

Impact of Leaf Age and Blanching on Phytochemical Composition and Antioxidant Activity of *Artocarpus lakoocha* Leaves

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Abstract

This study examined the effects of leaf age and blanching treatment on the phytochemical composition and antioxidant activity of *Artocarpus lakoocha* leaves (“Ma Had” in Thai), a plant recognized for its medicinal properties but with limited research on its foliage. The research methodology covered three main analytical areas: (1) qualitative phytochemical screening (2) quantification of total phenolic content and total flavonoid content and (3) a thorough assessment of antioxidant capacity. The antioxidant assessment employed 3 complementary in vitro assays: DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), and FRAP (ferric ion reducing antioxidant potential). Results indicated that fresh mature leaves contained lower levels of phytochemicals and exhibited reduced antioxidant activity compared to young leaves. Notably, blanched mature leaves demonstrated significantly higher total phenolic content (40.50 mg GAE/g DW) and total flavonoid content (73.90 mg QE/g DW) than blanched young leaves (27.52 mg GAE/g DW and 60.46 mg QE/g DW, respectively). This elevation in bioactive compounds corresponded with markedly enhanced antioxidant capacity, particularly in the ABTS assay, where blanched mature leaves (234.25 mg TE/g DW) substantially outperformed blanched young leaves (89.27 mg TE/g DW). These findings underscore the superior bioactive potential of mature leaves following blanching, highlighting their promising use in nutraceutical development due to enhanced antioxidant activity and retention of bioactive compounds.

Keywords: Medicinal plant, Thermal processing, Natural antioxidants, Functional ingredient

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Introduction

Medicinal plants are an important source of bioactive phytochemicals, such as phenolic compounds and flavonoids, which are valued for their antioxidant properties (Kumar & Pandey, 2013; Pandey & Rizvi, 2009). The primary function of these natural antioxidant compounds involves the neutralization of detrimental free radicals, which confers a protective effect against the onset of oxidative stress-induced diseases (Valko et al., 2007). Consequently, there is increasing scientific and commercial interest in identifying novel plant-based sources of these compounds for application in functional foods and pharmaceuticals.

Artocarpus lakoocha, a species indigenous to South and Southeast Asia, is historically acknowledged in traditional medicine for its wide range of therapeutic effects, which encompass antibacterial, antiviral, and antioxidant activities (Ganguly & Kumar, 2020). While research has confirmed the presence of valuable phenolics and flavonoids in its bark, fruit, and roots (Hossain et al., 2016; Pandey & Bhatnagar, 2009; Singhatong et al., 2010), the leaves have been largely overlooked, representing a significant gap in knowledge and an underutilized potential resource.

The concentration of phytochemicals in plants is dynamic and can be strongly influenced by the developmental stage of plant tissue (Isah, 2019). Leaf maturity, in particular, alters the profile and quantity of bioactive compounds as the plant's metabolic priorities shift from growth to defense (Nobossé et al., 2018). Understanding how phytochemical content differs between young and mature leaves is therefore essential for optimizing harvest time and potential utilization.

Post-harvest processing also plays a critical role in preserving the quality of plant materials (Tanko et al., 2005). Blanching, a brief thermal treatment in hot water, is a key pre-treatment for herbs and leafy vegetables. Its primary purpose is to inactivate degradative enzymes such as polyphenol oxidase and

peroxidase that cause the loss of color, flavor, and bioactive compounds during drying and storage. However, this thermal process can also modify the phytochemical profile of the final product, making it necessary to evaluate its overall impact (Gbadamosi et al., 2018; Nobosse et al., 2017; Xiao et al., 2017).

This study addresses these gaps by investigating the combined effects of leaf maturity (young vs. mature) and blanching on the phytochemical content and antioxidant activity of *A. lakoocha* leaves. The aim is to provide scientific evidence for optimizing the processing conditions. The findings are expected to establish the ideal leaf stage and pre-treatment approach to maximize bioactive compound retention, thereby promoting the valorization of these underutilized leaves as a functional ingredient for the nutraceutical and food industries.

