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FORMULATION AND EVALUATION OF PAPAYA SEED EXTRACT BASED HERBAL HAIR CARE SHAMPOO

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ABSTRACT:

Aim: This study aimed to formulate and evaluate a herbal hair care shampoo incorporating papaya seed extract.
Objectives: The objectives included quantifying the extract's active constituents, assessing its antifungal activity, and evaluating various shampoo formulations.
Results and Discussion: Soxhlet extraction yielded 23% papaya seed extract, with 46 grams obtained from 200 grams of seeds, demonstrating efficient bioactive compound extraction. The extract contained 34.48 mg QE/g flavonoids, exhibited 78.01% DPPH radical scavenging activity, and showed significant antifungal effects against Candida albicans, with inhibition zones of 12.8 mm at 500 mg and 13.5 mm at 1000 mg. FTIR and HPLC analyses confirmed the presence and stability of quercetin. Six shampoo formulations were developed and evaluated, all formulations displaying satisfactory pH (6-7), solids content (18-27%), and pseudoplastic viscosity. Among these, formulation F6 exhibited superior viscosity, foam stability, and antifungal activity, with a 15.8 mm inhibition zone, making it the most promising for further development and commercial production. **Conclusion:** Overall, the study highlighted the potential of papaya seed extract to provide significant antioxidant and antifungal properties, identifying formulation F6 as the best candidate for further development and commercialization.

Keywords: Herbal shampoo, *Carica papaya* seed extract, Quercetin, Antioxidant, Anti-fungal, Natural extracts.

1. INTRODUCTION

Shampoo is a portable cosmetic preparation that is used to wash the hair and scalp. Its primary goal is to remove remaining hair grooming products, debris from the scalp, and accumulated sebum from the hair. The World Health Organization (WHO) estimates that almost 80% of people all over the world, especially in developing countries, use herbal remedies for basic medical needs. Perhaps the most often used cosmetic product for routinely cleaning the scalp and hair is a shampoo. A shampoo is a mixture of detergent and water that has been appropriately enhanced for other uses, such as lubricating, feeding hair, or dispensing medicine (Bagade MJ et al., 2023). Singh A (2018) describes dandruff is a chronic, non-inflammatory scalp disease characterised by excessive scalp tissue scaling. Dandruff is treated using hair care treatments containing a variety of antifungal agents.

Carica papaya, sometimes known as papaya, papaw, or pawpaw, is the scientific name for this member of the Caricaceae family. Khan NH (2021) presents the *Carica papaya*, a tropical fruit, is one of the main crops grown in tropical and sub-tropical regions because of its juicy and delicious flesh. In addition, the fruit's high nutritional and therapeutic value contributes to its rising demand. Flavonoids are found in papaya seeds. Plant chemicals classified as flavonoids are well-known for having anti-microbial and antioxidant characteristics. Studies have revealed that papaya seeds contain a variety of flavonoids, including quercetin, kaempferol, and catechins, even if the precise kinds and amounts of flavonoids may differ slightly. The antioxidant and antibacterial qualities of papaya seeds are among its many possible health advantages, which are attributed to these flavonoids. Research has been done on the possible antifungal qualities of papaya seeds. They include substances that have been demonstrated in several studies to have antibacterial action, such as flavonoids, carpaine, and benzyl isothiocyanate. Particularly, benzoyl isothiocyanate has been shown to have potent antifungal properties against a variety of fungi, such as *Aspergillus* species, *Candida* species, and dermatophytes. According to research Papaya seed extract may prevent the formation of fungal infections by rupturing their cell membranes or interfering with their metabolic functions. As a result, papaya seeds may be used as a natural treatment for fungal infections that impact the skin, nails, and mucous membranes (Yogiraj V et al., 2014). The phytochemical screening revealed the presence of alkaloids, cardiac glycosides, anthroquinones, saponins, flavonoids, and carbohydrates (Augustine AU et al., 2014).

Synthetic shampoos hold a significant market share in the traditional shampoo industry. The most widely used detergents are those that include sodium lauryl sulphate, yet even within a manufacturer's product line, the concentration might differ significantly between brands. These synthetic-ingredient shampoos are dangerous, especially when used often. This results in extreme dryness of the hair shaft and hair, which damages budding hair shafts and causes hair to fall out. Herbal formulations or their constituent parts are good substitutes for synthetic agents. The table (Table 1) contains a list of plants used to make shampoo, as well as their common names and documented functions and uses. In this experiment, we attempted to make a stable antidandruff shampoo by removing all commonly used synthetic constituents and substituting them with appropriate natural components. A designed shampoo is meant to maintain its stability during its shelf life and match the consumer's expectations of a cosmetic product (Chandran S et al., 2016).

2. MATERIALS AND METHODS

All plant materials were bought from Hebsur Herbals at Hubballi, Karnataka. Papaya seeds were collected from Sadhguru Herbals and authenticated by a professor Head of Department

of botany, at the H. S. Kotambri Science Institute Hubballi. Plant materials were collected, processed, and kept under specific circumstances. Extracts were produced using known protocols and subjected to identification tests for various phytochemical ingredients.

Sl no.	Common name	Botanical name	Parts used	Category
1	Papaya	*Carica papaya*	Seeds	Anti-fungal activity
2	Nagarmotha	*Cyperus scariosus*	Root	Promotes hair growth
3	Hibiscus	*Hibiscus rosa-sinensis*	Flower	Conditioning agent
4	Shikakai	*Acacia concinna*	Powder	Detergent
5	Soap nut	*Sapindus mukorossi*	Fruit	Detergent
6	Xanthan Gum	*Xanthomonas campestris*	Powder	Thickening agent
7	Gaur gum	*Cyamopsis tetragonolobus*	Powder	Thickening agent

Table 1: List of ingredients and their category

2.1 Preparation of seed extract

In this study, papaya seeds were processed using the Soxhlet extraction method with 95% ethanol, chosen for its efficacy in extracting a wide range of compounds. The seeds, after being crushed, were placed in the Soxhlet extractor. Ethanol was passed through the seeds repeatedly, enabling continuous and thorough extraction. The resultant solution was then collected, filtered to remove impurities, and concentrated if necessary. After evaporating the solvent, a final extract rich in bioactive compounds was obtained. This Soxhlet extraction method provides a reliable and efficient means of isolating valuable chemicals from papaya seeds for potential pharmaceutical and biological applications (Al Badi K and Khan SA 2014).

