Revista de Ciências Agroveterinárias
Universidade do Estado de Santa Catarina (UDESC)

Research Article

Ultrasonic-Green Extraction of *Lansium domesticum* Leaves: Optimization, Phenolic and Flavonoid Content, Stability, and Antioxidant Activity

Extração ultrassônica-verde de folhas de Lansium domesticum: otimização, conteúdo fenólico e flavonoide, estabilidade e atividade antioxidante

Fitry Tafzi* (ORCID 0000-0002-6137-9662), **Addion Nizori** (ORCID 0000-0002-3874-8132), **Indriyani Indriyani** (ORCID 0000-0001-9576-9143), **Indra Lasmana Tarigan** (ORCID 0000-0002-7238-7780)

Universitas Jambi, Indonesia. *Author for correspondence: fitrytafzi@unja.ac.id

Submission: March 20th, 2025 | Acceptance: July 04th, 2025

ABSTRACT

Lansium domesticum, Duku, leaves contain bioactive compounds with significant health benefits, particularly as antioxidants. However, an efficient and sustainable extraction method is essential to maximize their potential. This study investigates ultrasonic-assisted green extraction (UAE) as an eco-friendly approach for optimizing the extraction of phenolic and flavonoid compounds. The optimization process involved three key parameters: solvent ratio (1:10, 1:20, 1:30, 1:40, 1:50), extraction temperature (40, 50, 60, 70, 80 °C), and extraction time (20, 40, 60, 80, 100 min), using a completely randomized design. The bioactive compound stability was assessed under various conditions, and antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH). The results demonstrated that solvent ratio and extraction temperature significantly influenced extract yield, phenolic and flavonoid content, and antioxidant activity, while extraction time affected yield and bioactive content but not antioxidant activity. The optimal extraction condition is a 1:20 powder-to-solvent ratio, 50 °C temperature, and 80 min extraction time. Yielded 5.98 ± 0.1% extract, 40.49 ± 0.9 mg AGE/g phenolic content, 185 ± 3.9 mg QE/g flavonoid content, and 38.28 ± 0.2% antioxidant activity. These findings highlight the UAE as a sustainable and effective method for extracting bioactive compounds, with promising applications in pharmaceuticals, nutraceuticals, and functional food.

KEYWORDS: Antioxidant. Green Extraction. *Lansium domesticum.* Optimization.

RESUMO

As folhas de *Lansium domesticum*, Duku, contêm compostos bioativos com benefícios significativos à saúde, particularmente como antioxidantes. No entanto, um método de extração eficiente e sustentável é essencial para maximizar seu potencial. Este estudo investiga a extração verde assistida por ultrassom (EAU) como uma abordagem ecologicamente correta para otimizar a extração de compostos fenólicos e flavonoides. O processo de otimização envolveu três parâmetros principais: proporção de solvente (1:10, 1:20, 1:30, 1:40, 1:50), temperatura de extração (40, 50, 60, 70, 80 °C) e tempo de extração (20, 40, 60, 80, 100 min), usando um delineamento completamente randomizado. A estabilidade do composto bioativo foi avaliada sob várias condições, e a atividade antioxidante foi avaliada usando ensaios DPPH. Os resultados demonstraram que a proporção de solvente e a temperatura de extração influenciaram significativamente o rendimento do extrato, o conteúdo fenólico e flavonoide e a atividade antioxidante, enquanto o tempo de extração afetou o rendimento e o conteúdo bioativo, mas não a atividade antioxidante. As condições ótimas de extração — proporção pó-solvente de 1:20, temperatura de 50 °C e tempo de extração de 80 min — renderam 5,98 ± 0,1% de extrato, 40,49 ± 0,9 mg AGE/g de conteúdo fenólico, 185 ± 3,9 mg QE/g de conteúdo flavonoide e 38,28 ± 0,2% de atividade antioxidante. Essas descobertas destacam a UAE como um método sustentável e eficaz para extrair compostos bioativos, com aplicações promissoras em produtos farmacêuticos e nutracêuticos.

PALAVRAS-CHAVE: Antioxidante. Extração Verde. *Lansium domesticum.* Otimização.

INTRODUCTION

Duku (*Lansium domesticum*) is one of the tropical fruits found in Indonesia. Duku varieties in Indonesia are known by several local names, such as langsat (*Lansium domesticum* 'Langsat-Lonkong Group') and kokosan (*Lansium domesticum* var. aquaeum) (ABDALLAH et al. 2022, HANUM et al. 2018). Duku is a superior horticultural commodity in Jambi Province, with the main production centres spread across Muaro Jambi, Batanghari, Bangko, and Bungo Regencies. Duku fruit is seasonal and generally bears fruit once a year (ERYANI et al. 2020, SYAMSUARDI et al. 2018). *Lansium domesticum*, commonly known as duku or langsat, is a tropical fruit valued not only for its sweet and refreshing taste but also for its rich nutritional profile. It contains essential vitamins such as vitamin A, vitamin C, and B-complex vitamins (ABDALLAH et al. 2022, LUBIS et al. 2022). Vitamins A and C serve as natural antioxidants that help neutralize free radicals and protect cells from oxidative stress (VENKATACHALAM 2019). In addition to its nutritional benefits, duku is a promising source of bioactive compounds, especially phenolics and flavonoids, which have been widely studied for their diverse biological activities.

Phenolic compounds such as gallic acid and ellagic acid have been identified in either peel or pulp extracts of the fruit and are known for their strong antioxidant, anti-inflammatory, and antimicrobial properties (ABDALLAH et al. 2022). Furthermore, flavonoids like quercetin, kaempferol, and luteolin have been detected in the seed and peel of the fruit. These flavonoids contribute to anti-inflammatory action by modulating key pathways, such as NF-κB and preventing lipid peroxidation, making them relevant for the prevention of chronic diseases, including cancer, diabetes, and cardiovascular disorders (JOMOVA et al. 2025). Therefore, the potential of *Lansium domesticum* goes beyond its nutritional value, positioning it as a natural source of nutraceuticals and phytopharmaceuticals that could be developed for use in functional foods and plant-based therapeutic agents.

In addition, various parts of the duku plant, such as leaves, fruit, and bark, are known to have potential pharmacological activity. Several studies have explored the pharmacological effects of duku leaves (LUBIS et al. 2022). Duku leaf extract shows anticancer activity against colon cancer cells and oral cancer cells (LUBIS et al. 2023). In addition, duku leaf extract also has antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli* (MUNIR et al. 2018). Another study revealed that duku leaf extract has antifeedant properties against insect pests, such as *Spodoptera litura* and *Epilachna varivestis* (OPIRRUMAPE et al. 2017).

