

Effect of Boiling on Total Phenolics and Antioxidant Activity (DPPH And ABTS) of *Carica Papaya* Flower Ethanolic Extracts

Khadijah^{*1}, Merlin¹, Ahmad Muchsin Jayali¹, Artati², Wa Reti La Maca¹, Wa Andini Ariyani¹, Sarni³, Ikram Hamid⁴

¹Department of Chemistry Education, Universitas Khairun, Ternate, Indonesia

²Department of Medical Laboratory Technology, Poltekkes Kemenkes Makassar, Makassar, Indonesia

³Department of Pharmacy, Politeknik BauBau, Kendari, Indonesia

⁴Department of Mathematic Education, Universitas Khairun, Ternate, Indonesia

*Corresponding : khadijah@unkhair.ac.id

Article Information: Received December 2025; Accepted June 2026; Published June 2026

ABSTRACT

Carica papaya flowers are widely found in North Maluku, Indonesia, and have potential as a source of phenolic compounds with antioxidant activity. However, information on the effect of boiling on their phytochemical profile and bioactive properties is still limited. This study aimed to compare the phytochemical profile, total phenolic content, and antioxidant activity of ethanol extracts from raw and boiled *C. papaya* flowers. Fresh flowers were prepared as raw and boiled samples at 90°C for 10 min, then extracted with ethanol. Qualitative phytochemical screening was carried out, total phenolic content was determined using the Folin-Ciocalteu method, and antioxidant activity was evaluated using DPPH and ABTS assays in triplicate. The qualitative phytochemical profile of both extracts showed the presence of alkaloids, flavonoids, phenolics, steroids, terpenoids, and saponins. Boiled *C. papaya* flower extract had higher total phenolic content than raw extract, with mean values of 14.42 mg TAE/g and 8.05 mg TAE/g, respectively. Boiled extract also showed stronger antioxidant activity, with lower IC₅₀ values in the DPPH assay (197.99 mg/L vs. 289.31 mg/L) and ABTS assay (94.37 mg/L vs. 134.48 mg/L). These results suggest that boiling may enhance the release of phenolic compounds and improve the antioxidant potential of *C. papaya* flowers. Further studies using compound identification, statistical validation, and in vivo testing are needed to confirm these findings and support their application in functional food development.

Keywords: *Carica Papaya* flowers, boiling, total phenolics, DPPH, ABTS, IC₅₀, functional foods, Antioxidant

INTRODUCTION

The rising prevalence of degenerative diseases—cancer, diabetes mellitus, and coronary heart disease has intensified the search for safe, effective natural antioxidants. These compounds neutralize excess free radicals, preventing oxidative stress that damages cells, tissues, and organs. While synthetic antioxidants (BHT, BHA, TBHQ) have been widely used, their long-term high-dose administration is increasingly restricted due to hepatotoxic, nephrotoxic, and neurotoxic effects¹. Research has thus shifted toward sustainable alternatives from medicinal plants and local agricultural resources.

The rising prevalence of degenerative diseases—cancer, diabetes mellitus, and coronary heart disease has intensified the search for safe, effective natural antioxidants. These compounds neutralize excess free radicals, preventing oxidative stress that damages cells, tissues, and organs. While synthetic antioxidants (BHT, BHA, TBHQ) have been widely used, their long-term high-dose administration is increasingly restricted due to hepatotoxic, nephrotoxic, and neurotoxic

effects¹. Research has thus shifted toward sustainable alternatives from medicinal plants and local agricultural resources.

Papaya (*Carica papaya*), abundant in tropical North Maluku, Indonesia, emerges as a promising candidate. Nearly all parts fruit, seeds, leaves, and flowers offer health benefits. Traditionally consumed as stir-fries or local dishes, *C. papaya* flowers provide nutrition plus empirical benefits: blood glucose reduction², anti-inflammatory effects³, hair growth promotion⁴, and antibacterial/antioxidant activity⁵. These flowers contain alkaloids (causing bitterness), flavonoids, phenolics, saponins, and tannins⁵. Phenolics neutralize radicals via hydrogen atom transfer or single electron mechanisms, with total phenolic content strongly correlating to antioxidant capacity⁶. For comparison, *C. papaya* leaves show DPPH IC₅₀ values of 0.072-0.080 mg/mL, validating phenolics as key predictors.

