Optimization of Phenolic and Flavonoid Extraction from Durian Peel: Effects of Solvent Type, Extraction Parameters, and Antioxidant Activity Evaluation

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Highlights:

- ✓ Optimal extraction achieved at 80°C, 15 minutes, and 70% ethanol.
- ✓ Ethanol extracts show superior antioxidant activity (IC50 = $68.61 \mu g/mL$).
- ✓ Durian peel extracts demonstrated strong DPPH radical scavenging activity.
- \checkmark Water offers an eco-friendly alternative for bioactive compound extraction.

Abstract

This study investigates the effects of extraction parameters, including temperature, time, solvent type, ethanol concentration, and solvent-to-material ratio, on the extraction efficiency of phenolic and flavonoid compounds from durian peels. Ethanol and water were utilized as solvents to evaluate their efficacy, and the antioxidant activity of the extracts was determined using the DPPH radical scavenging assay. Results revealed that ethanol extracts achieved superior phenolic and flavonoid yields and stronger antioxidant activity, with an IC50 value of 68.61 μ g/mL compared to 82.68 μ g/mL for water extracts. Optimal conditions for phenolic and flavonoid extraction were identified at 80°C and 15 minutes for both solvents, while 70% ethanol at a 30:1 solvent-to-material ratio demonstrated the highest extraction efficiency. The findings suggest that water can serve as a sustainable alternative to ethanol, offering an environmentally friendly and

cost-effective option for bioactive compound extraction. This study highlights the potential of valorizing durian peels as a source of natural antioxidants for pharmaceutical, cosmetic, and food applications.

1/ Introduction

Durian peels, often considered agricultural waste, are rich in bioactive compounds, including phenolics and flavonoids, which have significant potential for various applications (Zhan et al., 2021). Research indicates that these compounds possess antioxidant, antimicrobial, and antiinflammatory properties, making them valuable for use in pharmaceuticals, cosmetics, and food preservatives (Charoenphun and Klangbud., 2022). Utilizing durian peels for extracting these bioactives not only adds economic value but also addresses environmental concerns associated with agricultural waste disposal (Zhan et al., 2021). By converting durian peels into valuable products, waste is reduced, and environmental pollution is mitigated. This approach aligns with sustainable agricultural practices, promoting environmental stewardship while enhancing economic gains (Tran et al., 2023). Several phenolic compounds in durian exhibit strong antioxidant activity, highlighting their potential in health-related products (Charoenphun and Klangbud., 2022). Studies have demonstrated the antimicrobial properties of extracted flavonoids from durian peels, suggesting beneficial uses in food preservation and safety (Charoenphun and Klangbud., 2022). The strategic extraction of bioactive compounds from durian peels represents an innovative approach to transforming agricultural waste into high-value products, thereby alleviating waste disposal issues and reducing environmental pollution.

The efficiency of extracting phenolic and flavonoid compounds from durian (Durio zibethinus) peels is highly dependent on factors such as temperature, extraction time, ethanol concentration, and the ratio of ethanol to durian peel. Optimizing these parameters is essential to maximize yield and maintain the bioactivity of the extracts. Elevated temperatures enhance the solubility of phenolic compounds and improve mass transfer rates, thereby boosting extraction efficiency. However, excessive temperatures can degrade thermolabile compounds. Similarly, prolonged extraction times increase compound diffusion but may also lead to the oxidation or hydrolysis of phenolics and flavonoids. Thus, determining an optimal balance between temperature and time is crucial to achieving high yields while preserving compound integrity. The extraction of phenolics and flavonoids from Curcuma zedoaria leaves revealed that a temperature of 75 °C and an extraction time of 92 minutes were optimal for maximizing yield while preserving compound integrity (Azahar et al., 2017). Researchers highlighted that excessively high temperatures or prolonged extraction times could degrade thermolabile phenolic compounds, emphasizing the critical need for careful parameter optimization (Azahar et al., 2017). Similarly, the extraction of flavonoids from red and brown rice bran identified an ideal temperature of 60 °C and an extraction time of 40 minutes. The study noted that higher temperatures or extended durations could lead to the oxidation or decomposition of flavonoid structures, reinforcing the importance of precise parameter control to maintain compound stability (Ghasemzadeh et al., 2018).

The polarity of the solvent is another critical factor influencing the extraction of phenolic compounds. ethanol concentrations from 50 to 70% are highly effective for extracting phenolics

and flavonoids from plant materials, such as durian peels. The highest total phenolic content and the greatest antioxidant activity of the extracts were achieved using 50% ethanol at 40°C (Jiménez-Moreno et al., 2019). Tran et al. (2024) found that using 75% ethanol at 60°C yielded the highest flavonoid content from durian fruit rinds (Tran et al., 2024). Similarly, Bambang and Sani (2018) demonstrated that ultrasonic-assisted extraction using a 1:9 ratio of durian peel to 70% ethanol for 20 minutes resulted in significant antioxidant activity and elevated levels of total phenolics and flavonoids (Bambang and Sani., 2018).

