



Formulation and *In vitro* evaluation of herbal shampoo containing papaya seed extract

A Sudheer ^{1,*}, AV Rajyalakshmi ¹, N Audinarayana ¹, Babu BK ¹ and D Jothieswari ²

¹ Department of Pharmaceutics, Sri Venkateswara College of Pharmacy (Autonomous), RVS Nagar, Tirupati Road, Chittoor-517127, Andhra Pradesh, India.

² Department of Pharmaceutical Analysis, Sri Venkateswara College of Pharmacy (Autonomous), RVS Nagar, Tirupathi Road, Chittoor-517127, Andhra Pradesh, India.

GSC Biological and Pharmaceutical Sciences, 2025, 31(01), 239-255

Publication history: Received on 19February 2025; revised on 13 April 2025; accepted on 15 April 2025

Article DOI: <https://doi.org/10.30574/gscbps.2025.31.1.0128>

Abstract

Formulation and In Vitro Evaluation of Herbal Shampoo Containing Papaya Seed Extract. The objectives included quantifying the extract's active constituents, assessing its antifungal activity, and evaluating various shampoo formulations. Large-scale production with high extract yields was shown to be feasible using the Soxhlet extraction process. The shampoo's therapeutic qualities were attributed to a rich composition of advantageous components, including carbohydrates, phenols, flavonoids, alkaloids, and glycosides, which were discovered during a thorough phytochemical examination. FTIR, HPLC, and spectrophotometric studies verified quercetin's presence and structural integrity, ensuring accurate measurement and robust antioxidant properties. Shampoo formulations with desirable physical attributes, good washing capabilities, ideal viscosity, and foam stability were especially demonstrated by formulation F6. According to in-vitro antifungal testing, F6 exhibited superior viscosity and antifungal activity over time, demonstrating substantial inhibition against *Candida albicans*. The research highlights the potential of adding natural extracts, such as papaya seed, to personal care products, improving their overall efficacy and therapeutic qualities while fostering the creation of hair care products that are safe, efficient, and easy to use while also offering significant health advantages.

Key words: Herbal Shampoo; Papaya seeds; *In-Vitro* Evaluation; Seed Extract; Chemical Test.

1. Introduction

Herbal shampoo Shampoos are most probably used as beautifying It is a hair care product that is used for cleanse scalp and hair in our daily life. Shampoos are most likely utilized as beautifying agents and are sticky solution of detergents containing suitable additives preservatives and active Ingredients. It is usually applied on wet hair, Massaging into the hair, and cleansed by inking with water. The purpose of using herbal shampoo is used to remove dirt that is make up on the hair without stripping out much of the sebum. Many artificial shampoos are present in the current market both medicated and non-medicated, however, shampoo popularized due to natural origin which is safe , increases consumers demand and free from side effects. It should effectively and completely remove dust or soil, excessive sebum or other fatty Substances and loose corneal cells from the hair [1]. But popularity of the herbal shampoos among consumers is on rise because of the belief. The herbal shampoos are safe and free from side effect. Herbal shampoos is widely unstable product all over the world it has been used for many years .chemical herbal shampoos are prepared with several chemicals which can care hairs problems bur also responsible for damage of hairs. Some international researchable said that the chemicals of herbal shampoos also responsible for cancer herbal shampoos are defined as preparation of a surfactant in suitable form liquid , solid or powder which when used under the condition specified will remove surface grease , dirt & skin debries from the hair shaft & scalp[2].

*Corresponding author: A Sudheer

2. Materials and Methods

2.1. Preparation of seed extract

The Soxhlet extraction method with 95% ethanol was used in this work to process papaya seeds since it is effective at extracting a variety of chemicals. Following their crushing, the seeds were put into the Soxhlet extractor. The seeds were continuously and thoroughly extracted by passing ethanol through them several times. After that, the resulting solution was gathered, filtered to get rid of contaminants, and concentrated if needed. A final extract full of bioactive chemicals was produced when the solvent was evaporated. A dependable and effective technique for separating important compounds from papaya seeds for possible medical and biological uses is the Soxhlet extraction process [3].

2.2. Preformulation Studies

2.2.1. Phytochemical screening of seed extract:

Papaya seed extract is subjected to a variety of qualitative assays as part of phytochemical screening in order to identify the presence of various bioactive components. Here are several techniques for applying the designated tests to the testing of particular phytochemicals [4-9].

2.2.2. Alkaloids

Method: Dragendorff's Test

- **Procedure:** To the extract, add a few drops of Dragendorff's reagent, which is a potassium bismuth iodide solution.
- **Observation:** When alkaloids are present, a red or orange precipitate will occur.

Phenols

Method: Lead Acetate Test

- **Procedure:** Add a few drops of 10% lead acetate solution to the extract.
- **Observation:** A white precipitate indicates the presence of phenols.

Flavonoids

Method: Shinoda Test

- **Procedure:** To the extract, add a few drops of strong hydrochloric acid and a tiny bit of magnesium turnings.
- **Observation:** Flavonoids are indicated by the appearance of a pink, red, or magenta colour.

Tannins

Method: Ferric Chloride Test

- **Procedure:** Add a few drops of a 5% ferric chloride solution to the extract.
- **Observation:** The appearance of a dark blue, green, or black colour indicates the presence of tannins.

Saponins

Method: Foam Test

- **Procedure:** Shake the extract vigorously with water in a test tube.
- **Observation:** Saponins are present when foam forms persistently and lasts for at least ten minutes.

Carbohydrates

Method: Molisch's Test

- **Procedure:** Add concentrated sulphuric acid down the side of the test tube after adding a few drops of Molisch's reagent (α -naphthol in ethanol) to the extract.

- **Observation:** Carbohydrates are present when a violet ring forms at the interface between the two liquids.

2.2.3. Determination of absorption maxima

100 mg of quercetin was dissolved in methanol to reach a concentration of 10 µg/ml in order to ascertain the absorption maxima of quercetin. Scanning in the 200–600 nm wavelength range revealed that the solution had its maximum absorbance at 260 nm.

2.3. Compatibility Studies [10]

2.3.1. Fourier Transform Infrared Spectroscopy (FTIR) Studies

ATR model IR affinity 1S, Shimadzu, Japan, was used for FTIR spectroscopy on the pure chemicals and shampoo formulations. The spectroscopy's scanning range was established at 400 cm⁻¹ to 4000 cm⁻¹.

2.3.2. High Performance Liquid Chromatography (HPLC) Analysis

The food and pharmaceutical sectors rely heavily on the study of bioactive chemicals found in natural extracts. Papaya seed ethanol extract is analysed in this study using the HPLC method (Agilent 1200 Infinity system). A C18 column (4.6 x 250 mm) with a mobile phase of 100% methanol (HPLC grade) and a flow rate of 1.0 ml/min allowed us to successfully separate and elute compounds. Ideal for the investigation of UV-absorbing components often found in plant extracts, the sample, which consisted of 1000 mg of papaya seed ethanol extract dissolved in methanol, was injected at a volume of 20 µl and detected at a wavelength of 260 nm. The objective of this process was to determine and measure the bioactive components found in papaya seed extract [11].