2.2 Preformulation Studies

2.2.1 Phytochemical screening of seed extract (Augustine AU et al., 2014)

Phytochemical screening of papaya seed extract involves various qualitative tests to detect the presence of different bioactive compounds. Here are methods for testing specific phytochemicals using the specified tests (Table 6).

1. Alkaloids

Method: Dragendorff's Test

1. **Procedure:** Add a few drops of Dragendorff's reagent (a solution of potassium bismuth iodide) to the extract.
2. **Observation:** The formation of an orange or red precipitate indicates the presence of alkaloids.

2. Phenols

Method: Lead Acetate Test

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

3. Flavonoids

Method: Shinoda Test

1. **Procedure:** Add a small amount of magnesium turnings and a few drops of concentrated hydrochloric acid to the extract.
2. **Observation:** The appearance of a pink, red, or magenta colour indicates the presence of flavonoids.

4. Tannins

Method: Ferric Chloride Test

1. **Procedure:** Add a few drops of a 5% ferric chloride solution to the extract.

2. **Observation:** The appearance of a dark blue, green, or black colour indicates the presence of tannins.

5. Saponins

Method: Foam Test

1. **Procedure:** Shake the extract vigorously with water in a test tube.
2. **Observation:** Persistent foam formation that lasts for at least 10 minutes indicates the presence of saponins.

6. Carbohydrates

Method: Molisch's Test

1. **Procedure:** Add a few drops of Molisch's reagent (α-naphthol in ethanol) to the extract, followed by the addition of concentrated sulfuric acid down the side of the test tube.
2. **Observation:** The formation of a violet ring at the interface of the two liquids indicates the presence of carbohydrates.

2.2.2 Determination of absorption maxima

To determine the absorption maxima of quercetin, 100 mg of quercetin was dissolved in methanol to achieve a concentration of 10 µg/ml. The solution exhibited its highest absorbance at 260 nm, which was identified by scanning in the wavelength range of 200 to 600 nm (Aswad M et al., 2021).

2.2.3 Preparation of standard calibration curve of quercetin

A standard calibration procedure for quercetin involves preparing a standard stock solution containing a concentration of 1000 µg/ml by dissolving 50 mg of quercetin in 50 ml of methanol. A series of standard aliquots were prepared in the concentration range of 25–150 µg/ml and the absorbance was recorded on UV spectroscopy (Model UV-1900, Shimadzu) at the wavelength range of 200 to 800 nm. This equation is then used to calculate the concentration of quercetin in unknown samples using their absorbance measurements (Aswad M et al., 2021).

2.2.4 Fourier Transform Infrared Spectroscopy (FTIR) Studies

The pure compounds and shampoo formulations were subjected to FTIR spectroscopy using ATR model IR affinity 1S, Shimadzu, Japan. The scanning range for the spectroscopy was set from 400 cm^{-1} to 4000cm^{-1}.

2.2.5 High Performance Liquid Chromatography (HPLC) Analysis

The analysis of bioactive compounds in natural extracts is pivotal in the pharmaceutical and food industries. In this research HPLC method is used for analysing papaya seed ethanol extract (Agilent 1200 Infinity system). We successfully separated and eluted substances using 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. The sample, consisting of 1000mg papaya seed ethanol extract dissolved in methanol, was injected at a volume of 20 µland detected at a wavelength of 260nm, ideal for the analysis of UV-absorbing components commonly found in plant extracts. Through this procedure, we aimed to identify and quantify the bioactive constituents present in papaya seed extract. Analysis of the chromatogram revealed distinct peaks corresponding to various compounds within the extract. These findings provide valuable insights into the composition of papaya seed ethanol extracts, facilitating their potential applications in pharmaceutical and nutraceutical formulations (Savic IM et al., 2013).

2.2.6 Determination of total flavonoid content

To achieve a concentration of 100 µg/ml, 10 mg of seed extract was diluted in 100 ml of methanol. This concentration was diluted with 4 ml of water. 0.3 ml of 5% NaNO2 was added to the volumetric flask simultaneously, followed by 0.3 ml of 10% AlCl3 and 2 ml of 1 M NaOH was added to the solution. The volume was increased to 10 ml by adding 4.4 ml of distilled water. A spectrophotometer measured the absorbance at 510 nm. The procedure was repeated three times to obtain the mean absorbance value (Table 5). The TFC was calculated using a linear regression method based on quercetin's standard plot. It was expressed as mg QE/g (Zhao LJ et al., 2018).

2.2.7 *In-vitro* antioxidant activity

1. **Preparation of DPPH solution:**
   In a conical flask, dissolve 4 mg of DPPH in 100 ml of methanol. Cover the flask with aluminium foil to protect the solution from light. Allow the solution to sit at room temperature for 30 minutes.
2. **Preparation of standard ascorbic acid solution:**
   Dissolve 10 mg of ascorbic acid in 100 ml of methanol to prepare a stock solution with a concentration of 100 µg/ml. This standard solution is then diluted serially to create concentrations of 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml. From each of these diluted solutions, pipette 1 ml into separate 10 ml volumetric flasks. Add 3 ml of the DPPH solution to each flask, then fill to the 10 ml mark with methanol. Measure the absorbance of each solution at 517 nm.
3. **Preparation of extract solution:**
   Dissolve 10 mg of the leaf extract in 100 ml of methanol to prepare a standard solution with a concentration of 100 µg/ml. This solution is then serially diluted to obtain concentrations of 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml. From each of these diluted solutions, pipette 1 ml into separate 10 ml volumetric flasks. Add 3 ml of DPPH solution to each flask, and then fill to the 10 ml mark with methanol. Measure the absorbance of each solution at 517 nm (Mishra K et al., 2012).

Control: 6 ml methanol+ 3 ml DPPH solution

Formula:

$$\%\ \textbf{Inhibition} = \frac{(\textbf{Absorbance of control} - \textbf{Absorbance of sample})}{\textbf{Absorbance of control}} \times \mathbf{100}$$

2.3 Formulation of Herbal shampoo

In this study, herbal shampoo is formulated by using papaya seed extract for its potential hair care benefits. To formulate a herbal shampoo, mix the required quantity of xanthan gum or guar gum in glycerine to achieve the desired viscosity. Add this mixture to water while stirring continuously to form a thickening base. In another beaker, prepare a decoction by boiling the required quantities of Hibiscus, Reetha, Shikakai, and Nagarmotha in water. After boiling, filter the decoction to remove any solid residues, ensuring a clear herbal extract. Combine this filtrate with the thickening base, mixing thoroughly. Next, incorporate Papaya seed extract then add vitamin E as a preservative into the mixture, ensuring even distribution. 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 (Al Badi K and Khan SA 2014).

