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ORIGINAL ARTICLE

Fabrication, organoleptic evaluation and in vitro characterization of cream loaded with *Carica papaya* seed extract

Muhammad Sarfraz PhD[1] | Kanwal Shabbir MPhil[2] | Qazi Adnan PhD[2] | Haji Muhammad Shoaib Khan PhD[2] | Jafir Hussain Shirazi PhD[2] | Hamna Sabir Pharm D[2] | Nimrah Mehmood Pharm D[2] | Yousef A. Bin Jardan PhD[3] | Kifayat Ullah Khan PhD[4] | Abdul Basit PhD[5]

[1]College of Pharmacy, Al Ain University, Al Ain, United Arab Emirates

[2]Department of Pharmaceutics, Faculty of Pharmacy, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

[3]Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

[4]Quaid-e-Azam College of Pharmacy, Sahiwal, Punjab, Pakistan

[5]Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, Thailand

Correspondence
Muhammad Sarfraz, College of Pharmacy, Al Ain University, Al Ain, United Arab Emirates.
Email: muhammad.sarfraz@aau.ac.ae

Abstract

Objective: The current study aimed to provide preliminary insights into potential biopharmaceutical applications of *Carica papaya* seed extract by evaluating its phytochemical and biological profiles. Furthermore, the study aimed to develop a stable oil-in-water (O/W) emulsion for the effective delivery of antioxidant-rich biologicals for cosmetic purposes.

Methods: The hydroethanolic (ethanol 80%: 20% water) extract of *C. papaya* seeds was prepared via maceration technique. The chemical composition was carried out through preliminary phytochemical screening and estimation of total phenolic contents (TPC) and total flavonoid contents (TFC). The biological profile of the extract was explored using various in-vitro antioxidant methods. The homogenization procedure was used to create a cream of O/W and various tests were applied to assess the stability of the emulsion. By keeping the emulsion at different storage conditions (8 ± 0.5°C, 25 ± 0.5°C, 40 ± 0.5°C, and 40 ± 0.5°C ± 75% relative humidity [RH]) for a period of 28 days), the physical stability parameters of the emulsion, including pH, viscosity, centrifugation, phase separation, and conductivity, as well as rheological parameters and organoleptic parameters (odor, color, liquefaction, and creaming), were assessed.

Results: The preliminary phytochemical screening assay revealed the presence of various plant secondary metabolites including alkaloids, phenolics, flavonoids, tannins, saponins, and quinones. The extract was found to be rich in TPC and TFC. The in vitro antioxidant study gave maximum activity in the DPPH method. The plant extract containing cosmetic cream exhibited remarkable stability during the entire research. Data gathered indicated that no phase separation or liquefaction was seen after the experimental period. Throughout the experimental period, a small variation in the pH and conductivity values of the base and formulation was seen.

Conclusion: The findings suggest that the seed extract of *C. papaya* is a rich source of polyphenols with antioxidant potential and can be a promising alternative for the treatment of various ailments. The stability of emulsion paves the way for its utilization as a carrier for the delivery of 3% *C. papaya* seed extract and applications in cosmetics products.

KEYWORDS
antioxidant activity, *Carica papaya* seed, pharmaceutical emulsion, phytochemical analysis, stability evaluation

1 | INTRODUCTION

The bases for skincare formulations for both healthy and diseased skin are a variety of cosmetic emulsions. These cosmetics are in the form of a cream, lotion, body milk, or occasionally even as fluid for normal, dry, or oily skin.[1–3] Emulsions are typically used as cosmetic formulations due to their high ability of skin penetration, easy wash off, and variable viscosity.[4] Various pharmaceutical preparations used in the management of chronic skin diseases are formulated as emulsion systems.[5–7]

A thermodynamically unstable system known as an emulsion consists of two immiscible liquid phases, one of which is dispersed as a globule in the other liquid phase or as a continuous phase.[8,9] The introduction of an emulsifying agent can stabilize the entire system.[10,11] Emulsions consist of a dispersed system in which the internal phase is dispersed homogenously in the external phase in the form of small droplets and the whole system maintains its integrity with the aid of an emulsifying agent.[12] Emulsions can separate into two distinct phases and are thermodynamically unstable. Numerous processes, such as sedimentation, flocculation, creaming, phase inversion, or coalescence, would cause the system to become unstable.[13] Emulsions have been evaluated for their comprehensive rheological parameters. Depending on their rendered use, their rheology and stability are diverse widely. Thus pharmaceutical industries need a better understanding of the aspects which affect the rheological parameters and stability of their cosmetic products in order to maintain their desired physical characteristics.[14]