2.3.3. Determination of total flavonoid content

10 mg of seed extract was diluted in 100 ml of methanol to reach a concentration of 100 µg/ml. Four millilitres of water were used to dilute this concentration. In the volumetric flask, 0.3 ml of 5% NaNO₂ was added concurrently, followed by 0.3 ml of 10% AlCl₃ and 2 ml of 1 M NaOH. 4.4 ml of distilled water was added to get the volume up to 10 ml. The absorbance at 510 nm was measured using a spectrophotometer [12]. To determine the mean absorbance value, the process was carried out three times (Table 5). A linear regression approach based on the standard plot of quercetin was used to determine the TFC. The unit of measurement was mg QE/g.

2.4. In-vitro antioxidant activity [13]

- **Preparation of DPPH solution:** 4 mg of DPPH should be dissolved in 100 ml of methanol in a conical flask. To shield the solution from light, place aluminium foil over the flask. Let the mixture settle for half an hour at room temperature.
- **Preparation of standard ascorbic acid solution:** Make a stock solution with a concentration of 100 µg/ml by dissolving 10 mg of ascorbic acid in 100 ml of methanol. Following serial dilution, concentrations of 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml are produced. Pipette 1 ml of each of these diluted solutions into a different 10 ml volumetric flask. Each flask should contain 3 ml of the DPPH solution before being filled with methanol to the 10 ml mark. At 517 nm, measure each solution's absorbance.
- **Preparation of extract solution:** A standard solution with a concentration of 100 µg/ml can be made by dissolving 10 mg of the leaf extract in 100 ml of methanol. The concentrations of 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml are then achieved by serially diluting this solution. Pipette 1 ml of each of these diluted solutions into a different 10 ml volumetric flask. Each flask should contain 3 ml of DPPH solution before being filled with methanol to the 10 ml mark. At 517 nm, measure each solution's absorbance.

Control: 6 ml methanol+ 3 ml DPPH solution

Formula:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.4.1. Formulation of Herbal shampoo

Papaya seed extract is used in this study to make a herbal shampoo for possible hair care benefits. To create a herbal shampoo, combine the necessary amount of guar or xanthan gum with glycerine to get the right viscosity. Stir constantly as you add this mixture to water to create a thickening base. Boil the necessary amounts of Hibiscus, Reetha, Shikakai,

and Nagarmotha in water to make a decoction in a different beaker. To ensure a clean herbal extract, filter the decoction after boiling to get rid of any solid residues. Mix well to combine this filtrate with the thickening base [14]. Then, to ensure even distribution, add vitamin E as a preservative after incorporating papaya seed extract. Finally, adjust the pH of the shampoo by gradually adding citric acid until the desired pH level is reached. Mix well to ensure uniform consistency.

Table 1 Formulation of Herbal Shampoo

Ingredients	F1	F2	F3	F4	F5	F6
Papaya seed extract(g)	2	4	6	2	4	6
Herbal extract*(ml)	15	15	15	15	15	15
Xanthan gum(g)	0.1	0.15	0.2	-	-	-
Gaur gum(g)	-	-	-	0.15	0.3	0.45
Citric acid(g)	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin E(ml)	1	1	1	1	1	1
Glycerine(ml)	2	2	2	2	2	2
Water(ml)	30	30	30	30	30	30

Table 2 Ingredients of Herbal Extract

Ingredients	Parts	Quantity
Nagarmotha	Root	1 gm
Shikakai	Flower	2 gm
Hibiscus	Powder	1 gm
Soapnut	Fruit	3 gm

2.4.2. Evaluation of herbal shampoo formulation

The created formulation's quality was evaluated using a number of quality control tests, including conditioning performance, physiochemical control, visual appearance, and furthermore.

Physical appearance

The created compositions were evaluated for colour, consistency, clarity, and aroma [15].

Measurement of pH

To produce a sample with a 10% concentration, the generated mixture was diluted with distilled water [16]. A digital pH meter was then used to measure the pH of the generated sample at room temperature (302°C).

Determining of percent solid content

Four grammes of shampoo were placed to a weighed, dry, and clean porcelain dish. The precise weight of the shampoo was calculated when the dish containing it was weighed. The shampoo was placed in a porcelain dish and cooked on a hot skillet until the liquid dried [17]. We calculated the weight after drying.

$$\% \text{ Solid Content} = \frac{\text{Net wt of dry sampl (W1-W3)}}{\text{Net wt of test sample (W2 - W1)}} \times 100$$

Dirt dispersion

Put two drops of shampoo and 10 millilitres of distilled water in the big test tube. Put one drop of Indian ink in the test tube, put a stopper on it, and shake it ten times [18]. There were four different categories for the amount of ink in the foam: none, mild, moderate, and heavy.

Measurement of viscosity

The viscosity of the shampoo was measured using the Brookfield Viscometer. The viscosity of shampoo was measured using spindle number TL 6 at room temperature ($30\pm 2^\circ\text{C}$) with varying spindle speeds ranging from 1 to 5 rpms [19].

2.4.3. Analysis of Foaming Efficiency and Stability

With a few modest adjustments, the cylinder shaking method was used to test the foaming ability. A 10 ml graduated measuring cylinder was filled with 5 ml of the 1% shampoo solution, and it was manually closed. After one minute of shaking the measuring cylinder, the total amount of foam content was calculated. Five minutes were spent on this process. After shaking for one and four minutes, the foam's volume was measured to see how stable it was [20].

2.4.4. In-vitro anti-fungal efficacy

The agar-well diffusion method was used to evaluate the seed extract and shampoo formulation's antifungal efficacy. The fungal inoculum was dispersed throughout the media to inoculate the microorganism (*Candida albicans*) into Czapekdox agar. An 8-mm stainless-steel cork borer was used to create agar wells, which were then filled with 200 μl of plant extract [21]. On the same plate, control wells containing transparent solvents (negative controls) were also filled simultaneously. The diameter of the zone of inhibition was used to measure the antibacterial activity of the bacterial plates after they were incubated for 72 hours at 28°C .

2.4.5. Stability studies

The ICH guidelines for expedited stability studies were followed for conducting stability testing. The sample compositions were kept at room temperature ($40^\circ\text{C}\pm 2^\circ\text{C}$ and $75\pm 5\%$ relative humidity) for three months. The samples' physical properties, pH, viscosity, foam stability, and fungal activity were assessed [22].

3. Results and Discussion

3.1. Papaya seed extraction

Using 95% ethanol as the solvent, the Soxhlet extraction method produced a 23% yield of papaya seed extract in this investigation. 46 g of extract was successfully recovered from about 200 g of papaya seeds [Table 3].

3.2. Pre-formulation parameters

3.2.1. Phytochemical screening

Table 3 Plant Extract Phytochemical Screening

Phytochemicals	Papaya Seed Extract
Alkaloids (Drangendroff's Test)	+
Phenols (Lead acetate Test)	+
Flavonoids (Shinoda Test)	+
Saponins (Foam Test)	+
Tannins (Ferric chloride Test)	+
Carbohydrates (Molish's Test)	+

The presence of alkaloids, phenols, flavonoids, saponins, tannins, carbohydrates, and other components was confirmed by screening the produced papaya seed extract for a variety of phytochemicals.

3.2.2. Absorption maxima of Quercetin

Using methanol as a solvent, it was discovered that quercetin's absorption maxima was 260 nm (Figure 1).