However, processing affects bioactivity. Boiling the common preparation method induces chemical transformations varying by temperature, duration, and matrix⁷. Boiling serves as an effective pre-treatment for plant materials like *C. papaya* flowers by targeting multiple biochemical mechanisms that enhance phytochemical stability and bioavailability. Firstly, it inactivates oxidative enzymes such as polyphenol oxidase (PPO) and peroxidase (POD), which catalyze phenolic degradation and browning during storage or processing preserving total phenolic content (TPC) that might otherwise decline by 20-50% post-harvest. Secondly, thermal hydrolysis during boiling (typically 100°C for 5-15 min) disrupts cell wall matrices (pectins, hemicelluloses), releasing bound phenolics from insoluble complexes into free or esterified forms more amenable to solvent extraction. Matrix changes further facilitate this: heat-induced Maillard reactions or protein denaturation can improve solvent penetration, while mild boiling minimizes volatile loss compared to prolonged drying. These effects position boiling as superior to raw processing for antioxidant yield without introducing harsh chemicals.

Heat may degrade phenolics⁸ or enhance bioavailability by releasing bound forms⁹, creating contradictory outcomes. Given limited data on *C. papaya* flower phytochemicals, their antioxidant potential, and boiling effects, this study analyzes the phytochemical profile, total phenolic content, and antioxidant activity (DPPH and ABTS) of ethanol extracts from raw versus boiled flowers.

Considering the bioactive potential of *C. papaya* flowers, the scarcity of comprehensive data linking phytochemical profiles, total phenolic levels, and antioxidant capacity, coupled with no existing research on boiling's impact, this study employed tannic acid equivalents (TAE) rather than gallic acid equivalents (GAE) for total phenolic content (TPC) quantification via Folin-Ciocalteu method. Tannic acid (MW ~1701 Da), a reference hydrolyzable tannin, better matches the polyphenolic profile rich in tannins and condensed phenolics found in *C. papaya* flowers, yielding more accurate calibration curves ($R^2 > 0.99$) at 760 nm absorbance compared to gallic acid (MW 170 Da), which underestimates oligomeric/polymeric phenolics by 15-30%. While TAE enhances matrix-specific precision, it limits direct comparability to GAE-based literature (TAE values typically 1.2-2x higher), requiring conversion factors for cross-study benchmarking. This choice optimizes local relevance while acknowledging methodological trade-offs for future meta-analyses.

MATERIALS AND METHODS

Materials and Tool

Plant Materials

The *Carica papaya* L. (Caricaceae; voucher specimen: Khadijah 001, deposited at Biology Laboratorium, Universitas Khairun, Indonesia) flowers were collected from community plantations in West Obi District, South Halmahera Regency, North Maluku Province, during the dry season (March 2025, 8–10 AM harvest). Flowers were selected at the pre-anthesis bud stage (immature, unopened but showing color change; 1–2 days to bloom), fresh, undamaged, and light

green to yellowish (Figure 1). Samples comprised 50 buds from 10 trees across 3 garden plots (n=3 biological replicates, pooled prior to extraction) (Figure 1).



Figure 1. Plant of *C. papaya*

Chemical Materials

The materials used in this study were 70% ethanol, distilled water, phytochemical test reagents (FeCl₃, NaOH, HCl, Mg, Mayer's reagent, Dragendorff's reagent, Wagner's reagent), Folin-Ciocalteu reagent, Na₂CO₃, tannins, DPPH solution, and ABTS solution.

Tools

The tools used in this study are a set of glassware, an analytical balance, a rotary evaporator, a hotplate, and a Shimadzu UV-1800 UV-VIS spectrophotometer.

Method

Sample Preparation

Fresh *C. papaya* flowers (500 g fresh weight per biological replicate) were washed with tap water, surface-dried with tissue paper, and divided into two treatments (n=3 each):

Raw: Processed immediately without heating.

Boiled: 100 g flowers:1 L distilled water ratio, boiled in 2 L beaker with thermometer-controlled water bath maintaining 90-95°C for 10 min (internal flower temp ~85°C verified by probe).

Post-treatment, samples drained (cheesecloth), spread thinly (2 cm layer), and oven-dried at 45°C for 48 h until constant weight (final moisture <10%, verified by oven method AOAC 925.10).

Dried flowers ground (mesh 40-60, particle size 0.25-0.42 mm), sieved, and stored in airtight amber containers at 4°C until extraction (within 7 days)¹⁰.

Extraction (Maceration)

Simplicia powder (50 g, triplicate per treatment) macerated with 500 mL 70% ethanol (v/w = 10:1 mL/g, optimal for phenolic polarity) in Erlenmeyer flasks. Extraction repeated 3×24 h at 28±2°C (room temp Maluku), protected from light, with manual stirring (2× daily). Combined filtrates (Whatman No.1 filter paper) concentrated via rotary evaporator (Buchi R-100, 40°C, 150 mbar) to viscous extract (yield calculated). Extracts stored at -20°C; 70% ethanol selected for efficient extraction of polar phenolics/flavonoids (polarity index 4.4)¹¹.

