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Formulation And Evaluation of Herbal Anti-Aging Cream Containing *Carica Papaya* Leaves Extract

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ABSTRACT

OBJECTIVE: The aim of the present study was to formulate and evaluate an anti-aging herbal cream containing ethanolic extract of Carica papaya leaves and to assess its antioxidant activity. **METHODS:** Carica papaya leaves were authenticated, shade dried and extracted by maceration using 70% ethanol for 72 h. The extract was subjected to preliminary phytochemical screening, and total phenolic and flavonoid contents were determined by the Folin-Ciocalteu and aluminium chloride methods, respectively. An anti-aging cream was formulated in three concentrations of the extract (F1, F2, F3) by the oil-in-water emulsification method and evaluated for organoleptic, physical and chemical parameters, including pH, viscosity, spreadability, acid value and saponification value. Antioxidant activity of the extract and formulation was evaluated by the DPPH radical scavenging assay, and stability studies were carried out for 30 days under accelerated conditions (40 ± 2°C/75 ± 5% RH). **RESULTS:** Phytochemical screening confirmed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, steroids and tannins. The total phenolic content of the extract was 49.78 mg GAE/g extract and the total flavonoid content was 30.04 mg RE/g extract. The DPPH assay showed concentration-dependent radical scavenging activity, with the extract exhibiting an IC50 of 234.52 µg/ml and the formulation an IC50 of 4.58 mg/ml. Among the three batches, F3 (1.5 g extract) showed the best overall performance, with a pH of 7.2, spreadability of 19.8 g·cm/sec, viscosity of 24510 cp and good homogeneity. The formulation remained physically and chemically stable over 30 days of accelerated stability testing, with no significant change in colour, odour, pH or phase separation. **CONCLUSION:** Carica papaya leaf extract possesses significant antioxidant activity attributable to its flavonoid and phenolic content, and can be effectively incorporated into a stable, cosmetically acceptable anti-aging cream, supporting its potential as a natural alternative to synthetic anti-aging skincare formulations.

1. INTRODUCTION:

Free radicals are highly reactive, unstable molecular species that attack lipids, proteins and DNA; this ongoing oxidative injury is closely linked to premature skin aging as well as a range of chronic disorders. Antioxidant compounds counter this process by neutralising free radicals, thereby protecting cellular structures from oxidative damage.(Das Tama et al., 2020)

Because they are rich in bioactive constituents such as flavonoids, phenolic acids and alkaloids, medicinal plants remain an important natural source of antioxidant agents. Carica papaya leaves are one

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such source that has drawn considerable research interest, with several reports linking their pronounced antioxidant potential to their phenolic and flavonoid content.(Salla et al., 2016)

Among the methods available for assessing this activity, the DPPH radical-scavenging assay is widely used because it offers a straightforward measure of a compound's ability to neutralise free radicals. Earlier work has shown that papaya leaf extract scavenges free radicals effectively, supporting its suitability for incorporation into herbal formulations(Handayani et al., 2024).

Because many synthetic anti-aging creams carry a risk of skin irritation and allergic reactions, consumer demand has shifted toward herbal and natural alternatives perceived as safer and more environmentally friendly. Carica papaya leaves are a promising candidate for such formulations: their content of flavonoids and vitamins A, C and E confers antioxidant, antibacterial and wound-healing properties that can help counter premature skin aging by neutralising free radicals. Despite this potential, relatively few studies have examined papaya leaf extract in a topical dosage form. The present work was therefore undertaken to formulate a herbal anti-aging cream incorporating Carica papaya leaf extract and to evaluate its antioxidant activity for possible dermatological application.("EVALUATION OF HERBAL CREAMS FORMULATED USING ETHANOLIC EXTRACT OF CARICA PAPAYA LEAVES," 2022; Sekar et al., 2017)

2. MATERIALS AND METHODS:

2.2 Plant profile:

2.2.1 Synonyms: Papita (Hindi), Pappayi (Marathi), Fruit of Angel.

2.2.2 Biological source: A cultivated, fruit-bearing tree, *Carica papaya Linn.* Vertical incisions made on the half-ripe fruit yield a medicinal latex, which is the commercial source of the proteolytic enzyme papain.

Family: Caricaceae.

