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Research Article

Microwave assisted extraction of *Carica papaya* leaves for the formulation of herbal shampoo

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Abstract

Introduction: *Carica papaya* (*C.papaya*) leaves, which possess a large amount of tannin bioactive compounds, have been proven to have antimicrobial activity. The study aims to develop an anti-dandruff shampoo using *C. papaya* leaf extract via microwave-assisted extraction (MAE). The formulated shampoo was evaluated in accordance with the BIS certification. Methodology: The extraction of *C. papaya* leaf was carried out by MAE using water as the solvent, and the resulting extract was dried and evaluated for qualitative estimation of tannins. After estimating the tannin bioactive compound, the extract was checked for its base content. Four shampoos (F1, F2, F3, F4) were prepared by varying the concentration of surfactant and thickening agent. The shampoos were evaluated according to the BIS guidelines, which included cleansing ability, wetting time, viscosity, foaming, detergency, and stability. *In vitro* antifungal activity against *Candida albicans* (*C.albicans)* was assessed. Result: The extraction by MAE yielded 30 % of extract, and the qualitative analysis confirmed the presence of tannins. The base of the shampoo surfactant demonstrates stability and compatibility. The optimised shampoo F1 exhibited a significant detergency of 79.23 ± 0.43%, a quicker wetting time of 177s, and the hair tested on wigs was shiny and smooth, displaying pseudoplastic rheological behaviour. The zone of inhibition for *in vitro* antifungal activity against *C. albicans* for F1 was compared with that of ketoconazole shampoo, which was similar. Conclusion: The current study formulates and evaluates an anti-dandruff shampoo by extracting bioactive compounds from *C. papaya* leaf using water as a solvent and the MAE technique. The optimised F1 formulation possesses significant physicochemical properties in accordance with BIS grade and exhibits similar antifungal activity to the market formulation, ketoconazole.

Keywords: Shampoo, *Carica papaya*, Microwave-assisted extraction (MAE), Surfactant base, *In vitro* anti-fungal

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Introduction

Shampoos are hair care products used daily to clean hair and the scalp. The physical nature of these preparations is a viscous or slightly gel-like consistency, with a cleaner or coloured form, consisting of an approximate amount of detergent mixture, preservatives, and active chemicals, primarily used as cleansing agents. This product is used as a cosmetic to remove excessive sebum from the scalp layer, while environmental pollutants help remove dirt and debris from the scalp region. Typically, it is rubbed into wet hair after application, then rinsed with running water (1). Both medicated and nonmedicated shampoos are available on the market nowadays; nevertheless, herbal shampoos have gained popularity due to their safer, natural origin, which increases consumer demand and is free from side effects. These products play an essential role in today's market. In the Indian market, the fastest-growing segment under cosmetics is shampoo. From 2015 to 2020, the anti-dandruff segment had a CAGR of 17.26% under the shampoo category (2). The main drawback of these synthetic shampoos is that they harm the environment and lead to water pollution. There are two main types of shampoos: chemical-based anti-dandruff shampoos and herbal-based anti-dandruff shampoos.

These anti-dandruff shampoos are cosmeceuticals, products that fall between beauty, cleansing, and pharmacologically active pharmaceuticals, prescribed by dermatologists for the prevention or treatment of superficial skin and hair conditions associated with dandruff. Dandruff is the most prevalent dermatological skin disorder affecting the scalp. It is a persistent, non-inflammatory condition that causes extensive damage to scalp tissue (1).

Although the primary cause of dandruff is unknown, several factors can contribute to it, including an oily scalp, poor hygiene that can lead to a fungal infection, and not washing the hair for a

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week (3). Due to the increase in sebum release, which causes dandruff, its signs include skin discolouration, hair breakage, redness, flakes, itching on the scalp, and unexpected hair colours. *Malassezia furfur* is also a contributing factor to dandruff; the treatment or remedies for this condition often involve using anti-dandruff shampoo (3). Their anti-dandruff shampoo is typically formulated using the mentioned ingredients for their antibacterial or antifungal properties, such as Zinc pyrithione, selenium sulfide, and salicylic acid. The shampoos used in scalp treatments for dandruff on the market may have adverse effects because they contain chemicals. Side effects of synthetic shampoos include itchiness, irritation, hair loss, nausea, headaches, and increased scaling (4). The use of these synthetics has adverse side effects, including cancer, skin rashes, allergy symptoms, hair loss, skin discolouration, and allergic reactions (5).