Table 2: Formulation of Herbal Shampoo

Sl No.	Ingredients	F1	F2	F3	F4	F5	F6

1	Papaya seed extract(g)	2	4	6	2	4	6
2	Herbal extract*(ml)	15	15	15	15	15	15
3	Xanthan gum(g)	0.1	0.15	0.2	-	-	-
4	Gaur gum(g)	-	-	-	0.15	0.3	0.45
5	Citric acid(g)	0.05	0.05	0.05	0.05	0.05	0.05
6	Vitamin E(ml)	1	1	1	1	1	1
7	Glycerine(ml)	2	2	2	2	2	2
8	Water(ml)	30	30	30	30	30	30

Table 3: Ingredients of Herbal Extract

Sl no.	Ingredients	Parts	Quantity
1	Nagarmotha	Root	1gm
2	Shikakai	Flower	2gm
3	Hibiscus	Powder	1gm
4	Soapnut	Fruit	3gm

2.4 Evaluation of herbal shampoo formulation

Several evaluations for quality control were conducted to assess the prepared formulation's quality, including visual appearance, physiochemical control, conditioning performance, and so on.

2.4.1 Physical appearance

The colour, consistency, clarity and fragrance of the produced formulations were assessed.

2.4.2 Measurement of pH

The developed mixture was diluted with distilled water to create a sample with a 10% concentration. Then produced sample was evaluated for pH using a digital pH meter at room temperature (30±2℃).

2.4.3 Determining of percent solid content

A clean, dry porcelain dish was weighed, and 4 g of shampoo were added. The shampoo-filled dish was weighed and exact weight of shampoo has been calculated. The porcelain dish containing the shampoo was heated on a hot plate until the liquid got dried. The weight after drying was calculated (Dhayanithi S et al., 2021).

$$\% \textbf{ solid content} = \frac{\textbf{Net wt of dry sample } (\textbf{W3} - \textbf{W1})}{\textbf{Net wt of test sample } (\textbf{W2} - \textbf{W1})} \times \textbf{100}$$

2.4.4 Dirt dispersion

In the large test tube, add 10 mL of distilled water and two drops of shampoo. Add one drop of Indian ink to the test tube, cover it with a stopper, and shake ten times. The quantity of ink in the foam was classified as none, light, moderate, or heavy (Singh A 2018).

2.4.5 Measurement of viscosity

The Brookfield Viscometer was used to measure the shampoo's viscosity. The viscosity of shampoo was determined at room temperature (30±2℃) with different spindle speeds from 1 to 5 rpms and spindle no. TL 6 is used to determine viscosity (Saraswat N et al., 2020).

2.4.6 Analysis of Foaming Efficiency and Stability

The foaming ability was tested using a cylinder shaking method with minor modifications. 5 ml of the 1% shampoo solution was placed in a 10 ml graduated measuring cylinder and closed with a hand. The measuring cylinder was shaken for a minute and total quantity of foam content after 1 minute of shaking was determined. This procedure was performed for five minutes. The

stability of the foam was assessed by measuring its foam volume after 1 and 4 minutes of shaking (Shubhada S et al., 2023).

2.4.7 *In-vitro* anti-fungal efficacy

The antifungal activity of the seed extract and shampoo formulation was assessed using the agar-well diffusion method. In brief, the microorganism (*candida albicans*) was inoculated into czapek dox agar by spreading fungal inoculum across the media. Agar wells were made using an 8-mm stainless-steel cork borer and filled with 200 µl of plant extract. Control wells with clear solvents (negative controls) were also filled concurrently on the same plate. The plates of bacteria were incubated at 28 ℃ for a period of 72 hours, and antibacterial activity was assessed by determining the diameter of the zone of inhibition. The same approach was followed for ordinary Itraconazole (Inamdar P et al., 2014).

2.4.8 Stability studies

Stability tests were carried out in accordance with the ICH recommendations for accelerated stability study. For three months, the sample formulations were maintained at room temperature (40ºC±2℃ and 75±5% relative humidity) and samples were evaluated for their physical characteristics, pH, viscosity, stability of foam, and fungal activity (Singh A 2018).

3. RESULTS AND DISCUSSION

3.1 Papaya seed extraction

In this study, the yield of papaya seed extract obtained using Soxhlet extraction was 23%, with 95% ethanol as the solvent. Around 200 g of papaya seeds were used, and successfully extracted 46 g of extract.

3.2 PRE-FORMULATION PARAMETERS

3.2.1 Phytochemical screening

The prepared papaya seed extract was screened for various phytochemicals, confirming the presence of alkaloids, phenols, flavonoids, saponins, tannins, carbohydrates and other compounds (Table 4).

Table 4: Phytochemical Screening of Plant Extract

Sl. No	Phytochemicals	Papaya Seed Extract
1	Alkaloids (Drangendroff's Test)	+
2	Phenols (Lead acetate Test)	+
3	Flavonoids (Shinoda Test)	+
4	Saponins (Foam Test)	+
5	Tannins (Ferric chloride Test)	+
6	Carbohydrates (Molish's Test)	+

3.2.2 Absorption maxima of Quercetin

The absorption maxima of quercetin was found to be 260nm using methanol as a solvent (Figure 1).