Papaya, scientifically known as *Carica papaya* and belonging to the Caricaceae family, is a unique fruit within its family, exhibiting characteristics of the lactiferous group of plants. This lush, herbaceous plant is self-supporting, reproducing through self-pollination and displaying hermaphroditic, male, or female traits.[15–18] *C. papaya* thrives in tropical and subtropical zones, flourishing in temperatures ranging from 21 to 33°C. It is cultivated extensively in almost every country in Central and South America's tropical regions. Furthermore, it flourishes in excellent growing regions such as Sri Lanka, India, tropical Africa, and the Antilles.[19] Its leaves, fruit, shoots, flowers, roots, latex, seeds, and bark are employed to control and treat a diverse array of diseases. Rich in essential minerals such as calcium, potassium, magnesium, and iron, *C. papaya* also serves as a robust source of vitamins, including vitamin A, B, and C.[20,21] Unripe papaya extract contains a range of bioactive compounds, including steroids, carbohydrates, terpenoids, saponins, flavonoids, glycosides, and alkaloids. Leveraging these components, papaya is employed in the treatment of ailments such as dengue fever, cancer, malaria, heart conditions, gastrointestinal disorders, asthma, rheumatoid arthritis, and osteoarthritis.[22,23]

The phytochemical analysis of *C. papaya* seed extract and variations in physical and organoleptic parameters, such as color, liquefaction, odor, pH, conductivity, phase separation, viscosity, centrifugation, and cream in a cosmetic emulsion loaded with *C. papaya* seed extract, are described in the current study.

2 | MATERIALS AND METHODS

2.1 | Drugs and chemicals

The polyglyceryl-3 Methylglucose distearate (Tego Care 450) was purchased from the German company Evonik Goldschmidt. 2, 2-diphenyl-1-picrylhydrazyl DPPH was acquired from Sigma Aldrich in Germany, and liquid paraffin and cetostearyl alcohol were obtained from the Merck.

2.2 | Plant material collection and authentication

Papaya seeds of the *Carica* variety were gathered and seeds of *C. papaya were* dried under shade until a constant weight was obtained and then crushed in a grinder. The sample's coarse powder was maintained in an airtight container and kept in a cool, dry location for future use.

2.3 | Preparation of extract

A weighing scale was used to precisely measure 50 g of finely ground plant material, which was then carefully transferred to a beaker holding 500 mL of the solvent mixer, an 80% hydro-alcoholic solution. The pharmaceutical lab's beaker was wrapped in aluminum foil and left at room temperature for 3 days. The

extracted residue was removed using a method of filtration through several layers of muslin cloth to produce a coarse filtrate which was then processed three times through Whatman filter paper (No.1) to produce a filtrate that was particle free. When the solvent had been concentrated to one-third of the final volume, it was evacuated under vacuum at 40°C and 90 rpm. For upcoming analysis and/or characterization, a highly concentrated extract of *C. papaya* seeds was collected using a rotary evaporator and stored in a refrigerator at 4°C.[24]

2.4 | Phytochemical profiling

2.4.1 | Qualitative phytochemical screening

To analyze bioactive components such as alkaloids, glycosides, flavonoids, phenolics, saponins, tannins, quinones, reducing sugars, proteins, and terpenoids, *C. papaya* seed extract was employed.[25]

Alkaloid test

To detect the presence of alkaloids, 1 mL of Mayer's reagent was added to 1 mL of extract, followed by a few drops of iodine solution.

Glycosides test

Ten milliliters of H_2SO_4 (50%) were mixed with 1 mL of *C. papaya* extract. After 15 min of heating, Fehling solution (10 mL) was added and the mixture was boiled. Glycosides are present when a brick-red precipitate forms.

Flavonoid test

In a test tube with a magnesium ribbon piece inserted, 4 mg of the extract was ingested. Concentrated HCl was gradually introduced to the test tube. Flavonoids are present when the color changes from orange to red, and flavones are present when the color changes from red to crimson.

Terpenoids test

One milliliters of concentrated H_2SO_4 was added to 1 mL of extract, which was then given a water bath to incubate for 2–4 min. The presence of terpenoids is indicated by the presence of a gray tint.

Test for sugars

It was decided to take 1 mL of the extract and mix it with 1 mL of Benedict's solution. For 3–4 min, the sample was allowed to incubate in the water bath. The presence of reducing sugars is indicated by the appearance of an orange, blue, or green hue.

Quinones test

In 1 mL of extract, a few drops of NaOH were added, and the solution was neutralized by adding a few drops of HCl. Sample discoloration shows the presence of quinones.

Saponin test

A test tube was filled with 0.5 g of powdered *C. papaya* seeds, 5 mL of distilled water, and was violently shaken. The presence of saponins was indicated by a persistent froth development that persisted for 15 min.

Tannins test

A small amount of 2% $FeCl_3$ was added to 1 mL of the extract. Tanning results in the formation of black, green, or blue colors.

Protein test

A few drops of concentrated nitric acid were added to 1 mL of extract. The presence of proteins is shown by the color yellow.

