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Original article

A pilot study on the antioxidant activities of fresh and processed papaya leaves

Nalinrat Puangnil[1,2], Piyawadi Khaoiam[1,2], Arashaporn Uthairangsi[1,2], Praween Thosa[1,2], Rossarin Tansawat[1,2,*]

[1]Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceutical Science, Chulalongkorn University, Bangkok, Thailand
[2]Center of Excellence in Metabolomics for Life Sciences, Chulalongkorn University, Bangkok, Thailand

Abstract

***Background**:* Herbal plants and natural products have long been recognized for their antioxidant activity. Papaya is a popular plant in Thailand. Papaya leaf products are manufactured and sold in various forms. However, comparative studies on the antioxidant activity of papaya leaf products in different forms, such as fresh versus processed, remain limited.

***Objective**:* To investigate the total phenolic content (TPC) and antioxidant activity, and to compare the antioxidant capabilities of papaya leaves in different forms.

***Methods**:* Papaya leaves (n = 10) were collected from a papaya tree located in Thailand and prepared as freeze-dried leaf juice. Papaya leaf capsules and tea are supplements that manufactured and sold in Thailand. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity, ferric reducing antioxidant power (FRAP), and TPC were determined for the methanolic extract of papaya leaves and their products.

***Results**:* Freeze-dried papaya leaf juice exhibited the highest FRAP and TPC values, which were significantly higher than those of the other samples ($P < 0.05$). Meanwhile, papaya leaf capsules showed the highest antioxidant activity in the DPPH assay, but the difference was not statistically significant compared to freeze-dried papaya leaf juice ($P < 0.05$).

***Conclusion**:* The results indicate that the combination of compressed juice followed by freeze-drying is the most effective method for preserving the antioxidant activity of papaya leaves.

***Keywords**:* Antioxidant, antioxidant activity, *Carica papaya,* papaya leaf.

***Correspondence to: Rossarin Tansawat,** Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceutical Science, Chulalongkorn University, Bangkok 10300, Thailand.
E-mail: rossarin.t@pharm.chula.ac.th

Herbal plants and natural products have long been recognized for their therapeutic potential, particularly for their antioxidant properties.[1] Oxidative stress, caused by an imbalance between reactive oxygen species (ROS) and the body's antioxidant defenses, plays a significant role in aging and various diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions.[2,3] To evaluate the antioxidant capacity of herbal extracts and natural compounds, *in vitro* assays serve as essential tools in screening their effectiveness. These assays provide rapid, reproducible, and cost-effective methods to assess the ability of natural products to scavenge free radicals, reduce oxidative damage, and enhance cellular defense mechanisms.[1,4] *In vitro* antioxidant assays have been used to test various plants that are produced and widely marketed as herbal medicines or food supplements, such as *Andrographis paniculata*[5], *Tinospora cordifolia*[6], *and Curcuma longa*.[7]

Carica papaya Linn., commonly known as papaya, is cultivated in tropical and subtropical regions.[8] Various parts of the papaya plant possess therapeutic properties. For example, the enzyme papain aids in gastrointestinal health, its latex is used to treat dermatitis and psoriasis, and several studies suggest its potential benefits in managing dengue fever and malaria.[9] Nowadays, papaya leaves are processed and sold as food supplements in various forms, including capsules, tea, and traditional preparations such as extracted leaf juice. Consuming papaya leaf supplements is claimed to help maintain platelet levels, improve and protect against diabetes and cardiovascular diseases, provide strong antioxidant benefits, and aid digestion.[8,10] However, comparative studies on the antioxidant activity of papaya leaf products in different forms, such as fresh versus processed, remain limited. Further research is needed to understand how processing methods influence their antioxidant potential.

This study aimed to evaluate, the antioxidant activity of papaya leaves in various forms, including fresh leaves, juice, capsules, and tea, was evaluate using the ditphenlpicryhydrazy (DPPH) assay, ferric reducing ability power (FRAP) assay, and total phenolic content (TPC) analysis. Antioxidant analysis serves as a basic method for comparing the effects of different processing techniques. This study provides valuable information on the antioxidant potential of fresh and processed papaya leaves. Since papaya is widely cultivated in Thailand, the findings from this study can be applied to further research and development of papaya leaf products. This knowledge may also support proper product registration, enhance the economic value of papaya leaves, and facilitate the development of high-quality herbal products, thereby contributing to the improvement of Thailand's agricultural sector.