The efficiency of extracting phenolic and flavonoid compounds from plant materials, including durian peels, is greatly influenced by the polarity of solvents. Research indicates that solvents with intermediate polarity, such as ethyl acetate resulted in higher total phenolic and flavonoid contents, as well as superior antioxidant and antimicrobial activities, compared to more polar solvents like methanol and water (Palaiogiannis et al., 2023). For example, a study on *Avicennia officinalis* L. leaves found that acetone extracts had higher total phenolic and flavonoid contents than those obtained with methanol, ethanol, ethyl acetate, dichloromethane, or chloroform (Khalili et al., 2022). Similarly, research on red onion skin extracts showed that ethyl acetate produced greater phenolic and flavonoid contents along with superior antioxidant and antimicrobial activities compared to polar solvents like methanol and water (Khalili et al., 2022).

Additionally, the ratio of ethanol to durian peel significantly affects the concentration gradient between the solid and liquid phases, influencing the mass transfer of bioactive compounds. While a higher solvent-to-solid ratio can enhance extraction efficiency, diminishing returns are observed beyond a certain threshold. A study on ultrasonic-assisted extraction of durian peels found that varying the ratio of ethanol to peel affected both yield and total phenolic content, with optimal results achieved at specific ratios (Bambang and Sani., 2018).

In this study, we investigated the effects of various factors, such as extraction temperature and time, ethanol concentration, and the solvent-to-durian peel ratio, on the extraction efficiency of phenolic and flavonoid compounds from durian peel using ethanol and water. Additionally, the antioxidant activity of ethanol and water extracts was evaluated using DPPH, aiming to explore the potential of replacing ethanol with water as an extraction solvent. This approach aims to reduce production costs, minimize environmental impact, and improve health safety.

2/ Methods and chemicals

2.1/ Chemicals and reagents

Durian peels were sourced from Durian Shops, Ho Chi Minh City, Vietnam. The chemicals utilized for raw material analysis included aquadest, ethanol 95% (Vietnam), gallic acid (Merck), quercetin (Merck), Na₂CO₃ (China), AlCl₃ (China), DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma-Aldrich, 90%), and Folin-Ciocalteu reagent (China). The equipment employed in this study comprised UV-Vis spectrophotometer (Shimadzu), HPLC-UV (LC6000, SCION Instrument), an oven, and various glassware.

Durian peels were collected from some durian shops in Ho Chi Minh City and transported to the laboratory of Nguyen Tat Thanh University (333 Highway A1, District 12). At the laboratory, the durian peels were cut into small pieces and dried at 70°C until a constant weight

was achieved. After that, the dried durian peels were ground into smaller particles using a DE-300 grinder.

2.2/ Effect of extraction conditions on phenolics and flavonoids

2.2.1/ Effect of temperature and time

The study investigated the impact of temperature and time on the extraction efficiency of phenolics and flavonoids from durian peels. The extraction process using ethanol was conducted at varying temperatures (50, 60, 70, and 80°C), while water extraction was performed at temperatures of 70, 80, 90, and 100°C. Samples were collected at specific time intervals: 15, 30, 60, 90, 120, 180, 240, 360, and 480 minutes. The solvent-to-durian peel ratio was maintained at 20:1, with the ethanol concentration set at 70%. The temperature of the extract was monitored and controlled using a temperature sensor, with an accuracy deviation of approximately $\pm 2^{\circ}$ C.

2.2.2/ Effect of ethanol concentration

The extraction conditions involved using various ethanol concentrations (60%, 70%, 80%, and 95%) to extract phenolics and flavonoids from durian peels. Other initial parameters, including temperature, extraction time, and the ethanol-to-durian peel powder ratio, were kept constant. The extraction temperature and time were set based on the optimal values determined in step 2.2.1, with the ethanol-to-durian peel ratio maintained at 20:1 (V/W).

2.2.3/ Effect of ratio between solvents and durian peel (V/W)

The extraction conditions were investigated by testing different ratios of ethanol or water volume to durian peel powder weight (10:1, 20:1, and 30:1 (V/W)) to optimize the extraction of phenolics and flavonoids from durian peels. Meanwhile, other initial parameters, including ethanol concentration 95%. Temperature and extraction time were set based on the optimal values determined in steps 2.2.1 and 2.2.2 respectively.

2.3/ Phenolics analysis

The total phenolic content was determined using a colorimetric method with Folin-Ciocalteu reagent at 760 nm, using gallic acid as the standard (According to International Standard ISO 14502-1, 2005). The procedure involved mixing the extract with distilled water, Folin-Ciocalteu reagent, and Na₂CO₃ solution, followed by incubation at room temperature before spectrophotometric measurement. Standard and test samples were analyzed under identical conditions, with results calculated based on the gallic acid standard curve and expressed as mg GAE per gram of extract.

The total phenolic content (TPC) is calculated using the formula:

$$TPC = C^*k^*V$$

Where:

- > TPC: Total phenolic content (mg GAE/g).
- > C: The x-value from the gallic acid standard curve ($\mu g/mL$).
- \succ V: Volume of the extract solution (mL).
- \succ k: Dilution factor.