Materials and methods

1. Plant sample collection and identification

Leaves of *A. lakoocha* were collected in Kalasin Province, located in the Northeastern Thailand, between October and December 2020. Leaf maturity was categorized based on developmental stage, visual characteristics, and branch position. Young leaves (Fig. 1A) were defined as newly expanded leaves (first to third node from the apical tip), light green in color, with a pliable texture, and located near the apical tip of the branches. Mature leaves (Fig. 1B) were defined as fully expanded leaves (seventh to tenth node), dark green, and more sclerophyllous (leathery), obtained from the lower, more established portions of the same branches.

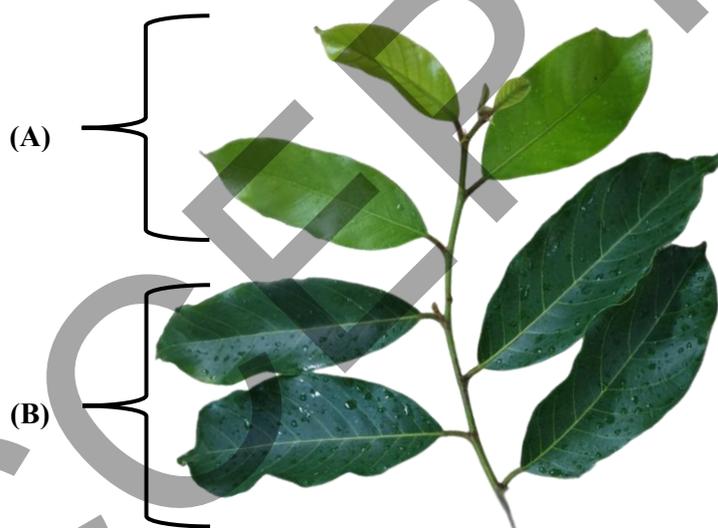


Fig. 1 Young leaf (A) and mature leaf (B) of *Artocarpus lakoocha*

2. Sample preparation

A. lakoocha leaves were washed and cut into pieces measuring 3 × 3 cm. A portion of the leaves was blanched in water at 100 °C for 30 sec and immediately cooled in cold water (Tadesse et al., 2023). All samples were dehydrated using a tray dryer (Model TD-10A, Owner Food Machinery Co., Ltd., Thailand) at 50 °C until the moisture content was reduced to below 10% and the water activity (a_w) was less than 0.6. The dried leaves were then ground into powder and stored at -20 °C for subsequent analysis.

3. Solvent extraction

Dried *A. lakoocha* leaf powder (1 g) was mixed with 9 mL of 70% ethanol. For fresh leaves, 2 g of finely chopped leaves were combined with 8 mL of 70% ethanol. Both samples were sonicated using an ultrasonic bath (Model LUC-405, Daihan Labtech Co., Ltd., South Korea) at a frequency of 40 kHz and a temperature of 5 °C for 30 min. The mixtures were subsequently refrigerated and centrifuged (Avanti J-E, Beckman Coulter, USA) at 5,000 rpm at 4 °C for 10 min. Low temperatures (4–5 °C) were maintained throughout extraction to minimize the thermal degradation of heat-sensitive phytochemicals. The resulting supernatant was collected, transferred to light-protected vials, and stored at -20 °C until further analysis.

4. Determination of total phenolic content (TPC)

The total phenolic content was determined using a modified Folin-Ciocalteu method, following Salih et al. (2021). For the assay, 5 μL of extract was mixed with 50 μL of Folin-Ciocalteu reagent and 1.5 mL of deionized water. After standing for 8 min, 50 μL of 20% sodium carbonate solution was added. The reaction was incubated for 30 min at room temperature, followed by the measurement of absorbance at 765 nm utilizing a UV-Vis spectrophotometer (Shimadzu UV-1800, Shimadzu, Japan). Quantification was achieved using a gallic acid standard curve, which was constructed over a concentration range of 50 to 175 $\mu\text{g}/\text{mL}$. The resulting data are reported as mg of gallic acid equivalent (GAE)/g of both fresh weight (FW) and dry weight (DW).