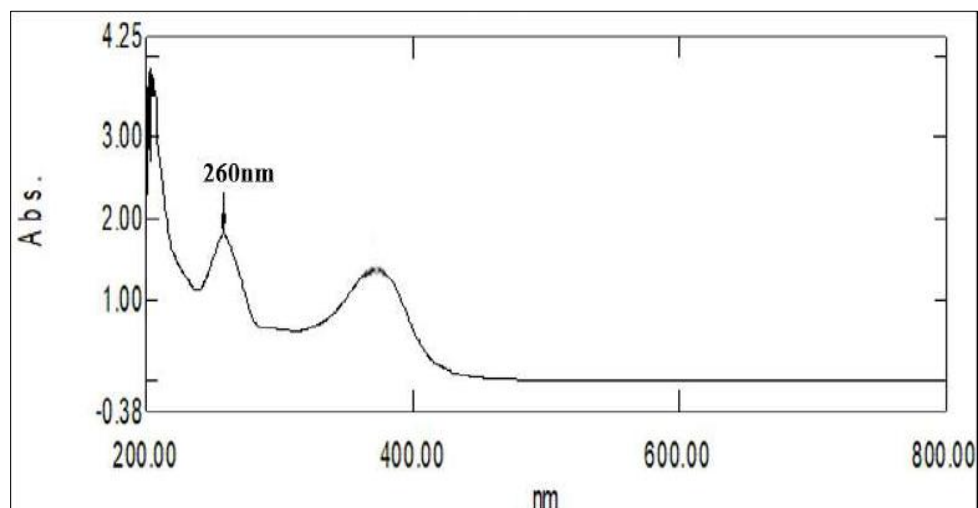


Figure 1 Absorption maxima of Quercetin

3.2.3. Compatibility study using FTIR spectroscopy

The presence of quercetin in papaya seed extract and its incorporation into a shampoo were verified by a Fourier Transform Infrared Spectroscopy (FTIR) investigation. These results validate quercetin's compatibility and stability in the formulation by showcasing its structural integrity and functional stability in both the extract and the mixture [Figure 2 to 6] & [Table 4 to 8].

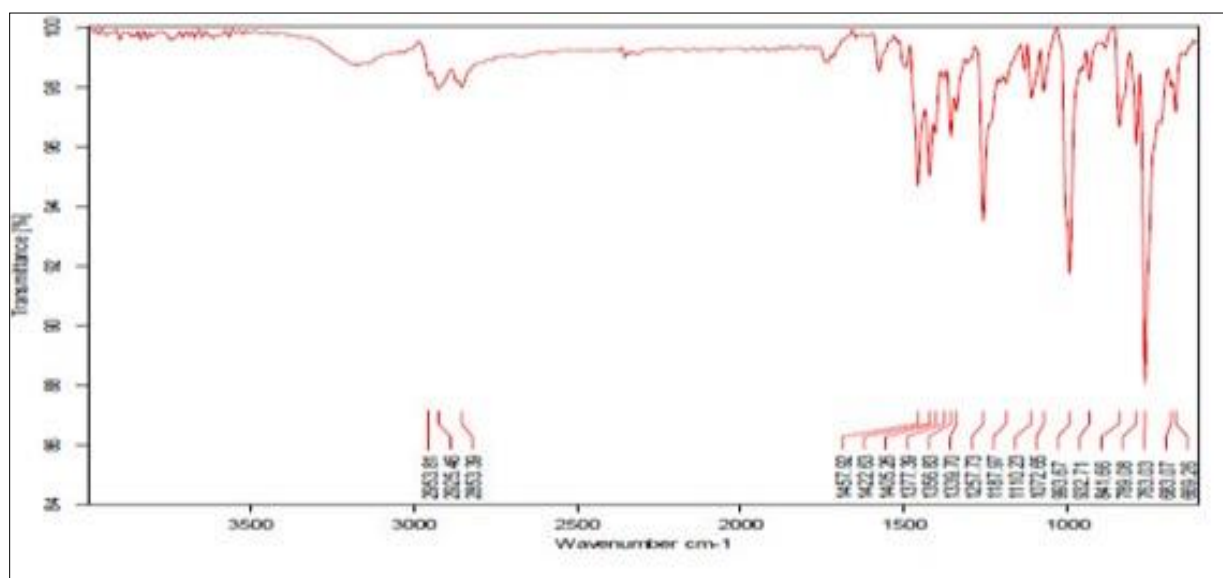
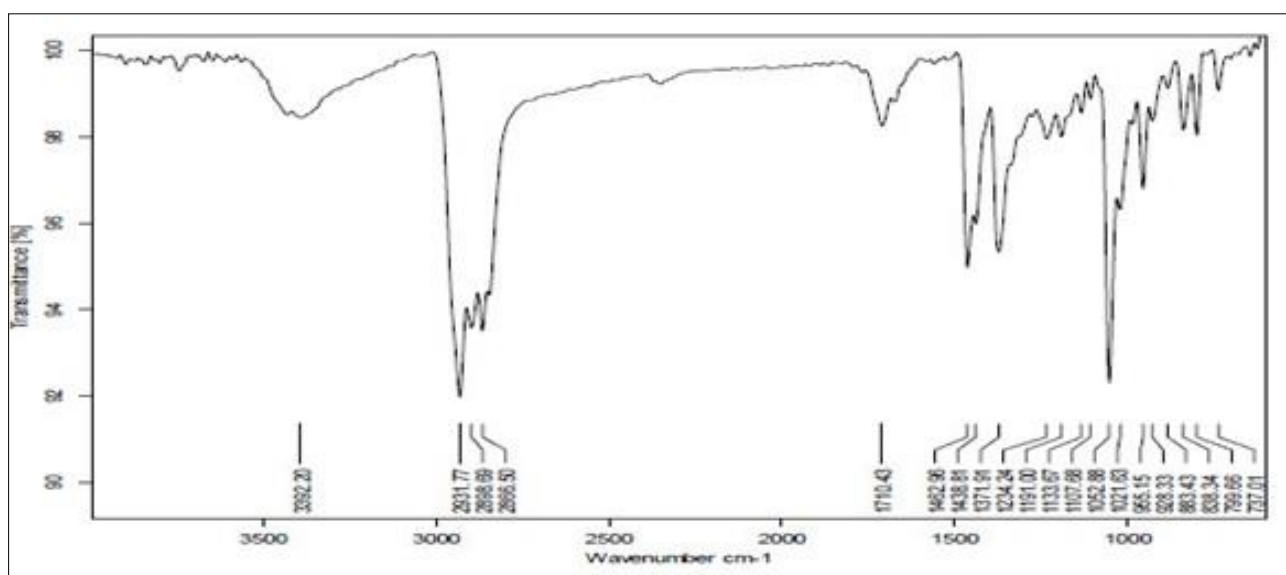


Figure 2 FTIR Spectrum of Herbal extract

Table 4 FTIR Herbal extract interpretation

S.NO	Interpretation	IR absorption bands (cm ⁻¹)	
		Characteristic peak	Observed peak
1.	O-H(stretch, free)	3700-3500	3735.48
2.	N-H stretch	3500-3180	3171.36
3.	Alkyl C-H Stretch	2950 - 2850	2868.62
4.	C, N triple bond stretch	~2250	2312.60
5.	C=O stretch	1810-1775	1714.21
6.	CH ₃ bend	~1375	1338.58
7.	C-C stretch	1300-1100	1108.91
8.	C-H bend (meta)	~880	885.16
9.	C-H bend (para)	850-800	840.62
10.	C-Cl Stretch	800-600	667.60