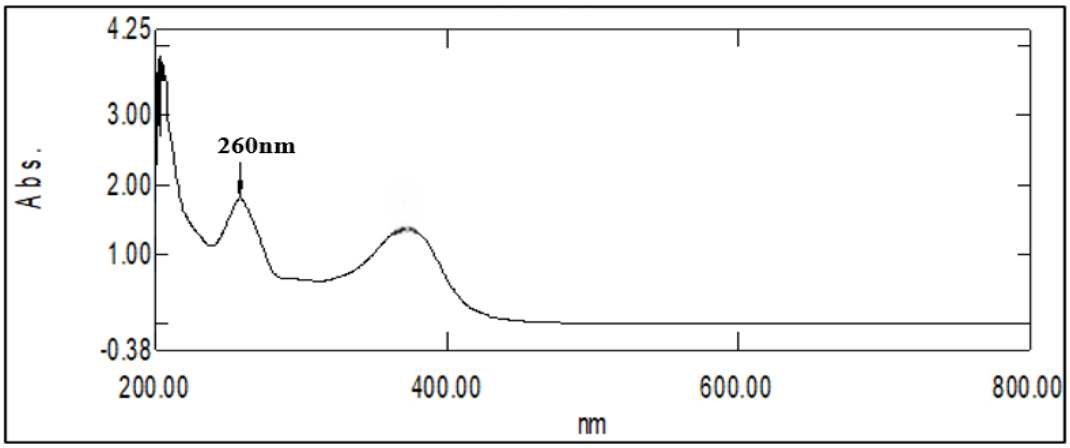


Figure 1: Absorption maxima of Quercetin

3.2.3 Standard calibration of Quercetin

Standard calibration of quercetin using an UV-Vis spectrophotometer that operates within the wavelength range of 200 to 800 nm. Quercetin was dissolved in methanol to create aliquots with concentration range from 25 to 150 µg/ml (Table 5). The calibration curve was created by measuring the absorption at 260 nm for each concentration and regression coefficient (R^2) is 0.9986 (Figure 2), revealing a linear relationship and verifying the reliability of the spectrophotometric approach for quantifying quercetin in methanol.

Table 5: Standard Calibration of Qurercetin

Sl No	Concentration (µg/ml)	Absorbance (nm)
1	25	0.141± 0.001
2	50	0.234± 0.001
3	75	0.343± 0.001
4	100	0.458± 0.001
5	125	0.582± 0.001
6	150	0.711± 0.001

**Mean ±SD, n=3*

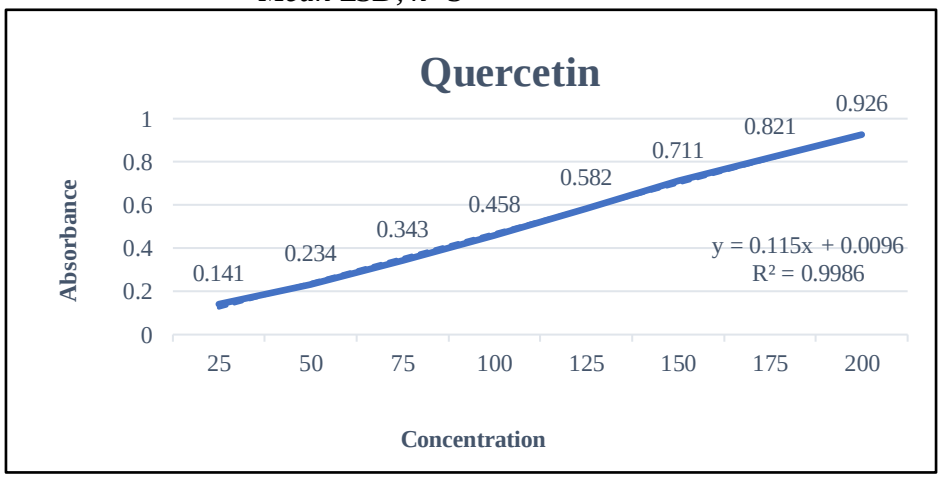


Figure 2: Calibration curve of Quercetin

3.2.4 Ftir

The FTIR study of quercetin revealed key characteristic bands that confirm its presence. The peak at 3400 cm^{-1} corresponds to the O-H stretching vibrational mode, indicating the presence of hydroxyl groups. The band at 1655 $cm^{-1}$is associated with C=O stretching, characteristic of carbonyl groups. Additionally, the peaks at 1600 $cm^{-1}$and 1500 $cm^{-1}$are attributed to C=C stretching in the aromatic rings (Figure 3). These findings confirm the molecular structure of quercetin and its functional groups, highlighting its potential antioxidant properties.

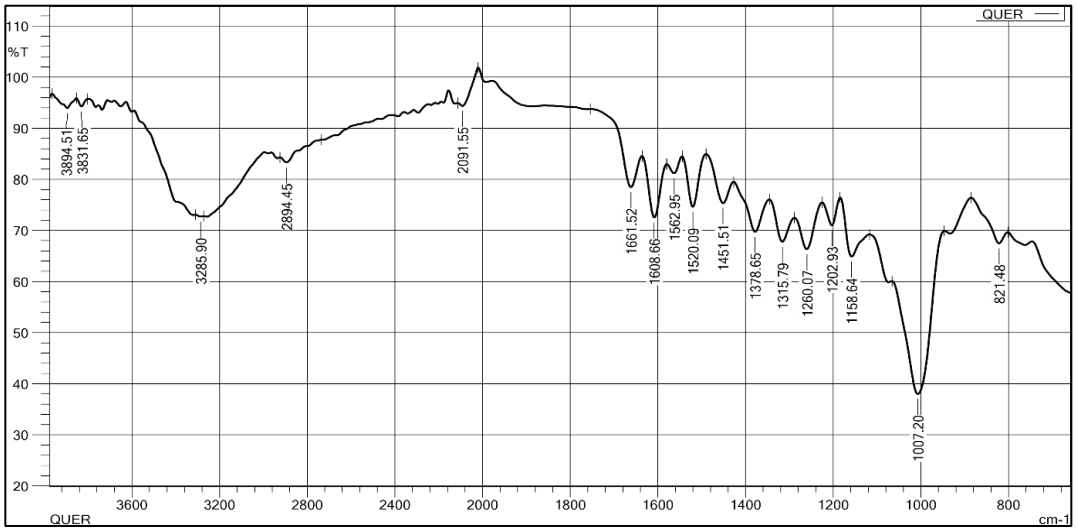


Figure 3: FTIR Spectrum of quercetin

3.2.4.1 Compatibility study using FTIR spectroscopy

A Fourier Transform Infrared Spectroscopy (FTIR) study confirmed the presence of quercetin in papaya seed extract and its integration into a shampoo. Significant absorption bands for hydroxyl (OH) stretching (3200–3550 cm-1), carbonyl (C=O) stretching (1650–1750 cm-1), and carbon-carbon double bond (C=C) stretching (1500–1600 cm-1) were identified, indicating the presence of quercetin's essential functional groups. Additionally, C-O-H stretching (1000–1200 cm-1) suggested alcohol or phenol moieties (Figure 4). These findings demonstrate quercetin's structural integrity and functional stability in both the extract and the shampoo, confirming its compatibility and stability in the formulation.

