids, terpenoids, and glycosides were present in all samples. Tannins were found in neem, aloe vera extracts, and clove oil but were absent in fenugreek seed extract.

Saponins were present only in fenugreek seed extract and were absent in neem, aloe vera, and clove oil. Clove oil uniquely contained quinones, while this compound was absent in the other plant extracts (Table 4).

Table 4: Phytochemical analysis of extracted compounds

S.no	Phytochemicals	Neem	Fenugreek seed	Aloe vera	Clove oil
1	Alkaloids	+	+	+	+
2	Flavonoids	+	+	+	+
3	Tannin	+	-	+	+
4	Saponin	-	+	-	-
5	Terpenoids	+	+	+	+
6	Glycosides	+	+	+	+
7	Quinone	-	-	-	+

*+ Present, - Absent

Characterization of plant extracts and clove oil:

Fourier transform infrared (FTIR) spectroscopy:

The functional group of plant extracts and clove oil was characterized by FTIR spectroscopy and shown in (Table 5 and Fig 5), in extracts, the peaks at 3340.09 cm^{-1} of the N-H stretch bond belong to the functional group of primary, secondary amines, and amides. The peaks at 2919.80 cm^{-1} of the O-H stretch bond belong to the functional group of carboxylic acids. The peaks at 1730.78 cm^{-1} of the C=O stretch bond belong to the functional group of Alpha, beta-unsaturated esters. The peaks at 1607.32 cm^{-1} of bond belong to the functional group of Primary amines. The peaks at 1512.75 cm^{-1} of the N-O asymmetric stretch bond belong to the functional group of Nitro compounds. The peaks at 1432.22 cm^{-1} of the C-C stretch (in-ring) bond belong to the functional group of aromatics. The peaks at 1232.69 cm^{-1} and 1028.42 cm^{-1} of the C-N stretch bond belong to the functional group of Aliphatic amines. The peaks at 600.81 cm^{-1} of =C-H bend the bond belong to the functional group of alkenes. The peaks at 573.00 cm^{-1} of C-Br stretch the bond belonging to the functional group of Alkyl halides. The highest peak shown at 1028.42 cm^{-1} of the C-N stretch bond belongs to the functional group of Aliphatic amines (Table 5).