2.4/ Flavonoids analysis

The total flavonoid content was measured using a spectrophotometric method involving the formation of a complex with AlCl₃ (Pękal and Pyrzynska., 2014). A 1 mL portion of the appropriately diluted extract was combined with 0.5 mL of 10% AlCl₃ solution and 0.5 mL of water. The mixture was left to incubate at room temperature for 10 minutes before being analyzed at a wavelength of 428 nm using a UV-Vis spectrophotometer. The same procedure was applied to both standard and test samples, with each experiment performed in triplicate to calculate the average. The total flavonoid content was determined using the quercetin standard curve and expressed as milligrams of quercetin equivalent (mg QE) per gram of extract.

The total flavonoid content (TFC) is calculated using the following formula:

$$\mathbf{TFC} = \mathbf{C}^* \mathbf{k}^* \mathbf{V}$$

Where:

- ➤ TFC: Total flavonoid content (mg QE/g extract).
- > C: The x-value from the quercetin standard curve ($\mu g/mL$).
- \succ V: Volume of the extract solution (mL).
- \succ k: Dilution factor.

2.5/ High-performance liquid chromatography-Ultravioliet (HPLC-UV) analysis

The analysis of phenolics (catechin, gallic acid, epigallocatechin gallate, epicatechin) and flavonoids (quercetin, kaempferol) using the HPLC model LC6000 involves preparing standard solutions for calibration and filtering extract samples through membranes to remove impurities. The analysis is conducted on a reverse-phase C18 column with a gradient mobile phase (water with 0.1% acid and methanol or acetonitrile) at 1.0 mL/min flow rate, 20 μ L injection volume, and 30°C column temperature. UV detection is set at 280 nm for phenolics and 360 nm for flavonoids, with a run time of 30-40 minutes. Results are determined by comparing peak areas to calibration curves and expressed as mg/g or μ g/mL. Each sample is analyzed in triplicate, and parameters like recovery (%) and RSD (%) are evaluated for reliability.

2.6/ Determination of antioxidant activity

Antioxidant activity was evaluated by assessing the free radical scavenging activity through the decolorization reaction of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol (Asekun et al., 2013). The reaction mixture consisted of 1 mL of 0.135 mM DPPH solution and 1 mL of the test solution at various concentrations. The mixture was shaken thoroughly and left in the dark for 30 minutes. The optical absorbance was measured at a wavelength of 517 nm using a UV-VIS spectrophotometer. Each experiment was performed in triplicate to calculate the average value. Quercetin was used as the positive control and was processed under the same conditions as the test samples, while methanol (MeOH) was used as the blank.

The DPPH free radical scavenging ability is determined using the following formula:

% Inhibition =
$$\frac{A_{0}-A}{A_0} * 100$$

Where:

> A₀: The absorbance of the control sample, consisting of DPPH and MeOH.

 \succ A: The absorbance of the test sample, consisting of DPPH and the extract.

Results are expressed as the percentage of DPPH free radical scavenging activity. IC_{50} is defined as the minimum concentration of the extract required to scavenge 50% of DPPH radicals. It is determined based on the linear relationship between the extract concentration and the percentage of DPPH scavenging activity.

The sigmoid (logistic) function is used to estimate the relationship between concentration and % inhibition:

$$Y = a + \frac{b-a}{1+e^{\frac{c-X}{d}}}$$

Where:

Y: % inhibition (inhibitory effect at concentration X).

- X: Concentration of the test substance ($\mu g/mL$).
- a: Minimum % inhibition value.

b: Maximum % inhibition value.

c: IC₅₀ (concentration causing 50% inhibition, to be determined).

d: Slope adjustment factor of the curve.

2.7/ Statistical analysis

All experiments for antioxidation activity were performed in triplicate, and the results are expressed as the mean \pm standard deviation (SD). To evaluate variations among the assays, analysis of variance (ANOVA) was conducted using Minitab 20 software (State College, Pennsylvania, USA), followed by Tukey's multiple comparison test with a significance level of P < 0.05.

3/ Results and discussion

3.1/Effect of temperature and extraction time on extracted efficiency of phenolics and flavonoids

3.1.1/Effect on phenolics extracted efficiency

Fig. 1 illustrates the phenolic extraction efficiency over time at different temperatures, using solvents including water (Fig 1a) and ethanol (Fig 1b). Phenolic extraction efficiency varied significantly with temperature and time for both ethanol and water. Using ethanol, phenolic extraction at room temperature remained minimal, with a maximum yield of 0.064 mg/L after 480 minutes, while at 80°C, it reached 1.33 mg/L within 15 minutes, followed by a gradual decrease. The efficiency at 80°C was 20.43 times higher than at room temperature. At 50°C, the phenolic concentration increased slowly, peaking at 0.98 mg/L after 120 minutes. Higher temperatures (60°C and 70°C) yielded faster phenolic increases, with maximum concentrations of 1.12 mg/L at 60°C (after 90 minutes) and 1.35 mg/L at 70°C (after 60 minutes).