**Figure 3** FTIR Spectrum of quercetin**Table 5** FTIR Spectrum interpretation of quercetin

S.NO	Interpretation	IR absorption bands (cm ⁻¹)	
		Characteristic peak	Observed peak
1.	O-H(stretch, free)	3400-3300	3392.20
2.	Alkyl C-H Stretch	2950 - 2850	2868.62
3.	C=O stretch	1810-1775	1710.43
4.	C=C stretch	~1475	1438.81
5.	CH ₃ bend	~1375	1371.91
6.	C-O-C stretch (dialkyl)	1300-1000	1052.88

7.	C-H bend (meta)	~880	883.45
8.	C-H bend (para)	850-800	838.34

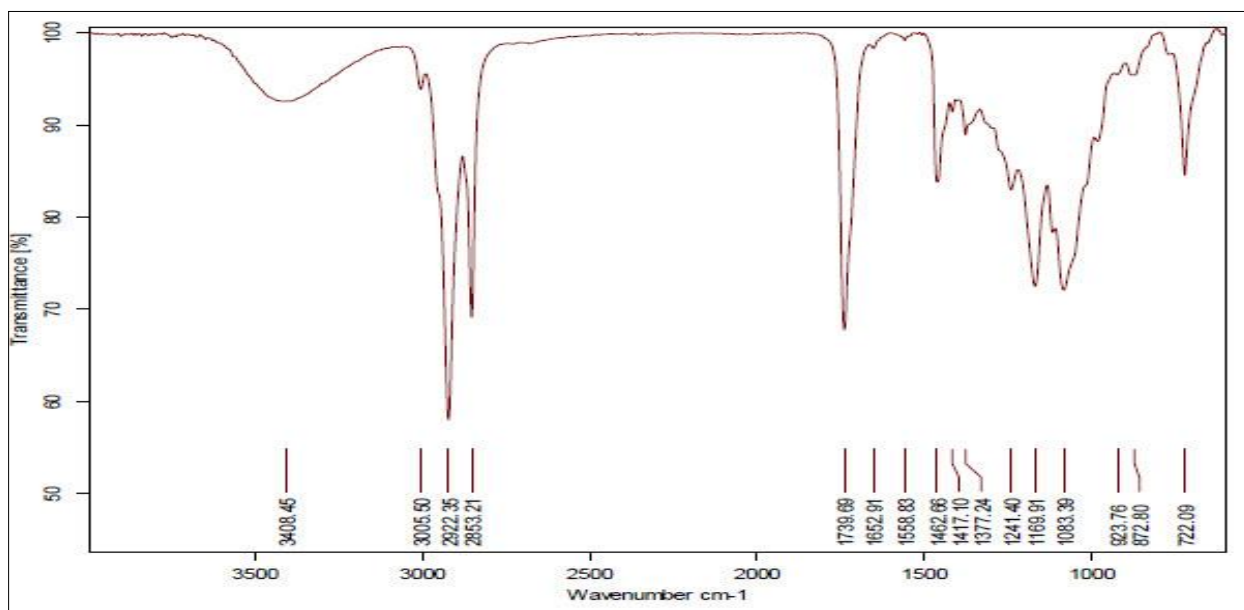


Figure 4 FTIR Spectrum of Xantham gum

Table 6 FTIR Spectrum interpretation of Xantham gum

S.NO	Interpretation	IR absorption bands (cm ⁻¹)	
		Characteristic peak	Observed peak
1.	O-H (stretch, free)	~3500	3408.45
2.	Alkyl C-H Stretch	2950 - 2850	2922.35
3.	C=O stretch	1810-1775	1739.69
4.	C=C stretch	~1475	1462.66
5.	CH ₃ bend	~1375	1377.24
6.	C-C stretch	1200-1100	1170.07
7.	C-O-C stretch (dialkyl)	1100-1000	1083.39
8.	C-H bend (meta)	~880	872.80

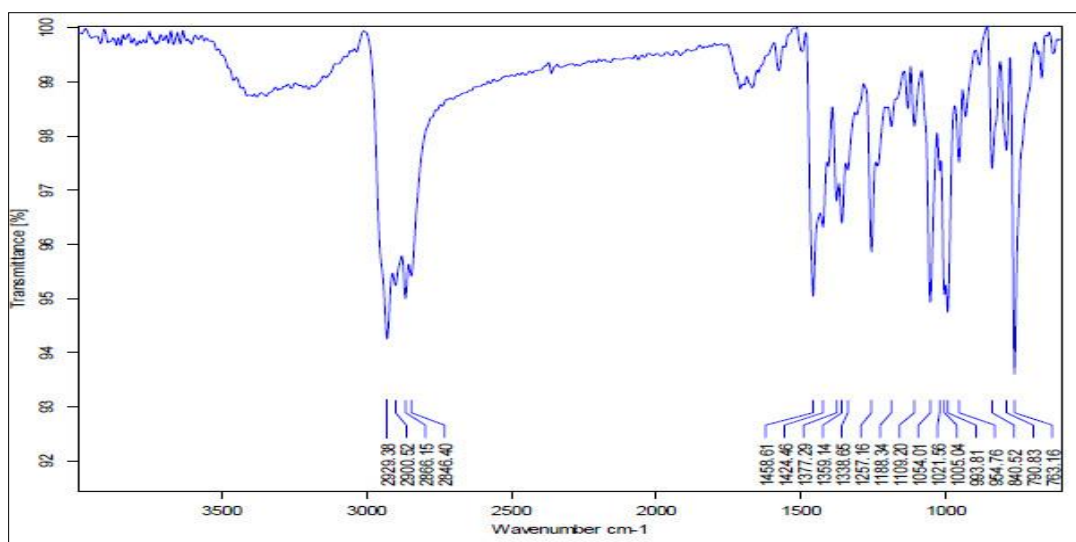


Figure 5 FTIR Spectrum of Guar Gum

Table 7 FTIR Spectrum interpretation of Guar Gum

S.NO	Interpretation	IR absorption bands (cm ⁻¹)	
		Characteristic peak	Observed peak
1.	Alkyl C-H Stretch	2950 – 2850	2929.38
2.	C=C stretch	~1475	1458.61
3.	CH ₃ bend	~1375	1377.29
4.	C-C stretch	~1100	1109.20
5.	C-O-C stretch (dialkyl)	1300-1000	1054.01
6.	C-H bend (para)	850-800	840.52
7.	C-Cl Stretch	800-600	763.16