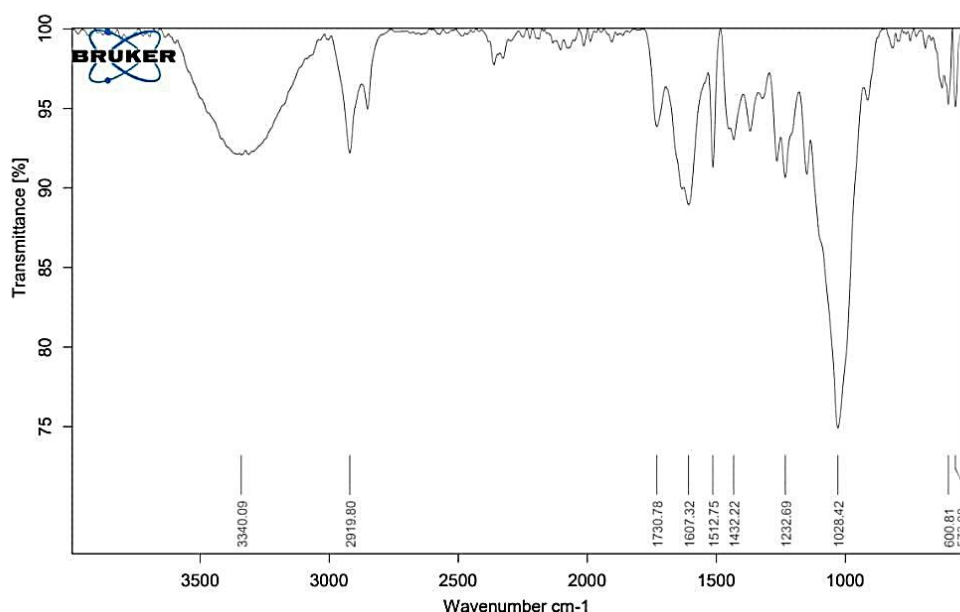


Fig 5: FTIR analysis of extracted compounds

Table 5: FTIR spectroscopic analysis of extracted compounds

S.no	Frequency cm ⁻¹	Range	Bond	Functional group
1	3340.09	3400-3250	N-H stretch	Primary, secondary amines, amides
2	2919.80	3300-2500	O-H stretch	Carboxylic acids
3	1730.78	1730-1715	C=O stretch	Alpha, beta-unsaturated esters
4	1607.32	1650-1580	N-H bend	Primary amines
5	1512.75	1550-1475	N-O asymmetric stretch	Nitro compounds
6	1432.22	1500-1400	C-C stretch (in-ring)	Aromatics
7	1232.69 1028.42	1250-1020	C-N stretch	Aliphatic amines
8	600.81	1000-650	=C-H bend	Alkenes
9	573.00	690-515	C-Br stretch	Alkyl halides

Assessment of formulated shampoo:

Antibacterial activity:

The antibacterial activity of the prepared shampoo was tested using the agar well diffusion method. The zones of inhibition were examined and measured for both formulations 1 and 2 (Fig 6 & 8). Formulation 2 had notable antibacterial action.

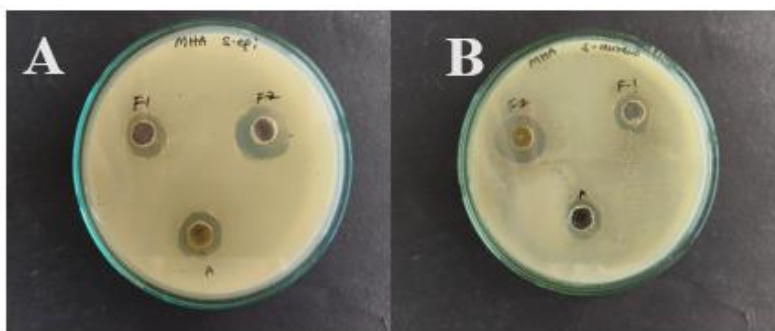


Fig 6: Antibacterial activity of formulations 1 & 2

A- *Staphylococcus epidermidis*, B- *Staphylococcus aureus*

Antifungal activity:

The agar well diffusion method was used to assess the antifungal efficacy of the shampoo formulation. The zones of inhibition were observed and measured (Fig 7 & 8). The maximum zones were observed in formulation – 2. This indicates that the ethanolic plant extracts possess potent antifungal properties against *Malassezia furfur*.



Fig 7: Antifungal activity of formulation – 1 & 2

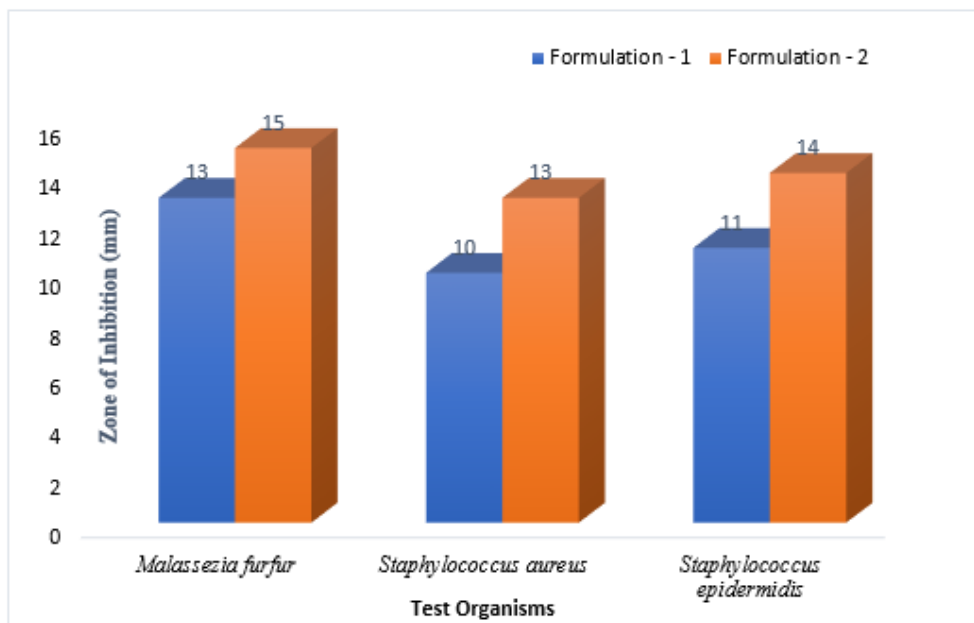


Fig 8: Antimicrobial activity of formulated shampoo 1 & 2

Characterization of formulated shampoo:

Organoleptic Properties:

The prepared anti-dandruff shampoos were examined for colour, odour, and texture and tabulated (Table 6).

