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Toxicity and Antioxidant Activity Test of Rambutan Seeds (*Nephelium lappaceum L.*)

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Abstract

Rambutan (*Nephelium lappaceum* L.) seeds have long been utilized in traditional medicine due to their potential therapeutic properties. This study aimed to determine the secondary metabolite profile, toxicity, and antioxidant activity of the methanolic extract and ethyl acetate fraction of rambutan seeds to strengthen their potential application in traditional medicine. The powdered of rambutan seed was extracted with a maceration technique using methanol, followed by fractionation using n-hexane and ethyl acetate. The methanol extract and ethyl acetate fraction were tested for toxicity using the Brine Shrimp Lethality Test (BSLT) and for antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Phytochemical screening was performed using standard qualitative method to identify secondary metabolites, which confirmed the presence of phenols, tannins, and flavonoids in both samples. Toxicity testing indicated LC_{50} values of 688.86 ppm for the methanol extract and 825.68 ppm for the ethyl acetate fraction. Antioxidant activity assessment revealed IC_{50} values of 328.61 ppm for the methanol extract and the higher activity for ethyl acetate fraction of 32.78 ppm. These results indicate that the ethyl acetate fraction of rambutan seeds may serve as a promising natural source of antioxidants for therapeutic development.

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INTRODUCTION

Rambutan (*Nephelium lappceum L.*) is a tropical fruit tree native to Southeast Asia and belongs to the Sapindaceae family (Bhattacharjee et al., 2022). It is widely across Indonesia, Malaysia, Thailand and the Philippines, and is known for it sweet, juicy fruit that is commonly consumed fresh or processed into value-added products (Sukmandari et al., 2017). Various part of the rambutan plant, including the leaves, bark, root, fruits, skin, pulp, and seeds have traditional utilized and are associated with significant therapeutic potential. The plant is well-known for its nutritional value and is reported to possess potentially significant amounts of bioactive constituent, including polyphenol, flavanoid, alkaloid, essential mineral, dietary fiber. These compounds are associated with various biological activities such as antioxidant, antimicrobial, anticancer, and antidiabetic (Afzaal et al., 2023; Bhat, 2019; Bhattacharjee et al., 2022; Gusman & Tsai, 2015; Lee et al., 2020; Mistriyani et al., 2021; Rojas et al., 2023; Sukmandari et al., 2017; Tsong et al., 2021).

The rambutan seed represents an underutilized component with considerable potential. Although the fruit pulp is widely consumed, the seeds are frequently discarded during processing, thereby contributing to agricultural waste. These by-products can serve as valuable

sources of functional compounds due to their low cost and wide availability (Sai-Ut et al., 2023). Recent studies have indicated that rambutan seeds may posses significant biological activities (Afzaal et al., 2023; Jahurul et al., 2020).

For instance, Fidrianny et al., (2015) reported that ethanolic seed extract from the Lebak Bulus variety demonstrated strong antioxidant activity with an IC_{50} value of 7 μg/mL and 7.34 μg/mL for ethyl acetate seed extract of the Rajah variety. Similarly, Soeng et al., (2015) reported the highest antioxidant activity of ethyl acetate of rambutan seed with IC_{50} value of 104.03 μg/mL. Consistent with earlier studies, the methanolic extract of rambutan seeds has also demonstrated potential antioxidant activity (Nguyen et al., 2019). The antioxidant potential of rambutan seed extract is mainly due to their rich content of secondary metabolites, including phenolic compounds as well as flavanoids and tannins (Afzaal et al., 2023; Jahurul et al., 2020; Lee et al., 2020; Nguyen et al., 2019; Nguyen et al., 2025; Tsong et al., 2021) Several studies have reported low IC_{50} values in antioxidant assay, indicating strong free radical scavenging activity of rambutan seed (Estrada-Gil et al., 2022; Kaptso et al., 2022).

Although numerous studies have demonstrated the antioxidant potential of rambutan seed extracts, the majority have focused on crude or ethanolic extracts. Limited data are available regarding the antioxidant activity and toxicity of other solvent fractions, such as methanol and ethyl acetate. This is a critical consideration, as solvent type can influence the profile of extracted bioactive compounds, potentially affecting both efficacy and safety. Furthermore, existing toxicity evaluations have primarily been conducted on ethanolic extracts, while the safety profiles of other fractions remain insufficiently explored. This research gap must be addressed to ensure the safe and effective use of rambutan seed extracts in pharmaceutical or nutraceutical applications. Therefore, this study aims to investigate the phytochemical composition, antioxidant activity, and toxicity of methanolic extract and ethyl acetate fraction of rambutan seeds.