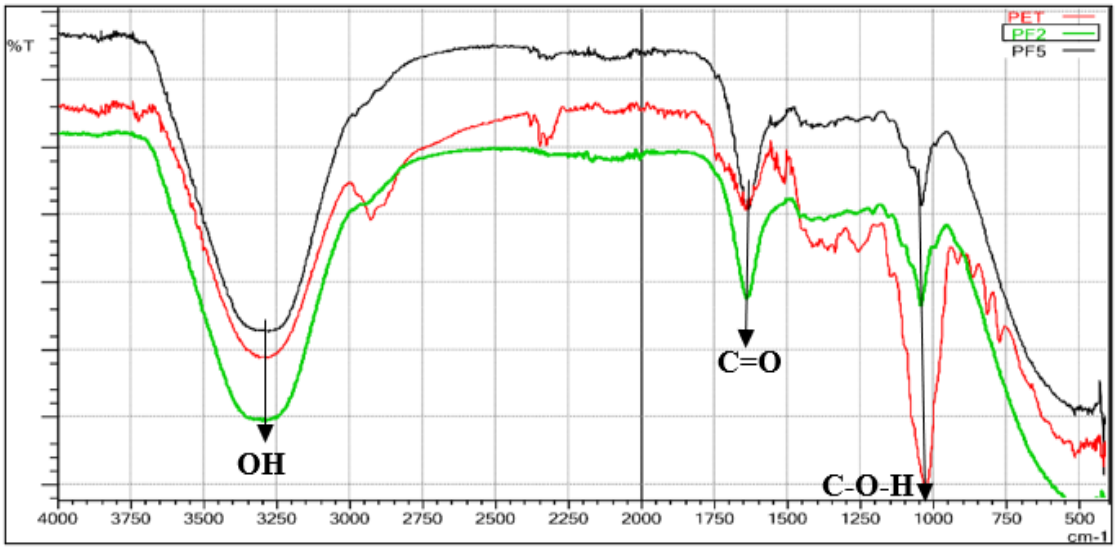


Figure 4: FTIR spectrum of Papaya seed extract, Shampoo formulation(F2), Shampoo formulation(F5)

3.2.5 HPLC

HPLC analysis of papaya seed extract, using a methanol mobile phase and C18 column at a wavelength of 260nm, revealed a retention time of 2.964 minutes and a peak area of 2.50343e4 mAUs. In comparison, the standard quercetin showed a retention time of 3.076 minutes and a peak area of 3.05769e4 mAUs. These results indicate the presence of quercetin in papaya seed extract, as evidenced by the similar retention times (Figure 5 and 6).