Dandruff is treated with various plants in the Indian medical system, including Brahmi, Bengal gram, Reetha, Hibiscus, Liquorice, and Marigold (6,7). There are numerous herbal shampoos on the market now that contain natural components, including plant extracts and essential oils (8). Drugs derived from herbal resources are readily available, affordable, secure, effective, and rarely cause adverse implications. As a result of its natural goodness and lack of side effects, the demand for herbal-based natural medications is significantly rising. These natural products are primarily extracted using traditional methods, which are tedious, low-yielding, and less efficient; the solvents employed may also harm the environment (6,7). In this current research, a herbal shampoo using papaya leaf extracts is being developed using Microwave-assisted extraction (MAE) and organic ingredients as the base. The herbaceous, laticiferous plant *Carica papaya L (C.papaya)*, commonly known as papaya, is a member of the family *Caricaceae*, genus *Carica*, and is well-known for its medicinal and dietary benefits. Active elements found in *C. papaya* leaves, including alkaloids, glycosides, tannins, saponins, and flavonoids, contribute to its therapeutic benefits. *C. papaya* leaf is used as a folk remedy for scalp infection with a mixture of other leaves, but its purgative smell is not popular in the present era. These leaf extracts have also been shown to possess antibacterial, antiviral, anticancer, hypoglycemic, and anti-inflammatory properties. The leaf extract contains a high tannin content and has been proven effective in treating various fungal skin infections. The high yield of tannins can be easily extracted from MAE using water as a solvent. MAE is a modern and efficient method to extract bioactive compounds from plant materials. This process involves using microwave radiation to heat the plant material, thereby releasing valuable compounds. MAE of *C. papaya* leaves has shown promising results in extracting bioactive compounds such as antioxidants. MAE on *C. papaya* leaves is a viable method for obtaining bioactive compounds. By utilising MAE, researchers have successfully extracted bioactive compounds from *C. papaya* leaves, including antifungal, antibacterial, and antioxidant compound*s*, more efficiently and effectively than traditional extraction methods. This method has several advantages, including reduced extraction time, a higher yield of compounds, and preservation of the plant's bioactivity (8). The present aim is to extract the bioactive tannin constituents from *C. papaya* leaves using the MAE technique. The extract is incorporated into a suitable base to develop an anti-dandruff shampoo, which is then evaluated and compared with market formulations. The *in vitro* antifungal activity against Candida albicans (C. Albicans) is assessed.

Materials and Methods

Materials

Plant

The *C. papaya* leaves used in the present study were collected from the college's garden and authenticated by a botanist. Sodium lauryl sulphate and hydropropyl methyl cellulose were purchased from Kemphasol. Glycerin and peppermint oil were purchased from Loba Chemie Pvt. Ltd., and benzyl alcohol was purchased from Yarrow Chemicals. All other chemicals used were of pharmaceutical grade.

Collection and extraction

Fresh leaves were collected, dried, and powdered in a suitable grinder, and then stored at room temperature in dark-colored plastic bags to prevent exposure to sunlight. The leaves of *C. papaya* were extracted in the Department of Pharmacognosy at the NGSM Institute of Pharmaceutical Sciences, Paneer, Deralakatte.

Extraction of *C. papaya* leaves by MAE

Dried leaves of *C. papaya* in the ratio of 1:10 (leaf: water) were soaked in distilled water. Then, place in the microwave and irradiate at 800W for 5 minutes. Sieved and 10 ml of extract was taken. A few drops of lemon juice were added with constant stirring as a stabiliser. The extract was concentrated by evaporating the solvent using a rotary evaporator flask and stored at 40°c. MAE was utilized for rapid and uniform heating, resulting in higher yields, energy savings, and high purity.

Qualitative analysis

The extracts of *C. papaya* leaves were subjected to preliminary qualitative phytochemical screening.

Tannins test

Gelatin test

The extract was treated with a 1% solution containing gelatin, and subsequently, 10% NaCl (sodium chloride) was added. The mixture was then observed for the formation of white or buff-colored gelatin precipitation, due to the ability of tannins to react with protein.

