Published Online: 25 JUL 2023

Physical Stability of Facial Serum Product Containing *Carica papaya* L. Leaf Extract

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Abstract

The leaves of *Carica papaya* L., which are generally considered as agricultural waste in Thailand, are reported as source of natural antioxidants. This study aimed to study the physical stability of a serum containing *Carica papaya* L. leaf extract which involves extracting and quantifying the phenolic and flavonoid contents in *C. papaya* leaves as well as their bioactivities. The ultrasonic-assisted extraction technique was used to prepare the extract in liquid form. Total phenolic and flavonoid contents were discovered at 5.94 ± 0.06 mg GAE/mL and 4.36 ± 0.10 mg QE/mL, respectively. Accordingly, the IC_{50} value of DPPH radical scavenging of the extract was 2.88 mg/mL and FRAP reducing power amount was 2.67 ± 0.06 mg AAE/mL. Antiaging facial serum containing 0.3% of *C. papaya* leaf extract was prepared and obtained as a homogeneous, transparent greenish-brown color solution. The product was stored at 4 °C, 25 °C and 45 °C for one month to investigate its stability. Color, pH value and viscosity of the formulation were determined. The color slightly changed during the investigation period, with total color difference (ΔE*) values ranging from 0.44 to 2.38. During the same period, the pH values increased slightly, from 5.21 (week 0) to an average of 5.35 (week 4) while the viscosity slightly decreased from 264.9 cP (week 0) to an average of 262.2 cP (week 4). The DPPH inhibition was initially obtained at 43.11 ± 0.95% and decreased during a one-month stability study in which the changes were about 3% - 9% compared with that of the beginning. The obtained results in this study suggested that the physical and bioactive stability properties of the product were

cosmetically acceptable and *C. papaya* leaves can serve as a valuable natural antioxidant source for use in cosmetic formulation.

Keywords: *Carica Papaya* L. Leaf, Antioxidant, Cosmetic, Stability, Serum

Introduction

Skin aging is one of the major skin concerns which can be caused by both intrinsic and extrinsic factors. Oxidative stress, induced by reactive oxygen species, plays an important role in the process of human skin aging (Naidoo & Birch-Machin, 2017). Therefore, incorporating antioxidants in cosmetic formulations presents an alternative approach to protecting the skin from oxidative damage and mitigating skin aging. Many natural sources, including plants, fruits, and vegetables, are abundant in well-known antioxidant compounds such as polyphenols and flavonoids (Hoang et al., 2021).

Carica papaya L. (*C. papaya*) is a widely cultivated plant in tropical and subtropical countries, including Thailand, where only its fruits are generally consumed. However, the leaves of *C. papaya* are used as traditional medicines in many Asian Pacific countries, such as India, Malaysia, Indonesia, Vietnam, Sri Lanka, and Pakistan (Nugroho et al., 2017). These leaves are rich in flavonoids, phenolic compounds, alkaloids and other phytochemicals (Ayoola & Adeyeye, 2010; Shubham et al., 2019). These compounds influence their bioactivities including antioxidant, peroxynitrite-scavenging, cytoprotective, anti-inflammatory, antibacterial and skin photodamage protective as well as therapeutic effects (Abdel-Halim et al., 2021; Khor et al., 2021; Nugroho et al., 2017; Owoyele et al., 2008; Seo et al., 2020; Singh et al., 2020).

To add value to the unutilized *C. papaya* leaves by using in anti-aging cosmetics, the aim of this study is to extract and quantify the phenolic and flavonoid contents in *C. papaya* leaves for using in anti-aging cosmetics. The evaluation of its biological activities and the cosmetic formulation was conducted along with a stability test.

Research Objectives

1. To extract bioactive compounds from *C. papaya* leaves.
2. To determine bioactive compounds and antioxidant activities of *C. papaya* leaf extract.
3. To study the application of *C. papaya* leaf extract in cosmetic formulation.

Scope of The Study

1. Extraction of *C. papaya* leaves using aqueous solvent.
2. Determination of total phenolic and total flavonoid contents of *C. papaya* leaf extract.
3. Quantification of DPPH radical scavenging and ferric reducing antioxidant power activities of the extract.
4. Formulation of facial serum containing the extract of *C. papaya* leaf and conducting stability test.