2.4.2 | Quantitative estimation of polyphenolic contents

Total Phenolic contents (TPC) determination

After some minor alterations, the 96-well microplate method which is based on the Folin–Ciocalteu method was used to determine the total phenolic content (TPC) of *C. papayas*. To complete the reaction, 10 mL of 10% Folin–Ciocalteu reagent (FCR) was added to 100 mL of plant extract (1 mg/mL), which was then given a 90 mL addition of 15% Na_2CO_3 solution. After 60 min at room temperature, the solution was tested for TPC at 765 nm using an automatic microplate absorbance reader. All the measurements were taken in triplicate. A calibration line was generated by using different dilutions (10–100 μg/mL) of gallic acid. Total phenolic contents were calculated by using a calibration curve which was reported as mgGAE/gram of the plant extract.

Determination of total flavonoid content (TFC)

Utilizing an aluminum chloride colorimetric technique as reported by Sembiring et al., with slight modifications, the total flavonoid contents (TFC) of the extract were quantified (Sembiring et al.).[25] To generate a calibration curve various concentrations (10–100 μg/mL) of quercetin were prepared by using absolute methanol. One hundred microliters of crude plant extract was mixed with 25 μL solution of 10% sodium nitrite in the well plate 10 μL of solution were added after the mixture was left at room temperature for 5 min to finish the reaction. The mixture above received 10% $AlCl_3$ and was once more left at room temperature for 5 min. Following this, 30 mL of methanol was used to dilute 35 μL of 1 M NaOH (4%) before being added. At room temperature, all reagents were combined and kept in the dark for roughly 30 min. The sample's absorbance was determined at 510 nm using an automated microplate absorbance reader. The calibration curve was generated by using standard quercetin. Total flavonoid contents were measured by calibration line which was reported as equivalents of mg quercetin (QE)/g of crude extract. All measurements were taken in triplicate.

2.5 | Determination of antioxidant potential

To examine the test extracts' capacity to scavenge free radicals, 2, 2-diphenyl 1-picrylhydrazyl (DPPH) radicals were reduced. By combining 24 mg of precisely weighed DPPH (the reagent) with 100 mL of methanol solution, a stock solution was created and placed in the freezer. Appropriate controls were maintained. Absorbance was maintained in the range (1–1.1 or 0.9–1.1) at the wavelength of 517 nm. Ten microliters of selected plant extract (5 mg/mL) was added in 90 μL of absorbance-maintained solution. After 30 min, 517 nm absorbance was measured against a control solution. As a benchmark, ascorbic acid (50 mg/mL) was employed. Increased scavenging activity is indicated by the decline in absorbance. According to Equation (1), the activity is expressed as a percentage of DPPH radical scavenging. Triplicate measurements of DPPH radical scavenging activity were made.

$$\%\,\text{inhibition} = \frac{(\text{abs. of control}) - (\text{abs. of test sample})}{\text{abs. of control}} \times 100, \quad (1)$$

where abs. of control means the absorbance of blank and abs. of test sample means activity in the presence of plant extract.

2.6 | Formulation development

2.6.1 | Ingredients of formulation

Cream of O/W was formulated by using the method of homogenization. An emulsion stabilized by 5% polyglyceryl-3 Methylglucose Distearate (TEGO® Care 450). The stable formulation consists of 3% of *C. papaya* seed extract, 3% of cetostearyl alcohol, 8% paraffin oil, and 81% distilled water. The same components and procedure were used to produce the base but without the soybean seed extract (Table 1).

2.6.2 | Preparation of formulation

The homogenization procedure was used to create a cream of O/W. The cream was prepared using two distinct phases: an oily phase and an aqueous phase. The oily phase was added to the aqueous phase to create an oil-in-water emulsion. The oily phase, which was heated to a temperature of 80 ± 0.5°C, contained paraffin oil, Tagore, and cetostearyl alcohol. The *C. papaya* seed extract was then added to the water-based aqueous phase, which had also been heated to the same temperature at the same time. Following that, the oily phase was gradually injected into the aqueous phase while being immediately stirred in a homogenizer for up to 10–15 min at 2000 rpm. The homogenizer speed was then decreased to 1500 rpm for 5 min after the cream had progressively cooled. To obtain a uniform cream the stirring was continued at a reduced speed of 1200 rpm and the temperature of the formed cream become equal to the temperature of the room. The same procedure was applied for the preparation of the base.[26]

2.7 | Stability studies

2.7.1 | Physical stability tests

For the length of the investigation, which lasted 28 days, the emulsions were stored at four distinct temperatures and relative humidity levels; 8 ± 0.5°C, 25 ± 0.5°C, 40 ± 0.5°C, and 40 ± 0.5°C. Throughout the 28-day study period, physical emulsion properties such as color, creaming, liquefaction, centrifugation, conductivity, rheology, and pH were noted at various intervals of time.

2.7.2 | Determination of type of emulsion

By diluting the emulsion with water and oil separately, the type of emulsion was determined.