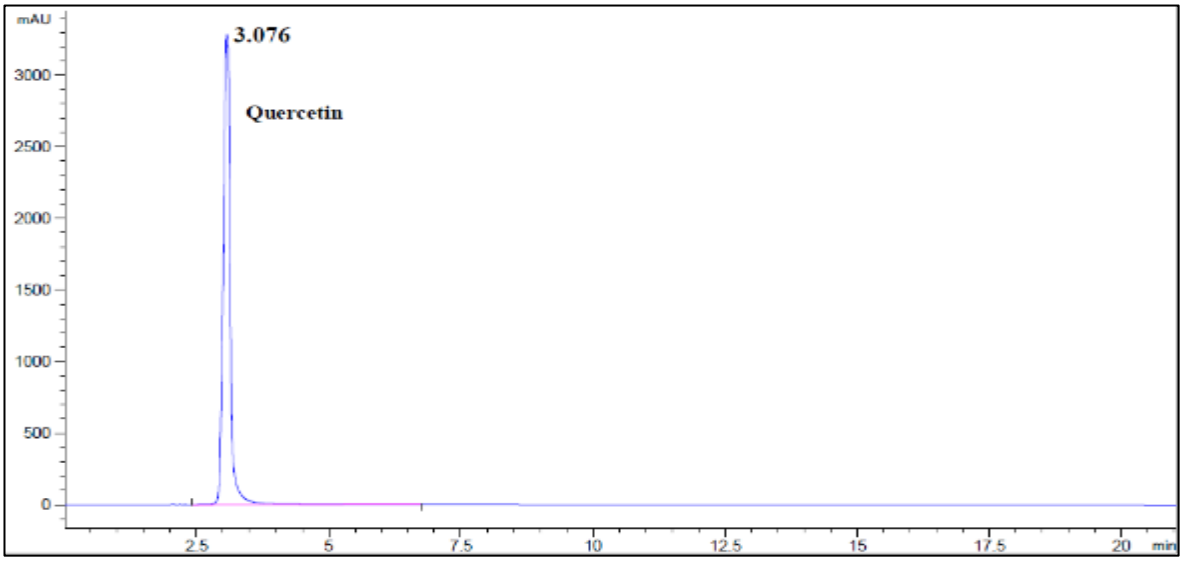


Figure 5: HPLC chromatogram of quercetin

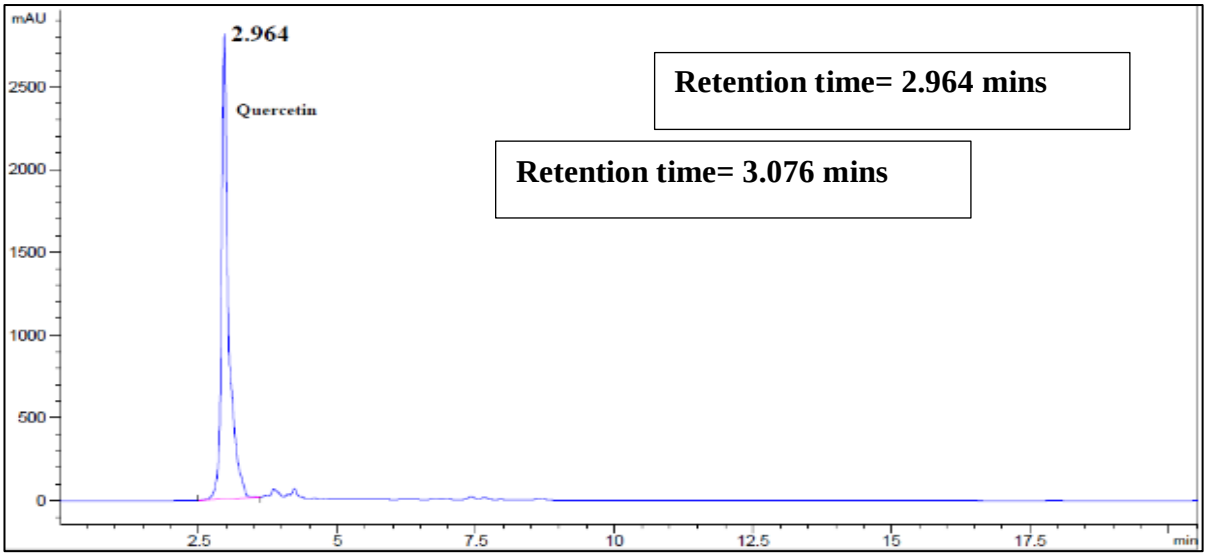


Figure 6: HPLC chromatogram of papaya seed extract

3.2.6 Total flavonoid Content

The quantitative determination of total flavonoids in the plant extract was based on a standard calibration curve of quercetin, demonstrating linearity within the 50-600 µg/ml concentration range. The total flavonoid content of the papaya seed extract was determined to be 9.03 mg QE/g of dry extract (Figure 7) (Table 6).

Table 6: Determination of Total Flavonoid Content

Parameters	Result
Total Flavonoid content	9.03 mg QE /g Extract

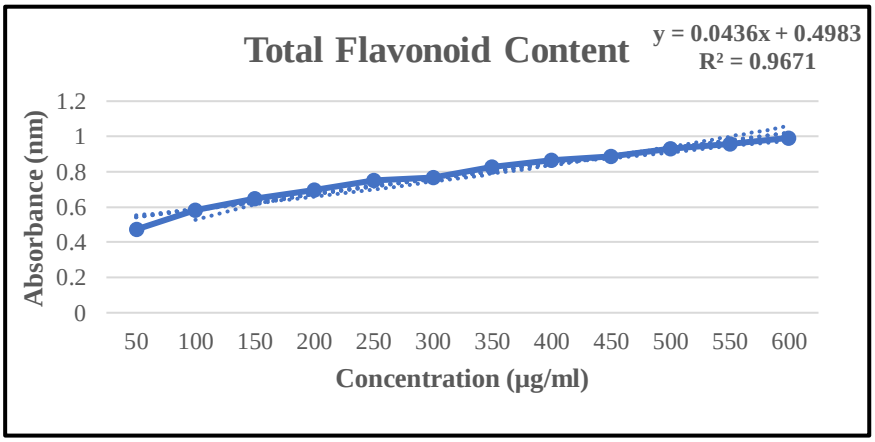


Figure 7: Total flavonoid content

3.2.7 *Invitro* antioxidant activity

At a concentration of 100 µg/mL, the papaya seed extract demonstrated 71.08% radical scavenging activity, indicating its high DPPH radical scavenging capabilities (Figure 8) (Table 7). This high antioxidant activity shows that integrating papaya seed extract into herbal shampoos could boost their protection against oxidative stress on the scalp and hair, thereby increasing overall hair health and durability.

Table 7: *In-Vitro* Antioxidant Activity of Standard and Plant Extract

Sl. no	Concentration(µg/ml)	Standard Ascorbic acid (% inhibition)	Plant extract (% inhibition)
1.	20	39.27	35.4
2.	40	44.33	43.37
3.	60	59.51	55.66
4.	80	70.6	66.5
5.	100	89.15	71.08

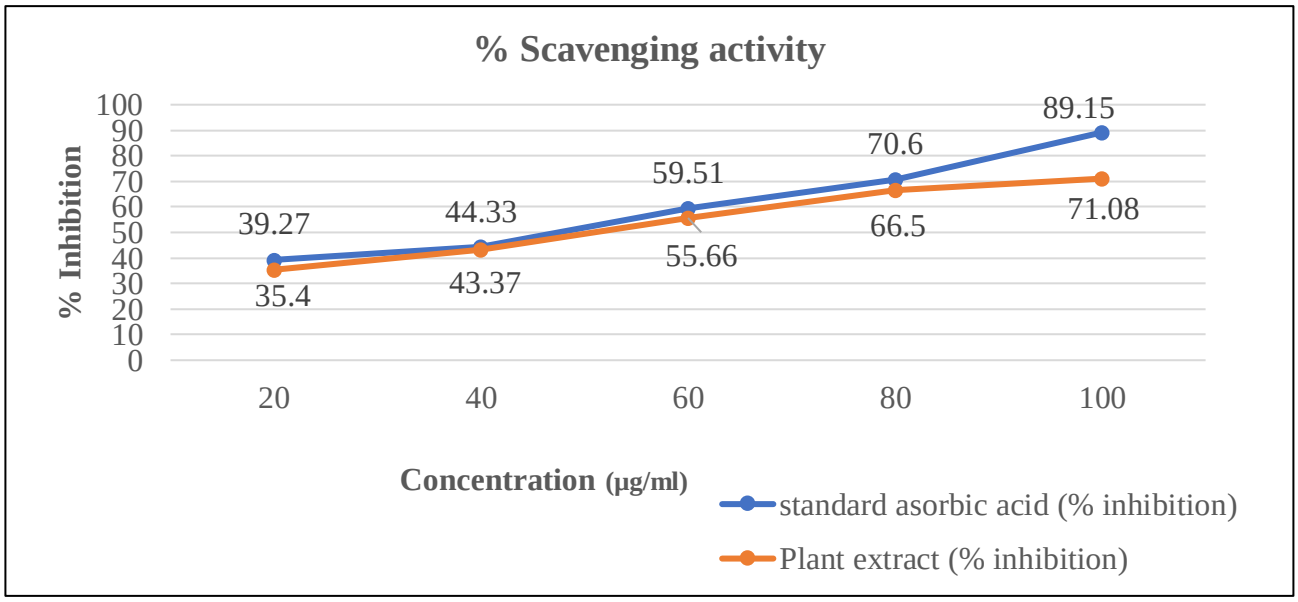


Figure 8: In-vitro antioxidant activity of standard and plant extract

3.2.8 Anti-fungal activity

A study investigated the antifungal activity of papaya seed extract against *Candida albicans* using Czapek Dox agar medium. The results demonstrated that the extract exhibited significant inhibitory effects on the fungal growth. At a concentration of 500 mg, the zone of inhibition measured 12.8 mm, while a higher concentration of 1000 mg produced a slightly larger zone of inhibition of 13.5 mm. (Fig No. 9) These findings indicate that papaya seed extract possesses dose-dependent antifungal properties, making it a potential candidate for natural antifungal agents.