Literature Review

Carica papaya L. belonging to family Caricaceae is generally known as papaya or pawpaw. It is originated from Mexico and northern South America and now has been widely cultivated in tropical and subtropical countries for its edible fruit for hundreds of years. Apart from its edible fruits, *C. papaya* leaves have traditionally been used for treatment of many diseases such as dengue, malaria, jaundice, hypoglycemia and immunomodulatory. It is also well known for its therapeutic properties including antiviral, reducing fever, antidiabetic, antidiarrhea, anticancer, anti-inflammatory, antioxidant, antimicrobial and wound healing (Sharma et al., 2020; Singh et al., 2020).

The total phenolic content (43-44 mg GAE/g) was identified in *C. papaya* leaf extracts obtained through ultrasonication-assisted and microwave-assisted extractions (Abdel-Halim et al., 2021), which are higher than those obtained through maceration, supercritical fluid extraction and freeze-dried method (2-29 mg GAE/g) (Khor et al., 2021). Although *C. papaya* leaves have been used as consumable medicines and nutritional herbs, no study has been found on their application in cosmetics.

Research Methodology

1. Plant material preparation

Fresh mature *C. papaya* leaves were collected from a private pesticide-free garden in Chiangrai, Thailand, in October 2022. The collected leaves were washed to remove dust particles and cut into small pieces. Then, they were dried in a convection oven at 50 °C for 20 hours. The dried *C. papaya* leaves were ground, using an electric grinder. The sample was stored in tightly closed containers.

2. Extraction of *C. papaya* leaves

The sample was soaked in deionized water (plant-to-solvent ratio; 1:5 w/v) in a sealed flask and subjected to sonication using a preheated ultrasonic bath. The extraction was conducted at 50 °C for 60 minutes. The extraction was repeated once using the same conditions, including any resulting sediment (dregs). The results of both extractions were then combined and the mixture was then allowed to cool down to room temperature before being filtered (Saifullah et al., 2020; Tsai & Lin, 2019). The filtrates were concentrated using a rotary evaporator to remove solvent under reduced pressure at 60 °C (Abdel-Halim et al., 2021; Vuong et al., 2013). The aqueous solvent was partially reduced to about 25% of the initial volume after 20 minutes, leaving an extract in a solution form. The extract solution was stored in a freezer for further tests and a preservative was added after the tests.

3. Determination of total phenolic content

Total phenolic content was measured using Folin-Ciocalteu method adapted from Obeng et al. (2020). The sample was mixed well with DI water (total 6 mL) and 0.5 mL of the Folin-Ciocalteu reagent and stood for 5 minutes. Sodium carbonate solution (20% w/v) at an amount of 1.5 mL was added and distilled water was then added to reach a volume of 10 mL. The solution was well mixed and then rested for 10 minutes at ambient temperature. After which, the absorbance was measured at 750 nm using UV-Vis Spectrophotometer (Thermo Fisher Scientific, USA). Gallic acid was applied as a standard for a calibration curve. The results were expressed as gallic acid equivalents mg GAE/mL extract.

4. Determination of total flavonoid content

Total flavonoid content was measured by aluminum chloride colorimetry following Obeng et al. (2020). The sample was mixed with DI water (total of 3.7 mL) and 150 μL of 5% (w/v) sodium nitrite solution. After incubation at room temperature for 5 minutes, 150 mL of 10% w/v aluminum chloride solution was added to the mixture and was left for 6 minutes at room temperature. Then, 1 mL of 1.0 M sodium hydroxide solution was added. The total mixture volume was made to 5 mL and vortexed. The absorbance was then measured at 510 nm (Thermo Fisher Scientific, USA), using quercetin as a standard for a calibration curve. The results were expressed as quercetin equivalents mg QE/mL extract.

5. DPPH radical scavenging assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined according to Kumar and Chaiyasut (2017). The diluted extract in 95% ethanol (total of 1 mL) was mixed with 1 mL of 0.2 mM DPPH (in 95% ethanol). The mixture was left in a dark place at room temperature for 30 minutes. The absorbance of the solutions was then measured at 517 nm (Thermo Fisher Scientific, USA). Ascorbic acid was used as a standard. The concentration giving 50% inhibition (IC_{50}, mg/mL) was reported.