For water, phenolic extraction at room temperature was negligible, with a maximum of 0.015 mg/L after 15 minutes. At 90°C, the concentration rapidly increased to 1.13 mg/L within 30 minutes, while at 70°C, 80°C, and 100°C, the maximum phenolic concentrations were 0.98, 1.06, and 1.17 mg/L, respectively, after 60 minutes. Extraction efficiency at 100°C was 76.56 times higher than at room temperature. These results highlight the significant impact of temperature on phenolic extraction efficiency for both solvents, with ethanol outperforming water in most conditions. Therefore, the optimal conditions for extracting phenolics from durian peels using solvents including water or ethanol were a temperature of 80°C and an extraction time of 15 minutes.

The results demonstrate a significant influence of temperature on phenolic extraction efficiency, with higher temperatures enabling faster and more effective extraction of phenolic compounds. Temperatures between 70°C and 80°C are identified as optimal for phenolic extraction, achieving high efficiency within a short duration. Higher temperatures enhance molecular motion, reduce material viscosity, increase water penetration, and improve contact area, thereby boosting extraction efficiency. Polar phenolic compounds were extracted at lower temperatures, while less polar phenolic compounds were extracted at higher temperatures (Ibañez et al. 2003). The decrease in water polarity at elevated temperatures enables it to dissolve non-polar compounds, facilitating their extraction. Thus, depending on the extraction temperature, phenolic compounds can be selectively extracted with high specificity (Antony and Farid, 2022).

At 50°C, the extraction process is slower, likely due to limited cell structure disruption at this temperature. In contrast, phenolic extraction is more vigorous at 60°C to 80°C, peaking during the initial stages before gradually declining due to thermal effects (Nafiunisa et al., 2019). The gradual decline in phenolic concentration after reaching a peak, particularly at higher temperatures such as 80°C, can be attributed to the thermal degradation of phenolic compounds when exposed to prolonged high temperatures. While high temperatures accelerate extraction initially, they can also lead to compound denaturation if extraction times are excessively long. This underscores the

importance of optimizing both temperature and time to prevent loss of valuable phenolic compounds.

The comparison of phenolics extraction efficiency between ethanol and water demonstrates distinct differences in yield under various conditions. Ethanol extraction showed higher efficiency across all tested parameters compared to water extraction, suggesting its superior solubilizing capability for phenolic compounds. Notably, as extraction temperatures increased, both solvents displayed an upward trend in phenolics yield, but ethanol consistently outperformed water, particularly at higher temperatures. This trend highlights ethanol's ability to disrupt plant cell matrices more effectively and solubilize phenolics at elevated temperatures.

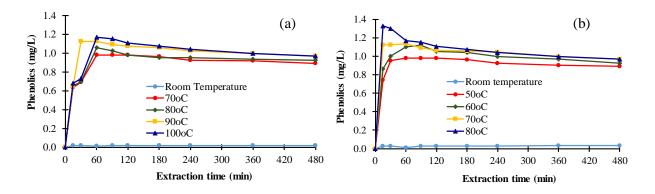


Figure 1: The effect of extraction temperature and time of water (a) and ethanol (b) on the phenolic extraction efficiency from durian peels.

3.1.2/ Effect on flavonoids extracted efficiency

Fig. 2a shows the flavonoid concentrations when water is used as the solvent at different temperatures (room temperature, 70°C, 80°C, 90°C, and 100°C) over 480 minutes. At higher temperatures (70°C-100°C), flavonoid concentrations increased rapidly within the first 60 minutes, followed by a gradual decline or stabilization. At 70°C, the peak concentration ranged between 0.9 and 1.0 mg/L after 90 minutes, while at 90°C and 100°C, the peak concentrations were 0.97 mg/L and 0.75 mg/L at 15 minutes, respectively, before declining over time. At room temperature, the flavonoid concentration remained very low, peaking at only 0.023 mg/L after 480 minutes. Fig. 2b illustrates the changes in flavonoid concentrations when ethanol is used as the solvent at various temperatures (room temperature, 50°C, 60°C, 70°C, and 80°C) over 480 minutes. The highest flavonoid concentrations were observed at 50°C (1.3 mg/L at 90 minutes) and 60°C (1.29 mg/L at 60 minutes), followed by a gradual decline over time. At 70°C and 80°C, the peak concentrations were lower, reaching 0.87 mg/L at 30 minutes and 0.92 mg/L at 15 minutes, respectively, before decreasing. At room temperature, the extraction process was slow and inefficient, with a maximum concentration of only 0.073 mg/L after 240 minutes. These results suggest that higher temperatures (above 60°C) initially accelerate flavonoid extraction but also promote degradation over time, whereas moderate temperatures (50°C and 60°C) yield more stable and efficient extraction. The optimal conditions for extracting flavonoids from durian peels using water or ethanol were a temperature of 60°C and an extraction time of 60 minutes.