The pharmacological effects of duku leaves are believed to be closely related to the phytochemical content contained therein. Several bioactive compounds that have been identified in duku leaves include flavonoids, alkaloids, and terpenoids, which are known to have various biological activities, such as antioxidants, antibacterials, and anticancer (MUNIR et al. 2018). Therefore, further exploration of the pharmacological potential of various parts of the langsat plant is still needed to support the development of broader natural ingredient-based drugs. The phytochemical constituents of duku include phenolic compounds, flavonoids, and triterpenoids (LUBIS et al. 2022). Phenolic compounds and flavonoids are widely distributed secondary metabolites in plants and are known for their diverse pharmacological properties, particularly as

antioxidants (ABDALLAH et al. 2022). Antioxidants play a crucial role in neutralizing free radicals that can cause cellular and tissue damage. Antioxidant activity of *Lansium parasiticum* water extract using DPPH and ABTS assay showed the highest total phenolic content of 152.910 ± 22.14 mg GAE/100 g, total flavonoid content of 1669.723 ± 370.091 mg QE/100 g, and antioxidant activity of DPPH of 68.51% ± 2.73 and ABTS of 6.063 U/mL ± 0.72 compared to other extracts. Total phenolics showed significant results on antioxidant activity (PITHONAH et al. 2023).

The quantity and quality of phytochemical constituents in plant extracts are significantly influenced by the extraction method employed. Conventional extraction techniques, such as maceration and soxhlet extraction, are frequently used to isolate bioactive compounds from plants. However, these methods have certain drawbacks, including prolonged extraction times and high energy consumption. Consequently, more efficient extraction techniques, such as ultrasonic-assisted extraction (UAE), are gaining popularity (AHMED et al. 2022). This technique employs ultrasonic waves to disrupt plant cell walls, thereby enhancing solvent penetration into the tissues and improving extract yield (ALTEMIMI et al. 2017).

UAE offers several advantages over conventional methods, including high reproducibility, reduced solvent usage, shorter extraction duration, and increased extract purity (ALTEMIMI et al. 2017). Previous research has demonstrated that the phenolic content in pistachio nut extracts is significantly higher when extracted using UAE compared to maceration and microwave-assisted methods. Furthermore, the UAE has been shown to enhance the levels of phenols and flavonoids in various ethanol-based plant extracts compared to maceration and soxhlet extraction (SHEN et al. 2023).

The extraction conditions of phenolic components, flavonoids, and antioxidant activity for each plant are not the same, due to differences in phenolic and flavonoid compounds in each plant. In addition to the type of compound, the conditions and extraction process carried out also affect the type of solvent (SHI et al. 2022), solvent ratio, temperature, extraction time, particle size, and pH. In addition, the antioxidant activity of the extraction process is also related to the solvent ratio condition (LIMA et al. 2023). The solvent ratio, temperature, and extraction time affect the yield and phenolic components and antioxidant activity (AHMED et al. 2022). This study aims to determine the optimal extraction conditions for extracting phenolic components, flavonoids, and antioxidant activity of duku leaf extract using the ultrasonic method and the stability of the extracted bioactive compounds.

MATERIALS AND METHODS

Chemicals and Instrumentations

The materials used in this study included duku leaves obtained from community plantations in Kota Karang Village, Kumpeh Ulu District, Muaro Jambi Regency. The chemicals used were ethyl acetate (Merck, Germany), ethanol (Sigma-Aldrich, USA), DPPH (Sigma-Aldrich, USA), 10% Na_2CO_3 (Merck, Germany), 80% Folin-Ciocalteu reagent (Merck, Germany), 5% $AlCl_3$ (Merck, Germany), 1 M CH_3COOK (Sigma-Aldrich, USA), gallic acid (Sigma-Aldrich, USA), quercetin (Sigma-Aldrich, USA), and distilled water. The equipment used included an ultrasonic sonicator (Branson

M2800H, USA), a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan), and standard laboratory glassware (Pyrex, USA) for sample preparation and analysis.

Sample Preparation and Extraction

The duku leaves were washed, air-dried, and ground into a fine powder to obtain simplicia. Extraction was conducted using the ultrasonic-assisted extraction (UAE) method in three sequential stages in order to find an optimum condition.

The solvent ratio effect: duku leaf powder was mixed with ethyl acetate solvent in varying ratios of 1:10, 1:20, 1:30, 1:40, and 1:50 (w/v). Each mixture was subjected to extraction by sonication using a Branson M2800H ultrasonic bath (USA) at 60 °C for 40 min. Following extraction, the solutions were filtered through Whatman No. 1 filter paper. The resulting filtrates were concentrated under reduced pressure using a rotary evaporator (Buchi R-300, Switzerland) at 40 °C to yield the ethyl acetate extracts of duku leaves. Subsequently, the extracts were analyzed for total phenolic content, total flavonoid content, and antioxidant activity.

The temperature effect: The effect of extraction time was evaluated at intervals of 20, 40, 60, 80, and 100 min. duku leaf powder was extracted with ethyl acetate at a solvent-to-material ratio of 1:20 (w/v). Each extraction was carried out by sonication using a Branson M2800H ultrasonic bath (USA) at a constant temperature of 50 °C. After extraction, the solutions were filtered through Whatman No. 1 filter paper, and the resulting filtrates were concentrated under reduced pressure using a rotary evaporator (Buchi R-300, Switzerland) at 40 °C to obtain the ethyl acetate extracts of duku leaves. The extracts were subsequently analyzed for total phenolic content, total flavonoid content, and antioxidant activity.

The extraction time effect: The extraction time was examined at 20, 40, 60, 80, and 100 min. Duku leaf powder was extracted with ethyl acetate at a ratio of 1:20. Each extraction was done by sonication (Branson M2800H, USA) at 50 °C. After extraction, the solution was filtered using Whatman No. 1 filter paper, and the resulting filtrate was concentrated using a rotary evaporator (Buchi R-300, Switzerland) at 40 °C to obtain the ethyl acetate extract of duku leaves. Furthermore, total phenol, total flavonoid and antioxidant activity were analyzed.

Effect of Temperature and pH on the Stability of Phenolic, Flavonoid Components, and Antioxidant Activity of *Lansium domesticum* Leaf Extract

This stage aimed to evaluate the stability of total phenolic content, total flavonoid content, and antioxidant activity of duku leaf extract when subjected to thermal and pH stress conditions. Assessing the influence of temperature and pH is essential to determine the suitability of the extract for further encapsulation and potential functional applications.

pH Stability

Buffer solutions with pH values of 2, 4, 6, 8, and 10 (3.6 mL each) were transferred into separate test tubes. Subsequently, 0.4 mL of the duku leaf extract solution was added to each tube. The mixtures were vortexed thoroughly and incubated for 2 hr at room temperature. After incubation, the samples were subjected to analyses of total phenolic content, total flavonoid content, and antioxidant activity.