2.2.3 Chemical constituents: The leaves are reported to contain the alkaloids carpaine and carpinine along with vitamins B and C, in addition to a range of flavonoids that includes quercetin, kaempferol 3-rutinoside, quercetin 3-(2G-rhamnosylrutinoside), quercetin 3-rutinoside, kaempferol 3-(2G-rhamnosylrutinoside) and myricetin 3-rhamnoside. (Ayodipupo Babalola et al., 2024)

2.2.4 Medicinal uses: Papain supports digestion and is used in managing indigestion, while the leaves themselves have been reported to aid wound healing and to show anticancer and antimicrobial effects. (Sharma et al., 2022)

2.3 Preparation of Ethanolic Extract of Carica Papaya

The collected leaves were shade dried and coarsely powdered. 25 g of the coarse powder was macerated in 250 ml of 70% v/v ethanol for 72 h with agitation at frequent intervals. The macerate was filtered through muslin cloth and the filtrate was oven dried at 70°C. The concentrated extract was stored in a porcelain dish, wrapped carefully in aluminium foil.("EVALUATION OF HERBAL CREAMS FORMULATED USING ETHANOLIC EXTRACT OF CARICA PAPAYA LEAVES," 2022) The percentage yield was calculated as:

Percentage yield = (weight of extract / weight of powdered leaves) × 100
= (2.8 / 25) × 100 = 11.2% w/w

2.4 Preliminary Phytochemical Screening

The extract was subjected to standard qualitative chemical tests to detect the presence of phytoconstituents such as alkaloids, flavonoids, glycosides, phenolics, saponins, steroids and tannins.(Oti Wilberforce & Nkechinyere Olivia, 2017)

2.5 Determination of Total Phenolic Content (TPC)

Carica papaya leaf extract at a concentration of 1 mg/ml was used for the analysis. The reaction mixture was prepared by mixing 0.5 ml of ethyl acetate solution of extract with 2.5 ml of 10% Folin-Ciocalteu's reagent, followed by addition of 2.5 ml of 7.5% $NaHCO_3$. A blank was concomitantly prepared using 0.5 ml water, 2.5 ml 10% Folin-Ciocalteu's reagent and 2.5 ml of 7.5% $NaHCO_3$. The samples were incubated at 45°C for 45 min and absorbance was measured at 765 nm using a UV-Visible spectrophotometer. Samples were analyzed in triplicate. A standard calibration curve of gallic acid (100-1000 µg/ml) was constructed as concentration vs. absorbance, and the phenolic content was expressed as mg gallic acid equivalent (GAE)/g of extract.(G et al., 2020)

2.6 Determination of Total Flavonoid Content (TFC)

The total flavonoid content was determined by the aluminium chloride colorimetric method using rutin as the reference standard. A stock solution was prepared by dissolving 10 mg of rutin in 10 ml of methanol (1000 µg/ml), and dilutions of 100-1000 µg/ml were prepared. To each aliquot, 4 ml distilled water, 0.3 ml of 5% $NaNO_2$, 0.3 ml of 10% $AlCl_3$ (after 5 min) and 2 ml of 1M NaOH (after 5-6 min) were added sequentially. The resulting red-coloured solution was incubated in the dark for 30 min and absorbance was measured at 510 nm. The flavonoid

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content was calculated from the calibration curve and expressed as mg rutin equivalent (RE)/g of extract.(G et al., 2020)

2.7 Formulation of Anti-aging Cream

The anti-aging cream was formulated using Carica papaya leaf extract at three different concentrations (F1: 0.5 g, F2: 1 g, F3: 1.5 g) by the oil-in-water emulsification method, as detailed in Table 1.

Table 1: Formulation Of Anti-Aging Cream (Quantity Per 50 G Batch)

Sr. No.	Ingredient	F1	F2	F3	Role
1	*Carica papaya* leaves extract	0.5 g	1 g	1.5 g	Active ingredient
2	Stearic acid	12 g	12 g	12 g	Emulsifier
3	Cetyl alcohol	0.5 g	0.5 g	0.5 g	Emollient
4	Glyceryl monostearate	0.5 g	0.5 g	0.5 g	Stabilizer
5	Propyl paraben	0.005 g	0.005 g	0.005 g	Preservative
6	Glycerine	5 ml	5 ml	5 ml	Humectant
7	Potassium hydroxide	0.5 g	0.5 g	0.5 g	pH adjuster
8	Methyl paraben	0.05 g	0.05 g	0.05 g	Preservative
9	Water	q.s. to 50 g	q.s. to 50 g	q.s. to 50 g	Vehicle