The observed effects of temperature and solvent type on flavonoid extraction efficiency are consistent with findings in existing literature. Studies have shown that higher extraction temperatures can enhance the yield of flavonoids due to increased solubility and diffusion rates. However, excessive temperatures may lead to the degradation of these compounds. For instance, Nikolić et al. (2018) reported that thermal degradation of flavonoids follows first-order kinetics, with significant losses occurring at elevated temperatures (Nikolić et al., 2018). Additionally, the choice of solvent plays a crucial role in extraction efficiency. Ghanimi et al. found that methanol and ethanol are more effective solvents for flavonoid extraction compared to water, likely due to their ability to better solubilize flavonoid compounds (Ghanimi et al., 2022). These studies corroborate the current observations that moderate temperatures and the use of ethanol as a solvent optimize flavonoid extraction while minimizing degradation.

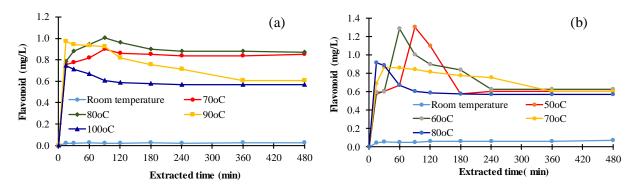


Figure 2: The effect of extraction temperature and time of ethanol (a) and water (b) on the flavonoids extraction efficiency from durian peels.

In general, the optimal conditions for extracting phenolics and flavonoids from durian peels using solvents such as water or ethanol in this study were a temperature of 80°C and an extraction time of 15 minutes.

3.1.3/ HPLC-UV analysis

Fig 3 presents HPLC chromatograms showing the identification and quantification of quercetin (Q) and kaempferol (K) from durian peel extracts under varying temperatures and extraction times. Panel (a) serves as the standard chromatogram for quercetin and kaempferol, with retention times of approximately 23.096 minutes and 25.062 minutes, respectively. Panels (b) and (b') illustrate the concentrations of Q and K extracted by ethanol at 80°C and water at 90°C after 30 minutes, showing notable differences in compound recovery between solvents. Similarly, panels (c) and (c') represent the concentrations of Q and K after 60 minutes, while panels (d) and (d') depict the results after 240 minutes for ethanol at 80°C and water at 90°C. Across the conditions, ethanol consistently demonstrated higher extraction efficiency compared to water, as

reflected by sharper peaks and higher intensities for both Q and K. Additionally, prolonged extraction times generally resulted in a decrease in compound concentration, likely due to thermal degradation or compound instability at higher temperatures. This emphasizes the importance of optimizing both temperature and time to maximize phenolic compound recovery while minimizing potential losses.

Fig. 4a shows the concentration changes of two flavonoid compounds, Quercetin and Kaempferol, extracted using water at 90°C over 360 minutes. Quercetin concentration increased rapidly, reaching its peak of 2.87 mg/L after 30 minutes, followed by a gradual decline. This indicates that Quercetin is efficiently extracted in the early stage but decreases due to degradation or loss during prolonged high-temperature extraction. In contrast, Kaempferol exhibited a much lower concentration, with a slight increase during the first 60 minutes, peaking at around 0.5 mg/L, and remaining stable thereafter. This suggests that Kaempferol is less affected by temperature or that the current extraction method is less effective for this compound. Overall, extraction at 90°C is effective for Quercetin in a short time frame, but prolonged extraction reduces its yield. For Kaempferol, adjustments to the method or extraction conditions may be necessary to improve recovery. Fig. 4b illustrates the concentration changes of Quercetin and Kaempferol during flavonoid extraction using ethanol at 80°C over 240 minutes. Both compounds showed a rapid increase in concentration during the first 60 minutes, followed by a slower but steady rise from 60 to 240 minutes. Quercetin reached a concentration of nearly 1.46 mg/L at 240 minutes, while Kaempferol peaked at approximately 1.35 mg/L. These results indicate that ethanol at 80°C is an effective solvent for extracting both Quercetin and Kaempferol, with a consistent upward trend in concentrations throughout the extraction process. However, Quercetin was extracted slightly more efficiently than Kaempferol, likely due to differences in their chemical properties and interactions with ethanol at this temperature.

The comparison of flavonoid extraction efficiency from durian peels highlights clear differences between water and ethanol as solvents at varying temperatures. The temperature at 90°C, Quercetin was extracted effectively in the initial stage, peaking after 30 minutes, but its concentration declined rapidly over time, indicating high initial efficiency but potential losses due to degradation with prolonged extraction. In contrast, the temperature at 80°C demonstrated a slower but more stable extraction process for both Quercetin and Kaempferol, with concentrations steadily increasing over 240 minutes without signs of decline. Temperature also plays a crucial role, with the temperaturer at 90°C enabling rapid but unsustainable extraction, while the temperature at 80°C offers greater stability and sustainability for flavonoid extraction from durian peels.