Temperature Stability

The duku leaf extract solution was heated in a water bath at temperatures of 50, 70, and 90 °C. Aliquots were collected at 30-minute intervals over a total period of 150 min. Each aliquot was analyzed for total phenolic content, total flavonoid content, and antioxidant activity.

Determination of Total Phenolic Content

A volume of 200 µL of extract solution was mixed with 1 mL of 10% (v/v) Folin–Ciocalteu reagent and allowed to stand for 1 min. Subsequently, 3 mL of 20% (w/v) sodium carbonate (Na_2CO_3) solution was added, and the mixture was vortexed. The reaction mixture was incubated in the dark at room temperature for 2 hr. The absorbance was measured using a UV-Vis spectrophotometer at 760 nm. Total phenolic content was calculated using a gallic acid standard curve and expressed as mg gallic acid equivalents per gram extract (mg GAE/g).

Determination of Total Flavonoid Content

A volume of 500 µL of extract solution was placed into a test tube, followed by the addition of 100 µL of 10% (w/v) aluminum chloride ($AlCl_3$) solution and 100 µL of 1 M potassium acetate (CH_3COOK). Subsequently, 4.3 mL of distilled water was added. The mixture was vortexed and incubated at room temperature for 30 min. The absorbance was measured at 415 nm using a UV-Vis spectrophotometer. Total flavonoid content was determined based on a quercetin standard curve and expressed as mg quercetin equivalents per gram extract (mg QE/g).

Determination of Antioxidant Activity

A total of 1 mL of sample was put into a test tube, then 5 mL of DPPH solution was added. The solution mixture was homogenized by vortex and stored for 30 min in a dark room. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. Antioxidant activity was calculated using the equation 1.

$$\text{Inhibition } (\%) = \frac{(\text{control absorbance} - \text{sample absorbance})}{\text{control absorbance}} x100\ \% \quad (1)$$

Statistical Analysis

Data obtained from the measurements of total phenolic content, total flavonoid content, and antioxidant activity were subjected to statistical analysis. Analysis of variance (ANOVA) was performed to evaluate the significance of differences among treatment groups. When significant effects were detected ($p < 0.05$), mean comparisons were further analyzed using Duncan's Multiple Range Test (DMRT) to identify specific differences between treatments. All statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) software.

RESULTS AND DISCUSSION

Solvent ratio affects the chemical constituents and antioxidant activity of the extract.

To evaluate the effect of the ratio of duku leaf powder to ethyl acetate solvent on extract yield, phenolic content, flavonoid content, and antioxidant activity, an experiment was conducted using solvent-to-powder ratios ranging from 1:10 to 1:50. The extraction process was performed at 60 °C for 40 min. The influence of solvent

ratio on extract yield, phenolic content, flavonoid content, and antioxidant activity is presented in Figure 1.

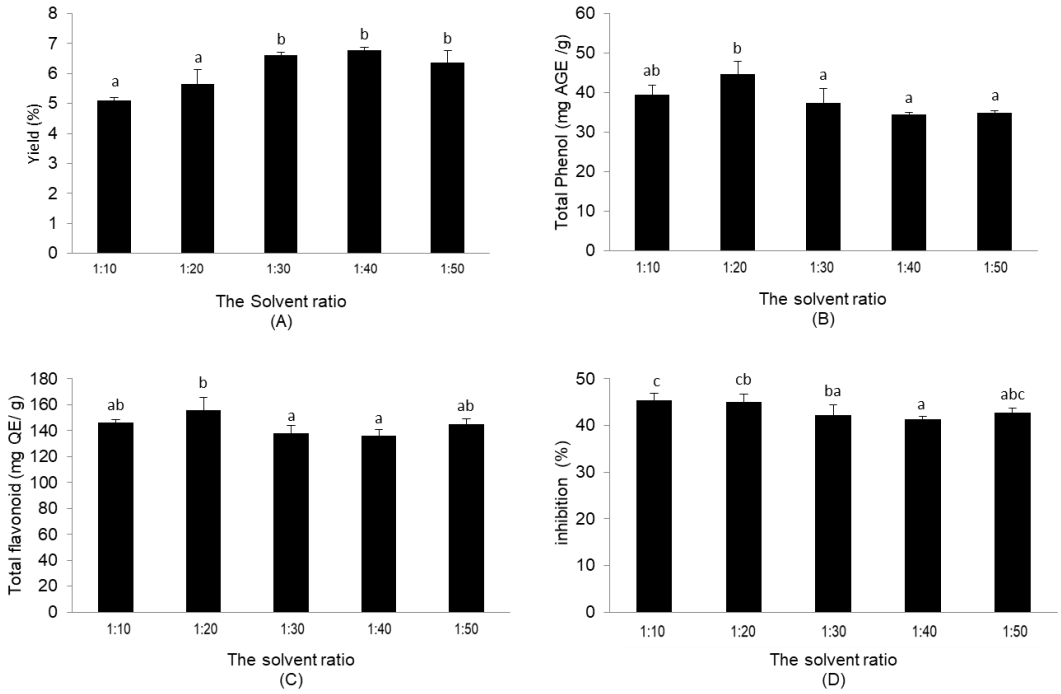

Figure 1. Effect of powder and ethyl acetate solvent ratio on yield (a), phenol content (b), flavonoid content (c), and antioxidant activity (d) of ethyl acetate extract of duku leaves. Extraction was carried out at 60 °C for 40 min. Mean values ± SD (n=3). Different lowercase letters indicate significant differences (P<0.05).

The powder-to-solvent ratio significantly influenced the yield of duku leaf ethyl acetate extract (Figure 1a). The highest extract yield was observed at a ratio of 1:40, reaching 6.77 ± 0.1%, whereas the lowest yield was obtained at a ratio of 1:10, measuring 5.10 ± 0.1%. An increasing in the solvent ratio from 1:10 to 1:40 resulted in a progressive increase in yield. This trend aligns with previous findings, which indicate that higher solvent volumes enhance extract recovery in ultrasonic-assisted extraction due to improved solvent penetration and mass transfer efficiency (BIN MOKAIZH et al. 2024). However, further increasing the solvent ratio to 1:50 led to a decline in yield, suggesting that an optimal solvent-to-powder ratio exists beyond which extraction efficiency no longer improves.

Statistical analysis using Duncan's multiple range test revealed that the yields obtained at ratios of 1:30, 1:40, and 1:50 were not significantly different, whereas these values differed significantly from those at ratios of 1:10 and 1:20. This finding suggests that increasing the solvent ratio up to 1:30 significantly enhances yield, but beyond this point, additional solvent does not contribute to further extraction efficiency. Previous studies have reported that an increase in solvent ratio initially enhances yield due to greater solvent penetration into plant cells, facilitating improved mass transfer of bioactive compounds from the plant matrix into the extraction medium (ANGGESTIA et al. 2024). However, once equilibrium is reached, mass transfer ceases, and further increases in solvent volume become ineffective.