2.7.3 | Centrifugation test

After preparation, centrifugal tests for emulsions were conducted. Centrifugal tests for emulsions were conducted again after 24 h, 48 h, 72 h, 7 days, 14 days, 21 days, and 28 days of preparation. The centrifugal tests were carried out using centrifugal tubes and 5 g of the sample at 25°C and 5000 rpm for 5 min.

2.7.4 | pH determination

A digital pH meter was used to measure the pH of freshly made emulsions and emulsions stored at various temperatures (8°C, 25°C, 40°C, and 40°C + 75% RH) over periods of zero, 24 h, 48 h, 72 h, 7 days, 14 days, 21 days, and 28 days.

2.7.5 | Electrical conductivity determination

Using a digital conductivity meter, conductivity tests were carried out on the prepared emulsion as well as on samples stored under

TABLE 1 Composition of base and formulation.

Cosmetic emulsion	Paraffin oil (%)	Tago care (%)	*C. papaya* seed extract (%)	Cetostearyl alcohol (%)	Distilled water (%)
Base	8	5	0	3	q.s. 100
Formulation	8	5	3	3	q.s. 100

various conditions. Conductivity tests were performed again on the emulsions 24 h, 48 h, 72 h, 7 days, 14 days, 21 days, and 28 days after initial preparation.

2.8 | Rheological parameters

The base and formulations' viscosity parameters were measured (in triplicate) using a programmable Brookfield rheometer and spindle number CP41 at 25°C. Version 2.6 of Rheocalc was utilized as a support tool. Freshly made (zero time) creams and all those placed at different temperatures, including 8°C, 25°C, 40°C, and 40°C + 75% RH, were subjected to rheological tests. The viscosity of all emulsion samples (0.5 ± 0.1 g) was measured after time intervals of zero, 24 h, 48 h, 72 h, 7 days, 14 days, 21 days, and 28 days at a spindle speed of 10–28 rpm.

2.9 | Statistical analysis

Using SPSS version 20.0, values obtained at various concentrations were examined. Two-way ANOVA was used to determine the importance of variation at various time intervals. For each mean value, the standard error of the mean was determined.

3 | RESULTS AND DISCUSSION

3.1 | Phytochemical composition

The extract of *C. papaya* subjected to phytochemical composition through polyphenolic quantification assays and preliminary phytochemical screening. Table 2 displays the findings from the papaya seed extract's phytochemical screening. According to the current research, the ethanolic extract lacks saponins and flavonoids whereas the botanical extract contains alkaloids, glycosides, terpenoids, reducing sugars, quinones, proteins, and tannins. The pharmacological properties of *C. papaya* are because of the various phytochemical constituents present in it.

TABLE 2 Phytochemical analysis of *C. papaya* seed extract.

Test	Observation	Inference
Alkaloids	The appearance of yellow color	+
Glycosides	Formation of brick red precipitate	+
Flavonoids	No color change from red to crimson	–
Terpenoids	The appearance of a grayish color	+
Carbohydrates	The appearance of green color	+
Quinones	Discoloration of test solution	+
Saponins	Froth formation	–
Tannins	The appearance of black-green color	+
Proteins	The appearance of yellow color	+

The therapeutic and restorative effects of botanical extracts are strongly tied to their phytochemical components, which can be divided into many broad categories, including saponins, alkaloids, tannins, terpenoids, reducing sugars, phenols, proteins, and quinones.[27] The total phenolic and flavonoid contents of *C. papaya* seed extract were 62.35 mg GAE/g and 10.5 mg QE/g, respectively. Phenolics or polyphenols are present in every medicinal plant and its products and are secondary metabolites.[28] Tannin-rich plants are used in phytotherapy to treat non-specific diarrhea, mild skin injuries, and mouth- and throat inflammations.[29] Additionally, saponins have expectorant action via activating an upper GI tract reflex.[30,31] This study explained that various medicinal plants containing alkaloids are used in several drugs because of their pharmacological actions however higher doses might be toxic. Plants rich in terpenoids have anti-fungal, antimicrobial, anti-spasmodic, antibacterial, and anti-allergic properties. Terpene-rich plants may be used to treat and prevent a variety of illnesses, including cancer.[32] Flavonoids are used in the treatment of all types of miscarriages because of their ability to prevent platelet aggregation (platelet stickiness).[33] Flavonoids anti-inflammatory properties help thin the blood and block the clotting pathway.[30]

3.2 | Antioxidant activity

Ascorbic acid (vitamin C) was utilized as a standard in the DPPH method to assess the antioxidant activity of medicinal plant extracts. To test the ability of extracts and their constituents to scavenge free radicals, a commonly used straightforward approach is used.[34] DPPH measurements of the botanical extract (3% of *C. papaya* seeds) revealed an antioxidant activity of 82.7%. Several bioactive substances, such as phenolic compounds, flavonoids, and isoflavonoids, are present in *C. papaya*. Phenolic metabolites contribute significantly to the antioxidant activity of *C. papaya*.[35] Phenolic compounds can scavenge radicals by giving hydrogen atoms to them resulting in the breakage of the lipid oxidation chain reaction. Thus phenolic compounds are responsible for antioxidant activity.[36]

3.3 | Stability studies

3.3.1 | Physical stability and organoleptic evaluation

Tables 3 and 4 displays the organoleptic evaluation of an O/W bases and formulation.