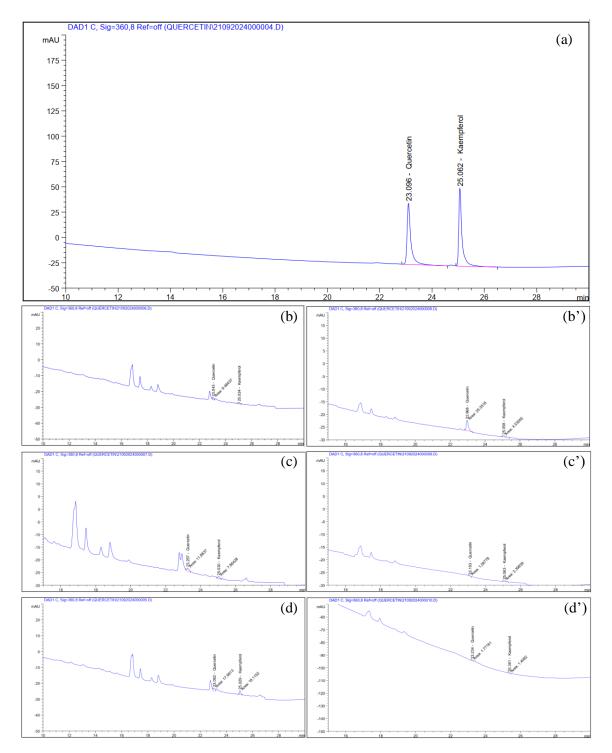


Figure 3: HPLC chromatogram for different temperature and extracted times of durian fruit peels. (a) standard for Quercetin (Q) and Kaempferon (K); (b) concentration Q and K by ethanol at 80°C after 30 min; (b') concentration Q and K by water at 90°C after 30 min; (c) concentration Q and K by ethanol at 80°C after 60 min; (c') concentration Q and K by water at 90°C after 60 min; (d) concentration Q and K by ethanol at 80°C after 240 min; (d') concentration Q and K by water at 90°C after 240 min;

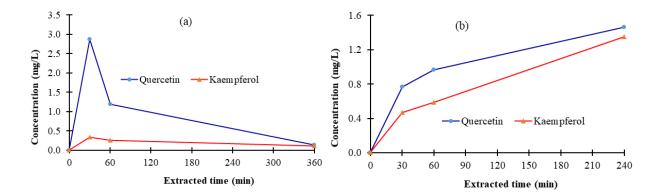


Figure 4: The extraction efficiency of phenolic compounds using water at 90°C (a) and ethanol at 80°C (b).

Fig. 5a demonstrates the changes in the concentrations of Catechin, Gallic acid, Epicatechin, and Epigallocatechin gallate when water was used as the extraction solvent at 90°C for 360 minutes. Catechin showed the highest increase, reaching 16.85 mg/L after 360 minutes, indicating its effective extraction under high-temperature water conditions. Epicatechin also gradually increased but to a lesser extent, reaching 3.64 mg/L. Gallic acid exhibited a much lower concentration, peaking at 0.579 mg/L after 30 minutes and then becoming undetectable from 60 to 360 minutes, suggesting it may degrade at high temperatures. Similarly, Epigallocatechin gallate was not detected throughout the extraction, indicating its susceptibility to thermal degradation at 90°C. These findings suggest that water at 90°C is effective for extracting Catechin and Epicatechin but not suitable for Gallic acid and Epigallocatechin gallate due to thermal instability. Fig. 5b illustrates the phenolic compound concentrations when ethanol was used at 80°C for 240 minutes. Catechin had the highest concentration, exceeding 40.15 mg/L, with a rapid increase during the first 60 minutes, followed by a gradual rise and stabilization. This highlights ethanol's effectiveness at 80°C for Catechin extraction. Other compounds, including Gallic acid, Epicatechin, and Epigallocatechin gallate, also showed increasing concentrations over time, reaching 6-7.05 mg/L, 3.26-9.04 mg/L, and 3.08-8.46 mg/L, respectively, after 240 minutes. Unlike water, Gallic acid and Epigallocatechin gallate were stable and steadily extracted in ethanol at 80°C. These results indicate that ethanol at 80°C is an effective solvent for extracting a broader range of phenolic compounds while maintaining their stability. Temperature affects the stability of Catechin, Gallic acid, Epicatechin, and Epigallocatechin gallate during the extraction process. Catechin and Epicatechin demonstrated thermal stability at 90°C, with their concentrations steadily increasing over time, peaking at 360 minutes. In contrast, Gallic acid and Epigallocatechin gallate were thermally unstable at 90°C, showing degradation under these conditions. However, all compounds exhibited stability at 80°C, indicating that a lower extraction temperature is more suitable for preserving the integrity of thermally sensitive compounds.

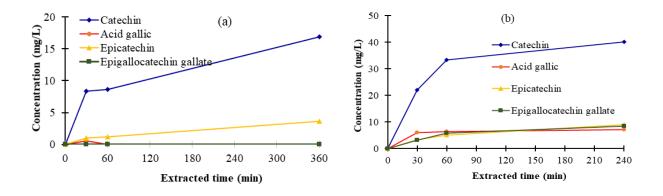


Figure 5: The extraction efficiency of phenolic compounds using water at 90°C (a) and ethanol at 80°C (b).