Formulation of Herbal Shampoo

a. Screening of the extract with different bases of shampoo

The different bases used for formulating *C. papaya leaf extract were a* gel base, clear shampoo, cream shampoo, & surfactant base shampoo, as shown in Figure 1. Based on the above, the surfactant-based shampoo was found to be compatible in terms of organoleptic, consistency, and physicochemical parameters.

b. Surfactant-Based Shampoo

The four different batches of shampoo bases were formulated by varying the concentration table 1 of surfactant and egg yolk in Table 1 to select a suitable shampoo composition. A weighed amount of egg yolk and sodium lauryl sulfate was added to a beaker. To this required amount of water was added and mixed. Then the necessary amount of benzyl alcohol, glycerin, hydroxypropyl methyl cellulose, and drug extract was added. For fragrance, peppermint oil was added and made up to volume with distilled water (9,10).

Table 1: Formulation of herbal Shampoo containing extract of *C. papaya* leaves

Ingredients	F1	F2	F3	F4
Extract	1gm	1 gm	1 gm	1 gm
SLS	5 gm	10 gm	5 gm	15 gm
HPMC	5 gm	5 gm	5 gm	5 gm
Egg yolk	5 ml	5 ml	15 ml	05 ml
Glycerin	15 ml	15 ml	5 ml	1 ml
Benzyl alcohol	0.1 ml	0.1 ml	0.1 ml	0.1 ml
Perfume (Peppermint oil)	0.01 ml	0.01 ml	0.01 ml	0.01 ml
Water upto	100 ml	100 ml	100 ml	100 ml

Evaluation of *C.papaya* leaf extract Shampoo

The product performance of the *C. papaya* leaf extract shampoo, encompassing organoleptic characteristics, pH, and physicochemical characterization, was assessed. All products were tested as part of a standard protocol to ensure the product's efficiency. All the results were carried out in accordance with the guidelines of the Bureau of Indian Standards (BIS) and compared with the specified standards.

Organoleptic evaluation

● Visual assessment

Colour, clarity, odour, and foam content of the produced mixture were all evaluated.

Physicochemical studies

● pH determination

pH is responsible for the hair's vibrant colour, shine, and overall health. The pH level of shampoo determines how the solution affects your hair. If the pH of shampoo is high, it can lead to cuticle damage because there is an increase in negatively charged electrons, which in turn increases friction between the fibres and causes splitting of the hair and damage to the hair fibres. At room temperature, the pH of the herbal shampoo in distilled water was measured using a digital pH meter (11-13).

● Wetting test

The time required for the canvas paper to absorb the water completely was used to determine the wetting time. The disc-shaped canvas paper was placed in a beaker over the formulated shampoo surface. With the help of a stopwatch, the time it took for the canvas paper to sink was noted. Wetting efficiency is higher only if the time taken is less for the canvas paper sinking (11-13).

● Determination of solid content percentage

The percentage of solid substance is given by adulterant or toxic metal ions. According to standard protocol, a weighed number of formulations were placed in a dry chain dish. The percentage of solid content was determined after the complete evaporation of the shampoo. The initial and final weight of the shampoo was noted, and the percentage of solid content was calculated (11-13).

$$\text{Percentage of solid content} = \frac{\text{final weight of shampoo}}{\text{initial weight of shampoo}} \times 100$$

● Foam ability and stability test

The method used to test the foam's stability is the cylinder shake. A small formulation solution was placed in a graduated cylinder and forcefully shaken. After a few minutes, the foam volume in the graduated cylinder was recorded to determine foam stability. The foams, or bubbles from the shampoo, dissolve the dirt, oil, and other contaminants present in hair and scalp. These bubbles lift out the hair's impurities and wash them away with water (11-13).

● Cleansing action

5 g of wool yarn was greased and placed in a 250 ml round-bottom flask with 200 ml of water, and 1 g of formulation was added. The flask's contents were kept at room temperature. The flask was shaken at 50 shakes per minute for 4 minutes. The wool was removed from the test formulation, dried using a hair dryer, and weighed (11-13).

● Surface tension measurement

The surface tension of the formulation was measured using distilled water as a standard using a stalagmometer at room temperature (11-13).

● Measurement of viscosity

The formulation viscosity not only affects the cleansing efficiency and customer perception of quality, but it also influences the product's properties, including foaming production, filling, stability, and storage. The viscosity of the shampoo was measured by a Brookfield viscometer at room temperature (11-13).