Materials and methods

Chemical reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH), iron (III) chloride hexahydrate, iron (II) sulfate heptahydrate, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), and gallic acid were purchased from Sigma Aldrich (St. Louis, MO, USA). Hydrochloric acid, acetic acid, methanol and Folin–Ciocalteu's reagent were obtained from Merck KGaA (Darmstadt, Germany). Sodium acetate and sodium carbonate were purchased from Qrec chemical (Auckland, New Zealand). All chemicals used in the experiment are analytical grade.

Sample preparation

Fresh papaya leaf

Leaves of *Carica papaya* Linn. were collected from 10 trees, washed 3–5 times with tap water, and air-dried. They were then ground using liquid nitrogen for further extraction in the next step.

Papaya leaf juice

The collected fresh papaya leaves (n = 10) were chopped into small pieces. A total of 10 g of the chopped leaves was weighed and placed into a juicer (Frutelia+, TEFAL®, China), with 40 mL of water added. The extracted papaya leaf juice was freeze-dried (Lyovapor™ L-200, BUCHI, Switzerland) before being used for extraction in the next step.

Papaya leaf capsule

Papaya leaf capsule supplements were obtained from 10 bottles. The powdered papaya leaf contents inside the capsules were used for analysis.

Papaya leaf tea

Dried papaya tea leaves were obtained from 10 tea bags (from 10 different boxes). The dried leaves inside the bag were used for the assays.

Sample extraction

All types of papaya leaf samples were extracted using a method slightly modified from Veeramohan R, et al.[11] For the analysis, the methanol extract of papaya leaves and their products was prepared by weighing 100 mg of the sample and adding 5 mL of

100% methanol. The mixture was continuously shaken (Rotamax 120, Heidolph, Germany) at 250 rpm for 17 h at room temperature. Afterward, the supernatant was collected and stored at -80°C (TSX series ultra-low freezer, Thermo Fisher Scientific, USA).

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay

The DPPH radical-scavenging assay was performed following the method of Jayasekera et al.[12] A 25 µL aliquot of the methanol extract of papaya leaves and its products was pipetted into a 96-well plate, followed by the addition of 250 µL of 0.2 mM DPPH in 95% ethanol. The mixture was incubated for 30 minutes, and absorbance was measured at 550 nm using a microplate reader (CLARIOstar®, advanced LVF Monochromators™, BMG LABTECH, Germany). All samples were analyzed in 3 replicates. Ascorbic acid was used as a positive control. A 0.1 mM ascorbic acid solution was prepared using water as the solvent.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed following the method of Jayasekera S, et al.[12] The FRAP working reagent was prepared by dissolving 10 mmol/L TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mmol/L HCl. A mixture of 10 mmol/L TPTZ, 20 mmol/L $FeCl_3$, and 300 mmol/L acetate buffer (pH 3.6) in a 1:1:10 ratio was prepared. The FRAP working reagent was stored at 37°C for 10 min. Then, 250 µL of the reagent was pipetted into a 96-well plate, followed by the addition of 8.5 µL of papaya leaf extracts and 25 µL of water. The mixture was left to stand for 30 min before analysis. Absorbance was measured at 595 nm using a microplate reader (CLARIOstar®, advanced LVF Monochromators™, BMG LABTECH, Germany). $FeSO_4 \cdot 7H_2O$ (1–12 mmol/L) was used to create the standard curve, with the results expressed as mmol $FeSO_4$/L. All samples were analyzed in 3 replicates.

Total phenolic content (TPC)

The TPC analysis was performed following the method of Jayasekera S, et al.[12] A 12.5 µL aliquot of the methanol extract of papaya leaves and their products was pipetted into a 96-well plate. Then, 250 µL of a 2% sodium carbonate solution was added, and the mixture was left to stand for 5 minutes at room temperature. Next, 12.5 µL of 50% Folin-Ciocalteu reagent was added, and the mixture was allowed to react for 30 minutes. Absorbance was measured using a microplate reader (CLARIOstar®, advanced LVF Monochromators™, BMG LABTECH, Germany) at 760 nm. A standard curve was created using a gallic acid solution (100-1000 µg/mL). The results were presented as milligrams of gallic acid equivalent (GAE) per unit of dry weight. All samples were analyzed in 3 replicates.