3.2/ Effect of ethanol concentration

The Fig. 6 illustrates the effect of varying ethanol concentrations (60%, 70%, 80%, and 95%) on the extraction efficiency of total phenolic content (TPC) and total flavonoid content (TFC), measured in mg/L. Ethanol (70%) yielded the highest extraction efficiencies, with TPC and TFC reaching approximately 1.3 mg/L and 0.7 mg/L, respectively. Both lower (60%) and higher ethanol concentrations (80% and 95%) resulted in decreased extraction efficiencies, suggesting that 70% ethanol is optimal for extracting these compounds.

These findings are consistent with previous research. For instance, a study on the extraction of phenolic compounds from grape stems found that a 50% ethanol solution at 40°C was most effective, highlighting the significant influence of ethanol concentration on extraction efficiency (Jiménez-Moreno et al., 2019). Similarly, research on flavonoid extraction from natural sources indicates that optimal yields are often achieved with ethanol concentrations ranging from 35% to 90%, depending on the specific plant matrix and compounds targeted (Chaves et al., 2020). The observed variations in extraction efficiency across different studies can be attributed to factors such as differences in plant materials, extraction temperatures, and solvent-to-solid ratios. Therefore, while a 70% ethanol concentration appears optimal in this context, the ideal conditions for extracting phenolic and flavonoid compounds may vary depending on the specific characteristics of the plant material and the extraction parameters employed.

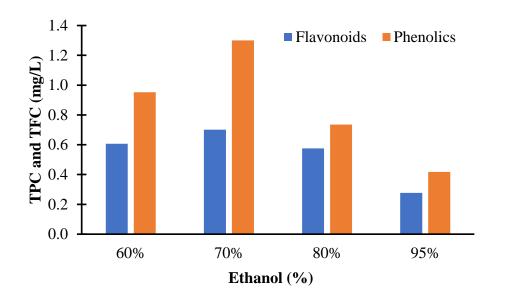


Figure 6: Effect of ethanol concentration on phenoloics and flavonoids extraction

3.3/ Effect of solvent and durian peel ratio

Fig. 7 show the Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) extracted from durian peels using water and ethanol at varying solvent-to-material ratios (10:1, 20:1, 30:1). Ethanol demonstrated superior extraction efficiency compared to water across all ratios, with TPC peaking at 1.3 mg/g and TFC at 1.0 mg/g at a 30:1 ratio. The observed trend that increasing the solvent-to-material ratio enhances extraction efficiency up to a certain point, beyond which additional increases yield minimal gains-is well-documented in the literature. For instance, a study published in BMC Chemistry examined the effects of temperature, time, and solvent ratios on the extraction of phenolic compounds. The researchers found that while increasing the solvent ratio improved phenolic extraction efficiency, there was a threshold beyond which no significant additional benefits were observed. This suggests that excessively high solvent-to-material ratios may lead to diminishing returns in extraction efficiency (Che Sulaiman et al., 2017). Similarly, research on the extraction of phenolic compounds from Lavandula stoechas using ultrasound-assisted extraction indicated that an optimal liquid-to-solid ratio exists. Beyond this optimal point, further increases in the solvent ratio did not result in significant improvements in extraction yield or total phenolic content (Ez zoubi et al., 2021). These findings align with the general understanding that while increasing the solvent-to-material ratio can enhance the extraction of phenolic compounds, there is an optimal point beyond which additional solvent does not significantly improve extraction efficiency. Ethanol's intermediate polarity allows it to dissolve both polar and slightly non-polar compounds effectively, whereas water's high polarity limits its capacity to extract less polar flavonoids. These findings align with Elboughdiri (2018) investigated the extraction of phenolic compounds from olive leaves and found that optimal conditions included a solvent-to-solid ratio of 30:1, an ethanol concentration of 80% (v/v), and a temperature of 40°C (Elboughdiri., 2018). These conditions were effective in maximizing the yield of total phenolic compounds.

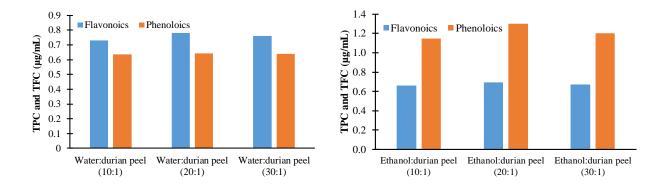


Figure 7: Effect of ratio of Ethanol/Water: durian peel on phenoloics and flavonoids extraction

3.4/ Antioxidant activity

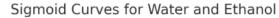
The results from Table 1 and Fig. 8 demonstrate the superior DPPH radical scavenging activity of ethanol extracts compared to water extracts from durian peels. Ethanol extract achieved a lower IC50 value of 68.61 μ g/mL, indicating greater efficiency in neutralizing DPPH radicals, and reached 99.95% inhibition at 100 μ g/mL. In contrast, the water extract had a higher IC50 value of 82.68 μ g/mL, with a maximum inhibition of 86.67% at the same concentration. The sigmoidal curves in Fig. 8 illustrate a dose-dependent relationship, with ethanol consistently outperforming water across all concentrations. The extract from durian peels using water also demonstrated good antioxidant activity compared to the ethanol extract. However, the antioxidant activity of the ethanol extract was approximately 1.2 times higher than that of the water extract. This suggests that water can be used as an alternative solvent to ethanol for extracting bioactive compounds from durian peels, contributing to enhanced economic efficiency and environmental protection.