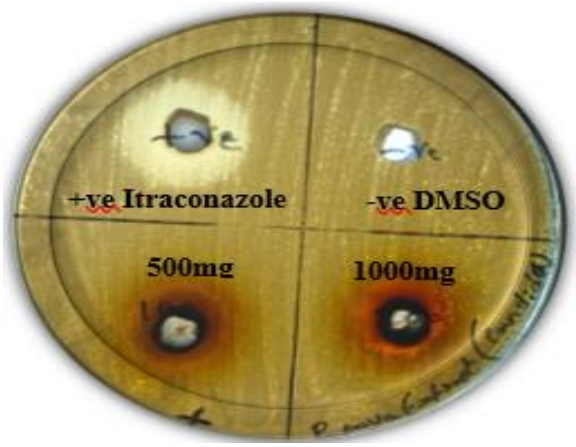


Figure 9: Zone of inhibition of papaya seed extract

3.3 Evaluation Parameters

3.3.1 Physical appearance

The shampoo has a brownish colour and a liquid consistency, giving it a natural and earthy appearance. Its clarity is opaque, suggesting the presence of nourishing ingredients and natural extracts. The fragrance is pleasantly derived from these natural extracts, providing a soothing and aromatic experience (Table 8).

3.3.2 PH determination

PH of the shampoo ranges from 5.5 to 7 pH and all the shampoo formulations are in the acceptable range (Table 8).

3.3.3 Percent solid content

The percent solids content, ranging from 18–27% (Table 2), ensured the shampoos were easy to apply and rinse out without being too thick or difficult to wash off (Table 8).

3.3.4 Dirt dispersion

The dirt dispersion test indicated that none of the six shampoo formulations caused ink to concentrate in the foam, ensuring that dirt remained in the water and would not redeposit on hair, signifying satisfactory quality (Table 8).

Table 8: 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

**Mean ±SD, n=3*

3.3.5 Viscosity

The viscosity of the herbal shampoo formulations ranges between 2000 and 9000 cps, ensuring optimal texture and performance. All six formulations are within this acceptable range for both rpm 1 and rpm 2. Additionally, as the rpm increases, the viscosity decreases, exhibiting typical shear-thinning behaviour (Figure 10) (Table 9).

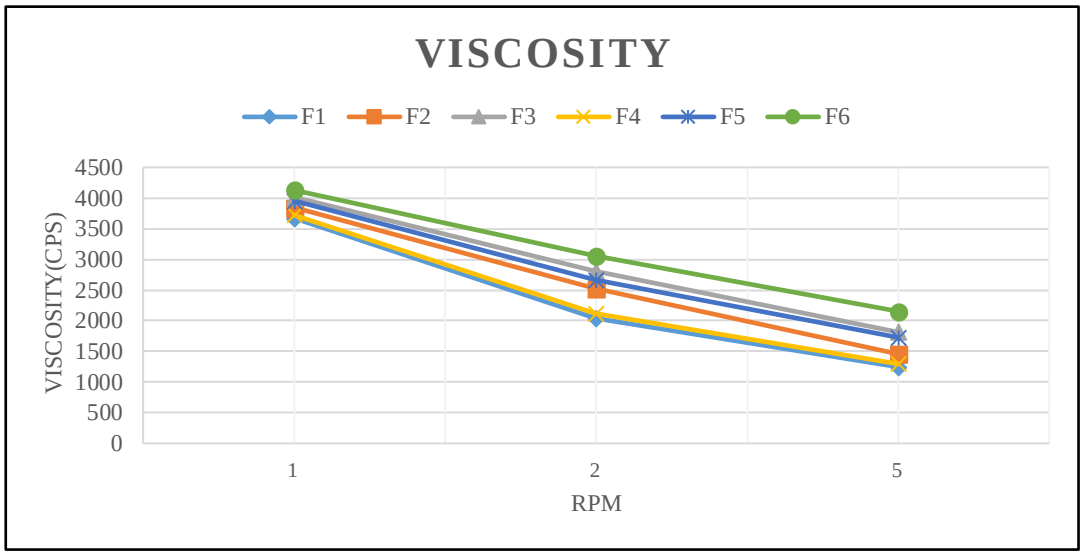
Table 9: Viscosity of Herbal Shampoo Formulations

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

**Mean ±SD, n=3*

Figure. 10: Viscosity of Herbal Shampoo Formulation



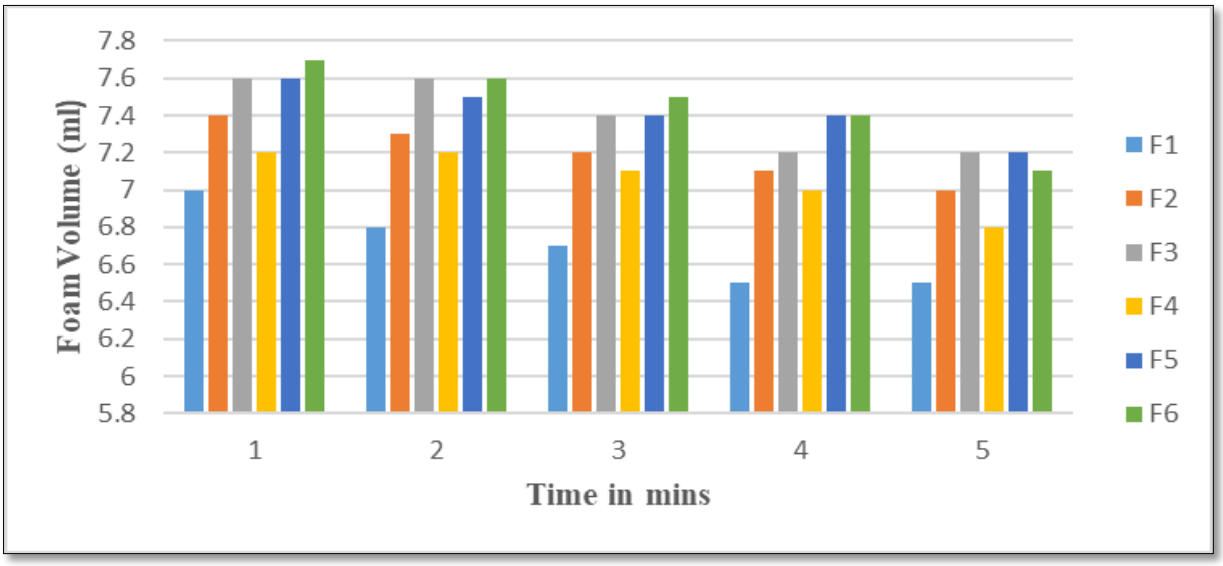
3.3.6 Foaming efficiency and foam stability

All six formulations exhibited similar foaming characteristics and foam retention in distilled water (Fig No. 11). The foam stability of the herbal shampoos, revealing stable foams with minimal changes in volume (Table 10). Notably, there is no direct correlation between detergency and foaming, confirming that a well-foaming shampoo does not necessarily clean better. The final formulations produced stable foams with consistent volume.