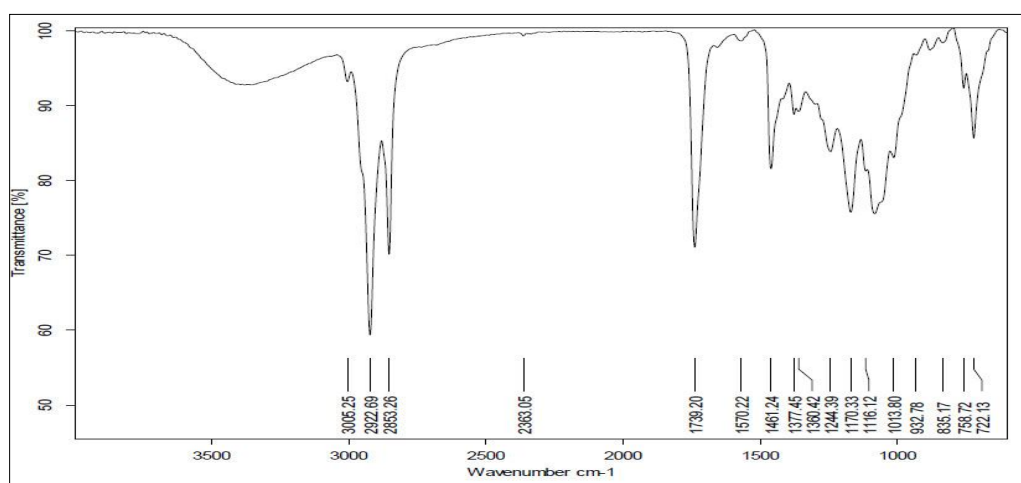


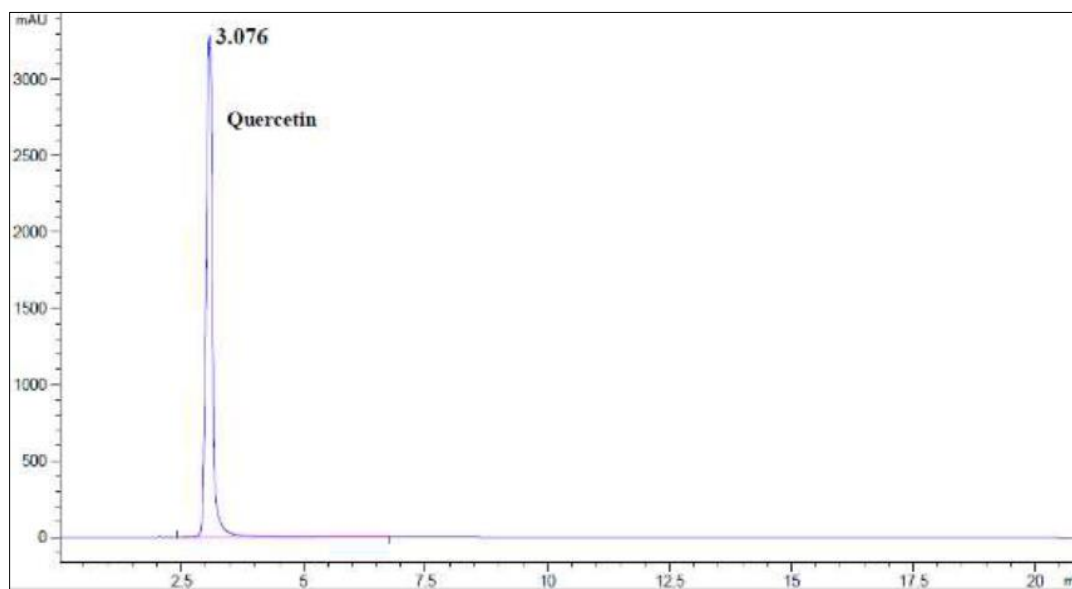
Figure 6 FTIR Spectrum of Mixture

Table 8 FTIR Spectrum interpretations of Mixture

S.NO	Interpretation	IR absorption bands (cm ⁻¹)	
		Characteristic peak	Observed peak
1.	O-H (stretch, free)	~3500	3408.45
2.	Alkyl C-H Stretch	~2950	2922.69
3.	C, N triple bond stretch	~2250	2363.05
4.	C=O stretch	1810-1775	1739.20
5.	C=C stretch	~1475	1461.24
6.	CH ₃ bend	~1375	1377.45
7.	C-C stretch	1200-1100	1170.33
8.	C-O-C stretch (dialkyl)	1100-1000	1013.80

3.2.4. HPLC

Using a C18 column and methanol mobile phase, an HPLC analysis of papaya seed extract at 260 nm showed a peak area of 2.50343e4 mAU and a retention period of 2.964 minutes. By contrast, the standard quercetin exhibited a peak area of 3.05769e4 mAU and a retention time of 3.076 minutes. The comparable retention periods in these studies suggest quercetin is present in papaya seed extract [Figure 7, 8].

**Figure 7** HPLC chromatogram of quercetin

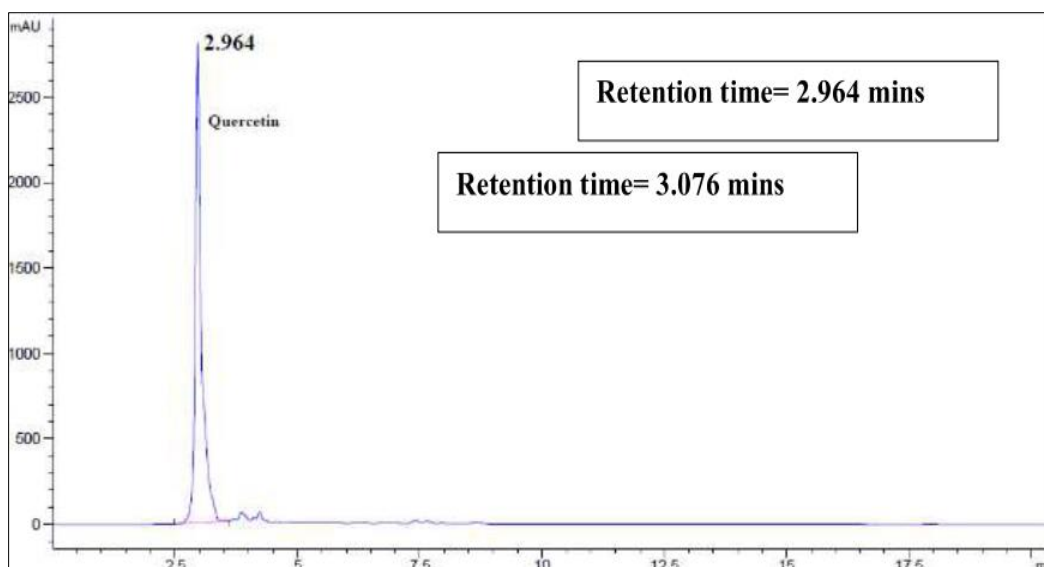


Figure 8 HPLC chromatogram of papaya seed extract

3.3. Total flavonoid Content

Total flavonoids in the plant extract were quantitatively determined using a standard calibration curve of quercetin, which showed linearity in the concentration range of 50–600 µg/ml. It was found that the papaya seed extract had a total flavonoid concentration of 9.03 mg QE/g of dry extract [Figure 9, Table 9].