5. Determination of total flavonoid content (TFC)

Determination of the total flavonoid content was accomplished through the application of the colorimetric method originally described by Nabi and Shrivastava (2016). For the reaction, equal volumes (1.5 mL) of the extract and the 10% aluminum chloride reagent were thoroughly mixed. This reaction solution was then subjected to a 30-min incubation period at room temperature, under conditions ensuring darkness. Absorbance was measured at 425 nm using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan). A calibration curve was prepared using quercetin as the reference standard, with concentrations ranging from 100 to 800 $\mu\text{g}/\text{mL}$. Results were expressed as mg of quercetin equivalent (QE)/g of fresh weight (FW) and dry weight (DW).

6. Determination of antioxidant activity

6.1 DPPH radical scavenging activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was assessed using a modified version of the methodology originally established by Brand-Williams et al. (1995) with slight modifications. For the assay, 100 μL of extract was combined with 2.0 mL of a 0.1 mM DPPH solution, which was freshly prepared in 95% ethanol. The mixture was incubated in darkness at room temperature for 30 min, and absorbance was measured at 517 nm against an ethanol blank.

A standard curve was generated using Trolox at concentrations ranging from 150 to 900 $\mu\text{g}/\text{mL}$. Antioxidant capacity was expressed as mg of Trolox equivalent (TE)/g of fresh weight (FW) and dry weight (DW).

6.2 ABTS radical cation decolorization assay

The ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) assay was executed following the methodology established by Re et al., (1999), with minor procedural adaptations. The highly colored ABTS^{•+} radical cation was prepared by chemically initiating the reaction between an ABTS stock solution and potassium persulfate. This mixture was allowed to stand in the dark for 12–16 h to ensure radical formation before experimental use. Subsequently, the ABTS^{•+} solution was diluted with ethanol, and a measured aliquot of the leaf extract was introduced. The resulting absorbance was recorded at 734 nm exactly 6 min after the addition of the extract. The quantification standard used was Trolox, with a calibration curve prepared across the concentration range of 150 to 900 $\mu\text{g}/\text{mL}$. The final results are presented as mg of Trolox equivalent (TE)/g of both fresh weight (FW) and dry weight (DW).

6.3 Ferric reducing antioxidant power (FRAP) assay

This method was conducted to evaluate the ability of antioxidants to reduce ferric ions following the method of Benzie and Strain (1999). An aliquot of leaf extract was reacted with FRAP reagent, producing a blue-colored complex. Absorbance was measured at 593 nm.

A standard calibration curve was prepared using ferrous sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) at concentrations ranging from 100 to 500 $\mu\text{g}/\text{mL}$. Antioxidant capacity was expressed as mg of ferrous sulfate equivalent (FE)/g of fresh weight (FW) and dry weight (DW).

7. Qualitative phytochemical screening

Leaf extracts were qualitatively screened for secondary metabolites, including phenolics, flavonoids, tannins, saponins, anthraquinones, steroids, and cardiac glycosides, using established methods.

7.1 Phenolics and tannins

The presence of phenolics and tannins was assessed using ferric chloride tests adapted from Sutoyo et al. (2021). An aliquot of extract (200 μL) was diluted with distilled water (1.0 mL), heated for 5 min, and filtered. The addition of 5% ferric chloride (FeCl_3) to the filtrate, producing a bluish-black color, indicated phenolics. A similar test with 1% FeCl_3 confirmed tannins if a blue, green, or brownish-green color appeared.

