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Influence of extraction techniques on the phytochemical contents and antioxidant properties of *Pometia pinnata* seed extracts

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Abstract. *Patilaya P, Sumantri IB. 2025. Influence of extraction techniques on the phytochemical contents and antioxidant properties of* Pometia pinnata *seed extracts. Biodiversitas 26: 3582-3589.* Matoa (*Pometia pinnata*) seeds contain phytochemicals with vigorous antioxidant activity. However, their antioxidant activity may be affected by extraction techniques. Here, we report the impacts of extraction techniques on the chemical constituents and antioxidant properties of *P. pinnata* seed extracts. The plant seeds were collected in Medan (Indonesia) in January 2020. The seed extracts, obtained by maceration and Soxhlation, were evaluated for their phytochemicals and antioxidant properties. GraphPad Prism 10 was used to analyze the data, with one-way ANOVA and Tukey's tests at a 5% significance level. The results demonstrated that the extraction yields of *P. pinnata* seeds obtained by maceration (10.41-38.63%) were slightly lower than those by soxhlation (15.95-49.78%). The total phenolic contents of extracts obtained by maceration (EM) and soxhlation (ES) were 25.29-46.88 mg g^{-1} and 46.46-93.26 mg g^{-1}, respectively. The total flavonoid contents of EM and ES were 0.90-11.77 mg g^{-1} and 3.25-20.78 mg g^{-1}, respectively. In the antioxidant assay, IC_{50} values of EM and ES were 63.95-1035.14 µg mL^{-1} and 37.25-102.02 µg mL^{-1}, respectively. The results suggest that soxhlation was more effective than maceration, yielding higher phytochemical contents and antioxidant properties of *P. pinnata* seed extracts.

Keywords: Antioxidant, maceration, matoa, *Pometia pinnata*, Soxhlet extraction

Abbreviations: DPPH: Diphenyl Picrylhydrazyl; EM: Extracts obtained by Maceration; ES: Extracts obtained by Soxhlation; EtOAc: Ethyl Acetate; EtOH: Ethanol; GAE: Gallic Acid Equivalence; IC_{50}: 50% Inhibitory Concentration; QE: Quercetin Equivalence; TFC: Total Flavonoid Content; TLC: Thin Layer Chromatography; TPC: Total Phenolic Content

INTRODUCTION

Free radicals are independent chemical species with an unpaired electron. They are highly reactive, short-lived, and capable of triggering chain reactions. They bind to chemical particles to form stable complexes. Free radicals interact with other molecules via redox mechanisms by donating or accepting their electrons. It may lead to human diseases, such as diabetes mellitus, inflammation, multiple sclerosis, cancer, cardiovascular disease, cataracts, rheumatoid arthritis, asthma, aging, and neurodegenerative disease (Martemucci et al. 2022). The diseases can be prevented by antioxidants (Rahaman et al. 2023).

Many antioxidants are found in plants (e.g., fruits and seeds). Among them is *Pometia pinnata* J.R.Forst. & G.Forst. (Figure 1.A), which belongs to the Sapindaceae family. *P. pinnata* is a tropical plant that grows up to 20-40 m in height. The ripe fruits have yellowish-green, green-reddish, or blackish-red skin (Yuniastuti et al. 2023). The plant has been used to treat several ailments, such as diarrhea, flu, fever, and infectious diseases (Janatiningrum et al. 2024). *P. pinnata* leaves exhibit biological activities such as antibacterial, antioxidant, anti-hypertension, nephroprotective, hepatoprotective (Utoro et al. 2022), antidiabetes (Wahyuni et al. 2022), analgesic (Santi et al. 2023), and larvicide (Ukratalo et al. 2024). The fruits exhibit antioxidant (Hajar et al. 2021) and cytotoxic properties (Bakhtra et al. 2022). The fruit peel and stem bark of *P. pinnata* show larvicidal activity against mosquitoes (Augusthinus et al. 2024; Moniharapon and Moniharapon 2025). The plant bark indicates antidiabetic activity in the in vivo study (Prihanti et al. 2020). Hajar et al. (2021) reported that *P. pinnata* seeds exhibited antioxidant and antibacterial properties. The plant seeds may also have potential benefits in cancer (Hanafi et al. 2020), diabetes mellitus and hypertension treatments (Nasution and Hadiati 2020), and nephroprotection therapy (Adrian et al. 2021).