Table 9 Total Flavonoid Content of Quercetin Herbal shampoo

Parameters	Result
Total Flavonoid content	9.03 mg QE /g Extract

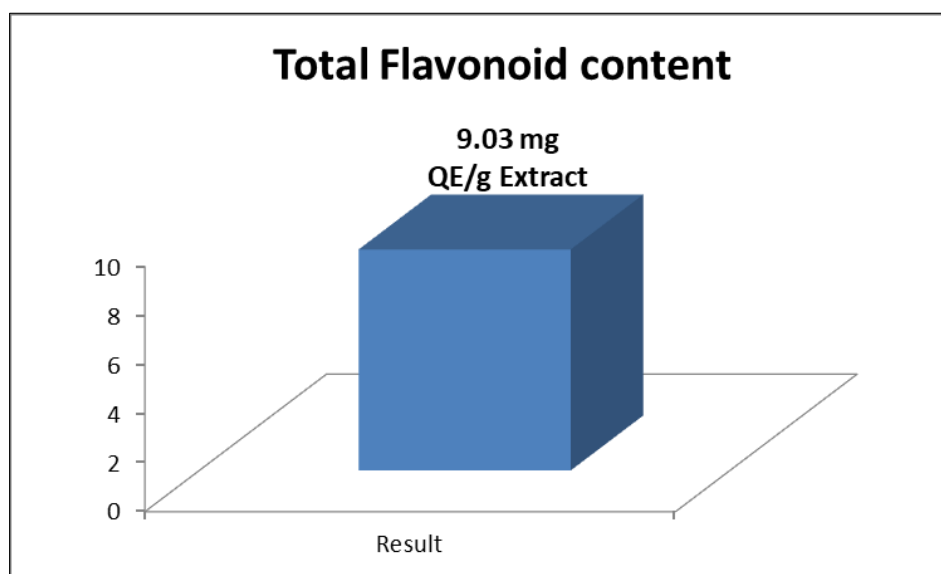


Figure 9 Total flavonoid content Quercetin Herbal shampoo

3.4. *In-vitro* antioxidant activity

Papaya seed extract showed 71.08% radical scavenging activity at 100 µg/mL, demonstrating its excellent DPPH radical scavenging capabilities (Figure 10) (Table 10). This high level of antioxidant activity indicates that adding papaya seed

extract to herbal shampoos may improve their ability to protect the scalp and hair from oxidative stress, improving the general health and longevity of hair.

Table 10 *In-Vitro* Antioxidant Activity of Standard and Plant Extract

Concentration ($\mu\text{g/ml}$)	Standard Ascorbic acid (% inhibition)	Plant extract (% inhibition)
20	38.26	34.5
40	43.32	42.36
60	58.50	54.65
80	71.08	65.51
100	88.14	72.09

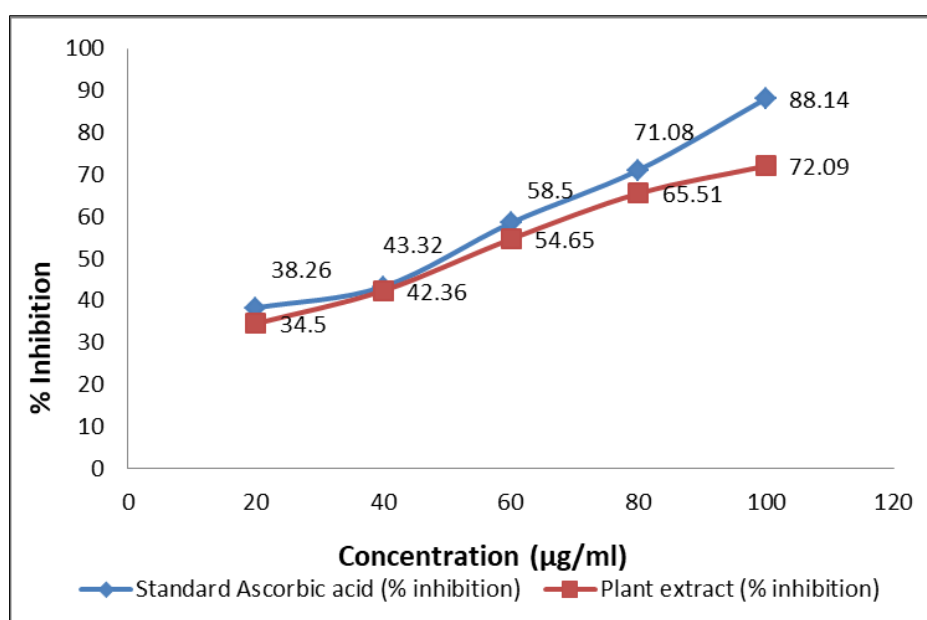


Figure 10 *In-vitro* antioxidant activity of standard and plant extract

3.5. Anti-fungal activity

Papaya seed extract's antifungal efficacy against *Candida albicans* was examined in a study employing Czapek Dox agar medium. According to the findings, the extract significantly inhibited the growth of the fungi. A zone of inhibition measuring 12.8 mm was obtained at a dosage of 500 mg, whereas a somewhat greater zone of inhibition measuring 13.5 mm was obtained. Papaya seed extract is a promising option for natural antifungal medicines because of these results, which show that it has dose-dependent antifungal effects.

3.6. Evaluation Parameters

3.6.1. Physical appearance

The shampoo appears earthy and natural due to its brownish colour and liquid consistency. Because of its opaque clarity, it appears to contain natural extracts and nourishing elements. These natural components provide a nice fragrance that is both calming and fragrant (Table 11).

3.6.2. PH determination

All shampoo formulations fall within the recommended pH range, which is between 5.5 and 7.

3.6.3. Percent solid content

The shampoos were easy to apply and rinse off without being very thick or challenging to wash off thanks to their solids content, which ranged from 18 to 27% (Table 11).

3.6.4. Dirt dispersion

A sufficient level of quality was demonstrated by the dirt dispersion test, which showed that none of the six shampoo formulas concentrated ink in the foam, guaranteeing that dirt stayed in the water and would not redeposit on hair (Table 11).

Table 11 Evaluation parameters for herbal shampoo formulations

Formulation code	Appearance	Colour	Clarity	pH	% Solid content	Dirt deposition
F1	Brownish, liquid and Characteristic	Brown	Slight opaque	6.5±0.025	20.2%	Light
F2	Brownish, liquid and Characteristic	Dark brown	opaque	6.8±0.020	22.2%	None
F3	Brownish, liquid and Characteristic	Dark brown	opaque	6.3±0.017	25.6%	None
F4	Brownish, liquid and Characteristic	Brown	Slight opaque	6.5±0.025	19.2%	Light
F5	Brownish, liquid and Characteristic	Dark brown	opaque	6.3±0.020	22.4%	None
F6	Brownish, liquid and Characteristic	Dark brown	opaque	7.0±0.017	26.1%	None
F7	Brownish, liquid and Characteristic	Dark brown	Slight opaque	7.1±0.016	25.4%	None
F8	Brownish, liquid and Characteristic	Dark brown	opaque	7.1±0.015	25.1%	None

3.6.5. Viscosity

For the best texture and performance, the herbal shampoo formulas include viscosities ranging from 2000 to 9000 cps. This permissible range applies to all six formulas for both rpm 1 and rpm 2 (Table 12). The viscosity also exhibits normal shear-thinning behavior, decreasing as the rpm increases (Figure 11).