● Wig test

The evaluation of test formulation was done for the following parameters:

a. Ease of application
b. Rinsing
c. Easy wet combing
d. Manageability
e. Luster

Hair wigs were used to test shampoo for its ideal properties. Before applying the shampoo to the hair, it was observed that it was viscous enough to remain in the hand, indicating that it would be simple to apply. Wet hair wig was given a shampoo application, and it was noted that the shampoo rapidly and readily covered the hair. After washing the hair with water, it was also noted that the test formulation's application time was quick, rinsed out easily, and left no tackiness or stickiness behind. After being washed, the hair was dried with a fresh cotton towel and combed with a standard nylon comb. The hair was seen to be readily combed through, untangled, and left in a manageable state. There was no sign of flyaway or fizziness. The hair was shiny and full-bodied (11-13).

***In vitro* antidandruff activity**

The well diffusion method is used to test antifungal activity on *Candida albicans*. The required amount of Sabouraud's agar medium was melted, and the medium was inoculated with 0.2 ml of a 72-hour-old *Candida albicans* suspension. The retri dish cups (10 mm diameter) were punched, filled with 0.05 mL of a sample solution dissolved in dimethyl sulfoxide (DMSO) (10 mL of shampoo in 100 mL of DMSO). The zone of inhibition, measured in millimetres, was determined after the incubation period (14).

Stability study

The herbal shampoo was examined for thermal stability in a closed sterile container, and it was kept at room temperature. They are organoleptic, and their physicochemical properties were evaluated every 30 days for an average of three months (15).

Results

Extraction Results

Percentage of yield obtained from solvent extraction. Results are presented in Table 2. 100 g of the 1000 mL of water was used, and the extract was 30 g.

Table 2: Percentage yield of *C.papaya*

Plant	Solvents
	Water(%w/w)
C.papaya	30

The percentage yield of *C. papaya* is 30% w/w, which is the highest compared to conventional extraction methods.

Qualitative phytochemical test

The test for qualitative analysis was performed, and the presence is confirmed in Table 3. The color changed from greenish to white precipitate, which indicates precipitation of tannins

Table 3: Tannin tests

Phytocompound	Solvent (Water)
Tannins	+ indicates the presence of tannins

Formulation of Herbal Shampoo

For selecting appropriate shampoo bases, namely liquid cream shampoo, clear liquid shampoo, surfactant base shampoo & gel shampoo. Three different shampoo bases were formulated to choose the suitable shampoo base. A surfactant-based shampoo was found to be effective, as indicated by the following results from the extracts. Results are mentioned in Table 4

Table 4: Results of formulation of different bases used in shampoo preparation

Sl No	Bases	Results
1	Liquid Cream Shampoo	Milky white appearances. Thick consistency & Incompatible with extract
2	Clear liquid Shampoo	Colourless, Less viscous & compatible with extract
3	Surfactant-based shampoo	Pale yellow, viscous & compatible with extracts
4	Gel shampoo	colourless, thick consistency

The above results indicated that for the preparation of shampoo, the surfactant-based shampoo will be more compatible in terms of physical compatibility.

Organoleptic Evaluation of Surfactant-Based Shampoo

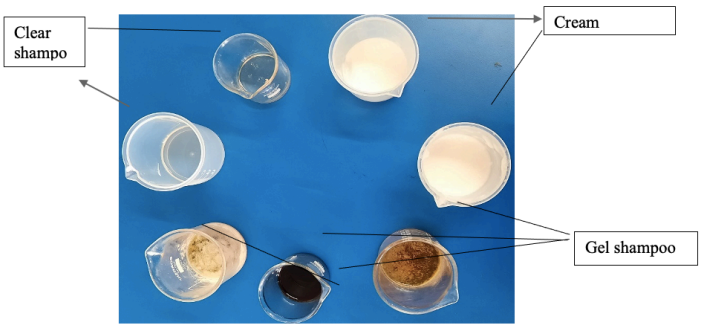
The organoleptic evaluation of shampoo includes macroscopic features of the surfactant-based shampoo, such as colour, taste, and odour. The result of the test is tabulated in Table 5 below.

Table 5: Features and Observations of Ingredients

Sl No	Test	Results
1	Appearance	Viscous, cream-colored liquid
2	Odour	Bitter almond
3	Phase separation	No phase separation was observed
4	Colour	Pale yellow
5	Texture	Fine

The formulated shampoo that has the above-mentioned organoleptic feature.

Fig 1: Different formulations of shampoo



Physicochemical Studies

The physicochemical features of the surfactant-based shampoo were as follows, as shown in Table 6.

The results are tabulated in Table 7 and Figure 2.