2.8 Method of Preparation: The oil phase, consisting of stearic acid, cetyl alcohol, glyceryl monostearate, and propyl paraben, was heated to **70°C** until a uniform mixture was obtained. Simultaneously, the aqueous phase containing glycerine, potassium hydroxide, methyl paraben, and distilled water was also heated to **70°C**. The heated oil phase was then added slowly to the aqueous phase with continuous stirring to form a stable emulsion. The ethanolic extract, previously dissolved in a small volume of water or propylene glycol, was incorporated into the emulsion under constant stirring. Finally, the preservatives were added, and the formulation was allowed to cool gradually to room temperature with continuous stirring to obtain a smooth and homogeneous cream.(Safta et al., 2023; Sekar et al., 2017)

3. Evaluation of the Formulated Cream

3.1Organoleptic evaluation: Colour, odour, texture and appearance were assessed by visual inspection.

3.2 pH: 0.5 g of cream was weighed, dissolved in 10 ml distilled water, and the pH was determined using a calibrated pH meter.(Shkreli et al., n.d.-a)

3.3 Spreadability: 1 g of cream was placed between two glass plates and a known weight was applied to the upper slide for 5 min to allow uniform spreading. A thread attached to the upper slide was pulled horizontally, and the time taken for the slide to move a fixed distance was noted. Spreadability (S) was calculated as $S = M \times L / T$, where M is the weight tied to the upper slide (g), L is the distance moved by the slide (cm) and T is the time taken (sec).(Patil et al., n.d.)

3.4 Viscosity: Determined using a Brookfield viscometer with spindle no. 64 at 20 rpm.

3.5 Acid value: 10 g of the sample was dissolved in 50 ml of an equal-volume mixture of ether and alcohol under a reflux condenser and slowly heated. 1 ml phenolphthalein indicator was added and the solution was titrated against 0.1 N NaOH until a faint pink colour appeared. Acid value = $56.1 \times n / w$, where n is the volume of NaOH consumed (ml) and w is the weight of substance taken (g).

3.6 Saponification value: 2 g of sample was refluxed with 25 ml of 0.5 N alcoholic KOH for 30 min. 1 ml phenolphthalein was added and the mixture was titrated immediately against 0.5 N HCl. Saponification value = $(b - a) \times 28.05 / w$, where b is the blank titration volume, a is the sample titration volume and w is the weight of the sample (g).(Akmal et al., 2024)

4. Antioxidant Activity by DPPH Assay

DPPH is a stable, deep violet-coloured free radical. When antioxidant molecules donate hydrogen atoms or electrons to DPPH, it is converted to a pale yellow product, and the resulting fall in absorbance at 517 nm serves as a direct index of the sample's antioxidant strength.

A 0.5 mM DPPH solution was prepared in methanol. Ascorbic acid (10 mg in 10 ml methanol) was used as the standard, from which a 100 µg/ml working dilution was prepared. Test samples included extract solutions at 100, 200 and 300 µg/ml, and formulation samples at 1, 2 and 3 mg/ml. 0.2 ml of each sample was mixed with 2 ml of DPPH solution; a control was prepared using 0.2 ml methanol with 2 ml DPPH solution. Reaction mixtures were incubated in the dark at room temperature for 30 min, and absorbance was measured at 517 nm. Percentage inhibition was calculated as:

$$\% \text{ Inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

where A control is the absorbance of the control and A sample is the absorbance of the test sample.

The optimized cream formulation was subjected to accelerated stability testing at 40 ± 2°C and 75 ± 5% relative humidity for 30 days as per ICH guidelines. Samples were withdrawn on day 0, 15 and 30 and

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evaluated for colour, odour, homogeneity, pH, phase separation, spreadability, washability, grittiness, viscosity and drug content.(Farida & Iswahyuni, 2018; G et al., 2020)

5.RESULTS AND DISCUSSION

5.1 Phytochemical Evaluation of Extract:

Preliminary phytochemical screening of the ethanolic extract of *Carica papaya* leaves confirmed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, steroids and tannins, as summarized in Table 2. These constituents, particularly the flavonoids and phenolic compounds, are known to contribute significantly to antioxidant and anti-aging activity.