Table 12 Viscosity of Herbal Shampoo Formulations

Formulations	Viscosity (cps)		
	1 RPM	2 RPM	5 RPM
F1	3671.2 ±9.14	2037.3 ±8.12	1246.1±9.17
F2	3846.1 ±8.24	2516.8 ±7.17	1357.5 ±8.12
F3	4015.3 ±9.18	2813.2 ±9.29	1413.6 ±8.48
F4	3721.6 ±7.89	2121.3 ±8.19	1396.2 ±8.97
F5	3956.1 ±8.32	2658.3 ±9.63	1827.5 ±8.28
F6	4128.7 ±8.13	3059.3 ±8.43	2153.1 ±9.25
F7	4115.6 ±8.06	3042.2 ±8.33	2125.2 ±9.18
F8	4122.5 ±8.07	3045.1 ±8.34	2123.1 ±9.21

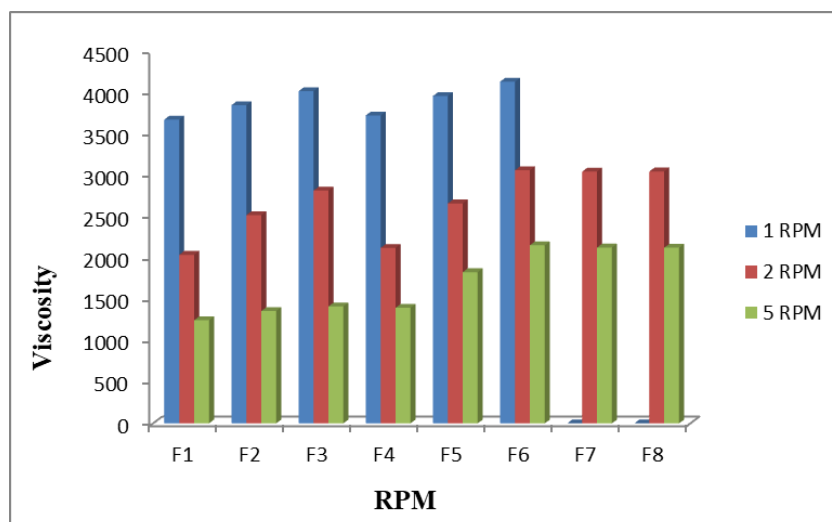


Figure 11 Viscosity of Herbal Shampoo Formulation

3.6.6. Foaming efficiency and foam stability

In distilled water, all six formulations showed comparable foaming properties and foam retention (Figure 12). The herbal shampoos' foam stability, which shows consistent foams with little volume variation (Table 13). Interestingly, detergency and foaming do not directly correlate, proving that a shampoo with higher foaming does not always clean better. Stable foams with a constant volume were formed by the finished compositions.

Table 13 Foam Stability of Herbal Shampoo Formulations

Time In mins	Foam volume(ml)							
	F1	F2	F3	F4	F5	F6	F7	F8
	7	7.4	7.6	7.2	7.6	7.8	7.6	7.7
	6.8	7.3	7.6	7.2	7.5	7.6	7.4	7.5
	6.7	7.2	7.4	7.1	7.4	7.5	7.3	7.5
	6.5	7.1	7.2	7.0	7.4	7.4	7.1	7.3
	6.5	7.0	7.2	6.8	7.2	7.1	7.2	7.2

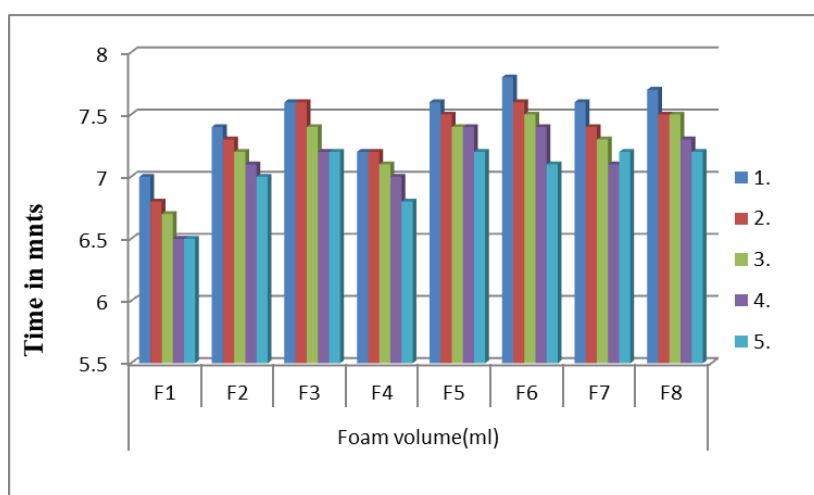


Figure 12 Foam Stability of Herbal Shampoo Formulations

3.7. Anti-fungal activity

Test results indicated that the shampoo formulations' antifungal efficacy against *Candida albicans* was inhibited to varied degrees. Formulations F1 and F2 each showed an inhibition zone of 12.2 mm, 13.1 mm, 14.1 mm, 13.9 mm, 15.2 mm, and 15.8 mm, respectively, with the highest inhibition in Formulation F6. In contrast, the negative control (placebo) displayed no inhibition, while the positive control revealed a 22 mm inhibition zone. According to these findings, all shampoo formulations contain antifungal qualities (Table 14, Figure 13) however the most effective ones against *Candida albicans* are F5 and F6.

Table 14 Antifungal Activity of Herbal Shampoo Formulations

Microbial strain	<i>Candida albicans</i>									
Shampoo Formulations	F1	F2	F3	F4	F5	F6	F7	F8	+ve control (Itracanazole 10mg/ml)	-ve control (placebo)
Inhibition (in mm)	12.4± 1	13.2 ± 1	14.2 ± 1	13.8± 1	15.4± 1	15.9± 1	15.8±2	15.6±1	22 ± 3	0

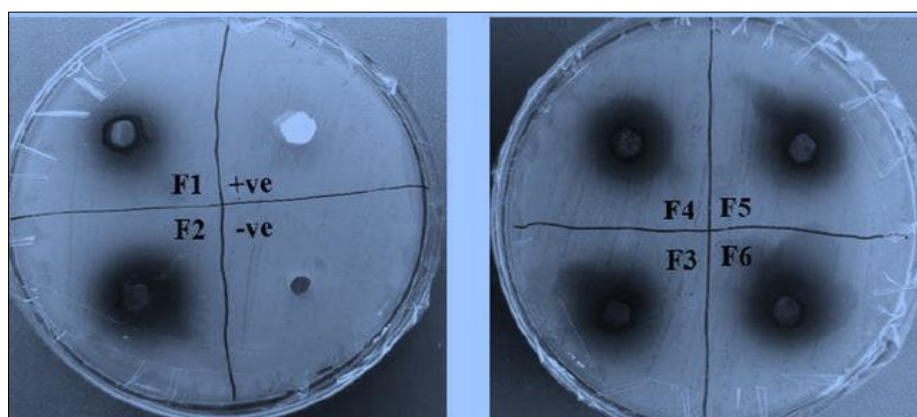


Figure 13 Zone of Inhibition of Herbal Shampoo Formulations on *Candida albicans*

3.7.1. Stability study

Three months were allotted for the stability investigation of the herbal shampoo formulations. The findings showed no discernible changes in foam ability, consistency, or appearance. The product's chemical makeup may have changed slightly, though, as seen by the tiny pH fluctuations and viscosity decrease. The formulations generally showed stability during the course of the investigation, with only slight changes in a few parameters. Formulation F6 was best for having strong antifungal action that persisted over time.