7.2 Flavonoids

Screening for flavonoids followed the method of Sutoyo et al. (2021). Extract (200 μL) was mixed with 50% ethanol (1.0 mL) and filtered. A magnesium ribbon and five drops of concentrated hydrochloric acid (36% HCl) were added to the filtrate, which was then heated for 5 min. Development of a deep yellow

color indicated a positive result.

7.3 Saponins and anthraquinones

Methods described by Ayoola et al. (2008) were used to test for saponins and anthraquinones. For the saponins, extract (200 μ L) was diluted in distilled water (5.0 mL), heated, and shaken vigorously. Persistent froth indicated a positive result. For anthraquinones, extract (200 μ L) was heated with 10% sulfuric acid, cooled and filtered. The filtrate was treated with 10% ammonium hydroxide (NH_4OH), with a pinkish-red color indicating their presence.

7.4 Steroids

Steroids were determined by the method of Ahmed et al. (2019). Extract (200 μ L) was dissolved in dichloromethane (1.0 mL) and filtered. The filtrate was treated with 0.5 mL of glacial acetic acid and 3 drops of concentrated sulfuric acid (98% H_2SO_4). A blue or blue-green coloration indicated a positive result.

7.5 Cardiac glycosides

The test for cardiac glycosides was adapted from Ayoola et al. (2008). Extract (200 μ L) was first dissolved in dichloromethane (1.0 mL), and 5 drops of glacial acetic acid and 5 drops of 1% ferric chloride solution were added. This mixture was carefully layered beneath 1.0 mL of concentrated sulfuric acid. The observation of a brown interfacial ring provided evidence for the existence of a deoxysugar, a structural feature typically found in cardenolides. The test was also considered positive if a violet ring appeared below the brown ring, or if a greenish ring formed in the acetic acid layer and gradually spread.

8. Statistical analysis

All experimental data were expressed as mean \pm standard deviations (SD) based on triplicate measurements for each treatment. A 2-way analysis of variance (ANOVA) was performed using a 2 \times 2 factorial arrangement in a completely randomized design (CRD). All statistical analyses were performed utilizing the SPSS software, version 29 (IBM Corp., USA).

Results and discussion

1. Phytochemical properties of fresh *Artocarpus lakoocha* leaves

Leaf maturity significantly influenced the phytochemical properties of *A. lakoocha* leaves, as summarized in Table 1. Fresh young leaves consistently exhibited a superior phytochemical profile compared to mature leaves. The total phenolic content in young leaves (13.14 mg GAE/g FW) was approximately 59% higher than that of mature leaves (8.28 mg GAE/g FW). A similar trend was observed for total flavonoid content. This pattern aligns with physiological studies showing that plants often allocate higher concentrations of protective secondary metabolites, such as phenolics and flavonoids, to younger and more vulnerable tissues (Zhang et al., 2018).

The elevated levels of bioactive compounds in young leaves translated into greater antioxidant capacity. Young leaves demonstrated significantly higher free-radical scavenging activity in both the ABTS assay (178.29 mg TE/g FW) and the DPPH assay (27.35 mg TE/g FW), as well as stronger reducing power in the FRAP assay (43.64 mg FE/g FW), compared to mature leaves. These findings suggest that for fresh applications, young leaves represent a more potent source of natural antioxidants.

Table 1 Phytochemical properties of fresh *Artocarpus lakoocha* leaves

Parameter	Young leaves	Mature leaves
Total phenolic content (mg GAE/g FW)	13.14 \pm 0.58 ^a	8.28 \pm 0.97 ^b
Total flavonoid content (mg QE/g FW)	51.52 \pm 1.14 ^a	41.35 \pm 1.05 ^b
DPPH Assay (mg TE/g FW)	27.35 \pm 1.14 ^a	16.91 \pm 1.08 ^b
ABTS Assay (mg TE/g FW)	178.29 \pm 3.53 ^a	139.01 \pm 2.98 ^b
FRAP Assay (mg FE/g FW)	43.64 \pm 6.47 ^a	31.89 \pm 3.38 ^b

Remark: Values are expressed as means \pm SD of triplicate samples ($n = 3$). Superscript letters (^{a, b}) indicate significant differences between young and mature leaves ($p < 0.05$).