Table 6: Evaluation of formulation for Physical appearance, pH, wetting test, and % of solid content

	Standard as per BIS	Formulation			
		F1	F2	F3	F4
Appearances	Colourless -pale	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow
pH	4-9	6.2	7	6.5	6
Wetting	60-180sec	177 Sec	200 sec	180 sec	190sec
% of solid content	10-20%	17.75%.	25%	22%	21%

sec= seconds, %= percentage.
The results of F1-F4 fall within the range specified by the BIS standard. The F1 formulation can be considered optimum since the pH, wetting time, and solid content ranges are within the BIS standard.

pH

For the shampoo, pH is determined at 25 °c The shampoos were found to be slightly acidic while shampooing on the alkaline side, compared to the pH of shampoos. The present formulation is alkaline to reduce hair damage or loss. The results are tabulated in Table 7 and Figure 2.

Wetting time

The wetting time results are represented in Table 7. Formulations F1-F4 are within the BIS standards. The results are tabulated in Table 6.

Percentage Solid Content

The result of the percentage solid content is tabulated in Table 6.

Foam ability and stability test

The shampoos were evaluated for their foam height and stability in distilled water. It was found to show good foaming characteristics in distilled water. Among shampoos, F1-F4, F1 foamed well in distilled water, and the results are tabulated in Table 7.

Surface tension measurement

The Surface tension of the shampoo tests its detergency. Table 7 represents the results of determining the surface tension of the shampoo formulation F1-F4. The decrease in surface tension of formulated shampoo in water was within prescribed limits, indicating good detergent action.

Cleansing action

The cleansing action of the formulated shampoo was evaluated by measuring the percentage of sebum removed after shampooing. Table 7 presents the results of the evaluation of the cleansing action. As seen from the results, shampoos F2 and F3 exhibited the lowest detergency. It may be regarded as a mild cleanser.

Table 7: Results of Foam ability and stability test, Surface tension measurement & Detergency of Test Shampoo

Testing Parameter	Formulation			
	F1	F2	F3	F4
Foam ability and stability test (5 minutes)	152± 0.04	145± 0.32	135± 0.02	120± 0.42
Surface tension measurement: (Dynes cm^{-1})	34.9 ± 0.32	37.7 ± 0.02	33.4 ± 0.04	33.1 ± 0.42
Detergency (%)	79.23 ± 0.43	65.7 ± 0.25	60.5 ± 0.44	62.6 ± 0.36
Results are Mean ± standard deviation.				

Viscosity

For the optimized formulation, the viscosity of shampoo F1 was measured using a Brookfield viscometer at different RPMs with spindle number 12. Results are tabulated in Table 8. The viscosity results increased with an increase in speed, indicating that the shampoos showed pseudoplastic behaviour.

Table 8: Viscosity profile of shampoo formulation at different rpm

Shampoo	RPM (Rotations Per Minute)		
	3	6	12
F1	2187	4999	1653

Wig test

The hair wig was washed with shampoo, and the results were promising. The shampoo had easy application, the foam formed easily, and was lustre-free. The wig is smooth and shiny, with reduced frizz, as shown in Figure 5.

Fig 2: Results of pH

Fig 3: Results of the percentage of solid content

Figure 4: Foam ability and stability test

Fig 5: Results of the wig test

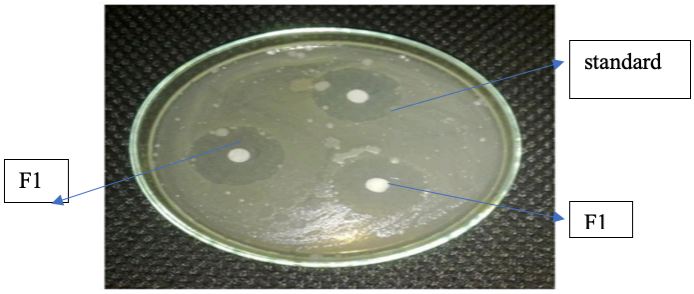
In vitro antifungal test

It was found to possess antifungal activity with a 14 mm zone of inhibition. The antifungal activity of the shampoo was determined against the species. The results for the test, summarized in Table 9 and Figure 6 for the zone of inhibition test, show the presence of a clear zone around the shampoo, suggesting that the shampoo possesses activity that can inhibit the growth of dandruff-causing pathogens. The visible clear zone produced by shampoo against various fungal species is shown in Figure 6 below

Table 9: Result of antifungal activity

Shampoo	Diameter of zone inhibition (mm)
Ketoconazole Shampoo	13
Extract + Surfactant Base Shampoo	14

Figure 13: The diameter of zone inhibition (mm)



Stability study

The stability study for the optimized formulation F1 was conducted at room temperature. All the organoleptic and physicochemical properties were evaluated. There was no change in organoleptic properties. The results are tabulated in Table 10.