Color

The formulation was off-white in color and the freshly made base was white. After the entire trial period of 28 days at varied temperatures, there was no change seen in the color of either the base or the formulation. The presence of polyphenols prevents microbial attack, which frequently results in color change as a result of

TABLE 3 Organoleptic evaluation of base at different time intervals.

	Color				Odor				Liquefaction				Phase separation			
Time	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
0 h	White	White	White	White	-	-	-	-	-	-	-	-	-	-	-	-
24 h	White	White	White	White	-	-	-	-	-	-	-	-	-	-	-	-
48 h	White	White	White	White	-	-	-	-	-	-	-	-	-	-	-	-
72 h	White	White	White	White	-	-	-	-	-	-	-	-	-	-	-	-
7 days	White	White	White	White	-	-	-	-	-	-	-	-	-	-	-	-
14 days	White	White	White	White	-	-	-	-	-	-	-	-	-	-	-	-
21 days	White	White	White	White	-	-	-	-	-	-	-	-	-	-	-	-
28 days	White	White	White	White	-	-	-	-	-	-	-	-	-	-	-	-

Note: -, no change; +, slight change; A, 8°C; B, 25°C; C, 40°C; D, 40°C + 75% RH.

TABLE 4 Organoleptic evaluation of formulation at different time intervals.

	Color				Odor				liquefaction				Phase separation			
Time	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
0 h	O.W	O.W	O.W	O.W	-	-	-	-	-	-	-	-	-	-	-	-
24 h	O.W	O.W	O.W	O.W	-	-	-	-	-	-	-	-	-	-	-	-
48 h	O.W	O.W	O.W	O.W	-	-	-	-	-	-	-	-	-	-	-	-
72 h	O.W	O.W	O.W	O.W	-	-	-	-	-	-	-	-	-	-	-	-
7 days	O.W	O.W	O.W	O.W	-	-	-	-	-	-	-	-	-	-	-	-
14 days	O.W	O.W	O.W	O.W	-	-	-	-	-	-	-	-	-	-	-	-
21 days	O.W	O.W	O.W	O.W	-	-	-	-	-	-	-	-	-	-	-	-
28 days	O.W	O.W	O.W	O.W	-	-	-	-	-	-	-	-	-	-	-	-

Note: -, no change; +, slight change; A, 8°C; B, 25°C; C, 40°C; D, 40°C + 75% RH; O.W, off-white.

microbial growth destroying formulation components.[37] A greater temperature's ability to separate the oily phase may also cause a color change.[38]

Odor

There was no smelly odor in the base and formulation when stored for a period of 28 days at various conditions of storage. Microorganisms are responsible for the smelly odor in the emulsion. Microorganisms spoil the formulation and cause a smelly odor. İn most cases fungus is responsible for spoilage of creams. Candida species are dominant to spoil the creams.[39]

Liquefaction

No liquefaction was noticed in any of the formulations retained for the course of the 28-day study period at 8°C, 25°C, 40°C, or 40°C ± 75% RH. Due to the high temperature, liquefaction occurs. As reported the viscosity decreases as the temperature increases resulting in liquefaction.[40]

Phase separation

Throughout the 28-day testing period, no phase separation was seen in the base or formulation stored at different temperatures (8°C, 25°C, 40°C, and 40°C + 75% RH). Under the influence of gravity, the density difference between the two phases causes creaming and sedimentation which results in phase separation. By using proper homogenization (decreasing the globule size) this drawback can be controlled.[41]

Centrifugation

To assess the physical stability of semisolid formulations held at various temperatures, centrifugation testing is typically utilized.[42] For samples which were stored under various storage conditions at elevated temperatures, such as 8°C, 25°C, 40°C, and 40°C + 75% RH, centrifugation tests were conducted. After completing a centrifugation test for 10 min at 5000 rpm, there was no phase separation visible, demonstrating that the formulations were stable at all temperatures.