The solvent-to-powder ratio significantly influenced the extraction of phenolic compounds. The highest phenolic content (44.75 ± 3.1 mg AGE/g) was obtained at a ratio of 1:20, while the lowest (34.41 ± 0.5 mg AGE/g) occurred at 1:40. Increasing the solvent ratio up to 1:20 enhanced phenolic yield; however, further increases to 1:30 and 1:40 led to a decline. Statistical analysis using Duncan's multiple range test indicated no significant difference in phenolic content between ratios of 1:10 and 1:20, but both were significantly higher than those at 1:30 to 1:50.

These findings are consistent with and further support the observed increase in the phenolic content of pistachio nut extract as the solvent-to-material ratio increases (SHI et al. 2022). Similarly, a previous study reported that the total phenolic content of *Inula helenium* root extract increased as the solvent ratio increased from 1:5 to 1:20, after which a further increase in the solvent ratio resulted in a decline in phenolic content. This observed decrease at higher solvent ratios may be attributed to excessive dilution, which reduces the concentration gradient between the plant matrix and the solvent, thereby limiting the efficiency of further extraction (ANGGESTIA et al. 2024)

The solvent-to-powder ratio also had a significant effect on the extraction of flavonoid compounds. The highest flavonoid content was observed at a ratio of 1:20, reaching 155.86 ± 10.1 mg QE/g extract, whereas the lowest flavonoid content was obtained at a ratio of 1:40, measuring 136.16 ± 4.7 mg QE/g extract. Duncan's multiple range test indicated that the flavonoid content at a ratio of 1:20 was not significantly different from that at 1:10, but it was significantly higher than the values obtained at ratios of 1:30 and 1:40. Increasing the solvent ratio enhanced the flavonoid content in corn silk extract, but further increases led to a decrease.

Similarly, another study also observed that increasing the solvent ratio did not significantly improve the flavonoid content of Rhodiola rosea rhizome extract (LI et al. 2023). The decline in flavonoid content at higher solvent ratios may be attributed to excessive dilution, which reduces flavonoid concentration in the extract and may lead to compound loss due to prolonged exposure to the extraction medium (SAI-UT et al. 2023).

Increasing the solvent-to-powder ratio from 1:10 to 1:20 led to an increase in the phenolic and flavonoid content of the duku leaf extract. This phenomenon can be attributed to the enhanced mass transfer of phenolic and flavonoid compounds from the plant matrix into the solvent, which continues until equilibrium is reached (PITHONAH et al. 2023). However, beyond this ratio, further increases in the solvent volume did not contribute to higher phenolic and flavonoid content in the extract. This decline may be associated with prolonged evaporation time required for larger solvent volumes, leading to greater losses of heat-sensitive phenolic and flavonoid compounds due to degradation or volatilization.

The solvent-to-powder ratio also significantly influenced the antioxidant activity of the ethyl acetate extract of duku leaves. Antioxidant activity was evaluated based on the extract's ability to scavenge DPPH free radicals. The highest antioxidant activity was observed at a ratio of 1:10, reaching 45.37 ± 1.6%, whereas the lowest antioxidant activity was recorded at a ratio of 1:40, measuring 41.36 ± 0.6%.

A negative correlation was observed between the solvent ratio and antioxidant activity, indicating that increasing the solvent volume led to a decline in the extract's

ability to neutralize free radicals. Statistical analysis using Duncan's multiple range test revealed that the antioxidant activity of the extract obtained at a ratio of 1:10 was not significantly different from that at 1:20 but was significantly higher than the activity observed at ratios of 1:30 and 1:40. An excessive solvent ratio reduces the ability of the extract to inhibit DPPH free radicals. The optimal solvent-to-powder ratio for obtaining maximum antioxidant activity was determined to be 1:20, as further increases in the solvent volume did not enhance antioxidant activity (PREDESCU et al. 2016).

The antioxidant activity of the extract is closely associated with its phenolic and flavonoid content, as these bioactive compounds play a crucial role in neutralizing free radicals (MUTHA et al. 2021). In this study, the highest phenolic and flavonoid concentrations were obtained at a ratio of 1:20, and beyond this point, their levels declined. Consequently, the antioxidant activity of the extract did not increase beyond this ratio. Based on these findings, subsequent experimental stages were conducted using a solvent-to-powder ratio of 1:20.

Effect of Extraction Temperature on % Yield, Total Phenol and Flavonoid, and Antioxidant Activity

The extraction temperature plays a crucial role in determining the yield, phenolic content, flavonoid content, and antioxidant activity of duku leaf extract obtained through ultrasonic-assisted extraction (Figure 2). In this study, extractions were performed using an optimal powder-to-solvent ratio of 1:20 for 40 min. A higher extraction temperature generally resulted in an increased extract yield (Figure 2). Extraction temperatures ranging from 40 to 80 °C led to an increase in yield from 4.92 ± 0.09% to 6.17 ± 0.25%.

Statistical analysis using Duncan's multiple range test indicated that raising the temperature from 40 to 50 °C significantly enhanced the yield. However, further increases from 50 to 70 °C did not produce statistically significant differences in yield. The highest yield (6.17 ± 0.25%) was observed at an extraction temperature of 80 °C, suggesting that higher temperatures facilitate the diffusion of bioactive compounds by reducing solvent viscosity and breaking down plant cell walls. The extraction temperature also significantly influenced the phenolic content of the duku leaf extract (Figure 2). Increasing the temperature from 40 to 50 °C enhanced the phenolic concentration in the extract. However, beyond 50 °C, the phenolic content declined, with the lowest value recorded at 80 °C (26.11 ± 0.3 mg AGE/g extract), compared to the highest value at 50 °C (33.95 ± 1.5 mg AGE/g extract). The Duncan test revealed that phenolic content at temperatures of 40–70 °C was not significantly different, but extraction at 80 °C resulted in a significant reduction.

Similarly, flavonoid content was affected by extraction temperature (Figure 2c). Increasing the temperature from 40 to 60 °C led to a rise in flavonoid concentration, reaching a peak at 60 °C (142.53 ± 2.1 mg QE/g extract). However, further increases in temperature to 70 and 80 °C resulted in a decline in flavonoid content, with the lowest recorded value at 80 °C (133.23 ± 2.6 mg QE/g extract). The Duncan test indicated no significant differences in flavonoid content across the 40–70 °C temperature range. These results align with the findings of several previous studies, which reported that increasing the extraction temperature up to an optimal point enhances flavonoid yield

in garlic and oregano extracts, whereas excessive temperatures lead to degradation of flavonoid compounds (ALTEMIMI et al. 2017, DO et al. 2014).