In addition, *P. pinnata* seeds contain flavonoids, tannins, saponins, and terpenoids. Such compounds are most commonly obtained through extraction with organic solvents, particularly by maceration (Hanafi et al. 2020) or soxhlation (Sholiha et al. 2024). Maceration is a simple extraction technique, but it is time-consuming and requires a large amount of solvent. However, because it is a cold extraction technique, it is suitable for extracting heat-sensitive compounds. Another method used to extract phytochemicals from plant seeds is sequential cold maceration and percolation. Those techniques can reduce the chemical complexity in the plant extracts. This simplifies the fractionation and isolation processes of the particular

phytochemical compounds (Bitwell et al. 2023). Conversely, soxhlation is a hot extraction technique. This technique involves a continuous hot solvent extraction. The heat may increase the solubility of the phytochemicals and the extraction yields. It may also degrade the desired bioactive compounds (Patel et al. 2019). Therefore, the comparison study is important, mainly to obtain an effective method for extraction (Mungwari et al. 2025). The proper extraction methods can produce good-quality extract. A change in the chemical contents of the extracts may directly affect their biological activities, including antioxidant properties (Ramesh et al. 2024). However, there have been no reports on the effects of different extraction techniques on *P. pinnata* seed extract's chemical and biological properties.

Furthermore, there are several studies related to the effects of maceration and soxhlation on the phytochemical and antioxidant properties of plant extracts, but the results vary (Risnadewi et al. 2019; Saptarini and Wardati 2020; Mansouri et al. 2021; Özmatara 2021). Consequently, there is a need to establish an extraction method for each plant sample individually (Wołosiak et al. 2021). This study aims to compare maceration and soxhlation techniques on *P. pinnata* seed extract properties, including the extraction yield, phytochemical content, and antioxidant activity.

MATERIALS AND METHODS

Materials

Pometia pinnata seeds (Figure 1.C) were collected from local garden (Medan, Indonesia) in January 2020. Analytical-grade chemicals from Merck (Rahway, NJ, USA), such as 96% ethanol, acetic acid, aluminum trichloride ($AlCl_3$), ascorbic acid standard, butanol, chloroform, ethyl acetate, diphenyl picrylhydrazyl (DPPH), gallic acid, hydrochloric acid, iron (III) chloride, isopropanol, magnesium powder, methanol, *n*-hexane, quercetin, sodium carbonate, sodium acetate, and distilled water, as well as Dragendorff, Meyer, Folin-Ciocalteu (FC), and Liebermann-Burchat reagents, were used in this study.

Extraction

Maceration technique

The *P. pinnata* ripe fruits (Figure 1.B) were separated from the pulp. The seeds were cleaned, oven-dried, and ground using an electric grinder. At room temperature, the seed powder (100 g) was soaked in 300 mL of *n*-hexane with occasional stirring for 72 h. The liquid portion was separated, and the residue was re-soaked in 300 mL of *n*-hexane twice. After all filtrates were combined, the solvent was evaporated to obtain the crude extracts of *P. pinnata* seeds. The dried residue was macerated in ethyl acetate and followed by 96% ethanol with the same procedure. The extraction yield was expressed as a percentage (%), the ratio between the dried extract and dried seed weights (Abdealsiede et al. 2020).

Soxhlation technique

Briefly, 100 g of dried seeds was heated in a Soxhlet apparatus containing 300 mL of *n*-hexane at 75°C for 48 h. The solution was concentrated to produce the crude extract. The residue was dried and re-extracted successively in ethyl acetate, followed by 96% ethanol using the same procedure. The extraction yield was also expressed as a percentage (%) (Mahire and Patel 2020).