2. Phytochemical analysis of *Artocarpus lakoocha* leaves powder

Qualitative phytochemical screening revealed a consistent profile across all leaf samples, regardless of maturity or blanching treatment, as detailed in Table 2. The uniform presence of key bioactive classes—including phenolics, tannins, flavonoids, saponins, and steroids—indicates that these compounds are thermally stable under the short blanching conditions (100 °C for 30 sec). Similar findings have been reported for various leafy vegetables, where brief blanching effectively preserves major phytochemicals such as phenolics and flavonoids (Kim et al., 2020; Ncube et al., 2022). This preservation is primarily attributed to the inactivation of polyphenol oxidase and peroxidase, enzymes responsible for phytochemical degradation, thereby enhancing the nutritional quality of the vegetables (Ijod et al., 2025).

The detection of these compound classes in both young and mature leaves further suggests that their biosynthetic pathways remain active throughout leaf development, a pattern observed in other plant species as well (Masa et al., 2016). In contrast, anthraquinones and cardiac glycosides were not detected in any sample, indicating that these compounds are either absent in *A. lakoocha* leaves or present at concentrations below the detection limits of the methods used.

Table 2 Phytochemical analysis of *Artocarpus lakoocha* leaves powder

Phytochemical class	Compound tested	Young leaves		Mature leaves	
		Not blanched	Blanched	Not blanched	Blanched
Phenolic compounds	Phenolics	+	+	+	+
	Tannins	+	+	+	+
	Flavonoids	+	+	+	+
Terpenoids	Saponins	+	+	+	+
	Steroids	+	+	+	+
	Cardiac glycosides	–	–	–	–
Others	Anthraquinones	–	–	–	–

Remark: + indicates presence; – indicates absence of phytochemical content.

3. Total phenolic and flavonoid content of *Artocarpus lakoocha* leaves powder

In contrast to the results from fresh samples, the analysis of dried *A. lakoocha* leaf powders revealed that leaf maturity significantly influenced phytochemical content (Table 3). Mature leaves exhibited higher concentrations of both total phenolics (TPC) and total flavonoids (TFC) than young leaves. For instance, the TPC in blanched mature leaves (40.50 mg GAE/g DW) was approximately 47% greater than that in blanched young leaves (27.52 mg GAE/g DW).

This reversal in phytochemical prevalence is likely attributable to the differential thermal stability of compounds during the dehydration process. Simpler, more labile phenolics typical of young leaves may be more prone to degradation, whereas the complex, polymerized compounds in mature leaves appear more resilient to thermal processing. This observation aligns with findings in other plant species, where older leaves often retain higher levels of phenolic and flavonoid compounds following post-harvest treatment (Pinto et al., 2022). Additionally, stable compounds, such as terpenoids, in mature leaves may contribute to the elevated bioactive content observed after drying, as these compounds are known to withstand processing conditions (Jirakitticharoen et al., 2023).

Blanching did not significantly affect phenolic or flavonoid content in either young or mature leaves, indicating the stability of these compounds under short blanching durations and moderate temperatures. In other studies, such as with bathua leaves, a slight decrease in total phenolic content was observed with prolonged blanching, while flavonoid levels initially increased before declining—suggesting partial resilience to the blanching process (Neeharika et al., 2019). Similarly, research on various leafy vegetables has shown that blanching-induced reductions in phenolic and flavonoid levels are generally modest and do not substantially alter the nutritional profile (Hong & Ahn, 2005).

Nutrient retention during blanching depends on factors such as temperature, duration, and chemical additives. For example, blanching at 80 °C for one min in potassium metabisulphite effectively preserved

the nutritional quality of green leafy vegetables, including phenolics and flavonoids (Saranya et al., 2017). Moreover, reusing blanching water containing leached phytochemicals may help retain these compounds during food preparation (Sicari et al., 2020).