METHOD

Materials

Rambutan fruit was obtained from Desa Tanjung Labu, Rantau Pulung, Kutai Timur, Indonesia. The chemical used are *n*-hexane, ethyl acetate, methanol, distilled water, dimethyl sulfoxide, reagen Mayer, reagen Wagner, reagen Dragendroff, hydrochloric acid, sulphuric acid, Iron (III) chloride, Mg tape, 2,2-diphenyl-1-picrylhydrazyl (DPPH), *Artemia salina* leach.

Preparation of Methanol Extract

The preparation of extract was began with separating rambutan seeds from the skin and pulp, followed by wet sortation to select the seeds with poor quality and impurities. The seeds were chopped and dried at 45°C. The dried seeds were then weighed and grinded using an electrical blender and proceed to the extraction step. The powder of rambutan seed was extracted using the maceration method using methanol. The maceration was carried out for 3 x 24 h while stirring every 24 h. The filtrate was then filtered and collected, followed with the re-maceration process untill the filtrate obtained is transparent. The filtrate was concentrated with a rotary evaporator. The concentrated extract was collected then the solvent remaining in the extract was evaporated by aerating and stored in desiccator (Mistriyani et al., 2021).

Fractionation of Methanolic Extract of Rambutan Seed

The methanolic extract (100 mg) was dissolved with distilled water. The solution was then put in a separating funnel and added with 100 mL of *n*-hexane, then vigorously shaken and left to

rest until two layers are formed. The bottom layer was collected for further fractionation using ethyl acetate, while in the top layer was collected as *n*-hexane fraction. In each fractionation process on each solvent was repeated until the top layer looks transparent and then concentrated with a rotary evaporator (Mistriyani et al., 2021).

Phytochemicals Screening Test

Alkaloids Test

The methanol extract (2 mL) and ethyl acetate fraction (2 mL) were added with distilled water, NaCl and three drops of concentrated hydrochloric acid. The mixture was then added with three drops of each reagents, Dragendroff, Meyer and Wagner. Positive results of alkaloids were indicated by formation of a white precipitate on Meyer's reagent, orange precipitate on Dragendroff's reagent and brown on Wagner's reagent (Dubale et al., 2023).

Flavonoid Test

The methanol extract (2 mL) and ethyl acetate fraction (2 mL) were heated for 5 minutes. Then added with 0.5 cm of Mg tape and 5 drops of concentrated HCl. The positive result was indicated by a change of the solution from yellow to red color (Dubale et al., 2023).

Triterpenoid/Steroid Test

The methanol extract (2 mL) and ethyl acetate fraction (2 mL) were added by three drops of concentrated HCl and one drop of concentrated H_2SO_4. If the solution formed a red or purple, it was positive to contain triterpenoids, and if it formed a green or blue solution indicating there was a ring which was positive for steroids.

Tannin Test

The tannin test was carried out by heating the methanol extract (2 mL) and ethyl acetate fraction (2 mL) for 5 minutes. Then added a few drops of $FeCl_3$ 1%, the positive results was indicated by a forming of a greenish brown solution (Dubale et al., 2023).

Saponin Test

Saponin test was carried out by foam test in hot water. Foam that was stable for 10 minutes and did not disappear on the addition of one drop of 2 N HCl indicates saponin (Dubale et al., 2023).

***Antioxidant Assay* (DPPH *Assay*)**

Extract and fraction (50 mg each) were dissolved in methanol and diluted to 50 mL to obtain a 1000 ppm stock solution. Serial dillution were prepared: 200-600 ppm for methanolic extract and 20-60 ppm for ethyl acetate fraction, each in triplicate. DPPH solution was prepared by dissolving 45 mg of DPPH in methanol and diluting to 50 mL (1000 ppm) in a brown volumetric flask. The maximum absorption wavelength was determined at 515 nm using UV-Vis spectrophotometry. For the assay, 2 mL of each extract and fraction dilution were mixed with 2 mL of DPPH solution, then vortexed and incubated in the dark for 30 minutes. A blank solution consisted of 2 mL methanol and 2 mL DPPH solution treated identically. Absorbance was measured at 515 nm. The IC_{50} values were calculated using linear regression analysis (Baliyan et al., 2022).