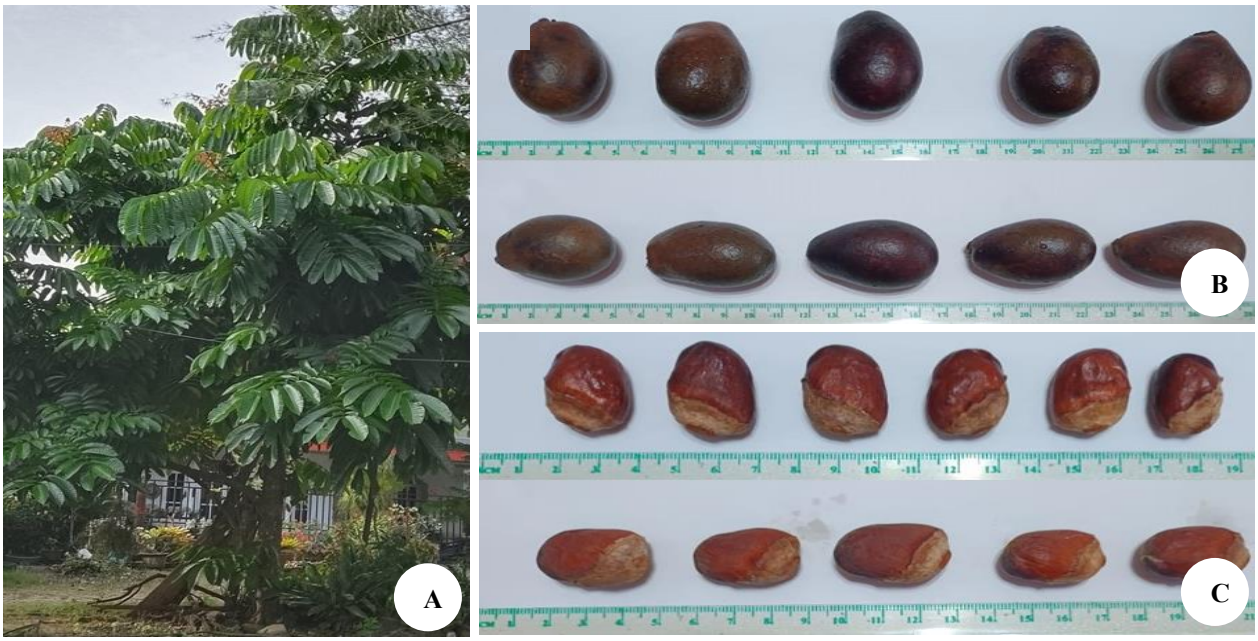


Figure 1. *Pometia pinnata* used in the study: A. Tree, B. Fruits, C. Seeds

Phytochemical screening

The screening procedures by Dubale et al. (2023) were used in this study. Alkaloid, flavonoid, glycoside, saponin, tannin, and terpenoid in the *P. pinnata* seeds were evaluated as follows:

Alkaloids

One hundred milligrams of the sample were combined with 2% hydrochloric acid (5 mL). The mixture was heated in a water bath. After filtration, the filtrate was divided into four tubes. Meyer's, Dragendorff's, and Wagner's reagents were added into the first, second, and third tubes, respectively. The fourth tube was used as a control. Alkaloids were present if a yellowish-white, reddish-brown, and brown precipitate was formed in the first, second, and third tubes, respectively.

Flavonoids

A sample (100 mg) was extracted in ethanol (5 mL). The filtrate was divided into two tubes. Mg powder and 2 N hydrochloric acid (0.5 mL) were added to tube 1, while tube 2 was used as a control. The formation of dark red, green, or blue colors in tube 1 revealed the appearance of flavonoid compounds.

Glycosides

The sample (100 mg) was extracted in distilled water (5 mL). The aqueous extract was placed in a tube and combined with NaOH solution. Glycosides were considered present if a yellow color was formed in the solution.

Saponins

A sample (0.5 mL) was combined with hot distilled water (5 mL). After cooling, the mixture was shaken vigorously for about 10 sec. Saponin was present if foam was formed in the tube. The foam has remained after adding 2 N HCl.

Tannins

The sample (100 mg) was boiled in distilled water (10 mL). The aqueous extract was placed in a tube and mixed with 0.1% ferric chloride. Tannin was present if the mixture turned blue-black.

Triterpenoids

The sample (0.5 mL) was combined with chloroform (2 mL) and sulfuric acid (3 mL). A reddish-brown color indicated the presence of triterpenoids.