Statistical analysis

One-way analysis of variance (ANOVA) with post-hoc Tukey's test used to evaluate the significant difference between mean values at the confidence level of 95% ($P < 0.05$). Descriptive analysis, one-way ANOVA and data visualization were performed by JASP 0.18.3.0.

Results

DPPH radical-scavenging assay

The DPPH radical-scavenging activity was tested in the methanolic extract of fresh and processed papaya leaf products. The box plot presenting the experimental results is shown in **Figure 1**. Fresh and processed papaya leaves exhibited a statistically significant difference ($P < 0.05$) in antioxidant capacity compared to 0.1 mM ascorbic acid (positive control). Fresh papaya leaves, juice, and capsules demonstrated higher antioxidant capacity than positive control, while papaya leaf tea had a lower DPPH scavenging activity. The highest DPPH scavenging activity was observed in papaya leaf capsules (94.4 ± 1.0%), followed by freeze-dried papaya leaf juice (90.966 ± 1.949%), fresh papaya leaves (90.0 ± 1.9%), and papaya leaf tea (58.1 ± 4.9%) (**Figure 1**). According to the post-hoc analysis, papaya leaf tea exhibited the lowest DPPH scavenging activity, with a significant difference from the other samples. On the other hand, the papaya leaf capsule demonstrated the highest scavenging activity, though the difference was not significant when compared to freeze-dried leaf juice ($P < 0.05$).

Ferric reducing antioxidant power (FRAP) assay

The antioxidant activity was evaluated based on the capacity to reduce ferric (III) iron to ferrous (II). The FRAP results are calculated from the standard curve of $FeSO_4 \cdot 7H_2O$, as shown in **Figure 2A**. In **Figure 2B**, the results show the highest antioxidant capacity was observed in freeze-dried papaya leaf juice (1,284.9 ± 74.4 µM Fe (II)/g extract), followed by papaya leaf capsules (531.4 ± 48.7 µM Fe (II)/g extract), papaya leaf tea (379.6 ± 46.9 µM Fe (II)/g extract), and fresh papaya leaves (323.8 ± 41.0 µM Fe (II)/g extract), respectively. The antioxidant capacity of papaya leaves, and their products significantly differed ($P < 0.05$), except for fresh leaves compared to dry tea leaf.

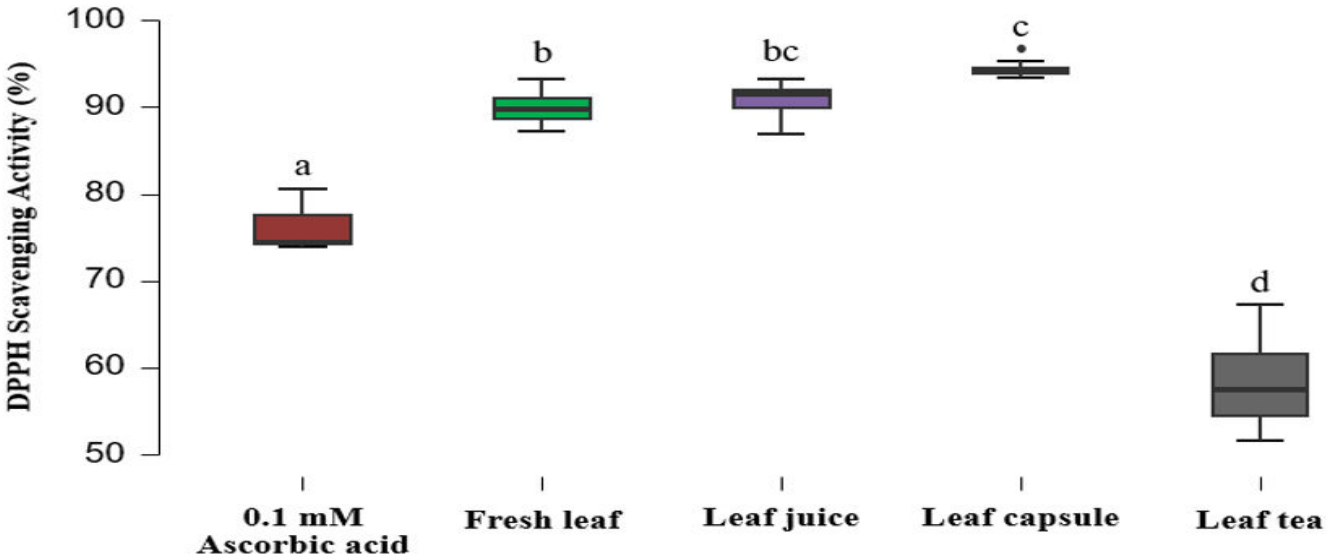

Figure 1. Post-hoc analysis compares DPPH scavenging activity (%) across 0.1 mM ascorbic acid (positive control) and four groups of papaya leaves. Groups with the same letter are considered not significantly different ($P < 0.05$).