Table 10: Results of stability study for 90 days

Testing parameter	30 days	60 days	90 days
Appearance	Viscous, cream-colored liquid	Viscous, cream-colored liquid	Viscous, cream-colored liquid
Odour	Bitter almond	Bitter almond	Bitter almond
Phase separation	No phase separation was observed	No phase separation was observed	No phase separation was observed
Colour	Pale Green	Pale Green	Pale Green
Texture	Fine	Fine	Fine
pH	6	6	6.2
% of solid content	18%	18.5%	18%
Foam ability and stability test (5 minutes)	145± 0.21	140± 0.18	150± 0.12
Detergency (%)	65.23 ± 0.03	60 ± 0.05	60 ± 0.28

Discussion

The MAE method of extraction requires only a few seconds to half an hour, compared to other traditional methods and green synthesis, in which radiation is used as a heat source. This heat source will significantly reduce the time required compared to other conventional extraction methods. The thermolabile chemical constituents can be easily extracted by this method. It also prevents thermal degradation and oxidation, and enhances yield in both quantitative and qualitative terms. The additional benefit is that MAE requires fewer solvents, resulting in a higher extraction yield than other traditional extraction methods and enhanced efficiency. The MAE is environmentally friendly and non-toxic, and it can be easily scaled for larger-scale production in industries. Compared with different shampoo formulation bases, the surfactant-based shampoo was found to be the most stable for formulation, as determined by organoleptic and macroscopic studies. The physicochemical analysis parameters, including pH, wetting test, and determination of the solid content percentage, were conducted for the surfactant-based shampoo. The pH of shampoo plays a crucial role in enhancing and improving hair health, while also helping to prevent eye irritation and maintaining the biological balance of the scalp. The percentage solid content of the shampoo was within the limit set by BIS, indicating the absence of heavy metal or toxic metal ions. The wetting time of a shampoo depends on its concentration; in other words, wetting time is inversely proportional to detergency. Accordingly, shampoos with maximum detergency should have a minimum wetting time, which helps improve the shampoo's cleansing property. The ability of shampoo to produce foam is a crucial criterion, as it reduces the interfacial tension between dirt, dead cells, or excess sebum and the scalp layer, thereby improving the cleansing ability. The above parameters depend on the concentration of surfactant added, a characteristic suitable for shampoo formulations. Shampoo F1 had fair detergency and was interpreted as a good cleanser. However, shampoo F1 is an active cleanser, as it showed maximum detergency and is considered more suitable for normal hair. The shampoo's decrease in surface tension of the water, from 72.8 dynes/cm to 32-37 dynes/cm, is an indicator of its good detergent action. By comparing the results of formulations from F1 to F4, F1 is considered optimized. The result of F1 was considered optimized due to its shorter wetting time, high detergency ability, and other parameters, which met the BIS certification standards for shampoo. The F1 formulation also showed pseudoplastic flow similar to the marketed formulation. The F1 formulation was tested for antifungal activity by the agar diffusion method and compared with the marketed formulation. It inhibited the growth of *Candida albicans,* providing the anti-dandruff activity against Seborrheic dermatitis. It can also be used for anti-dandruff shampoo. The inhibition of shampoo was like a market formulation. Furthermore, the stability study over 90 days revealed no changes in the organoleptic and physicochemical parameters of the shampoo.

Conclusion

C. papaya leaves were extracted using MAE, and the yield was higher than that of the traditional extraction method. The extract had high quantities of tannin, which is responsible for its antifungal activity. Tannins can be easily extracted using the MAE method. The studies have proven that surfactant-based shampoos are suitable for formulating herbal shampoos. By varying the different concentrations of SLS and HPMC, four different compositions were formulated. The organoleptic and physicochemical analyses were conducted in accordance with BIS standards for the four formulations. The stability study indicated that the formulation was not physically incompatible at room temperature. The room temperature is suitable for storing the formulation. This method of extraction and formulation is more straightforward for the scale-up process. Further research is needed on the toxicity of shampoo at the molecular level using standard *in vitro* cell line tests, which can be easily scaled up for industrial applications. The natural product has numerous components that have fewer side effects, are non-toxic, biodegradable, and environmentally friendly. Extensive research is needed to develop these products for technology transfer and safer use.

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