Analysis of phenolic and flavonoid contents

A procedure of Nayeem et al. (2022) was applied for determining the TPC and TFC in *P. pinnata* seed extracts. A solution of standard gallic acid (1 mg mL^{-1}) was made in 80% methanol (10 mL). This gallic acid solution was diluted in distilled water to obtain the calibration standards (100-500 µg mL^{-1}). The extract solution (1 mg mL^{-1}) was prepared by dissolving 100 mg of the crude extract in 100 mL of 80% methanol. Next, each solution (0.1 mL) was mixed with distilled water (7.9 mL) and FC reagent (0.5 mL). This mixture was combined with 20% sodium carbonate (1.5 mL) and incubated for 90 min. Subsequently, the absorbances of the mixture were checked with a UV/VIS spectrophotometer (Shimadzu-UV1800) at 754 nm. The calibration curve of gallic acid is presented in Figure 2.A. TPCs were reported as gallic acid equivalents in the dried extract (mg GAE g^{-1}).

To quantify TFC, the calibration solutions (5-30 µg mL^{-1}) were provided by further diluting a quercetin standard stock solution in distilled water. The extract solution of *P. pinnata* seeds (1 mg mL^{-1}) was used for this quantification. Next, those solutions (2 mL) were combined with 10% $AlCl_3$ (0.1 mL), 1 M CH_3COONa (0.1 mL), and distilled water (2.8 mL). The mixtures were allowed for 40 min. Finally, their absorbances were checked with a UV/VIS spectrophotometer (Shimadzu-UV1800) at 431 nm. The calibration curve of quercetin is presented in Figure 2.B. TFCs were recorded as quercetin equivalent in the dried extract (mg QE g^{-1}).

Thin layer chromatographic analysis of plant extracts

Thin Layer Chromatographic (TLC) analysis of plant extracts was conducted by using Mustarichie et al. (2019) and Aljubiri et al. (2021) methods. About 2 µL of the ethyl acetate solution of the extracts (100 mg mL^{-1}) were spotted on silica gel TLC plates (Merck). The TLC plates were dried in a fume hood. The plates were transferred to the saturated chamber containing *n*-hexane and ethyl acetate in a ratio of 8.5:1.5. Once the eluent reached the marked line on the TLC plate, the plate was removed from the developing chamber. The plates, after being dried, were sprayed with Liebermann-Burchard's reagent and subsequently observed under 366 nm ultraviolet light. Other plates were developed in a mobile phase involving butanol-acetic acid-distilled water (4:1:5). The dried plates were visualized with 10% AlCl3. Each plate was then examined under 366 nm ultraviolet light. The phytochemical compounds were detected by observing the Rf values and color of bands.

Evaluation of antioxidant activity

The antioxidant properties of *P. pinnata* seed extracts were evaluated using a method adapted from Herawati et al. (2021). Briefly, a solution of 0.1 mM DPPH (2.5 mL) was combined with 0.5 mL of each plant extract and ascorbic acid (antioxidant standard), each with concentrations ranging from 20 to 100 µg mL^{-1}. Those mixtures were then kept for 30 min at 30°C, and their absorbances were determined at 517 nm. The antioxidant properties were evaluated according to the DPPH inhibition percentage. It was calculated by multiplying the ratio of the difference between the control and sample absorbances to the control absorbance by 100%.

Then, the 50% inhibitory concentration (IC_{50}) was determined using a regression equation derived by plotting the inhibition of free radicals (%) against sample concentrations. The IC_{50} was defined as the extract or ascorbic acid concentrations that inhibited 50% of DPPH.

Statistical analysis

The data were expressed as the mean and standard error. GraphPad Prism 10 was used to conduct a One-Way

ANOVA and Tukey's Post Hoc Test on the data. A significant difference was recognized if the *p*-value<0.05. Conversely, it was not significantly different if the *p*-value>0.05.

RESULTS AND DISCUSSION

Extraction yield and phytochemical compounds

The extraction yields of *P. pinnata* seeds obtained by maceration and soxhlation techniques using three different solvents, n-hexane, Ethyl Acetate (EtOAc), and Ethanol (EtOH), are summarized in Table 1. The extraction yields were used to assess the effectiveness of different extraction techniques. Using maceration, *n*-hexane produced the highest yield of extraction, outperforming EtOH and EtOAc. A similar pattern was observed with the soxhlation method. Soxhlation produced an extraction yield approximately 1.29 to 1.53 times greater than that achieved by maceration. Statistical analysis indicated that extraction techniques significantly affect the yields of *P. pinnata* seed extracts (p<0.05). Soxhlation outperformed maceration in terms of extraction yields.