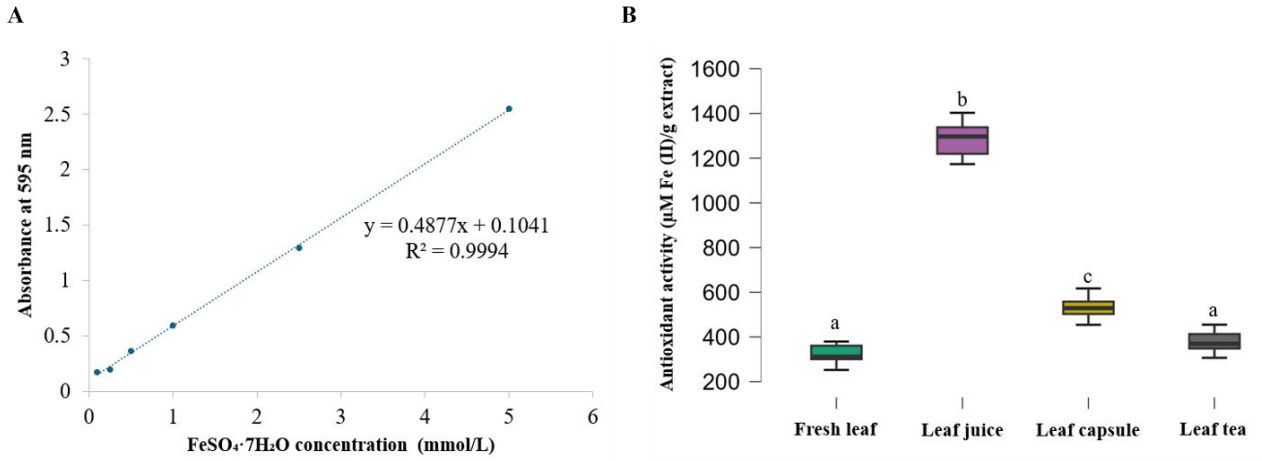

Figure 2. (A) $FeSO_4 \cdot 7H_2O$ standard curve. **(B)** Post-hoc analysis compares antioxidant activity (µM Fe (II)/g extract) across four groups of papaya leaves. Groups with the same letter are considered not significantly different ($P < 0.05$).

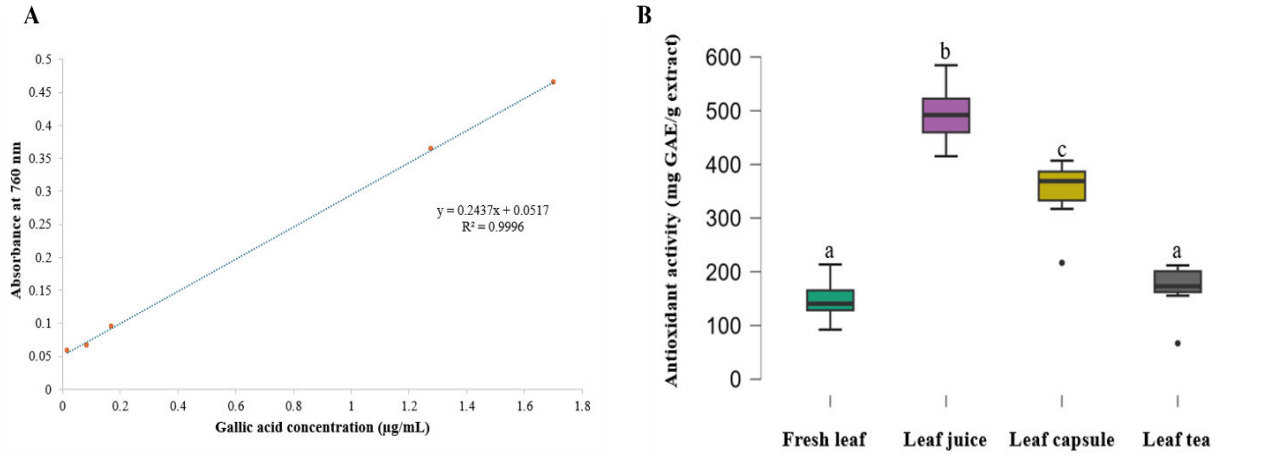

Figure 3. (A) Gallic acid standard curve. **(B)** Post-hoc analysis compares antioxidant activity (mg GAE/g extract) across four groups of papaya leaves. Groups with the same letter are considered not significantly different ($P < 0.05$).