pH evaluation

Any cosmetic product's stability and effectiveness can be evaluated using the key parameter of pH.[43] Human skin has a pH range of 4.5–6.0, with an average pH of 5.5. As a result, the pH of compositions meant for human skin application should fall within this range.[44] Freshly made base and formulation with an extract from *C. papaya* seeds have pH values of 5.98 and 5.61, respectively, which are in the

same range as skin pH. For a period of 28 days, the pH values of the base stored at several temperatures, including 8°C, 25°C, 40°C, and 40°C + 75% RH, decreased constantly (Table 5). Base pH values at 8°C, 25°C, 40°C, and 40°C + 75% RH decreased from 5.98 to 5.2, 5.37, 5.39, and 5.44, respectively, after the study period. While the pH of the formulation decreased from 5.61 to 5.28, 5.26, 5.25, and 5.19, respectively, at 8°C, 25°C, 40°C, and 40°C + 75% RH (Table 6). It was determined that the change in pH of various base and formulation samples was significant at various time intervals and temperatures by utilizing a two-way ANOVA test with a 5% threshold of significance. For the duration of the 28-day testing period, a minor but consistent decline in the pH values of the base and formulation was seen. It was determined that the pH values of base and formulation changed significantly throughout the study period, and that variation in the pH values under various storage circumstances may have been caused by paraffin oil oxidation.[45] The use of paraffin oil in the formulation runs the risk of oxidation, which produces organic acids and aldehydes and lowers pH levels.[46] Additionally, it has been suggested that pH lowering may be caused by the breakdown of any chemical into its metabolites over time and at an increased temperature.[38]

Electrical conductivity

Conductivity is a crucial factor to consider when evaluating a cosmetic product's durability. The proportion of water grows in the lower part of the emulsion due to creaming, while the proportion of oil rises in the higher section due to the increase in oil proportion. Because water makes up their continuous phase, water in oil emulsions transmit electricity well, whereas oil in water emulsions does not because oil makes up their continuous phase.[47]

Freshly made base and formulation had conductivity values of 65.34 and 76.43, respectively. The conductivity values of the base showed a small decrease. The observed conductivity values of the base held at various accelerated temperatures (8°C, 25°C, 40°C, and 40°C + 75% RH) were 59.21, 58.24, 57.92, and 56.36, respectively, at the end of the study period of 28 days (Table 7). While the formulation's conductivity value indicates a small increase during the course of the trial. Conductivity values of the formulation at various storage conditions (8°C, 25°C, 40°C, and 40°C + 75% RH) were found to be 87.28, 88.64, 90.17, and 93.62, respectively, after the investigation (Table 8).

The change in electrical conductivity is significant at various levels of time and temperature, according to a two-way analysis of variance (ANOVA) study with a 5% level of significance. Previous research has shown that the clustering of cream droplets and the migration of charged particles within aggregates are responsible for the increase in conductivity values.[48] Another study demonstrates that when compared to formulations that aren't loaded with drugs, drug-laden formulations have greater conductivity values.[49]

Rheological analysis

Rheology is an important tool to interpret the viscosity characterization and flow properties of a formulation. Better results of a formulation for viscosity give a sign for better spreadability of a formulation. Cosmetic products vary regarding their rheological

TABLE 5 pH of base and storage conditions at 25°C, 40°C, and 40°C + 75% RH.

Time	8°C	25°C	40°C	40°C + 75% RH
Fresh	05.98 ± 0.02	05.98 ± 0.03	05.98 ± 0.01	05.98 ± 0.02
24 h	05.85 ± 0.01	05.89 ± 0.01	05.87 ± 0.03	05.88 ± 0.01
48 h	05.71 ± 0.05	05.8 ± 0.02	05.78 ± 0.06	05.81 ± 0.03
72 h	05.62 ± 0.02	05.75 ± 0.02	05.69 ± 0.02	05.76 ± 0.05
7 days	05.52 ± 0.01	05.64 ± 0.01	05.62 ± 0.01	05.69 ± 0.02
14 days	05.4 ± 0.02	05.56 ± 0.04	05.52 ± 0.04	05.63 ± 0.03
21 days	05.29 ± 0.04	05.48 ± 0.05	05.44 ± 0.04	05.53 ± 0.01
28 days	05.2 ± 0.02	05.37 ± 0.02	05.39 ± 0.05	05.44 ± 0.03

TABLE 6 pH values of formulation at various storage conditions at 8°C, 25°C, 40°C and 40°C + 75% RH.

Time	8°C	25°C	40°C	40°C + 75% RH
Fresh	05.61 ± 0.02	05.61 ± 0.03	05.61 ± 0.01	05.61 ± 0.03
24 h	05.54 ± 0.01	05.56 ± 0.02	05.55 ± 0.02	05.55 ± 0.07
48 h	05.48 ± 0.02	05.51 ± 0.02	05.53 ± 0.06	05.48 ± 0.02
72 h	05.44 ± 0.03	05.45 ± 0.01	05.48 ± 0.02	05.4 ± 0.04
7 days	05.4 ± 0.04	05.39 ± 0.04	05.43 ± 0.01	05.33 ± 0.01
14 days	05.36 ± 0.01	05.35 ± 0.03	05.39 ± 0.05	05.27 ± 0.02
21 days	05.31 ± 0.02	05.3 ± 0.02	05.33 ± 0.02	05.23 ± 0.05
28 days	05.28 ± 0.03	05.26 ± 0.04	05.25 ± 0.01	05.19 ± 0.01

TABLE 7 Conductivity of base at various storage conditions 8°C, 25°C, 40°C and 40°C + 75% RH.