Table 1 also presents the phytochemical constituents of *P. pinnata* seeds. The phytochemical analysis of *P. pinnata* seed extracts demonstrated the presence of secondary metabolites, such as alkaloids, flavonoids, glycosides, saponins, and triterpenoids. Flavonoids, glycosides, and triterpenoids were present in all extracts. However, alkaloids and saponins were exclusively identified in the ethanolic extracts produced by both maceration and soxhlation. Consequently, it appears that the extraction techniques did not have a significant impact on the phytochemical composition of the seed extracts.

Phenolic and flavonoid contents of *P. pinnata* extracts

The Total Phenolic Contents (TPCs) of *P. pinnata* seed extracts were determined by a regression line of gallic acid ($y = 0.002x + 0.0128$ with $r^2 = 0.9990$) (Figure 2.A). The highest TPC was observed in ethanolic extracts obtained by maceration, followed by EtOAc and *n*-hexane extracts (Table 2). A similar trend was also seen in extracts obtained by soxhlation, except that the TPCs in soxhlation extracts were 1.84-2.81 times higher than the maceration extracts (p<0.05). The results indicated that the extraction techniques affected the TPCs of *P. pinnata* seed extracts.

The Total Flavonoid Contents (TFCs) of seed extracts were obtained from a regression equation of quercetin ($y = 0.032x + 0.0187$ with $r^2 = 0.9991$) (Figure 2.B). The maximum TFCs were observed in the ethanolic extracts produced by both maceration and soxhlation, followed by EtOAc and *n*-hexane extracts (Table 2). Consistently, the TPCs of extracts from soxhlation were 1.49-3.61 times higher than those from maceration. When statistically analyzed at a 95% confidence level, the TFCs of maceration and soxhlation extracts were significantly different (p<0.05).

Table 1. Extraction yield and phytochemical constituents of *Pometia pinnata* seed extracts

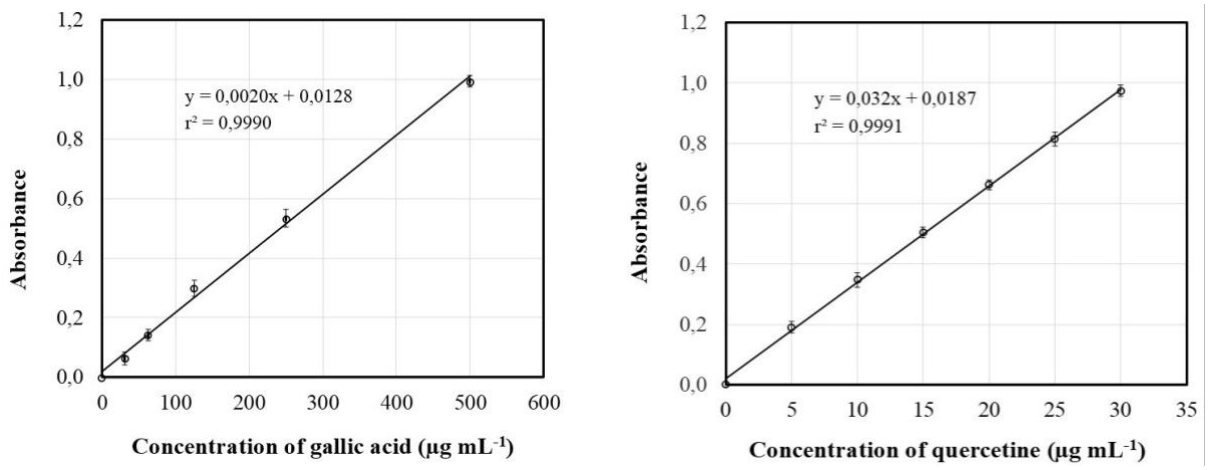
Extraction technique	Sample	Extraction yield (%)	Phytochemical compounds					
			Alkaloids	Flavonoids	Glycosides	Saponins	Tannins	Triterpenoids
Maceration	*n*-Hexane extract	38.63±0.223[a]	-	+	+	-	-	+
	Ethyl acetate extract	10.41±0.060[b]	-	+	+	-	-	+
	Ethanolic extract	13.05±0.075[c]	+	+	+	+	-	+
Soxhlation	*n*-Hexane extract	49.78±0.287[d]	-	+	+	-	-	+
	Ethyl acetate extract	15.95±0.092[e]	-	+	+	-	-	+
	Ethanolic extract	17.64±0.102[e]	+	+	+	+	-	+
-	Dried seed	-	+	+	+	+	-	+