Phytochemical Screening

Qualitative tests were performed on the ethanol extract of *C. papaya* flowers, including alkaloids, flavonoids, phenolics, terpenoids, steroids, and saponins. A positive result was indicated by a color change or the formation of a precipitate according to the characteristics of each reagent. The semi-quantitative grading in Table 1 was based on the observable intensity of the reaction, where (+) indicated a weak response, (++) a moderate response, and (+++) a strong response.

Alkaloid Test

The ethanol extract (1 mL) was placed in a test tube, followed by the separate addition of 2–3 drops each of Dragendorff's, Mayer's, and Wagner's reagents. Positive reactions were observed as follows: orange-red precipitate with Dragendorff's reagent, white precipitate with Mayer's reagent, and brown precipitate with Wagner's reagent, confirming the presence of alkaloids¹².

Flavonoid Test

A 0.5 mL sample extract was placed in a test tube, followed by the addition of 5 drops of 1% NaOH solution. The color that appeared was yellow and turned colorless after the addition of 5 drops of 1 M HCl solution, indicating the presence of flavonoid compounds¹⁰.

Phenolic Test

The sample extract (0.5 mL) was placed in a test tube, then 10 drops of 1% FeCl₃ were added. The extract contains phenol if it produces a green, red, purple, blue, or dark black color¹².

Steroid and Terpenoid Test

The sample extract (1 mL) was placed in a test tube, then 2 mL of chloroform, 10 drops of acetic anhydride, and 3 drops of concentrated sulfuric acid were added (Liebermann-Burchard reaction). A positive reaction for the presence of steroids is indicated by the formation of a greenish-blue solution, and the formation of an orange-red or purple color indicates the presence of terpenoids¹³.

Saponin Test

The sample extract was added to a 2 mL test tube, then 10 mL of hot distilled water was added, and the mixture was shaken vigorously for 30 seconds. The formation of a stable foam 1-10 cm high for 10 minutes that does not disappear with the addition of one drop of 2 N HCl indicates the presence of saponin compounds¹¹.

Determination of Total Phenolic Content

Total phenolic content was determined by the Folin-Ciocalteu method (Chanwitheesuk et al., 2005) with modifications. 5 mL of the extract (initial concentration: 1 mg/mL) was mixed with 0.25 mL of Folin-Ciocalteu reagent (50%, v/v) and 0.5 mL of saturated sodium carbonate (Na₂CO₃) solution in 10 mL test tubes. The final volume was made up to 10 mL with distilled water. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer after 30 min incubation at room temperature. Tannic acid (analytical grade, ≥98% purity, Sigma-Aldrich) was used as standard (concentration range: 0-100 µg/mL; R₂ = 0.9986). Results were expressed as mg tannic acid equivalents (TAE)/g dry extract (ppm = mg/L; % = g/100 g extract).

Antioxidant Activity Test

DPPH Method

Antioxidant activity was determined using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay. A DPPH solution was prepared in methanol at a concentration of 0.01 mM, and

the final volume was adjusted to 5 mL. The absorbance of the DPPH control was measured at 515 nm. For sample analysis, 200 mg of extract was dissolved in 5 mL of methanol and vortexed for 1 hour. Then, 1 mL of the sample solution was mixed with 1 mL of 0.01 mM DPPH solution, and the mixture was adjusted to a final volume of 5 mL with methanol before absorbance measurement at 515 nm. The inhibition concentration (IC₅₀) was calculated using the following equation.

$$\% \text{ Inhibition} = \left(\frac{\text{Abs control} - \text{Abs Sample}}{\text{Abs control}} \right) \times 100\% \dots\dots\dots 14$$

The IC₅₀ value is obtained from the intersection of the line between the inhibitory power and the concentration axis, then entered into the equation $y = a + bx$, where $y=50$ and the x value represents the IC₅₀^{11,12}.