Total phenolic content (TPC)

Phenolic compounds are important components in plants with redox properties that play a key role in antioxidant activity. The TPC results are presented as gallic acid equivalent (GAE) per unit of dry weight, which must be calculated from the standard curve of gallic acid, as shown in **Figure 3A**. The results show that the highest total phenolic content was observed in freeze-dried papaya leaf juice (405.8 ± 41.4 mg GAE/g extract), followed by papaya leaf capsules (244.5 ± 27.9 mg GAE/g extract), papaya leaf tea (84.8 ± 25.4 mg GAE/g extract), and fresh papaya leaves (66.116 ± 27.5 mg GAE/g extract) (**Figure 3B**). According to the post-hoc analysis, the total phenolic content of papaya leaves, and their products shows a significant statistical difference ($P < 0.05$), with the exception of fresh papaya leaves and papaya leaf tea, where no significant variation was observed.

Discussion

This study found that papaya leaves show potential as antioxidants, and the processing of papaya leaves enhances their antioxidant activity. Based on the DPPH-radical scavenging activity, fresh leaves, leaf juice, and capsule supplements exhibited a statistically significant higher in antioxidant capacity compared to 0.1 mM ascorbic acid (positive control) ($P < 0.05$). Overall, papaya leaves in juice form demonstrated the highest antioxidant activity and TPC values, significantly surpassing fresh leaves and other processed forms (capsule supplement powder and dried leaf).

Antioxidant activity testing is categorized into two main mechanisms: hydrogen atom transfer (HAT) and single electron transfer (SET). The Mechanism of action of HAT involves antioxidants transferring a hydrogen atom to free radicals, while SET is based on redox reactions, where antioxidants donate an electron to stabilize radicals.[13,14] The DPPH assay uses 1,1-diphenyl-2-picrylhydrazyl as an organic radical to evaluate the antioxidant capacity of a sample by using colorimetry. The mechanism of the DPPH assay involves both SET and HAT. [4] The DPPH exhibits a purple color, and when it receives a hydrogen atom from an antioxidant, it is reduced to DPPH-H, which is colorless. This color change in the solution serves as an indicator for assessing antioxidant activity.[4,14] The methanol extract of papaya leaf capsules showed the highest average DPPH scavenging activity, followed by freeze-dried papaya leaf juice, with no significant difference between them. Several studies reported the effect of particle size on antioxidant activity. Zhao X, et al. reported the effect of grinding on antioxidant activity. The study found that when the particle size of grape pomace powder decreases, its DPPH radical-scavenging capacity increases. The highest scavenging activity was observed with particles smaller than 18.83 ìm, while an increase in particle size led to a reduction in scavenging ability.[15] Similarly, Wu Z, et al. reported that the smallest sieve size (M400) of Sanchi flower powder exhibited the highest DPPH radical-scavenging activity, while the lowest scavenging activity was found in the largest sieve size (M60).[16] A study on the effect of particle size on the antioxidant activity of *Sargassum cristaefolium* ethanol extracts reported that smaller particle sizes exhibited higher antioxidant activity, as demonstrated by the results of DPPH, ABTS, hydroxyl radical scavenging assays, and FRAP analysis.[17] In addition, freeze-drying has also been investigated for its potential effects on antioxidant activity, particularly as measured by the DPPH assay. According to Gomes et al., the results from DPPH and ABTS assays indicated that freeze-dried papaya pulp exhibited higher antioxidant activity compared to both fresh samples and those processed by spray drying.[18] Kittibunchakul S, et al. compared the effects of hot-air drying and freeze-drying on the phytochemical content, antioxidant activity, and safety of maoberry fruits. Their findings indicated that freeze-dried samples showed higher DPPH scavenging activity compared to those subjected to high-temperature hot-air drying.[19] In this study, capsule supplements and papaya leaf juice exhibited the highest DPPH scavenging activity. The capsule contains papaya leaves in the form of fine powder, while the juice was made from comminuted fresh leaves that were freeze-dried before analysis. Both forms were produced by reducing the particle size of the papaya leaves, which aligns with previous studies suggesting that grinding significantly impacts the breakdown and disruption of cell wall components. This process leads to an increased release of phenols and flavonoids bound in the cell wall, which are plant metabolites with antioxidant properties.[16] As reported by Kittibunchakul S, et al., higher temperatures during hot-air drying resulted in decreased DPPH scavenging activity. Consistent with this finding, the dried papaya leaf tea in this study exhibited the lowest antioxidant activity as measured by the DPPH assay. Therefore, temperature appears to be a factor influencing antioxidant activity.[19]