This result reflects ethanol's superior ability to extract phenolic and flavonoid compounds, which are primary contributors to radical scavenging activity. The sigmoidal relationship between concentration and % inhibition observed in both extracts suggests a dose-dependent response, where higher concentrations yield increased DPPH inhibition. The observed results align with previous studies on the effectiveness of ethanol as a solvent for antioxidant compound extraction. This study compared various assays for estimating antioxidant activity from guava fruit extracts and found that ethanol-based extracts exhibited significantly higher antioxidant activity compared to water extracts. The authors attributed this to ethanol's intermediate polarity, which allows for the dissolution of both polar and non-polar phenolic compounds (Lohvina et al., 2022). Similarly, Amina et al. (2018) reported enhanced phenolic and flavonoid extraction using 70% ethanol, leading to lower IC50 values in DPPH assays compared to pure water (Amina et al., 2019). In addition, the IC50 value of the ethanol extract in this study compares favorably with other plantbased studies. Ashraf et al. (2024) evaluated the antioxidant potential of citrus peel extracts and found that the IC50 values for DPPH radical scavenging activity ranged from 19.53 to 41.88 mg/mL, depending on the citrus variety and extraction solvent. The lowest IC50 value was observed in mussambi (Citrus sinensis) peel extracts using 90% methanol as the solvent. These findings suggest that the antioxidant activity of durian peel ethanol extracts is comparable to or

better than some citrus peel extracts, depending on the extraction conditions and citrus variety. The water extract's IC50 value, while higher, is also consistent with the trend observed in studies like Elboughdiri et al. (2018), where water-based extractions yielded weaker antioxidant activities due to the limited solubility of less polar compounds. Overall, this study highlights the importance of solvent selection in antioxidant extraction, with ethanol demonstrating greater efficiency in extracting bioactive compounds that contribute to DPPH radical scavenging. Future work could explore mixed solvent systems or optimize extraction conditions to further enhance antioxidant activity.

Table 1: DPPH scavenging activity of the ethanol and water extract and fractions from durian peels.

Solvents	DPPH Inhibition (%)					
	20 (µg/mL)	40 (µg/mL)	60 (µg/mL)	80 (µg/mL)	100 (μg/mL)	IC ₅₀ (µg/mL)
Water	36.44	45.45	47.32	66.24	86.67	82.68
Ethanol	40.59	58.67	60.92	79.38	99.95	68.61



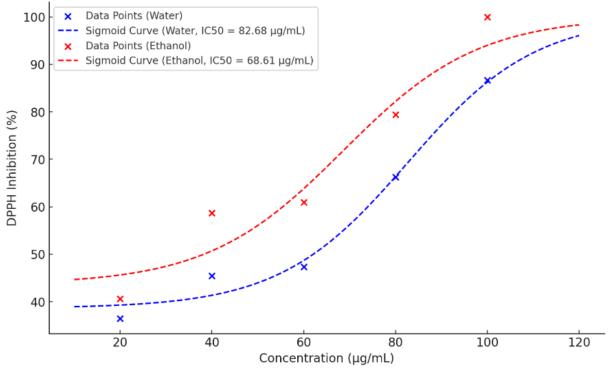


Figure 9: The sigmoid curve describes the relationship between concentration (μ g/mL) and % DPPH inhibition for both solvents (water and ethanol).

4/ Conclusion

This study highlights the effectiveness of different extraction parameters on the recovery of phenolic and flavonoid compounds from durian peels, with a focus on comparing water and ethanol as solvents. The results demonstrated that ethanol, particularly at a concentration of 70% and a 30:1 solvent-to-material ratio, significantly outperformed water in extracting bioactive compounds and exhibited stronger antioxidant activity, as evidenced by its lower IC50 value. However, water also showed promising potential as a sustainable and eco-friendly solvent for phenolic and flavonoid extraction, albeit with slightly lower antioxidant efficacy. Optimal extraction conditions for both solvents were achieved at 80°C and 15 minutes.

These findings suggest that durian peels, an agricultural byproduct, can be effectively valorized as a source of natural antioxidants for various applications in the food, cosmetic, and pharmaceutical industries. Additionally, the use of water as a solvent offers a more environmentally friendly and cost-efficient alternative to ethanol, supporting sustainable extraction practices. Further research is recommended to explore mixed solvent systems and to scale up extraction processes for industrial applications.

Declaration of Competing Interest

The authors affirm that they do not possess any identifiable financial interests or personal relationships that might have influenced the findings presented in this paper.

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