ABTS Method

The ABTS radical cation was prepared by reacting a 7 mM ABTS solution with 2.45 mM potassium persulfate in ethanol, followed by incubation in the dark at room temperature for 12–16 h. The resulting stock solution was then diluted with ethanol until an absorbance of 750 nm was obtained. For the assay, 1 mL of ethanol extract from raw and boiled *C. papaya* flowers at concentrations of 10, 20, 40, 80, and 160 mg/L was mixed with 1 mL of ABTS solution, incubated in the dark for 6 min at room temperature, and the absorbance was measured at 750 nm using a UV-Vis spectrophotometer. Antioxidant activity was expressed as IC₅₀.

$$\% \text{ Inhibition} = \left(\frac{\text{Abs blank} - \text{Abs sample}}{\text{Abs Blank}} \right) \times 100\% \dots\dots\dots (\text{Andry et al., 2025})$$

RESULTS

Table 1. Extraction Yield of Raw and Boiled *C. papaya* Flower in Ethanol Extracts

Sample	Extraction method	Solvent	Sample weight	Extract weight	Extraction yield
Raw <i>C. papaya</i> flower	Maceration	Etanol 70%	115 g	14 g	12,17 %
Boiling <i>C. papaya</i> flower	Maceration	Etanol 70%	115 g	26,15 g	22,74 %

Table 2. Phytochemical Screening of Raw and Boiled *C. papaya* Flower Ethanol Extracts

Phytochemical Test	Raw <i>C.papaya</i> flower	Boiled <i>C. papaya</i> flower	Description
Alkaloid			
• Dragendorff	+	+++	Brick red sediment
• Mayer	+	+	White sediment
• Wagner	+	+++	- Brown sediment
Flavonoid	+	+++	The solution is yellow and becomes colorless after adding HCl.

Phytochemical Test	Raw <i>C.papaya</i> flower	Boiled <i>C. papaya</i> flower	Description
Phenolik	+	+	Brownish-green solution
Steroid	+	+	There is a bluish-green solution.
Terpenoid	+	++	There is a brick-red solution.
Saponin	+	+++	The steady foam persisted for 10 minutes following the addition of HCl.

Note: (-) : negative; (+) : positive but weak; (++) : Strong; (+++) : Positive and very strong

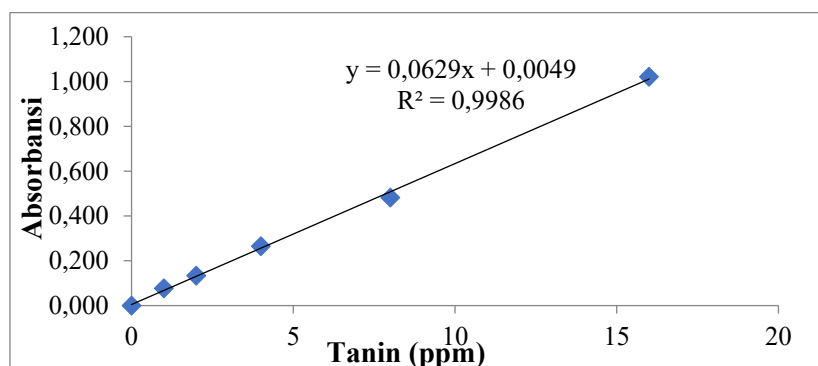


Figure 1. Tanin curve standard

Tabel 3. Total Phenolic Content of *C. papaya* Flower Ethanol Extract

Sample	A ($\lambda = 765$ nm)	FP	Total Phenolics (ppm)	Mass of sample (g)	mg Equivalen of tanin/g Sample	Total Phenolics (%)	Average of mg Equivalen of tanin/g Sample
Raw <i>C. papaya</i> flowers	0,404 0,405 0,405	50 50 50	317,25 318,04 318,04	0,3947 0,3945 0,3947	8,038 8,058 8,058	0,804 0,806 0,806	8,051 ± 0.012
Boiled <i>C. papaya</i> flowers	0,376 0,38 0,378	50 50 50	294,99 298,17 296,58	0,2057 0,2057 0,2057	14,341 14,495 14,418	1,434 1,450 1,442	14,418 ± 0.039

Notes : FP stands for the dilution factor (50×) used to prepare the extract solution prior to analysis. The total phenolic content was determined using the tannic acid calibration curve and represented as milligrams of tannic acid equivalent (TAE) per gram of dry weight (DW) of the sample. Percentage figures were calculated using the same methodology. Recurrent or substantially like values signify consistent repeat measurements and conversion processes, rather than useless duplication

Table 4. Results of Antioxidant Activity Measurement of Raw and Boiled *C. papaya* Flower Ethanol Extract Using the DPPH Method

Sampel	Concentration of extract (mg/L)	Absorbance ($\lambda=515$ nm)	Antioksidan activity (%)	IC ₅₀ (mg/L)
Raw <i>C.papaya</i> flower	25	0,962	11,25	289,31
		0,969	10,61	
		0,966	10,89	
	50	0,870	19,74	
		0,882	18,63	
		0,876	19,19	
	100	0,830	23,43	
		0,838	22,69	
		0,834	23,06	
	200	0,661	39,02	
		0,665	38,65	
		0,663	38,84	
	400	0,378	65,13	
0,386		64,39		
0,382		64,76		
Blanko	1,084			
Boiling <i>C. papaya</i> flower	25	0,903	16,70	197,99
		0,911	15,96	
		0,907	16,33	
	50	0,879	18,91	
		0,884	18,45	
		0,882	18,63	
	100	0,739	31,83	
		0,725	33,12	
		0,732	32,47	
	200	0,523	51,75	
		0,535	50,65	
		0,529	51,20	
	400	0,117	89,21	
0,119		89,02		
0,118		89,11		
Blanko	1,084			