Table 10: Foam Stability of Herbal Shampoo Formulations

Time In mins	Foam volume(ml)					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
1	7	7.4	7.6	7.2	7.6	7.8
2	6.8	7.3	7.6	7.2	7.5	7.6
3	6.7	7.2	7.4	7.1	7.4	7.5
4	6.5	7.1	7.2	7.0	7.4	7.4
5	6.5	7.0	7.2	6.8	7.2	7.1

Figure 11: Foam Stability of Herbal Shampoo Formulations



3.3.6 Anti-fungal activity

The antifungal activity of the shampoo formulations against *Candida albicans* was tested, and the results showed varying degrees of inhibition. Formulation F1 had an inhibition *zone* of 12.2 mm, F2 had 13.1 mm, F3 had 14.1 mm, F4 had 13.9 mm, F5 had 15.2 mm, and F6 had the maximum inhibition at 15.8 mm. In comparison, the positive control (Itraconazole 10 mg/ml) indicated a 22 mm inhibition zone, whereas the negative control (placebo) showed no inhibition. These results show that all shampoo formulations have antifungal properties, with F5 and F6 being the most effective against *Candida albicans* (Figure 12) (Table 11).

Table 11: Antifungal Activity of Herbal Shampoo Formulations

**Mean ±SD, n=3*

Microbial strain	***Candida albicans***							
Shampoo Formulations	F1	F2	F3	F4	F5	F6	+ve control (itracanazole 10mg/ml)	-ve control (placebo)
Inhibition (in mm)	12.2± 1	13.1± 1	14.1± 1	13.9± 1	15.2± 1	15.8± 1	22±2	0

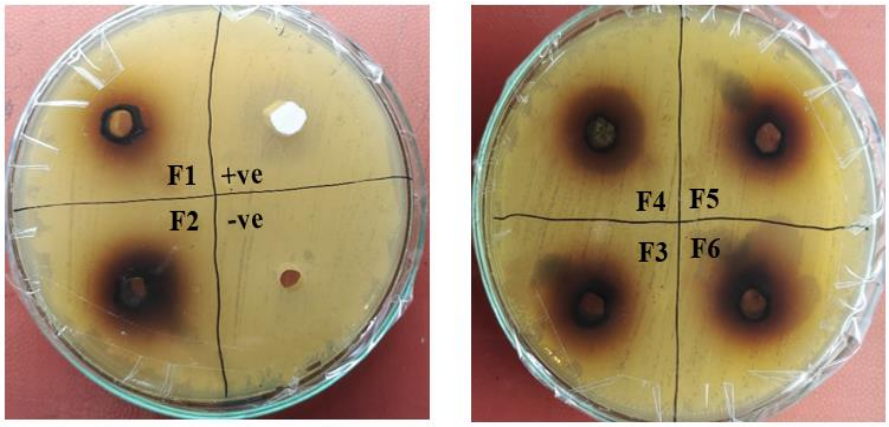


Figure 12: Zone of Inhibition of Herbal Shampoo Formulations on Candida albicans

3.3.7 Stability study

The stability analysis of the formulated herbal shampoos was conducted over three months. The results indicated no visible changes in appearance, consistency, or foam ability. However, there were slight changes in pH and a minor decrease in viscosity, suggesting a slight alteration in the product's chemical composition. Overall, the formulations demonstrated stability throughout the study period, with only minor variations in some parameters. Notably, formulation F6 exhibited good antifungal activity, maintaining its effectiveness over time (Table 12).

Table 12: Stability results for 3 months

Sl No.	Evaluation Parameters	Shampoo formulations (F1-F6) Observations (40ºC±2℃ and 75±5% relative humidity)				
		Initial		1 month	2 months	3 months
1	Physical parameters	No change		No change	No change	No change
2	Phase Separation	No Phase Separation		No Phase Separation	No Phase Separation	No Phase Separation
2	Foam stability	Good		Good	Good	Good
3	pH	6.56 ±0.014		6.5 ±0.035	6.3 ±0.091	6.2 ±0.012
4	Viscosity	3889.83 ±9.18		3586.83 ±5.38	3462.23 ±8.17	3163.68 ±2.13
5	Anti-fungal activity	F1	12.2 ±1	No Inhibition	No Inhibition	No Inhibition
		F2	13.1 ±1	12.6 ±1	No Inhibition	No Inhibition
		F3	14.1 ±1	13.5 ±1	12.8 ±1	12.5 ±1
		F4	13.9 ±1	No Inhibition	No Inhibition	No Inhibition
		F5	15.2 ±1	14.2 ±1	12.1 ±1	10.2 ±1
		F6	15.8 ±1	15.5 ±1	15.2 ±1	14.6 ±1

**Mean ±SD, n=3*

4. CONCLUSION

In conclusion, the study confirms that *Carica papaya* seed extract is a highly viable ingredient for formulating herbal shampoos, offering significant antioxidant and antifungal benefits while ensuring stability and user satisfaction. The Soxhlet extraction method demonstrated feasibility for large-scale production with high extract yields. Comprehensive phytochemical analysis revealed a rich composition of beneficial compounds, including carbohydrates, phenols, flavonoids, alkaloids, and glycosides, contributing to the shampoo's medicinal properties. Spectrophotometric, FTIR, and HPLC analyses confirmed the presence and structural integrity of quercetin, ensuring reliable quantification and high antioxidant potential. Formulated shampoos, particularly formulation F6, exhibited appealing physical characteristics, effective cleansing properties, optimal viscosity, and foam stability. In-vitro antifungal tests showed strong inhibition against *Candida albicans*, with F6 maintaining superior viscosity and antifungal activity over time. The study underscores the potential of incorporating natural extracts like papaya seed into personal care products, enhancing their therapeutic properties and overall effectiveness, and supporting the development of natural, effective, and user-friendly hair care products with substantial health benefits.

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