Table 6: Organoleptic properties

S.no	Organoleptic properties	
1	Colour	Green
2	Texture	Gel
3	Odour	Rose oil

Determination of pH:

The pH values of shampoo are 4.5-5.5. the pH of the formulation shampoos is mentioned in the table (Table 7).

Solid Contents Determination:

The solid content of formulated shampoo- 1 and formulated shampoo- 2 were given in the table. The formulation shampoo-2 has a low solid content compared to formulation shampoo-1 (Table 7 & Fig 9).



Fig 9: Solid content of formulated shampoo 1 & 2

Dirt Dispersion:

Shampoos that concentrate the ink into the foam were considered substandard, leaving dirt in the water. The dirt that remains in the foam becomes stuck in the hair and is hard to remove. In, formulation -1 moderate dirt was left in the foam, and in formulation -2 no dirt was left in the foam (Table 7).

Wetting Time:

Wool yarn was meticulously timed using a stopwatch to determine when it began to float at the shampoo solution's surface and when it began to sink. The wetting time of Formulation -1 was found to be 14 sec and formulation -2 was found to be 12 sec which is good (Table 7).

Table 7: Characterization of formulated shampoo

S.no	Characterization	Formulation -1	Formulation -2
1	pH	6	5
2	Solid content (%)	21.22±0.02	16.45±0.02
3	Dirt Dispersion	Moderate	Light
4	Wetting Time (sec)	14	12

Foam stability:

The total volume of foam after 1, 2, 3, and 4 min of shaking was recorded immediately using the graduates of the cylinder. Formulations 1 and 2 showed significant foam stability (Fig 10).

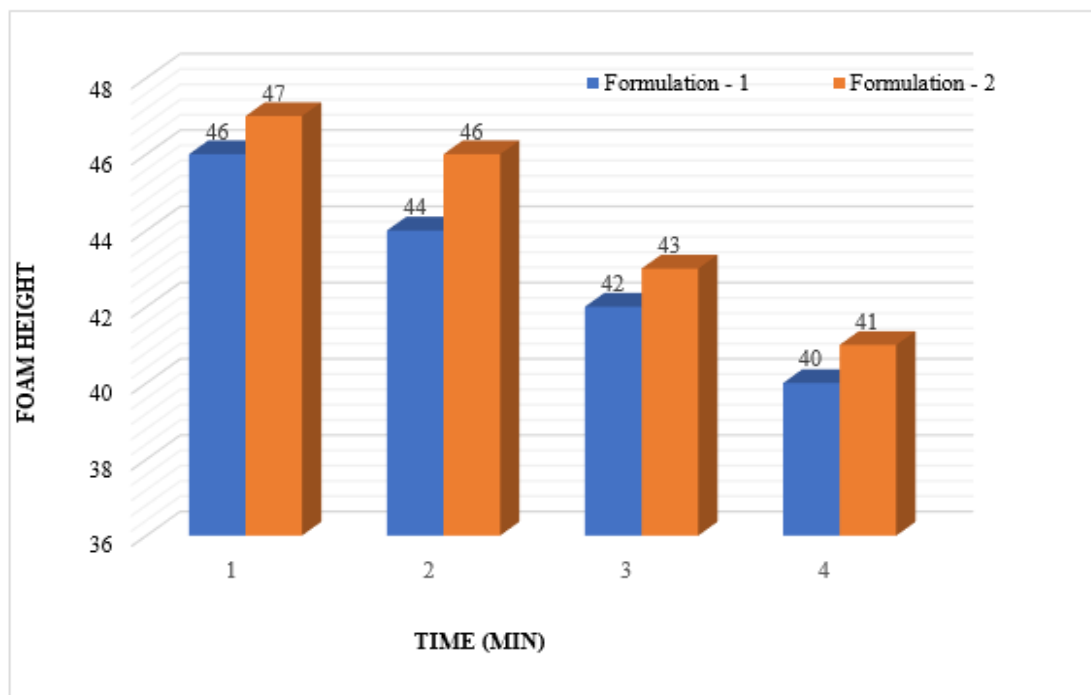


Fig 10: Foam stability of formulated shampoo 1 & 2

Stability studies:

The stability of the shampoo formulations was assessed by storing them at room temperature for a duration of three weeks. The results of this evaluation concluded that both formulations 1 and 2, remained chemically and physically stable throughout the entire storage period at room temperature.