6. Ferric reducing antioxidant power assay (FRAP)

The ferric reducing antioxidant power (FRAP) of *C. papaya* leaf extract was determined using the method adapted from Obeng et al. (2020). FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), a solution of 10 mM TPTZ solution in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ using the ratio of 10:1:1 (v/v/v). The amount of 3 mL of the FRAP reagent was then mixed with the diluted extract to reach a total volume of 3.1 mL. After the reaction mixture was incubated at 37 °C for 30 minutes, the absorbance of the solution at 593 nm was measured (Thermo Fisher Scientific, USA). The results were expressed as ascorbic acid equivalents mg AAE/mL extract.

7. Preparation of facial serum

The facial serum was formulated by adding 0.3% of *C. papaya* leaf extract based on its antioxidant activity, and the stability of the product was studied. The formulation followed the components listed in Table 1.

Table 1 Ingredient and ratio list of the facial serum with *C. papaya* leaf extract.

Ingredient	Amount (%w/w)
Part A	
1. Disodium EDTA	0.05
2. Hydroxyethyl cellulose	0.2
3. Xanthan gum	0.1
4. Glycerin	2.0
5. Butylene glycol	5.0
6. Honey	0.5
7. Panthenol	0.5
8. Sodium hyaluronate	0.3
9. DI water	89.45
Part B	
1. *C. papaya* leaf extract	0.3
2. Phenoxyethanol	0.5
3. Diazolidinyl Urea (and) Iodopropynyl Butylcarbamate (and) Propylene Glycol (Liquid Germall™ Plus)	0.3
4. Polysorbate 20	0.8

Preparation procedure: In Part A, disodium EDTA was dissolved in DI water and heated to 70 ± 10 °C, then hydroxyethyl cellulose was correctly dispersed in the mixture until it transformed to be clear and thick. Meanwhile, xanthan gum was dispersed in a separate container containing glycerin, butylene glycol, honey, and panthenol while stirring continuously. At the same time, sodium hyaluronate was allowed to swell in hot DI water. After cooling to room temperature, all mixtures in

Part A were combined. Then, the remaining ingredients in Part B were added to the mixture with continuous stirring.

8. Physical stability study of serum

The formulation underwent an accelerated stability test at different storage conditions for 1 month, including 4 °C ± 2 °C in a refrigerator, 45 °C ± 2 °C in a hot oven, and 25 °C ± 2 °C (room temperature). The following parameters were recorded weekly: physical appearances, pH value (measured by pH Meter - QIS, Netherlands), color (evaluated by Spectrophotometer CM-700d - Konica Minolta, Japan, identifying L*, a*, and b* values; where L* is a lightness value determining white or black, a* is a value determining the color of green (-) or red (+), and b* is a value determining the color of blue (-) or yellow (+)), and viscosity (measured by Viscometer - Brookfield, USA).

9. Antioxidant activity study of serum

Antioxidant extraction from a serum containing *C. papaya* leaf extract was performed using DI water as a solvent at a ratio of 1:1 (w/w). The extraction was carried out under ultrasonic conditions at a temperature of 40°C for 10 minutes. Further examination was then conducted by determining DPPH radical scavenging activity during a stability experiment. The results were recorded weekly and reported as a percentage of inhibition.

Results and Discussion

1. *C. papaya* leaf extract

The extract in a liquid or solution form is convenient in developing cosmetic formulations as it is easy to incorporate with other ingredients. The obtained extract has a dark brown color. The final weight was reduced to 24 ± 1% compared to the initial weight at 77.34 ± 1.35 g.