Table 3 Total phenolic and flavonoid content of *Artocarpus lakoocha* leaves powder

Sample	Total phenolic content (mg GAE/g DW)	Total flavonoid content (mg QE/g DW)
Young leaves		
Not blanched	28.67±1.12 ^b	60.79±3.09 ^b
Blanched	27.52±1.11 ^b	60.46±5.34 ^b
Mature leaves		
Not blanched	38.85±1.07 ^a	70.99±2.73 ^a
Blanched	40.50±1.44 ^a	73.90±1.56 ^a

Remark: Values are expressed as means ± SD of triplicate samples ($n = 3$). Superscript letters (^{a, b}) indicate significant differences between young and mature leaves ($p < 0.05$).

4. Antioxidant activity of *Artocarpus lakoocha* leaves powder

The antioxidant activity of dried *A. lakoocha* leaf powders, presented in Table 4, generally correlated with the quantitative phytochemical results, with mature leaves exhibiting superior antioxidant capacity. This is primarily attributable to their significantly higher concentrations of total phenolics and flavonoids, as shown in Table 3, since these compounds are key contributors to antioxidant activity (Chin et al., 2023).

The enhanced antioxidant potential of mature leaves was most evident in the FRAP assay, where their reducing power was significantly greater than that of young leaves. A similar trend was observed in the DPPH assay, although the difference was not statistically significant. Notably, the ABTS assay revealed an interactive effect between leaf maturity and blanching. Blanching significantly increased the ABTS radical scavenging activity of young leaves, elevating it to a level comparable with that of blanched mature leaves. This suggests that the blanching process may have released bound phenolic compounds or activated specific phytochemicals within the young leaf matrix that are particularly effective against the ABTS radical cation (An et al., 2022; Magangana et al., 2021).

Table 4 Antioxidant activity of *Artocarpus lakoocha* leaves powder

Sample	DPPH assay ^{ns} (mg TE/g DW)	ABTS assay (mg TE/g DW)	FRAP Assay (mg FE/g DW)
Young leaves			
Not blanched	33.96±5.60	221.00±2.71 ^c	49.55±1.37 ^b
Blanched	30.68±6.13	234.57±4.06 ^a	46.82±1.40 ^b
Mature leaves			
Not blanched	42.64±4.59	228.06±2.06 ^b	59.47±1.66 ^a
Blanched	42.97±8.87	234.25±3.83 ^a	57.56±1.87 ^a

Remark: Values are expressed as means ± SD of triplicate samples ($n = 3$). Superscript letters (^{a-c}) indicate significant differences between young and mature leaves ($p < 0.05$). ns = not significant.

Conclusion

This study demonstrates that leaf maturity is a critical determinant of the phytochemical and antioxidant potential of processed *Artocarpus lakoocha* leaves. The most notable finding was a reversal of the trend observed between fresh and processed samples. Whereas fresh young leaves exhibited higher bioactivity, processed mature leaves contained markedly greater concentrations of total phenolics and flavonoids, which translated into superior overall antioxidant capacity.

Blanching proved to be a viable pre-treatment method that did not degrade the major phytochemical classes and, in some cases, enhanced the antioxidant activity. Accordingly, for the development of shelf-stable nutraceuticals and functional food ingredients, mature *A. lakoocha* leaves represent the recommended strategy to maximize the yield of bioactive compounds and antioxidant potential. These findings provide a valuable foundation for the valorization of this underutilized resource.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Declaration of generative artificial intelligence in the writing process

During the preparation of this manuscript, the authors used SCISPACE (<https://scispace.com/>) to search for relevant literature and Gemini (<https://gemini.google.com/>) to check grammar and polish the wording. The final content was reviewed and edited by the authors, who take full responsibility for the accuracy and integrity of the publication.

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