DISCUSSION

Dandruff is a serious health issue since it affects more than 50% of the people in the world. Although synthetic treatments such as shampoos and lotions have been effective in treating the condition, they may have adverse effects like irritation and dryness (Zoya *et al.*, 2016). The objective of the present study is to formulate an anti-dandruff herbal shampoo incorporating plant extracts to inhibit *Malassezia furfur*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*, which are responsible for a majority of dandruff cases.

Dandruff specimens were inoculated on Emmons' agar medium, where they resulted in the formation of cream-white colonies, which were further subjected to other biochemical tests for the identification of *Malassezia furfur*, which included catalase tests, esculin hydrolysis, and Tween assimilation tests. All these organisms, however, were more susceptible to ethanolic extracts of neem, fenugreek, aloe vera, and clove oil than to the extracts with methanol, distilled water, and chloroform (Putra *et al.*, 2023). The extracts were shown to have many phytoconstituents such as alkaloids, flavonoids, terpenoids, glycosides, and tannins, while clove oil has quinones. FTIR analysis showed the presence of functional amides, aromatic groups, and carboxylic groups.

Two shampoo formulations were formulated using neem, aloe vera, fenugreek, clove oil, xanthan gum, reetha, vitamin E, rose oil, and lemon juice, following (Kushwaha *et al.*, 2025). The antimicrobial tests performed indicated a clear zone of inhibition in formulation-2 compared to inhibition in formulation-1, and the characterization tests (pH, foaming stability,) also have better results, consistent with Selvakumar *et al.* (2012). Stability tests indicated stability in both formulations with time of storage.

Hence, herbal shampoo offers a safer, healthier, and more effective option to chemical-based products, with formulation 2 being better in efficacy. In addition, the use of herbal ingredients attracts consumers who prefer natural and holistic hair care products.

CONCLUSION

The creation and evaluation of the anti-dandruff shampoo with herbal ingredients showed that it is naturally effective in combating dandruff. This study incorporates a scientific advancement and the indigenous knowledge of the people in coming up with a formulation that seeks to satisfy the growing demand for herbal cosmetic products by selectively sourcing and combining active herbs like neem, fenugreek seeds, aloe vera, and clove oil. Phytochemical screening of the extracts of the plants and clove oil fulfilled the aim with the identification of active ingredients such as alkaloids, flavonoids, terpenoids, glycosides, and tannins, which have known effects against microbes. Saponins were only found in the fenugreek extract, distinguishing it from other plant extracts. Clove oil demonstrated the presence of quinones, which was also different from the other plant extracts. The FTIR analysis offered a chemical description of the extracts with particular absorption bands indicating the presence of functional groups associated with the reported biological activities. The extracts were effective against *Staphylococcus aureus*, *S. epidermidis*, and *Malassezia furfur*. The most effective antibacterial and antifungal effects amongst the extracts were observed in the neem ethanolic extract, followed by extracts of fenugreek, aloe vera, and clove oil. These findings prove the capability of each component, both individually and when combined, to combat the microbe's causing dandruff. Herbal shampoo was prepared in two variants (formulation-1 and formulation-2) and tested against the same microorganisms by using the well diffusion method. Out of the formulations, Formulation-2 showed better antimicrobial activity, especially against *Malassezia furfur*, the major causative organism of dandruff. The characterization of formulated shampoos was done based on the organoleptic properties, pH analysis, solid content determination, foam volume measurement, dirt dispersal,

wetting time, and cleaning efficacy evaluation, which showed that both formulations met the acceptable quality, although Formulation-2 exhibited significant property. With the rise of the herb-based products, the new anti-dandruff shampoo seems a hopeful replacement for the chemical products that result in irritations and itching of the scalp. The inclusion of medicinal plant extracts with established antimicrobial action in the formulation delivers an effective, environmentally safe, and sustainable antidandruff solution that is both curative and preventive in nature.

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Conflict of Interest

There is no conflict of interest among the authors for publishing this manuscript.

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