The prepared solution extract of *C. papaya* leaves was measured for its total phenolic and flavonoid contents, DPPH radical scavenging activity (IC_{50}) and FRAP reducing power before being applied to the cosmetic formulations. The results were summarized in Table 2 which indicated the potential antioxidant capacity of *C. papaya* leaf extract in solution form. The DPPH radical scavenging activity of the solution extract was weaker than the methanolic crude extract of *C. papaya* leaves using ultrasonic-assisted extraction (IC_{50}, 0.377 ± 0.014 mg/mL), reported by Soib et al. (2020). On the other hand, *C. papaya* leaf extract in this study had higher antioxidant activity compared with *Brassica oleracea* var. *botrytis* (cauliflower) leaves and *Murraya koenigii* (curry tree) leaves crude extracts, which reported IC_{50} values of 27.26 ± 0.07 - 472.5 ± 0.01 mg/mL (Bhatt et al., 2020). The amount of solution extract was then applied in facial serum formulation relative to the IC_{50} value which was considered as a minimum amount of extract required in the formulation to obtain 50% DPPH inhibition.

Table 2 Physical properties, biological compounds and activities of *C. papaya* leaf extract in solution form

Properties	**Results**
pH (100% Concentration)	6.52 ± 0.02
Appearance	Dark brown liquid
Odor	Weak burning leaf smell
Total phenolic content (mg GAE/mL extract)	5.94 ± 0.06
Total flavonoid content (mg QE/mL extract)	4.36 ± 0.10
DPPH (IC_{50}, mg/mL)	2.88 ± 0.00
FRAP antioxidant (mg AAE/mL extract)	2.67 ± 0.06

2. Formulation of facial serum

The appearance of the serum with 0.3% *C. papaya* leaf extract was shown in Figure 1, and the formulation was found to be homogeneous, transparent greenish-brown color, and had a light texture with a weak natural scent influenced by the odor of *C. papaya* leaf extract. The pH and viscosity values are 5.21 and 264.9 cP, respectively.

Figure 1 Serum containing 0.3% of *C. papaya* leaf extract

3. Physical stability study of the serum

The physical appearance of the serum is illustrated in Figure 2. The appearances of the serum under all storage conditions were unable to be noticed by visual observation for any physical change. The pH values increased slightly after one month of study, from 5.21 (week 0) to an average of 5.35 (week 4) under all conditions. However, the pH values remained cosmetically acceptable, ranging from 5.15 to 5.38, as the human skin's pH typically ranges from 4.5 to 6.0 (Jagtap et al., 2022; Smaoui et al., 2017). During the same period, the viscosity of the formulations slightly decreased from 264.9 cP (week 0) to an average of 262.2 cP (week 4). The final values remained close to the initial value indicating only a small alteration. The pH and viscosity values suggested that the formulations were stable throughout the study period.

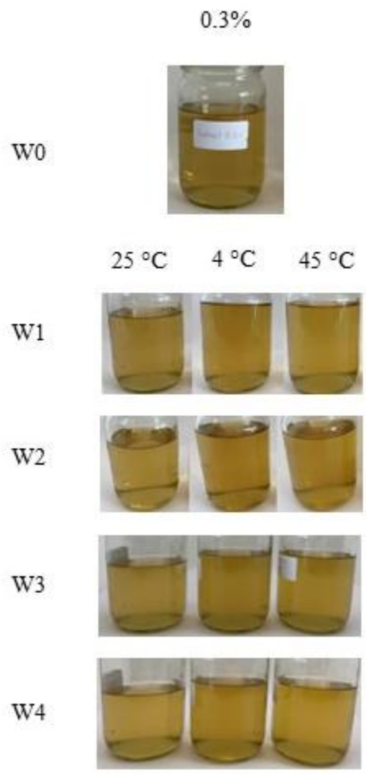

Figure 2 Serums containing 0.3% *C. papaya* leaf extract during the stability study

Furthermore, color measurements of the serum were summarized in Table 3. During the study period, there were slight changes in color measurements of the serum. The L* values insignificantly decreased under all conditions. The a* values slightly increased under 25 °C and 4 °C, indicating more redness of the products, while the value slightly decreased under 45 °C. There was an increase in the b* values, which was more significant under 45 °C condition, followed by 4 °C and less under 25 °C. The b* values indicated a more yellow tone of the formulation. Finally, the total color difference or ΔE* was calculated, and the overall values were less than 3.5 (ranging from 0.44 - 2.38), implying that no significant difference in color was noticed (Mokrzycki and Tatol 2011).