Note: Extraction yield is represented by the mean value of three repetitions (n=3). Different superscript letters on data indicate a statistically significant difference (p<0.05) between the corresponding groups. Conversely, similar superscript letters indicate no statistically significant difference. +: present, -: absent

Table 2. TPC, TFC, and DPPH IC_{50} of *Pometia pinnata* seed extracts

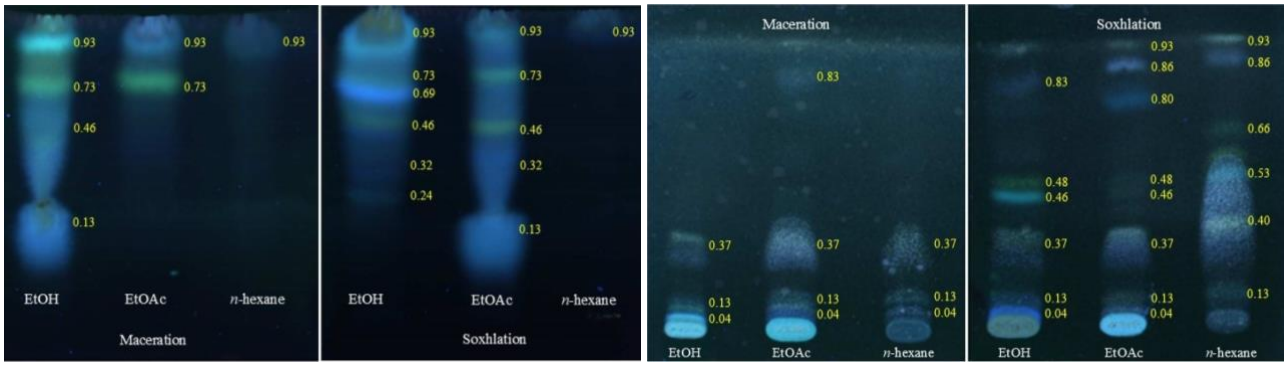
Extraction technique	Sample	TPC (mg GAE g^{-1})	TFC (mg QE g^{-1})	IC_{50} (µg mL^{-1})
Maceration	*n*-Hexane extract	25.29±0.054[a]	0.90±0.053[a]	1035.14±50.137[a]
	Ethyl acetate extract	30.90±0.081[b]	4.91±0.023[b]	137.30±2.452[b]
	Ethanolic extract	46.88±0.134[c]	11.77±0.074[c]	63.95±0.922[c]
Soxhlation	*n*-Hexane extract	46.46±0.066[c]	3.25±0.273[d]	102.02±1.142[b]
	Ethyl acetate extract	86.85±0.028[d]	7.30±0.030[e]	44.01±0.134[c]
	Ethanolic extract	93.26±0.022[e]	20.78±0.037[f]	37.25±0.152[d]
-	Dried seed	-	-	11.88±0.421[d]

Note: TPC, TFC, and IC_{50} are presented as the mean of three repetitions (n=3). Different superscript letters on data indicate a statistically significant difference (p<0.05) between the corresponding groups. Conversely, similar superscript letters indicate no statistically significant difference



A **B**

Figure 2. Calibration curves of: A. Gallic acid, and B. Quercetin standards are generated using triplicate measurements (n=3)



A **B**

Figure 3. TLC chromatogram of *Pometia pinnata* seed extracts using the eluent system of butanol-acetic acid-water (4:1:5) sprayed with: A. Liebermann-Burchard's reagent, and B. *n*-hexane-ethyl acetate (8.5:1.5) sprayed with $AlCl_3$