Time	8°C	25°C	40°C	40°C ± 75% RH
Fresh	65.34 ± 0.05	65.34 ± 0.02	65.34 ± 0.02	65.34 ± 0.03
24 h	64.25 ± 0.01	64.87 ± 0.01	64.92 ± 0.02	64.47 ± 0.06
48 h	63.45 ± 0.03	64.11 ± 0.03	64.22 ± 0.01	63.24 ± 0.02
72 h	62.76 ± 0.02	63.48 ± 0.02	63.56 ± 0.02	62.57 ± 0.04
7 days	62.15 ± 0.01	62.59 ± 0.01	62.67 ± 0.03	60.89 ± 0.02
14 days	61.16 ± 0.03	61.37 ± 0.04	61.49 ± 0.05	59.73 ± 0.01
21 days	60.22 ± 0.02	59.93 ± 0.06	58.82 ± 0.03	58.22 ± 0.05
28 days	59.21 ± 0.06	58.24 ± 0.01	57.92 ± 0.02	56.36 ± 0.03

TABLE 8 Conductivity of formulation at various storage conditions 8°C, 25°C, 40°C and 40°C + 75% RH.

Time	8°C	25°C	40°C	40°C + 75% RH
Fresh	76.43 ± 0.02	76.43 ± 0.04	76.43 ± 0.01	76.43 ± 0.03
24 h	77.74 ± 0.04	77.85 ± 0.02	77.93 ± 0.04	78.04 ± 0.02
48 h	78.45 ± 0.03	78.79 ± 0.02	79.35 ± 0.01	80.27 ± 0.04
72 h	79.98 ± 0.01	80.31 ± 0.01	81.73 ± 0.05	82.46 ± 0.05
7 days	82.65 ± 0.02	82.19 ± 0.02	83.94 ± 0.02	85.81 ± 0.02
14 days	84.34 ± 0.05	84.63 ± 0.03	85.66 ± 0.02	88.69 ± 0.03
21 days	85.57 ± 0.04	86.47 ± 0.05	88.72 ± 0.05	91.55 ± 0.04
28 days	87.28 ± 0.02	88.64 ± 0.02	90.17 ± 0.03	93.62 ± 0.02

analysis due to their components. Rheology study helps in the characterization of cream by describing any change in the formulation which is induced by temperature, time, and shared stress to predict the stability of the formulation. Rheological behavior of formulations influenced by phospholipids, polymers, and surfactants.[50]

The rheogram of share rate versus viscosity was plotted for the base at 24 h (Figure 1) and formulation at 24 h (Figure 2) and the rheogram of base at Day 28 (Figure 3) and formulation at Day 28 (Figure 4) at various conditions of storage 8°C, 25°C, 40°C, and 40°C + 75% RH. Rheogram represents that by increasing the share rate, the viscosity decreases. An inverse correlation between share stress and viscosity is seen as a result of the share rate. The creation of aggregates in the formulation, when there was no tension applied, is the reason for the inverse relationship between viscosity and shared stress, which leads to a drop in viscosity when stress is introduced. Systems used for share thinning are non-Newtonian systems.[51] Power law was applied to analyze the programs. The values of the flow index indicate that emulsion acts as a non-Newtonian fluid. A marked non-Newtonian behavior was observed in emulsions containing different plant extracts, most vegetables, paste and fruits are pseudoplastic and their flow index behavior varies between 0 and 1.[52] All emulsions' rheograms displayed non-Newtonian behavior and pleasant rheological characteristics, with flow indices of under 1. Emulsions with pseudoplastic behavior produce a coherent coating on the skin, and this property of the emulsion is very helpful for improving the fortification of the skin's surface with phenolic antioxidants. When shear stress was applied, viscosity changes were seen. Viscosities

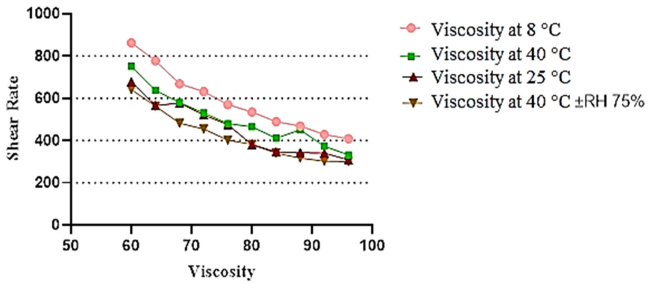

FIGURE 1 Rheogram of the base at 24 h.