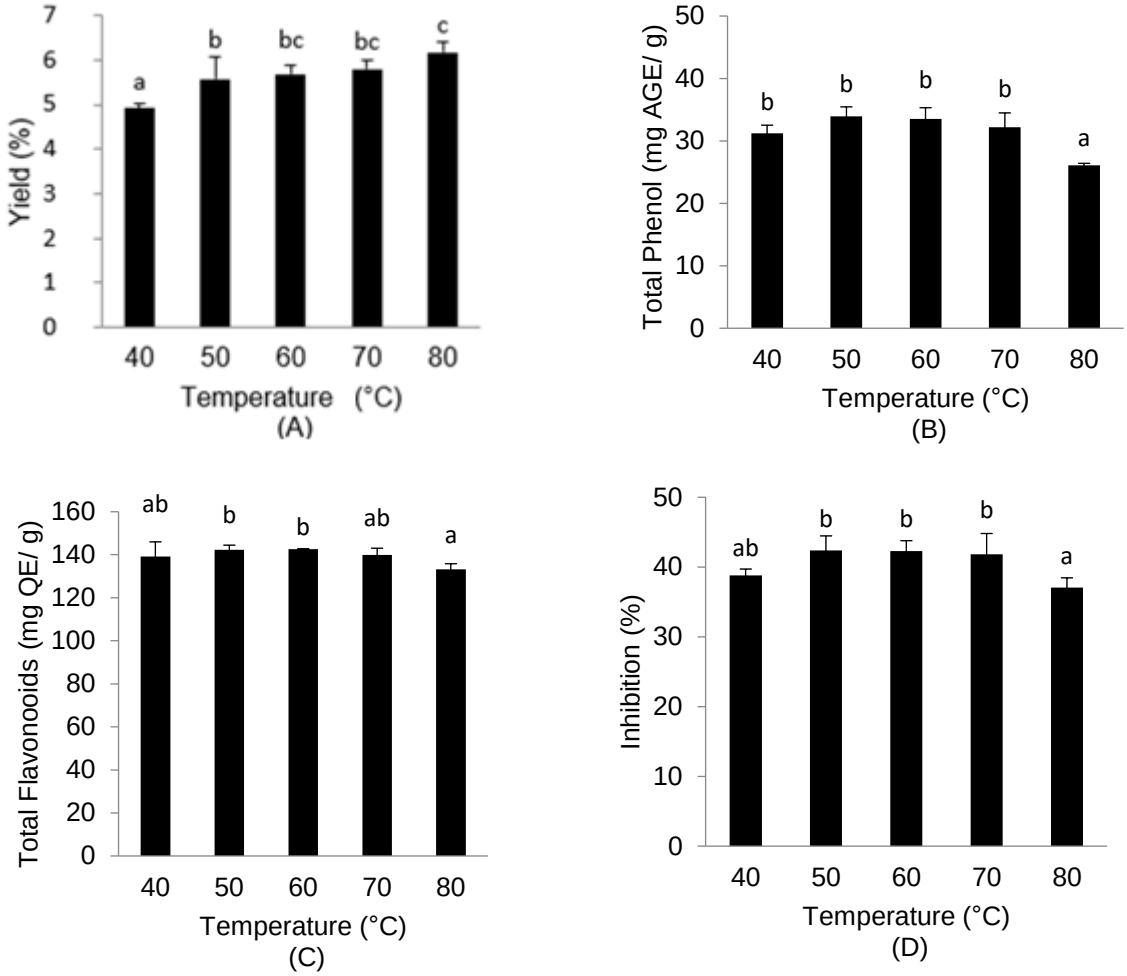

Figure 2. Effect of extraction temperature on yield (A), phenol content (B), flavonoid content (C), and antioxidant activity (D) of ethyl acetate extract of duku leaves. Extraction was carried out with a powder and solvent ratio of 1:20 for 40 min. Mean values ± SD (n=3). Different lowercase letters indicate significant differences (P<0.05).

Temperature is a critical factor affecting the efficiency of phenolic and flavonoid extraction. Higher temperatures facilitate the breakdown of plant cell walls and decrease solvent viscosity, thereby improving the diffusion of phenolic and flavonoid compounds into the solvent. However, excessive temperatures can lead to the thermal degradation of these bioactive compounds, reducing their overall yield (XU et al. 2021).

Extraction temperature also influenced the antioxidant activity of duku leaf extract (Figure 2d). The highest antioxidant activity was recorded at 50 °C (42.36 ± 2.1%), whereas the lowest was observed at 80 °C (37.05 ± 1.4%). Duncan's test showed that antioxidant activity remained statistically unchanged across the 40–70 °C range, but a significant decline occurred at 80 °C. This indicates that excessive heat exposure during extraction negatively impacts the antioxidant potential of the extract. Antioxidant activity is closely related to the phytochemical composition of plant extracts, and many bioactive compounds, including phenolics and flavonoids, are thermolabile (SACI et al. 2017). As shown in Figure 2, extraction at 80 °C resulted in a substantial reduction in

phenolic and flavonoid content, consequently diminishing the extract's antioxidant activity. Based on these findings, the optimal extraction temperature for obtaining high phenolic and flavonoid content, as well as strong antioxidant activity, was determined to be 50 °C. Therefore, subsequent extraction experiments were conducted at this temperature to ensure maximum bioactive compound recovery.

Effect of Extraction Time on Yield, Phenolic and Flavonoid Content, and Antioxidant Activity

Extraction time significantly influences the yield, phenolic content, and flavonoid content of duku leaf extract obtained using ultrasonic-assisted extraction; however, it does not significantly affect the antioxidant activity of the extract (Figure 3d). In this study, extractions were performed at an optimal temperature of 50 °C with a powder-to-solvent ratio of 1:20.

Extraction time plays a crucial role in determining the yield of ethyl acetate extract (Figure 3a). Prolonging the extraction duration led to a higher yield, with the highest yield (6.02 ± 0.1%) obtained at 100 min, whereas the lowest yield (5.02 ± 0.2%) was recorded at 20 min. Increasing the extraction time from 20 to 60 min significantly enhanced the yield. However, extending the extraction to 80 and 100 min did not result in a statistically significant increase in yield. This indicates that equilibrium had been reached in the extraction medium, where further prolongation did not contribute to additional solubilization of bioactive compounds.

Furthermore, extraction time also significantly influenced the phenolic and flavonoid content of the extract (Figures 3b and 3c). Increasing the extraction duration from 20 to 80 min enhanced the phenolic and flavonoid content. However, extending the extraction time to 100 min resulted in a marked decline. The highest phenolic and flavonoid content was observed at 80 min, with values of 40.49 ± 0.9 mg AGE/g extract and 185.20 ± 3.9 mg QE/g extract, respectively. Conversely, the lowest values were recorded at 100 min, with phenolic and flavonoid contents of 24.23 ± 1.5 mg AGE/g extract and 116 ± 6.6 mg QE/g extract, respectively.