Notes : A_c = absorbance of the DPPH control; A_s = absorbance of the sample. Percentage inhibition was calculated as $(A_c - A_s) / A_c \times 100$. Concentration levels were selected to provide a response range approaching 50% inhibition for descriptive support of IC₅₀ estimation.

Table 5. Results of Antioxidant Activity Measurement of Raw and Boiled *C. papaya* Flower Ethanol Extract Using the ABTS Method

Sample	Extract concentration (mg/L)	Absorbance ($\lambda=750$ nm)	Antioxidant activity (%)	Value of IC ₅₀ (mg/L)
Raw <i>C.papaya</i> flower	10	0,848	1,05	134,48
		0,851	0,70	
		0,849	0,93	
	20	0,805	6,07	
		0,811	5,37	
		0,808	5,72	
	40	0,716	16,45	
		0,720	15,99	
		0,718	16,22	
	80	0,580	32,32	
		0,584	31,86	
		0,582	32,09	
	160	0,358	58,23	
		0,361	57,88	
		0,359	58,11	
Blanko	0,857			
Boiled <i>C.papaya</i> flower	10	0,808	26,34	94,37
		0,806	26,53	
		0,807	26,44	
	20	0,744	32,18	
		0,738	32,73	
		0,741	32,45	
	40	0,704	35,82	
		0,699	36,28	
		0,702	36,01	
	80	0,606	44,76	
		0,600	45,31	
		0,603	45,03	
	160	0,354	67,73	
		0,357	67,46	
		0,356	67,55	
Blanko	0,857			

Table 6. Table of Ascorbic Acid Standard

No	Concentration (mg/L)	Absorbance (A) $\lambda = 750 \text{ nm}$	Antioxidant Activity (%)	IC-50 (mg/L)
1	0.2	0.778	16.43	
2	0.4	0.739	20.62	
3	0.8	0.694	25.46	2.24
4	1.6	0.552	40.71	
5	3.2	0.324	65.20	
6	Control	0.931		

DISCUSSION

Phytochemical screening of raw and boiled *Carica papaya* flower extracts showed the presence of the same key classes of secondary metabolites such as alkaloids, flavonoids, phenolics, steroids, terpenoids and saponins. These results suggest that the qualitative phytochemical profile of the extract was unaffected by boiling. In other words, they proved that the major bioactive groups were still present following heat treatment, but this screening only showed the presence or absence of bioactive groups, not the amount of each chemical left. Similar observations were made in other plant materials, where thermal treatment reduced the amount of several phytochemicals but not their qualitative presence¹⁵. Flavonoids and phenolics are chemicals of particular importance, due to their close relationship with antioxidant activity. These compounds chemically have the ability to donate electrons or hydrogen atoms to neutralize free radicals which can be a feasible explanation for the antioxidant activity demonstrated in this study⁵. In addition, steroids like β -sitosterol in *C. papaya* are known to aid in cholesterol regulation and anti-inflammatory effects, and terpenoids such as α -pinene and limonene are reported to have anti-inflammatory, antimicrobial and anticancer properties providing further evidence for the therapeutic potential of *C. papaya*¹⁶. Saponins were also identified and this chemical class has been connected with antibacterial and antidiabetic activities and interactions with microbial cell membranes¹⁵.

The total phenolic content of *C. papaya* flower extract was determined using the Folin-Ciocalteu method with a UV-Vis spectrophotometer at a maximum wavelength (λ_{max}) of 765 nm. Tannin was used as the standard because it is a polyphenolic compound containing multiple phenolic hydroxyl groups. These hydroxyl groups react with Folin-Ciocalteu reagent and, after the addition of sodium carbonate (Na_2CO_3), produce a blue color. The redox interaction between phenolic compounds and the Folin-Ciocalteu reagent in an alkaline medium forms a blue molybdenum-tungsten complex. The greater the amount of phenolic compounds, the more phenolate ions are converted into this complex, resulting in a deeper blue color (Lamuella-Raventós, 2017). Total phenolic content was expressed as mg tannic acid equivalent (TAE) per g of sample using a tannin calibration curve.