Table 2: Phytochemical Evaluation Of Extract

Sr. No.	Test	Inference
1	Test for alkaloids	+
2	Test for flavonoids	+
3	Test for glycosides	+
4	Test for phenols	+
5	Test for saponins	+
6	Test for steroids	+
7	Test for tannins	+

5.2 Total Phenolic Content

Table 3: Calibration Curve Of Gallic Acid

Sr. No.	Concentration (µg/ml)	Absorbance
1	100	0.113
2	200	0.203
3	400	0.440
4	600	0.653
5	800	0.863
6	1000	1.104

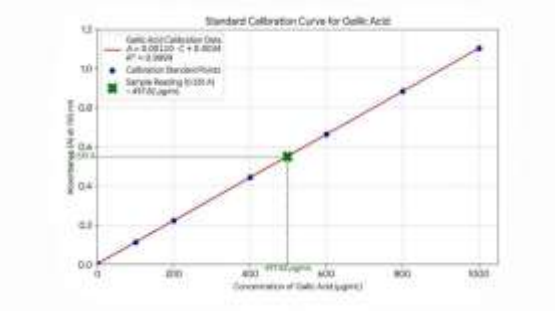

Fig. 2: calibration curve of gallic acid

Using the linear regression equation obtained from the calibration curve (y = 0.00110x + 0.0034), the absorbance of the test solution (0.551) corresponded to a gallic acid equivalent concentration of 497.8 µg/ml, i.e., 49.78% for the extract taken. The total phenolic content of the extract was therefore found to be 49.78 mg GAE/g extract.

5.3 Total Flavonoid Content

Table 4: Calibration Curve Of Rutin

Sr. No.	Concentration (µg/ml)	Absorbance
1	100	0.157
2	200	0.303
3	400	0.595
4	600	0.887
5	800	1.179
6	1000	1.471

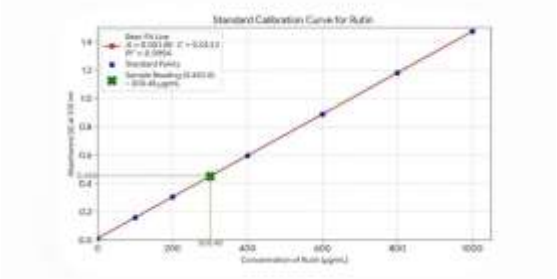

Fig. 3: calibration curve of rutin

Using the calibration equation (y = 0.00146x + 0.0113), the absorbance of the test solution (0.450) corresponded to a rutin equivalent concentration of 300.4 µg/ml, i.e., 30.04% for the extract taken. The total flavonoid content of the extract was therefore found to be 30.04 mg RE/g extract.

5.4 Organoleptic Evaluation of the Optimized Cream (F3):

Table 5: Organoleptic Evaluation

Sr. No.	Parameter	Observation
1	Colour	Light green
2	Odour	Acceptable
3	Texture	Smooth
4	Appearance	Creamy
5	Fragrance	Mild, refreshing
6	Homogeneity	Homogeneous

5.5 Chemical Evaluation of the Cream:

Table 6: Chemical Evaluation

Sr. No.	Parameter	Observation
1	Acid value	0.112 mg KOH/g
2	Saponification value	0.224 mg KOH/g

5.6 Physical Evaluation of the Cream:

Table 7: Physical Evaluation

Sr. No.	Parameter	Observation
1	pH	7.2
2	Spreadability	19.8 g·cm/sec
3	Viscosity	24510 cp
4	Washability	Easily washable

With a pH of 7.2 ± 0.05, the optimized formulation (F3) falls within the physiological range of the skin, so the risk of irritation on application is expected to be low. Its spreadability (19.8 g·cm/sec) and viscosity (24510 cp) point to a consistency well suited to topical use, and the cream also rinsed off easily.

5.7 Antioxidant Activity:

Both the extract and the cream formulation showed DPPH radical scavenging that rose with increasing concentration, consistent with the free-radical scavenging capacity contributed by the phenolic and flavonoid constituents of Carica papaya leaves.

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Table 8: Antioxidant Activity Of Extract And Formulation

Sr. No.	Sample	Concentration	Absorbance	% Antioxidant activity
1	Control	—	0.851	—
2	Standard (ascorbic acid)	100 µg/ml	0.150	82.0 %
3	Extract D1	100 µg/ml	0.631	24.8 %
	Extract D2	200 µg/ml	0.450	47.1 %
	Extract D3	300 µg/ml	0.343	59.6 %
4	Formulation F1	1 mg/ml	0.720	15.4 %
	Formulation F2	2 mg/ml	0.653	22.7 %
	Formulation F3	3 mg/ml	0.551	35.2 %