TLC profile

Thin Layer Chromatography (TLC) of *P. pinnata* seed extracts, developed using butanol-acetic acid-water (4:1:5) as an eluent system and Liebermann-Burchard's reagent as a visualizing agent, showed some variations in the phytochemical contents of the ethanolic, ethyl acetate, and *n*-hexane extracts (Figure 3.A). The ethanolic extract from maceration gave four major bands with Rf values of 0.13 (sky blue), 0.46 (greenish blue), 0.73 (emerald green), and 0.93 (turquoise). The ethyl acetate extract mostly gave two bands with Rf values of 0.73 (emerald green) and 0.93 (blue). The *n*-hexane extract gave only one band with a sky-blue color at an Rf value of 0.93. Interestingly, the band increased when the seed extracts were obtained using soxhlation, especially in the ethanolic and ethyl acetate extracts (Figure 3.A). On the other hand, the *n*-hexane extract shows only one sky blue band with an Rf value of 0.93.

TLC analysis of the seed extracts, developed using *n*-hexane-EtOAc (8.5:1.5) and visualized with $AlCl_3$ (Figure 2.B), further confirmed the chemical content variation between those extracts. Thus, this study showed that the extracts from soxhlation generally contained more compounds than those from maceration.

Antioxidant activity

The antioxidant activity of *P. pinnata* extracts was measured by their scavenging DPPH radical (Herawati et al. 2021). The most potent antioxidant activity was recognized in the ethanolic extract from maceration with an IC_{50} value of 63.95±0.922 µg mL^{-1} (Table 2). This was followed by the ethyl acetate extract (IC_{50} = 137.30±2.452 µg mL^{-1}) and *n*-hexane extract (IC_{50} = 1035.14±50.137 µg mL^{-1}). Statistical analysis indicated significant differences between the IC_{50} values of the maceration extracts (p<0.05). Therefore, it may be concluded that solvents used in the extraction can directly influence the antioxidant power of the extracts of *P. pinnata* seeds. This study also demonstrated that the antioxidant capacity of *P. pinnata* seed extract from maceration significantly increases with increasing solvent polarity. A similar phenomenon was also observed in the extracts from soxhlation, albeit the differences were less dramatic (Table 2).

Moreover, significant differences between the IC_{50} values of extracts from maceration and those from soxhlation

(p<0.05) were observed, suggesting that the extraction techniques also affected the antioxidant activity of *P. pinnata* seed extracts. Overall, extracts derived via soxhlation demonstrated lower IC_{50} than those derived via maceration. Interestingly, while the IC_{50} of the ethanolic extract of *P. pinnata* seed obtained by soxhlation was higher than that of ascorbic acid (IC_{50} = 11.88±0.421 µg mL^{-1}), they are not statistically different (p>0.05).

Discussion

The present research compared the effects of two conventional extraction techniques, maceration and soxhlation, on the phytochemicals and antioxidant properties of *P. pinnata* seed extracts. The results showed that different extraction techniques significantly affect extract properties, such as extraction yield, phytochemical composition, and antioxidant activity. Soxhlation has been reported to be a more effective method than maceration, as a higher temperature used during soxhlation may increase the solubility of phytochemicals more than in the maceration technique (Abduh et al. 2023). While we did observe that soxhlation gave higher extraction yields of *P. pinnata* seed extracts than maceration, they were less than those obtained in previous studies (Nasution and Hadiati 2020). The present study also showed that *P. pinnata* seeds are rich in nonpolar compounds. According to Sholiha et al. (2024), the plant seeds contain fatty acid methyl ester compounds, such as methyl arachidate, methyl linolenic, methyl eicosadienoate, and methyl eicosatetraenoate. Those compounds are Poly-Unsaturated Fatty Acid (PUFA) types with potential antioxidant activity (Gawron-Skarbek et al. 2023).