The statistical analysis showed no significant differences in phenolic and flavonoid content for extraction times between 20 and 60 min. However, extending the extraction time to 80 min increased the phenolic and flavonoid content by 27.49% and 24.98%, respectively. Further prolongation to 100 min led to a sharp decrease in phenolic and flavonoid content by 40.16% and 37.25%, respectively.

This trend aligns with findings from previous research, which reported that phenolic content initially increases with prolonged extraction but subsequently declines due to thermal degradation and oxidation (ANTONY & FARID 2022). The initial increase in phenolic and flavonoid content can be attributed to the time required for the solvent to penetrate leaf tissues, dissolve bioactive compounds, and facilitate their diffusion into the extraction medium (SACI et al. 2017). However, excessive extraction time exposes the extract to prolonged heat and oxygen exposure, leading to the degradation of thermolabile phenolic and flavonoid compounds, thereby reducing their overall content in the extract.

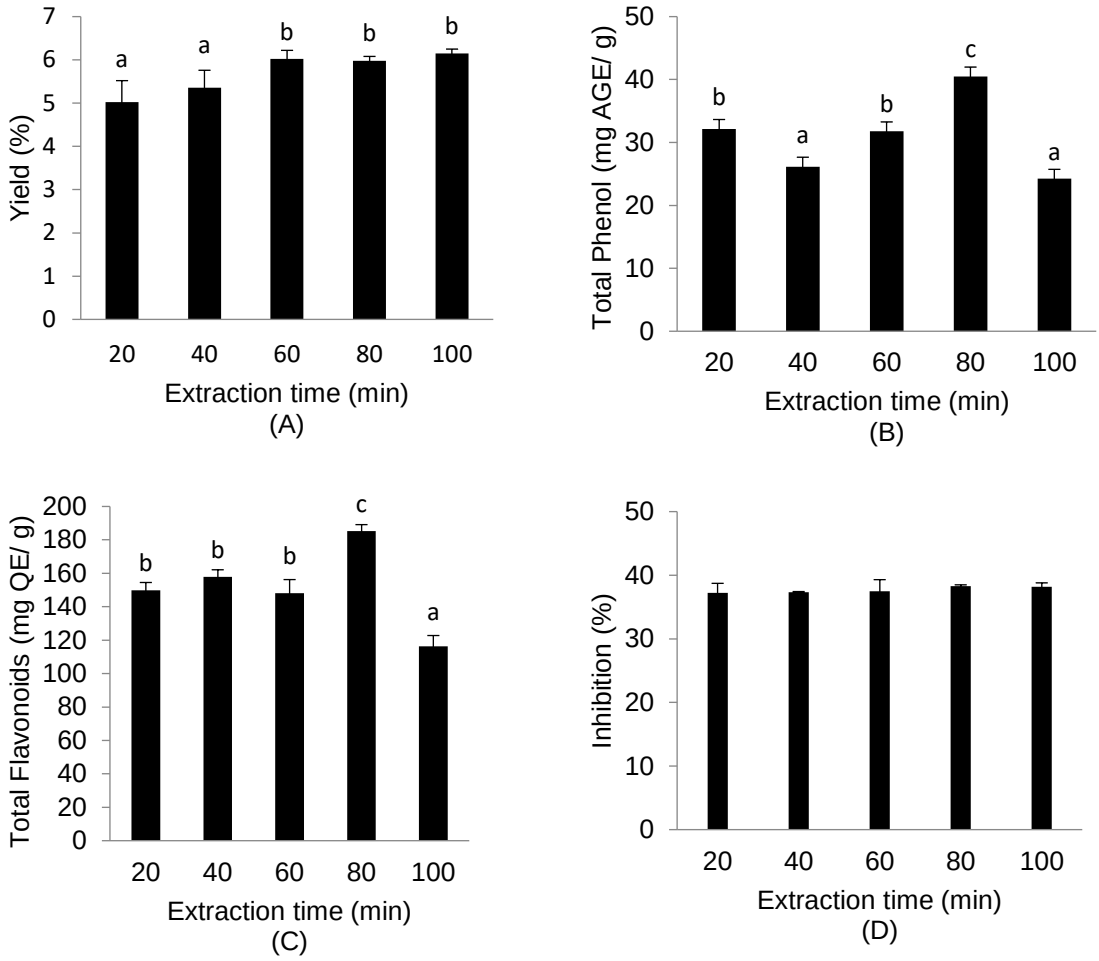

Figure 3. Effect of extraction time on yield (a), phenol content (b), flavonoid content (c), and antioxidant activity (d) of ethyl acetate extract of duku leaves. Extraction was carried out with a powder and solvent ratio of 1:20 at a temperature of 50 °C. Mean values ± SD (n=3). Different lowercase letters indicate significant differences based on Duncan's test (P<0.05).

Interestingly, extraction time between 20 and 100 min at 50 °C did not significantly affect the antioxidant activity of the extract. This finding contrasts with the study by SACI et al. (2017), which reported that extraction time influenced the antioxidant activity of carob extract. The absence of a significant effect in this study suggests that extraction at 50 °C does not degrade the key antioxidant compounds present in duku leaves. Furthermore, as shown in previous sections, the phenolic and flavonoid content at 50 °C is higher than at other extraction temperatures, reinforcing the stability of these antioxidant compounds under the selected conditions.

A supporting precedent is found in the work of YIM et al. (2023), who optimized hot-water extraction of Schizophyllum commune aqueous extract using response surface methodology. They observed that while temperature had significant linear and quadratic effects on DPPH scavenging activity and total phenolic content (TPC), extraction time did not have a significantly interacting effect with temperature on DPPH activity. Their results showed that optimal antioxidant performance was primarily governed by temperature (around 35–42 °C), and extending extraction time beyond this range did not further enhance DPPH activity. Together, these results affirm that at moderate extraction temperatures (40–50 °C), extraction time plays a minor role in

antioxidant efficacy. In our case, the high phenolic and flavonoid content found at 50 °C already noted in earlier sections corroborates this pattern, confirming that these compounds remain stable and functionally active under controlled thermal conditions.

Effect of Temperature and pH on the Stability of Phenolic, Flavonoid Components, and Antioxidant Activity of *Lansium domesticum* Leaf Extract

In the second stage, the stability of phenol, flavonoid components, and the activity of duku leaf extract were tested at various heating temperatures and pH solutions. The stability of phenol flavonoid components and antioxidant activity of duku leaf extract at various temperatures are presented in Figure 4.