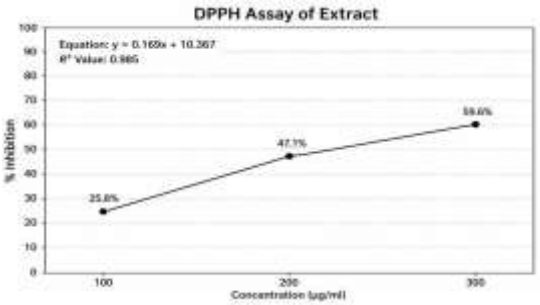

Fig. 3: antioxidant activity of extract

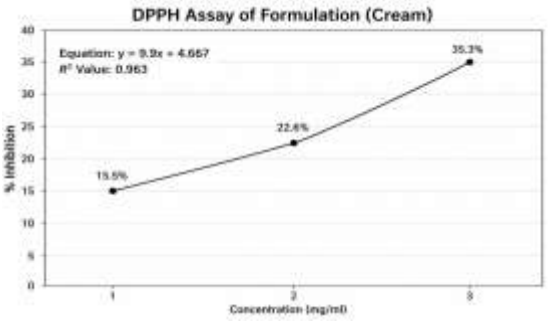

Fig. 4: antioxidant activity of cream formulation

Table 9: Ic50 Values

Sr. No.	Sample	IC50 Value
1	Extract	234.52 µg/ml
2	Formulation	4.58 mg/ml

The extract exhibited an IC50 of 234.52 µg/ml, while the formulated cream showed an IC50 of 4.58 mg/ml. This shift to a higher IC50 for the formulation relative to the pure extract is not unexpected, since the active constituents are diluted within the cream base; even so, the data show that the extract's antioxidant activity is retained once it is incorporated into the topical formulation.

5.8 Stability Studies:

The optimized cream formulation (F3) was subjected to accelerated stability testing at 40 ± 2°C and 75 ± 5% RH for 30 days.(Shkreli et al., n.d.-b) No significant changes were observed in colour, odour, homogeneity, pH, phase separation, spreadability, washability or drug content over the study period, confirming that the formulation is physically and chemically stable, as summarized in Table 10.

Table 10: Stability Testing Of Cream Formulation

Sr. No.	Parameter	Day 0	Day 15	Day 30	Result
1	Colour	Light green	No change	No change	Stable
2	Odour	Acceptable	No change	No change	Stable
3	Homogeneity	Uniform	Uniform	Uniform	Excellent
4	pH	7.2 ± 0.05	7.2 ± 0.05	7.2 ± 0.05	In range
5	Phase separation	None	None	None	Stable
6	Spreadability	19.8 g·cm/sec	19.8 g·cm/sec	19.8 g·cm/sec	Stable
7	Washability	Easy to wash	Easy to wash	Easy to wash	No change
8	Grittiness	Absent	Absent	Absent	Smooth
9	Viscosity	24510 cp	24510 cp	24510 cp	Stable
10	Drug content (%)	79.6 %	79.8 %	80.0 %	Stable

6. CONCLUSION:

The present study was successfully carried out using Carica papaya leaves, which were authenticated and confirmed under the family Caricaceae. The ethanolic extract was prepared by maceration using 70% ethanol for 72 h, with a percentage yield of 11.2% w/w, indicating efficient extraction of bioactive constituents. Phytochemical screening revealed the presence of flavonoids, phenols, alkaloids, glycosides, saponins, tannins and steroids, which contribute to the antioxidant and anti-aging properties of the extract. Quantitative analysis showed a total phenolic content of 49.78 mg GAE/g extract and a total flavonoid content of 30.04 mg RE/g extract, confirming strong antioxidant potential. The DPPH assay showed percentage inhibition increasing with concentration, with an IC50 of 234.52 µg/ml for the extract and 4.58 mg/ml for the formulation.

The optimized anti-aging cream (F3) exhibited a light green colour, acceptable odour, smooth texture, creamy appearance and good homogeneity, with a pH of 7.2 ± 0.05, good spreadability (19.8 ± 1.71 g·cm/sec), appropriate viscosity (24510 ± 1507 cp) and easy washability. Accelerated stability studies at

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40 ± 2°C/75 ± 5% RH for 30 days showed no significant changes, confirming good stability of the formulation. Overall, the study concludes that Carica papaya leaf extract possesses significant antioxidant activity and can be effectively incorporated into a stable and cosmetically acceptable anti-aging cream, with potential application as a herbal cosmetic product.

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8. CONFLICTS OF INTEREST:

Nil

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