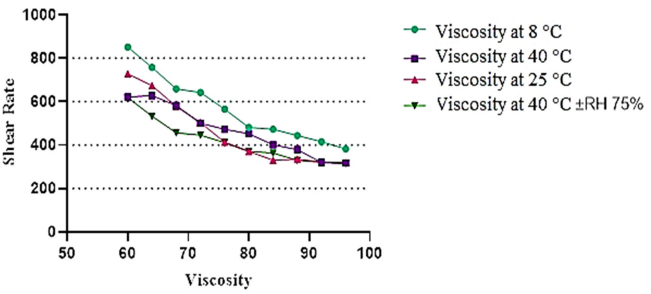

FIGURE 2 Rheogram of formulation at 24 h.

were observed to fall in tandem with rising share rates. Higher temperatures that is, 40°C and 40°C ± 75% RH are also responsible to decrease the viscosity. By increasing the temperature, viscosity decreases. By increasing the temperature, flow properties of molecules increases among the interface which indicated a decrease in the viscosity.[5]

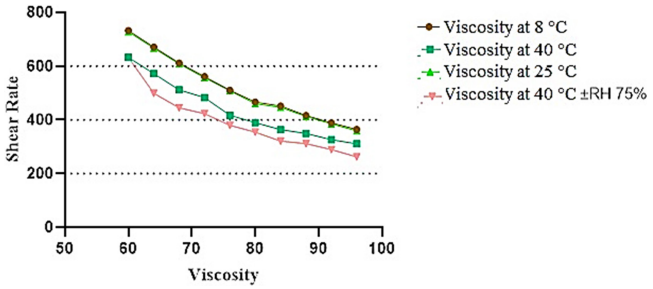

FIGURE 3 Rheogram of the base at Day 28.

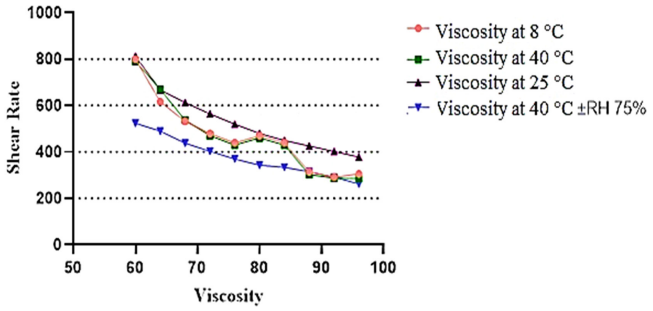

FIGURE 4 Rheogram of formulation at Day 28.

4 | CONCLUSION

The findings of the current investigation demonstrated that the presence of a significant amount of phenolic chemicals in the papaya seed ethanolic extract resulted in excellent antioxidant activity. The hydroxyl group found in phenolic compounds may be the cause of the extract's antioxidant effect. 3% *C. papaya* seed extract demonstrated great pharmaceutical stability and outstanding physical features when loaded in an oil-in-water emulsion, providing a special delivery method for a range of cutaneous issues. To evaluate the emulsion in the commercial market, in vivo research should be conducted on the skin of human test subjects using non-invasive biophysical methods.

AUTHOR CONTRIBUTIONS

Muhammad Sarfraz: Conceptualization and Supervision. Kanwal Shabbir, Jafir Hussain Shirazi, Nimrah Mahmood, Abdul Basit: Data Curation. Kanwal Shabbir, Haji Muhammad Shoaib Khan, Hamna Sabir, Kifayat Ullah Khan, Abdul Basit: Formal Analysis. Kanwal Shabbir, Haji Muhammad Shoaib Khan: Investigation. Qazi Adnan, Haji Muhammad Shoaib Khan, Jafir Hussain Shirazi, Hamna Sabir, Nimrah Mahmood, Yousef A Bin Jardan: Formal Analysis, Validation, Reviewing and Editing of Manuscript. Abdul Basit: Validation. Qazi Adnan, Haji Muhammad Shoaib Khan: Visualization. Muhammad Sarfraz, Qazi Adnan, Kifayat Ullah Khan: Writing Review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors report no conflict of interest.

DATA AVAILABILITY STATEMENT

Not applicable.

ETHICS STATEMENT

Authors declare human ethics approval was not needed for this study.

ORCID

Muhammad Sarfraz https://orcid.org/0000-0002-0516-4966
Qazi Adnan https://orcid.org/0000-0002-3172-2570
Haji Muhammad Shoaib Khan https://orcid.org/0000-0002-0727-3453
Jafir Hussain Shirazi https://orcid.org/0000-0001-5331-655X
Hamna Sabir https://orcid.org/0009-0001-0396-9840
Yousef A. Bin Jardan https://orcid.org/0000-0003-3302-4036
Kifayat Ullah Khan https://orcid.org/0000-0002-8108-5880
Abdul Basit https://orcid.org/0000-0002-0012-8931

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