The boiled extract contained a higher total phenolic content than the raw extract. The boiled *C. papaya* flower extract showed 14.418 mg TAE/g sample, whereas the raw extract showed 8.051 mg TAE/g sample. This suggests that boiling may have facilitated the release of phenolic compounds from the plant matrix. One possible explanation is that heating softened the plant tissue and improved the extraction of phenolic compounds into the solvent¹⁷. In addition, boiling has previously been reported to enhance the extractability of bioactive compounds by weakening the plant tissue matrix and promoting the diffusion of secondary metabolites from cell vacuoles into the solvent, thereby increasing the availability of active compounds, although some

thermal degradation may also occur¹⁸. However, these explanations remain speculative in the present study because no direct enzyme assays or phenolic profiling were performed.

The results showed that boiling increased total phenolic content and reduced IC₅₀ values in both DPPH and ABTS assays. Because only two treatment groups were compared, this pattern should be interpreted descriptively rather than as a formal correlation. The TPC of the raw extract was 8.051 ± 0.012 mg tannin equivalents/g sample, whereas the boiled extract showed 14.418 ± 0.039 mg tannin equivalents/g sample. The boiled extract showed a significantly higher TPC than the raw extract ($t = 257.2$, $p < 0.001$). This trend is consistent with previous reports showing that phenolic-rich extracts often exhibit stronger radical-scavenging activity.

An increase in total phenolic content was accompanied by a decrease in IC₅₀ values in both DPPH and ABTS assays, indicating that the boiled extract had stronger antioxidant activity. This trend suggests that higher phenolic content is associated with greater free radical scavenging capacity. The antioxidant activity of ethanol extracts of raw and boiled *C. papaya* flowers was evaluated using DPPH and ABTS assays to assess their ability to neutralize free radicals. These assays were selected because they complement the TPC results and provide a broader view of the relationship between phenolic content and antioxidant potential. Antioxidant activity was expressed as IC₅₀, defined as the concentration required to inhibit 50% of radical activity; thus, lower IC₅₀ values indicate stronger antioxidant activity. Ascorbic acid was used as the reference compound, and the absorbance measurements at 515 nm for DPPH and 750 nm for ABTS are presented in Tables 3 and 4.

The DPPH assay showed that the IC₅₀ values of the raw and boiled *C. papaya* flower ethanol extracts were 289.31 mg/L and 197.99 mg/L, respectively. The ABTS assay showed a similar trend, with IC₅₀ values of 134.48 mg/L for the raw extract and 94.37 mg/L for the boiled extract. These results indicate that the boiled extract had stronger antioxidant activity than the raw extract in both assays. However, the IC₅₀ values differed between the two methods, which is expected because DPPH and ABTS are based on different radical systems and reaction mechanisms. Despite these differences, both assays consistently showed an increase in antioxidant capacity after boiling.

The increased antioxidant activity was consistent with the higher total phenolic content of the boiled extract. The results showed a positive correlation between total phenols and antioxidant ability, with higher phenolic levels corresponding to lower IC₅₀ values. Similar findings were reported in previous studies including a study by Khadijah et al. (2021) which revealed that antioxidant activity increased with increased total phenolic content¹¹. Phenolic and flavonoid molecules are known to be electron or hydrogen atom donors and can neutralize free radicals and minimize oxidative processes. They are able to bind transition metal ions such as Fe²⁺ and Cu²⁺ involved in oxidation processes⁵. Moreover, certain chemicals such as kaempferol have been suggested to be responsible for the antioxidant action of papaya flowers⁴.

Antioxidant activity of boiling extract may possibly be due to heat effects during processing. Heating may help to break down the cell wall structures and liberate the phenolic chemicals previously bound in the plant matrix and therefore make them more available for reaction with free radicals. This is in line with the notion of thermal enhancement of phenolic release where heat treatment improves the release of bioactive compounds by decreasing the non-covalent connections with cellular macromolecules¹⁹. Therefore, boiling not only maintains but also increases the antioxidant capacity of *C. papaya* flower extract.

These findings are relevant to the development of functional foods based on indigenous plant resources, particularly *Carica papaya* flowers from North Maluku. The higher total phenolic content observed in the boiled extract (14.418 mg TAE/g) compared with the raw extract (8.051 mg TAE/g), together with its stronger antioxidant activity, suggests that boiling may enhance the functional potential of this locally available material. This is particularly important in the context of Indonesia's increasing burden of non-communicable diseases, which account for a large

proportion of mortality and highlight the need for preventive nutrition strategies. At the same time, growing interest in natural antioxidants reflects concern over the improper use of synthetic antioxidants, encouraging the search for safer plant-based alternatives. Because papaya is widely cultivated in Indonesia, including in local agricultural systems, its flowers may represent an accessible and sustainable raw material for the development of health-oriented products.