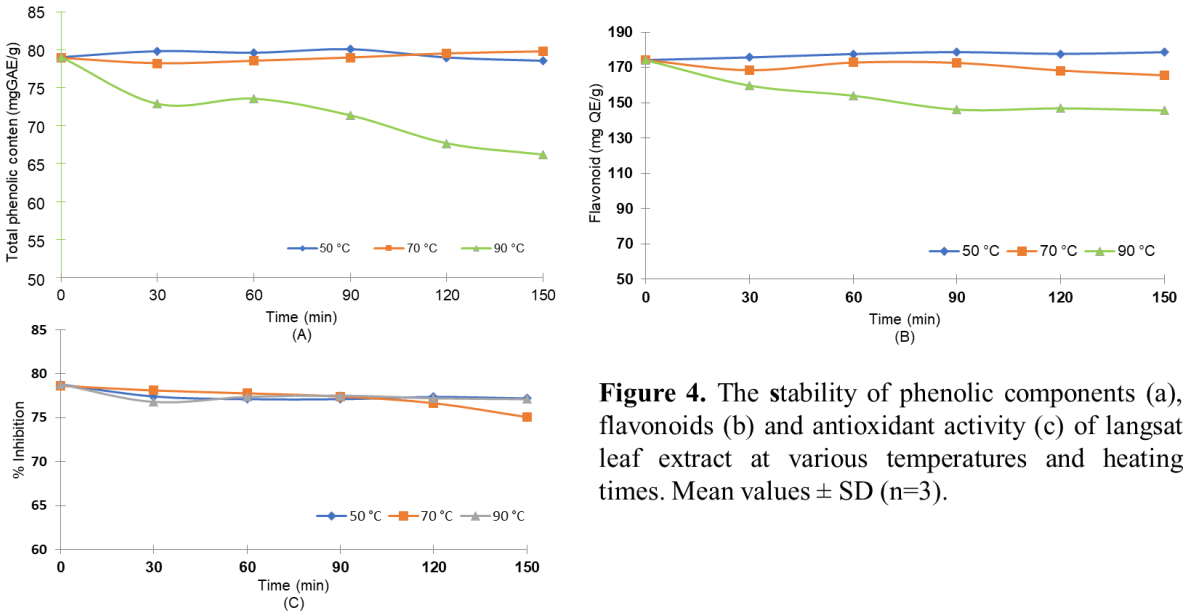

Figure 4. The stability of phenolic components (a), flavonoids (b) and antioxidant activity (c) of langsat leaf extract at various temperatures and heating times. Mean values ± SD (n=3).

The ANOVA indicated that temperature significantly affected the phenolic content of duku leaf extract. In the initial phase of heating, temperature did not significantly influence phenolic content. However, its effect became evident between 60 and 150 min. A higher temperature resulted in a greater reduction in phenolic content. Duncan's test revealed that at 60 min, the phenolic content of the extract heated at 50 °C was not significantly different from that at 70 °C, but was significantly different from that at 90 °C. At 150 min, the phenolic content of the extract at each temperature level showed significant differences.

The most substantial decline occurred at 90 °C after 150 min, with a reduction of 16.27%, whereas at 50 °C, the decrease was only 0.61%. These findings align with previous studies on various plant extracts, which have demonstrated that prolonged heating at high temperatures leads to a decline in phenolic acid content. Similarly, temperature significantly affected the flavonoid content of the extract. Higher temperatures resulted in lower flavonoid levels. Duncan's test indicated that flavonoid content was significantly different across all tested temperatures (50, 70, and 90 °C).

The most rapid degradation occurred at 90 °C, where flavonoid content decreased by 8.23% within 30 min and by 16.32% after 150 min. At 70 °C, flavonoid content declined by 5.04% after 90 min of heating. In contrast, heating at 50 °C for 150 min resulted in a 2.51% increase in flavonoid content, suggesting that mild heating may facilitate the release of bound flavonoids before degradation occurs.

The analysis of variance further confirmed that temperature significantly influenced the antioxidant activity of the extract, with its effect becoming evident after 60 min of heating. According to Duncan's test, at 60 min, the antioxidant activity of the extract heated at 50 °C was not significantly different from that at 90 °C but was significantly different from that at 70 °C. The overall changes in antioxidant activity after 150 min of heating at 50, 70, and 90 °C were 2.01%, 4.61%, and 2.14%, respectively. Interestingly, the decline in antioxidant activity was lower than the decrease in phenolic and flavonoid content. This discrepancy can be attributed to variations in the thermal stability of different phenolic compounds, as some phenolic constituents may remain active despite partial degradation.

This study also investigated the stability of phenolic, flavonoid components, and the antioxidant activity of duku leaf extract under different pH conditions. The results, presented in Figure 4, illustrate variations in phenolic and flavonoid content as well as antioxidant activity across different pH levels. These findings provide valuable insights into the optimal conditions for preserving the bioactive properties of the extract.

Effect of pH on Phenolic, Flavonoid Content, and Antioxidant Activity of *Lansium domesticum* Leaf Extract

The analysis of variance (ANOVA) indicated that the pH of the solution significantly affected the phenolic content of duku leaf extract. When the pH was reduced to 4, the phenolic content decreased by 6.31%. However, further acidification to pH 2 increased phenolic content. A similar pattern was observed under alkaline conditions; when the pH was increased to 8, the phenolic content decreased by 3.8%, but at pH 10, it increased again. These findings suggest that extreme acidic or alkaline conditions may influence the solubility and stability of phenolic compounds, leading to variations in their measured concentrations.

Statistical analysis also confirmed that pH significantly affected the flavonoid content of the extract. According to Duncan's test, there were significant differences in flavonoid content between pH 2 and pH 10, whereas no significant differences were observed between pH 4 and pH 8. In highly acidic conditions (pH 2), flavonoid content decreased by 10.73%, whereas in highly alkaline conditions (pH 10), it increased by 5.2%. These results suggest that flavonoids may be more susceptible to degradation in acidic environments but more stable or extractable under alkaline conditions.

The pH of the solution also significantly influenced the antioxidant activity of the extract. As pH increased, antioxidant activity decreased, whereas more acidic conditions enhanced antioxidant activity. The antioxidant activity of the extract increased by 19.28% when the pH was lowered from 7 to 2. Conversely, raising the pH from 7 to 10 resulted in a 13% decrease in antioxidant activity. This decline may be attributed to the instability of phenolic and flavonoid compounds in alkaline environments, leading to their degradation and subsequent reduction in antioxidant potential. These findings highlight the importance of pH in maintaining the stability and bioactivity of duku leaf extract, providing valuable insights into its optimal storage and application conditions.

CONCLUSION

The solvent ratio and extraction temperature affect the yield, phenol, flavonoid content, and antioxidant activity of the ethyl acetate extract of duku leaves. The extraction time affects the yield, phenol, and flavonoid content of the ethyl acetate extract of duku leaves, but does not affect the antioxidant activity. The best extraction conditions for extracting phenol compounds, flavonoids, and antioxidant activity from duku leaves with ethyl acetate solvent are the powder and solvent ratio of 1:20, extraction temperature of 50 °C for 80 min. Under these conditions, the extract yield was 5.98 ± 0.1%, phenol content of 40.49 ± 0.9 mg AGE/g extract, flavonoid content of 185 ± 3.9 mg QE/g extract, and antioxidant activity of 38.28 ± 0.2%. Moreover, our finding exhibited the importance of pH in maintaining the stability and bioactivity of duku leaf extract, providing valuable insights into its optimal storage and application conditions.