Table 3 Color measurement of the serum containing 0.3% of *C. papaya* leaf extract under different conditions

Condition	Week	Color Value			
		L*	a*	b*	ΔE*
25 °C	0	59.62 ± 0.16	0.48 ± 0.01	3.84 ± 0.62	
	1	59.85 ± 0.14	0.42 ± 0.01	4.44 ± 0.3	0.65
	2	59.52 ± 0.01	0.56 ± 0.01	4.93 ± 0.01	1.10
	3	59.45 ± 0.15	0.48 ± 0.01	4.25 ± 0.13	0.44
	4	59.57 ± 0.04	0.53 ± 0.01	5.05 ± 0.02	1.21
4 °C	0	59.62 ± 0.16	0.48 ± 0.01	3.84 ± 0.62	
	1	59.30 ± 0.08	0.49 ± 0.01	5.8 ± 0.01	1.99
	2	59.13 ± 0.12	0.59 ± 0.01	5.71 ± 0.02	1.94
	3	59.11 ± 0.01	0.56 ± 0.02	5.78 ± 0.01	2.01
	4	59.26 ± 0.05	0.57 ± 0.01	5.65 ± 0.01	1.85
45 °C	0	59.62 ± 0.16	0.48 ± 0.01	3.84 ± 0.62	
	1	59.12 ± 0.14	0.31 ± 0.01	4.97 ± 0.54	1.25
	2	59.45 ± 0.07	0.41 ± 0.00	5.86 ± 0.04	2.03
	3	59.24 ± 0.13	0.41 ± 0.01	6.05 ± 0.02	2.24
	4	58.87 ± 0.13	0.45 ± 0.02	6.1 ± 0.04	2.38

4. Antioxidant activity study of the serum

Table 4 shows the percentages of inhibition of DPPH radical scavenging activity of the serum. The base formula without *C. papaya* leaf extract was also tested to identify the antioxidant activity that might influence the activity of the serum. The base formula showed no antioxidant capacity (less than 0% inhibition), indicating that only *C. papaya* leaf extract influenced the serum's antioxidant activity. The formulation showed a slight decrease in antioxidant activity during the investigation, with final results differing by about 2.8% - 8.6% from the initial results. The extraction method may have contributed to the variation in obtaining antioxidants from the formulation, thereby affecting the difference in antioxidant capacity. The result indicates that the serum containing 0.3% C. *papaya* leaf extract was stable and cosmetically acceptable.

Table 4 DPPH radical scavenging activity of serum containing 0.3% *C. papaya* leaf extract under different conditions

Week	DPPH Radical Scavenging Activity (% inhibition)		
	25 °C	4 °C	45 °C
0	43.11 ± 0.95	43.11 ± 0.95	43.11 ± 0.95
1	41.51 ± 0.16	40.27 ± 0.90	41.79 ± 1.00
2	40.14 ± 1.76	40.51 ± 1.25	41.95 ± 1.68
3	39.85 ± 2.93	42.71 ± 2.99	37.08 ± 3.25
4	39.39 ± 0.68	40.20 ± 1.63	41.92 ± 2.59

Conclusion

In this study, *C. papaya* leaves were extracted using ultrasonication-assisted technique. The solution extract was deliberately concentrated to about 24-25% of its initial weight. The aqueous solvent effectively extracted polyphenols and flavonoids from *C. papaya* leaves. Accordingly, antioxidant potency was detected from *C. papaya* leaf extracts through DPPH radical scavenging activity and FRAP reducing power. The resulting extract showed potential antioxidant capacity, and anti-aging facial serums were formulated with 0.3% of *C. papaya* leaf extract. The serum with *C. papaya* leaf extract was further tested for its stability, and although there were slight changes in appearance and antioxidant activity during the one-month stability study, it was considered cosmetically acceptable.

Overall, the results suggest that *C. papaya* leaves can be a valuable natural antioxidant source for use in cosmetic formulations. However, further studies on phytochemical constituents, quantitative analysis, standardization and long-term stability tests are encouraged to be conducted. Further efficacy tests and clinical trials of the serum containing *C. papaya* leaf extract are also suggested to be carried out to ensure its effective application in cosmetic uses.

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