Table 15 Stability results for 3 months

Evaluation Parameters	Shampoo formulations (F1-F8) Observations (40°C ± 2 and 75 ± 5% relative humidity)			
	Initial	1 month	2 months	3 months
Physical parameters	No change	No change	No change	No change
Phase Separation	No Phase Separation	No Phase Separation	No Phase Separation	No Phase Separation
Foam stability	Good	Good	Good	Good
pH	6.56 ±0.014	6.5 ±0.035	6.3 ±0.091	6.2 ±0.012
Viscosity	3889.83 ±9.18	3586.83 ±5.38	3462.23 ±8.17	3163.68 ±2.13

4. Conclusion

The study concludes that *Carica papaya* seed extract is a very practical component for making herbal shampoos, maintaining stability and user pleasure while providing notable antifungal and antioxidant advantages. FTIR, HPLC, and spectrophotometric studies verified quercetin's presence and structural integrity, ensuring accurate measurement and robust antioxidant properties. Shampoo formulations with desirable physical attributes, good washing capabilities, ideal viscosity, and foam stability were especially demonstrated by formulation F6. According to in-vitro antifungal testing, F6 exhibited superior viscosity and antifungal activity over time, demonstrating substantial inhibition against *Candida albicans*. The research highlights the potential of adding natural extracts, such as papaya seed, to personal care products, improving their overall efficacy and therapeutic qualities while fostering the creation of hair care products that are safe, efficient, and easy to use while also offering significant health advantages.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest, financial or otherwise.

Funding Support

The authors declare that they have no funding for this study.

Author Contribution

All authors made substantial contributions to the conception, design, acquisition, analysis, or interpretation of data for the work. They were involved in drafting the manuscript or revising it critically for important intellectual content. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work, ensuring its accuracy and integrity.

References

- [1] Bagade, MJ. Ambre, SP., Arote, CY, Barve, CS., Bhavar, AR. and Shinde, MPS. An Overview and Introduction on Herbal Shampoo. (2023) (12):251–7. 2.
- [2] Jaya preeti P. Padmini K., Srikanth J, Lohita M, Swetha K Vengalrao p., a review on H Herbal Shampoo and its Evaluation, Asian J. Pharm. Ana. 3(4): 2013; 153-156 3.
- [3] Khan, NH. Phytochemical Analysis, Antioxidant and Antibacterial Activity Determination of Ethanolic Extract of *Carica Papaya* Seeds. 2021, Biomed J Sci Tech Res.33(5).
- [4] Yogiraj, V., Goyal, PK., Chauhan, CS., Goyal, A. and Vyas, B. *Carica papaya* Linn: an overview. Int J Herb Med. 2014, 2(5 Part A):1–8.
- [5] Eke, ON., Augustine, AU. and Ibrahim, HF. Qualitative Analysis of Phytochemicals and Antibacterial Screening of Extracts of *Carica papaya* Fruits and Seeds. Int J Mod Chem. 2014, 6(1):48–56.
- [6] Mishra, K., Ojha, H. and Chaudhury, NK. Estimation of antiradical properties of antioxidants using DPPH- assay: A critical review and results. Food Chem. 2012, 130(4):1036– 43.
- [7] Inamdar, P., Jalamvazir., Desai, S., Patel, D. and Meshram, D. (2014) Phytochemical screening and in vitro antifungal activity of *camellia sinensis*. Int J Pharm, Pharm Sci. 6(5):148–50.
- [8] Bautista Ban- OS S, Barrera-Necha LL, Bravo – Luna I, Bermudes TL. Antifungal activity of leaf and stem extracts from various plant species on the incidence of *colletorichumgloesporoides* of papaya and mango fruit after storage. Rev MexFitopatol (2002);20(8):12.
- [9] Chavez-QP, Goonzalez-FT, Rodriguez-BI, Gallegos- TS. Antifungal activity in ethanolic extracts of *carica papaya* L, cv. Maradol leaves and seeds. Indian J microbial (2011); 51(1):54-60.
- [10] Khan JA, Yadav J, Srivastava Y, Pal PK. In vitro evaluation of antimicrobial properties of *carica papaya*. Int J Bio Pharm Ali sci(2012);1(7):933-45.
- [11] Anibijuwon II, UdezeAo. Antimicrobial activity of papaya on same pathogenic organism of clinical origin from south -western Nigeria. Ethanobotanical leaflets (2009); 7:850-64.

- [12] M, N., A, R. J. ., RS, L. ., M, L. ., KV, M. ., M, L. ., & Shanmugapandiyan , P. A review: the therapeutic power of natural extracts in wound recovery. *Future Journal of Pharmaceuticals and Health Sciences*, 2024, 4(4), 94–101.
- [13] M, Suchitra, Harinadha Baba K, Yamini R, Sri Harshitha P, Surekha G, Pavani T, and Sakshi Jain. To Develop and Evaluate the in-Vitro Anti-Oxidant and Anti-Inflammatory Activity of Polyherbal Gel. *Future J. Pharm. Health. Sci*, 2023; 3 (3):377-86.
- [14] Praveen Gujjula, Mettu Nikhila, Gande Sowmya, Gadivenuka Saraswathi, & Dasari Divija. (2022). Drug-Induced Hepatotoxicity and Hepatoprotective Agents and their Herbal Scenes. *Int. J. of Clin. Pharm. Med. Sci*, 2(2), 68–78.
- [15] Pasupuleti, C., manisha, C., Suraj choudhary, Singaram neeraj, Eerankadi Anusha, Sakilahammed mullah, & Mohdubaidullah khan. (2023). Utilising the Ethanolic Extracts of Leaves Garcinia Cambogia and Commiphora Mukul Herbal Pills Prepared for Treating Anti-Obesity Effects. *Int. J.Exp. Biomed. Res*, 2(1), 33–40.
- [16] kavya B, Ramesh Y, Chandra YP, Penabaka V, Review On Pharmacy Informatics, *Asian Journal of Pharmaceutical Research and Development*. 2025; 13(1):104-109
- [17] Chandrani D, Lubaina SZ and Soosamma M, A review of antifungal effect of plant extract vs. chemical substances against malassezia spp., *Int J Pharm Bio Sci*, 3(3), 2012, 773 – 780.
- [18] Mansuang Wuthi-udomlert, Ployphand Chotipatoomwan, Sasikan Panyadee and Wandee Gritsanapan, Inhibitory effect of formulated lemongrass shampoo on Malassezia furfur: a yeast Associated with dandruff, *Southeast Asian j trop med public health*, 42(2), 2011, 363-369.
- [19] M, Alagusundaram, PriyankaKeshri, Mansi Tyagi, Nem Kumar Jain, Goli Venkateshwarlu, and Divya Gupta. Formulation Development and Characterization of Floating Drug Delivery System of Rosiglitazone Maleate. *Future J. Pharm. Health. Sci*, 2023; 3 (3):332-40.
- [20] Anil Kumar Dindigala, P Anitha, Anantha Makineni and V Viswanath. A review on amorphous solid dispersions for improving physical stability and dissolution: Role of polymers. *GSC Advanced Research and Reviews*, 2024, 19(03), 296–302.
- [21] Saravana kumar K, Bhavani Guvvala, Shilpaja Chella, Prudhvi Raj Vadamala, SwamyCharan Dontha, Floating Drug Delivery Systems - Challenges & Approaches, *YMER*, 2022; 21(11): 997-1009.
- [22] S.K. Rubina et.al. A Research Article on Formulation and Evaluation of Poly Herbal Shampoo. *European J of BioMed and Pharma Sci* 2017;6(12):234-242.