The FRAP is the SET test along with the use of colorimetry. This assay is used to determine the reduction ability from Fe^{3+} to Fe^{2+} of antioxidants. This colorimetric technique measures the capacity of antioxidants to reduce the [Fe^{3+}-(2,4,6-Tris(2-pyridyl)-s-triazine),]$^{3+}$ complex (colorless) to the [Fe^{2+}-(TPTZ),]$^{2+}$ complex (blue) in an acidic condition.[20,21] The highest reducing ability was observed in freeze-dried papaya leaf juice. The results show that freeze-dried papaya leaf juice exhibited significantly higher values than the other forms. Similar to the findings of Kittibunchakul et al., antioxidant activity measured by the FRAP assay indicated that freeze-dried maoberry fruits exhibited higher radical scavenging activity compared to fruits dried at high temperatures.[19] Chan EWC, et al. reported that the freeze-dried leaves of *Alpinia zerumbet* and *Etlingera elatior* showed significantly higher FRAP values compared to those of fresh leaves ($P < 0.05$).[22] Kumar D, et al. investigated the effect of drying methods on the antioxidant activities of dropped *Citrus sinensis* L. Osbeck fruits. The results showed that the dried fruit using the hot air oven method demonstrated lower radical scavenging capacity compared to the freeze-dried fruits.[23] These findings suggest that freeze-drying may contribute to the preservation of bioactive compounds, such as phenolics, while retaining a high level of radical scavenging activity.[19,23]

The antioxidant capacity is attributed to the chemicals in plants, and the processing methods have an impact on the alterations of these chemical levels. Consequently, the TPC was assessed in the papaya leaf samples to evaluate whether the concentrations of phenolic compounds in the samples are consistent with their antioxidant capacities. Phenolic compounds, as the secondary metabolites in plants, have redox properties that play a role in antioxidant activity.[24,25] The TPC is a common test used for plants and food products to assess their phenolic content through antioxidant activity. In the present study, papaya leaf juice showed the highest total phenolic content, which significantly higher than the other forms. Khor BK, et al. reported that the phenolic content of freeze-dried papaya leaves and ethanolic extracts was 10 to 16 times higher than that of hexane extracts and papaya leaves extracted using supercritical carbon dioxide, both with and without 5% ethanol.[26] Freeze-drying is a method that uses low temperatures, which helps stabilize phenolic compounds that degrade with heat. Another possible mechanism is that freezing can accelerate the activity of polyphenol reductase, leading to an increase in H^+ levels, which affects pH levels and enzyme activity in the environment. All of these factors can influence the levels of phenolic compounds in the sample.[27]

Limitations of this study include the relatively small sample size due to its pilot-scale nature and the inability to fully identify additives in the papaya leaf's commercial products. These unknown components may influence the results of antioxidant activity. Future studies should focus on a detailed investigation of the chemical profile of papaya leaves, including the quantification of active compounds. Such information is essential for research and development of high-quality papaya leaf products in the future.

Conclusion

Our results indicate that the combination of compressed juice followed by freeze-drying is the most effective method for preserving the antioxidant activity of papaya leaves. Since papaya trees can easily grow in Thailand's climate, this study highlights the potential for utilizing papaya leaves as a valuable agricultural resource. The findings can contribute to the development of papaya leaf-based products, creating economic opportunities in Thailand's agricultural and pharmaceutical sectors.

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Conflicts of interest statement

The authors declare no conflicts of interest related to this work.

Data sharing statement

The datasets generated or analyzed during the current study are included in this published article. Additional supporting data are available from the corresponding author upon reasonable request.

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