This indicates that boiling could be a valuable pre-extraction procedure to improve the recovery of bioactive chemicals. Hence, boiled *C. papaya* flowers have good potential to be used as a natural source of antioxidants for herbal preparations and functional health products.

However, there are certain limitation in this study. The phytochemical screening was primarily qualitative, so the exact chemicals responsible for the observed action were not identified. No extensive phenolic profiling or chemical identification was done and extract yield was not determined. Also the positive control was not fully displayed in the findings section which limits comparison. Finally, the antioxidant tests were performed solely in vitro, thus future investigations are needed to determine if the same effects occur in biological systems.

CONCLUSION

The average values obtained from the phytochemical screening, total phenolic content analysis and antioxidant assays revealed that the ethanol extracts of raw and boiled *Carica papaya* flowers had the same major classes of secondary metabolites which include alkaloids, flavonoids, phenolics, steroids, terpenoids and saponins. Boiling did not remove the primary bioactive components of *C. papaya* flowers. Moreover, in both DPPH and ABTS assays, the mean total phenolic content and mean IC₅₀ values of the boiling floral extract were greater and lower, respectively, than the raw extract indicating higher antioxidant potential under the studied conditions. The results imply that boiling could be a suitable pre-treatment for enhancing the extraction of phenolic chemicals from *C. papaya* flowers. However, the results should be considered preliminary because the present investigation was performed on the basis of a limited comparison and did not involve compound identification or standardized boiling optimization. In the future, effort should be done to analyze statistically significant differences, the most appropriate boiling conditions and the standardization of processing parameters for better development of *C. papaya* flowers as a natural antioxidant source.

ACKNOWLEDGMENT

Thank you to Universitas Khairun for funding this research under Grant Agreement No 021/PEN-PKUPT/PG.12/2025

CONFLICT OF INTEREST

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

Khadijah : Conceptualization, Methodology, Investigation, Formal Analysis, Data Curation, Writing – Original Draft.

Merlin : Investigation, Data Curation, Formal Analysis, Writing – Review & Editing.

Ahmad M Jayali : Validation, Methodology, Visualization, Writing – Review & Editing.

Artati : Resources, Data Curation, Visualization, Writing – Review & Editing.

Wa Reti La Maca : Supervision, Validation, Writing – Review & Editing.

Wa Andini Ariyani : Supervision, Project Administration, Writing – Review & Editing, Final Approval.

Sarni : Resources, Supervision, Validation, Writing – Review & Editing.

Ikram Hamid: Supervision, Project Administration, Writing – Review & Editing, Final Approval.

COLLECTIVE STATEMENT

All authors made significant contributions to the research and preparation of the manuscript entitled “Effect of Boiling on Total Phenolics and Antioxidant Activity (DPPH and ABTS) of *Carica papaya* Flower Ethanolic Extracts” All authors have read and approved the final version of the manuscript and agree to be accountable for the integrity, accuracy, and validity of the data and findings presented in this study.