This study also showed that higher quantities of phenolics and flavonoids were found in the *P. pinnata* seed extracts from soxhlation than those from maceration. It may also be due to the increased solubility of the phenolic and flavonoid compounds in hot solvents (Abduh et al. 2023). However, the TPC and TFC extracted from *P. pinnata* seeds varied, depending on the solvent used. The ethanol produced higher TPC and TFC values than ethyl acetate, which in turn yielded more than n-hexane (Table 2). According to Aatif (2023), polyphenolic compounds can decompose into phenolic acids and flavonoids. Small-molecule phenolic compounds are soluble in water. Nevertheless, condensation with other molecules renders these compounds insoluble in water. Flavonoids are commonly soluble in organic solvents but insoluble in water. However, their solubility in highly polar solvents may increase when coupled with polar compounds, e.g., with sugars, to form glycosides (Zhao et al. 2019). A previous study indicated that while flavonoids were absent in the n-hexane and ethyl acetate extracts of *P. pinnata* seeds, they were present in the methanol extract (Hanafi et al. 2020). The effects of extraction techniques on phytochemical content in other Sapindaceae species were reported. The soxhlation yields a *Sapindus rarak* fruit extract with a higher polyphenolic content than the maceration (Fitria et al. 2024). The phenolic content in *Nephelium lappaceum* (*rambutan*) peel initially increases with higher temperatures but decreases when temperatures reach or exceed 80°C (Tingting et al. 2022). In *Dimocarpus longan* peel, the TPC and TFC are highest in methanol extract, followed by ethanol and acetone extracts (Alam et al. 2023). In addition, *Litchi chinensis* peel extracts exhibit higher phenolic content when produced by modern extraction techniques, such as microwave and ultrasound-assisted extractions, compared to maceration (Deshmukh et al. 2024).

TLC analysis of *P. pinnata* seed extracts was conducted using different spraying agents, such as Liebermann-Burchat and $AlCl_3$. It was conducted to visualize the specific compounds of plant extracts. Liebermann-Burchat was used for the identification of terpenoids and steroids (Wutsqa et al. 2021). While $AlCl_3$ was used for the identification of flavonoids (Sultana et al. 2024). TLC profiling of the *P. pinnata* seed extracts showed the presence of various compounds with different Rf values and colors (Figure 3). Under UV light at 366 nm, triterpenes and sterols produce different colors of bands, such as blue, brown, green, pink, and yellow. Flavonoids, especially flavones, isoflavones, and flavanones, react with $AlCl_3$ to form a blue complex (Kaboré et al. 2022). In other studies, flavonoids produced yellow (Sammanta et al. 2024) and yellowish-blue bands (Mustarichie and Runadi 2021). This study also showed that the chemical constituents of extracts from soxhlation were greater than those from maceration. The most phytochemical spots had Rf values below 0.5. It indicates that the *P. pinnata* seed extracts contain more nonpolar compounds (Akhtar et al. 2022).

This study also revealed that the soxhlation-derived extracts were more potent in antioxidant activity than the maceration extracts. Interestingly, the ethyl acetate and ethanol extracts obtained by soxhlation exhibited powerful antioxidant activity with IC_{50}<50 µg mL^{-1} (Alam et al. 2020). The increase in antioxidant activity of plant extracts at higher temperatures can be attributed to three main mechanisms. The first is due to the increase in the activity of antioxidant enzymes (Zhou et al. 2019), such as catalase, superoxide dismutase, and glutathione peroxidase (Sundar et al. 2024). The second is the inactivation of polyphenol enzymes, which leads to the inhibition of polyphenolic degradation. The third is the formation of antioxidant agents via the Maillard reaction (Shamsul and Zahrah 2019). The increase in antioxidant activity of the plant extracts is also related to the increase in the TPC and TFC (Praptiwi et al. 2022; Asyhar et al. 2023).

Overall, different extraction methods, including maceration and soxhlation, resulted in varied phytochemical profiles and antioxidant activities in *P. pinnata* seed extracts. While we found that soxhlation is a better extraction method than maceration concerning the phytochemical contents and antioxidant capacity of extracts from *P. pinnata* seeds, how it fares against modern extraction techniques, such as microwave, ultrasonic, or supercritical extractions, remains to be investigated.

In conclusion, this work demonstrates that the extraction techniques significantly affect the yield of extraction, phytochemical composition, and antioxidant activity of *P. pinnata* seed extracts. Soxhlation proved to be a more

effective method than maceration, yielding higher extraction efficiency, richer phytochemical content, and more potent antioxidant properties. This technique can be considered for application in the pharmaceutical and food fields. However, further studies using modern extraction techniques are needed to obtain a complete overview of the effects of extraction techniques on the phytochemical and antioxidant properties of *P. pinnata* seed extract.

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