AUTHOR CONTRIBUTIONS

Conceptualization, FT, AN, and II; methodology, FT, AN; software, FT, ILT; validation, FT and ILT; formal analysis, FT, AN; investigation, FT, II; resources, FT; data curation, FT. and ILT; writing original draft preparation, FT and ILT; writing review and editing, FT, AN, II; visualization, FT, ILT; supervision, FT; project administration, FT; funding acquisition, FT. All authors have read and agreed to the published version of the manuscript.

FUNDING

This work was supported by the Indonesian Ministry of Research, Technology, and Higher Education for funding this research, number: 174/SP2H/LT/DRPM/2019.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable for studies not involving humans or animals.

INFORMED CONSENT STATEMENT

Not applicable because this study did not involve humans.

DATA AVAILABILITY STATEMENT

The data can be made available under request.

ACKNOWLEDGEMENTS

Thank you to the Department of Agricultural Product, Faculty of Agriculture, Universitas Jambi, for the research facilities.

CONFLICTS OF INTEREST

The author declares there is no conflict of interest.

REFERENCES

ABDALLAH HM et al. 2022. *Lansium domesticum* A Fruit with Multi-Benefits: Traditional Uses, Phytochemicals, Nutritional Value, and Bioactivities. Nutrients, 14: 1–42.

AHMED T et al. 2022. Optimization of ultrasound-assisted extraction of phenolic content & antioxidant activity of hog plum (*Spondias pinnata* L. f. kurz) pulp by response surface methodology. Heliyon 8: e11109.

ALTEMIMI A et al. 2017. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants 6: 42p.

ANGGESTIA W et al. 2024. Effect of Solvent Type on the Amount of Yield from Maceration of Moringa Plants (*Moringa oleifera*). Interdental Jurnal Kedokteran Gigi (IJKG) 20: 164–169.

ANTONY A & FARID M. 2022. Effect of Temperatures on Polyphenols during Extraction. Applied Sciences. 12: 2107.

BIN MOKAIZH AA et al. 2024. Ultrasonic-assisted extraction to enhance the recovery of bioactive phenolic compounds from *Commiphora gileadensis* leaves. Ultrasonics Sonochemistry 105: 106852.

DO QD et al. 2014. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. Journal of Food and Drug Analysis 22: 296–302.

ERYANI et al. 2020. Effect of Green Fertilizer of Gamal Plant (*Gliricidia sepium* (Jacq) DC.) On the Growth of Duku (*Lansium domesticum* Corr.). International Journal of Ecophysiology 2: 55–61.

HANUM L et al. 2018. Science & Technology Indonesia Morphological Diversity of *Lansium domesticum* Corr in South Sumatra. Sci. Technol. Indonesia 3: 41–44.

JOMOVA K et al. 2025. Flavonoids and their role in oxidative stress, inflammation, and human diseases. Chemico-Biological Interactions 413: 111489.

LI P et al. 2023. Extraction Optimization, Preliminary Identification, and Bioactivities in Corn Silk. Evidence-Based Complementary and Alternative Medicine 2023: 18p.

LIMA RC et al. 2023. Green ultrasound-assisted extraction of bioactive compounds of babassu (*Attalea speciosa*) mesocarp: Effects of solid-liquid ratio extraction, antioxidant capacity, and antimicrobial activity. Applied Food Research 3: 100331

LUBIS MF et al. 2022. A Review on Phytochemicals and Pharmacological Activities as Ethnomedicinal Uses of Duku (*Lansium domesticum* Corr.). Open Access Macedonian Journal of Medical Sciences 10: 57–65.

LUBIS MF et al. 2023. Cytotoxic activity of the purified extracts from duku (*Lansium domesticum* Corr.) Leaf against MCF-7 and HTB-183 cell lines, and the correlation with antioxidant activity. Journal of Medicinal and Pharmaceutical Chemistry Research 5: 1159–1172.

MUNIR T et al. 2018. An Overview of the Antibacterial Implications of *Lansium domesticum*. Journal of Basic & Applied Sciences 14: 206–209.

MUTHA RE et al. 2021. Flavonoids as natural phenolic compounds and their role in therapeutics: an overview. Future Journal of Pharmaceutical Sciences 7: 13p.

OPIRRUMAPE et al. 2017. The application of Mahoni, *Lansium domesticum* and *Lasium parasiticum* leaf from Gorontalo as natural pest insecticide on soybean plant. International Journal of ChemTech Research 10: 513–521.

PITHONAH NFM et al. 2023. Study on phenolic content, flavonoid content, and antioxidant capacity of extracts from *Lansium parasiticum* (Osbeck). Mediterranean Journal of Chemistry 13: 13.

PREDESCU NC et al. 2016. The influence of solid-to-solvent ratio and extraction method on total phenolic content, flavonoid content, and antioxidant properties of some ethanolic plant extracts. Revista de Chimie 67: 1922–1927.

SACI F et al. 2017. Optimization of phenolic compound recovery and antioxidant activity from carob pulp using response surface methodology. International Food Research Journal 24: 1094–1101.

SAI-UT S et al. 2023. Optimization of polyphenolic compounds from *Gossampinus malabarica* flowers by microwave-assisted extraction technology. Future Foods 8: 100271.

SHEN L et al. 2023. A comprehensive review of ultrasonic assisted extraction (UAE) for bioactive components: Principles, advantages, equipment, and combined technologies. Ultrasonics Sonochemistry 101: 106646.

SHI L et al. 2022. Extraction and characterization of phenolic compounds and their potential antioxidant activities. Environmental Science and Pollution Research 29: 81112–81129.

SYAMSUARDI S et al. 2018. Analysis of Genetic Impurity of An Original Cultivar Duku (*Lansium parasiticum* (Osbeck.) K.C. Sahni & Bennet.), from Jambi, Indonesia Using ITS and MatK Gene. International Journal of Environment, Agriculture and Biotechnology 3: 441–446.

VENKATACHALAM K. 2019. Bioactive Compounds of Longkong Fruit (*Lansium domesticum* Corr.). Reference Series in Phytochemistry: 1–16.

XU Z et al. 2021. Optimization of extraction of bioactive compounds from *Baphicacanthus cusia* leaves by hydrophobic deep eutectic solvents. Molecules 26: 13p.

YIM HS et al. 2013. Optimization of extraction time and temperature on antioxidant activity of *Schizophyllum commune* aqueous extract using response surface methodology. Journal of Food Science and Technology 50: 275–283.