REFERENCE

1. Ren J, Li Z, Li X, Yang L, Bu Z, Wu Y, et al. Exploring the Mechanisms of the Antioxidants BHA, BHT, and TBHQ in Hepatotoxicity, Nephrotoxicity, and Neurotoxicity from the Perspective of Network Toxicology. 2025. doi:10.3390/foods14071095
2. Pongoh AF, Queljoe E De, Rotinsulu H. Uji Antidiabetik Ekstrak Etanol Bunga Pepaya (*Carica papaya* L.) terhadap Tikus Putih Jantan (*Rattus norvegicus*) yang Diinduksi Aloksan. *Pharmacol.* 2020;9(1):160–9. doi:10.35799/pha.9.2020.27423
3. Veersain, Kumar A, Kumar M, Thilagam P, Yadav R, Rajpoot S, et al. A Comprehensive Review of Papaya’s Multidimensional Impact on Health and Wellness. *International Journal of Statistics and Applied Mathematics.* 2023;8(5):1065–71. doi:10.22271/math.2023.v8.i5So.1327
4. Manalo-cabalinan RAM, Louis G, Torre T Dela, Atienza AA, Arollado EC. *Carica papaya* Flower Extracts Possess Antioxidant and 5 α -Reductase Inhibitory Activities. *Acta Med Philipp.* 2024;58(19). doi:10.47895/amp.vi0.8002
5. Dwivedi MK, Sonter S, Mishra S, Patel DK, Singh PK. Antioxidant, Antibacterial Activity, and Phytochemical Characterization of *Carica papaya* Flowers. *Journal of Basic and Applied Sciences.* 2020;9(23):1–11. doi:10.1186/s43088-020-00048-w
6. Gaye AA, Ibn O, Cisse K, Ndiaye B, Ayessou NC, Cisse M, et al. Evaluation of Phenolic Content and Antioxidant Activity of Aqueous Extracts of Three *Carica papaya* Varieties Cultivated in Senegal. *Food Nutr Sci.* 2019;10:276–89. doi:10.4236/fns.2019.103021
7. Sławińska N, Olas B. The Current State of Knowledge About Thermal Processing of Edible Seeds; A Special Emphasis on Their Bioactive Constituents and Antioxidant Activity. *Food Chem.* 2024;458:1–12. doi:10.1016/j.foodchem.2024.140526
8. Silva MDO, Janser R, Castro S De. First-Order Degradation Kinetics of Phenolic Compounds and Antioxidant Properties of Fresh and Enzymatically Hydrolyzed Seriguela Pulp (*Spondias purpurea* L.). *ACS Food Science & Technology.* 2025;5:3520–9. doi:10.1021/acsfoodscitech.5c00554
9. Kim MY, Yoon N, Lee YJ, Woo KS, Kim HY, Lee J, et al. Influence of Thermal Processing on Free and Bound Forms of Phenolics and Antioxidant Capacity of Rice Hull (*Oryza sativa* L.). *Prev Nutr Food Sci.* 2020;25(3):310–8. doi:10.3746/pnf.2020.25.3.310

10. Baturante N, Khadijah K, Tahar M. Penentuan Total Flavonoid dan Total Fenolik Ekstrak Metanol Daun Gofasa (*Vitex cofassus*) dengan Metode Spektrofotometer UV-Vis. *SAINTIFIK@: Jurnal Pendidikan MIPA*. 2024;8(2):21–6. doi:10.33387/saintifik.v8i2.7333
11. Khadijah K, Soekamto NH, Firdaus F, Syah YM. Total Content of Phenol and Antioxidant Activity from Crude Extract Methanol of Brown Algae (*Padina* sp.) Collected from Kayoa Island, North Maluku. *J Phys Conf Ser*. 2021;1–9. doi:10.1088/1742-6596/1899/1/012034
12. Khadijah, Soekamto NH, Maisuri S, Chalid T, Rafidah NF. Total Phenol Content and Activities of Antioxidant Extracts Methanol Limes (*Citrus aurentifolia*) by UV-Vis Spectrophotometry. In: *E3S Web of Conferences*. 2021. p. 1008. doi:10.1051/e3sconf/202132801008
13. Zulfikar T, Sutriana A, Rozaliyana A. Phytochemical Screening of Three Extraction Process of *Calotropis gigantea*. In: *IOP Conference Series: Earth and Environmental Science*. 2024. p. 1–6. doi:10.1088/1755-1315/1356/1/012082
14. Andry M, Karo-karo SU, Pertiwi I, Syahfitri E. Antioxidant Activity Test of Avocado Leaf Kombucha (*Persea americana* Mill.) Based on Fermentation Duration Using the ABTS Method. *JPS*. 2025;8(4):2609–22. doi:10.36490/journal-jps.com
15. Ranjan S, Himani D, Mukopadayay S. A Review Article on Phytochemical and Pharmacological Activities of *Carica papaya*. *Int J Health Sci (Qassim)*. 2022;6(S3):11077–88. doi:10.53730/ijhs.v6nS3.8557
16. Kumarasinghe HS, Kim JH, Kim SL, Kim KC, Perera RMTD, Kim SC, et al. Bioactive Constituents from *Carica papaya* Fruit: Implications for Drug Discovery and Pharmacological Applications. *Appl Biol Chem*. 2024;67(103):1–23. doi:10.1186/s13765-024-00962-y
17. Başar V, Şimşek A, Turan E. The Thermal Stability of Phytochemicals and Physicochemical Properties of Kiraz and Findik Cherry Laurel Fruits (*Laurocerasus officinalis* L.) and Their Molasses. *Gida*. 2024;49(3):421–38. doi:10.15237/gida.GD23141
18. Palermo M, Pellegrini N, Fogliano V. The Effect of Cooking on the Phytochemical Content of Vegetables. *J Sci Food Agric*. 2014;94:1057–70. doi:10.1002/jsfa.6478
19. Călinoiu LF, Vodnar C. Thermal Processing for the Release of Phenolic Compounds from Wheat and Oat Bran. *Biomolecules*. 2019;10(21):1–14. doi:10.3390/biom10010021