***Toxicity Test* (BSLT *Assay*)**

Approximately 4 mg of *Atemia salina* eggs were incubated in seawater with continous aeration and light for 48 h to obtain nauplii. Methanolic extract and ethyl acetate fraction (200 mg each) were dissolved in seawater with 5 drops of DMSO and dilluted to 100 mL to obtain a 2000 ppm stock solution. From this, serial concentration of 600, 700, 800, 900, and 1000 ppm were

prepared, each in five replicates. Ten nauplii were introduced into 10 mL vials containing the test solution, then added with seawater to 10 mL and exposed to light for 24 h. Mortality was recorded, and LC_{50} values were calculated using Probit nalysis. A contol group without extract was also prepared (Pohan et al., 2023).

RESULTS AND DISCUSSION

The qualitative phytochemical screening of rambutan (*Nephelium lappaceum L.*) seed extracts was carried out using standard chemical reagent test for the detection of major classes of secondary metabolites. This technique involved color changes or precipitate formation as indicators of the presence of specific phytochemicals. The results revealed that both the methanol extract and ethyl acetate fraction contained phenolic compounds, flavonoids, and tannins, while other compounds such as alkaloids, terpenoids, steroids, and saponins were not detected (Table 1). These results are in agreement with previous studies. Fidrianny et al., 2015 and Yunusa et al., 2018 reported similar pyhtochemical profiles in rambutan seed extract, where phenolics and flavonoids were dominant secondary metabolites (Fidrianny, 2015; Kabiru Yunusa et al., 2018). The similarity of metabolites detected in methanol and ethyl acetate solvents indicates that the compounds are quite polar and can be extracted by both solvents (Nawaz et al., 2020).

Table 1. Qualitative screening of secondary metabolites in methanol extract and ethyl acetate fraction of rambutan (*Nephelium lappaceum L.*) seed.

Secondary Metabolites	Samples	
	Methanol extract	Ethyl acetate fraction
Alkaloids	-	-
Tannins	+	+
Flavonoids	+	+
Fenols	+	+
Terpenoids	-	-
Steoroids	-	-
Saponin	-	-

The antioxidant activity of both samples was evaluated using DPPH radical scavenging assay. The ethyl acetate fraction exhibited significanlly higher antioxidant activity, with an IC_{50} value of 32.78 ppm, compared to the methanol extract which showed an IC_{50} of 328.61 ppm (Table 2). The ethyl acetate fraction falls into the very strong antioxidant activity, while the methanol extract is considered to have weak antioxidant activity. In line with the previous study, reported that the ethyl acetate extract exhibited the highest IC_{50} values for antioxidant activity (Budikafa et al., 2019; Fidrianny, 2015) .

Table 2. IC_{50} and LC_{50} values of Rambutan (*Nephelium lappaceum L.*) seed methanol extract and ethyl acetate fraction based on DPPH assay.

Samples	IC_{50} (ppm)	LC_{50} (ppm)
Methanol extract	328.61	688.86
Ethyl acetate fraction	32.78	825.68

This results are consistent with the phytochemical screening data, as both samples contain flavonoids and phenolics and similarly with some previous studies (Afzaal et al., 2023; Jahurul et al., 2020; Lee et al., 2020; N. M. P. Nguyen et al., 2019; T. N. Nguyen et al., 2025; Tsong et al., 2021). The antioxidant activity of secondary metabolites is influenced by specific functional group in their structure. For instance, phenolic compounds have hydroxyl (-OH) groups that donate hydrogen atom to neutralize free radical. Flavonoid often contain catechol

groups, which stabilize free radicals through electron delocalization. Additionally, carbonyl groups and double bonds enhance this activity by distributing unpaired electrons evenly (Fathiah et al., 2024; Gulcin & Alwasel, 2023; Hassanpour & Doroudi, 2023; Mehmood et al., 2022). The higher activity of the ethyl acetate fraction is its semi-polar solvent, which may allow it to selectively extract semi-polar compounds such as flavonoid aglycone and low-molecular weight phenolics that are often associated with strong radical-scavenging activity (Nawaz et al., 2020). This could account for the lower IC_{50} value obtained in the DPPH assay compared to the methanolic extract. Solvent polarity is a key factor that influences the efficiency of phytochemical extraction and the resulting antioxidant activity of plant extracts (Herrera-Pool et al., 2021; Nawaz et al., 2020; Yulianti et al., 2023).

The toxicity evaluation using the Brine Shrimp Lethality Test (BSLT) revealed that the methanol extract exhibited an LC_{50} value of 688.86 ppm, while the ethyl acetate fraction showed an LC_{50} value of 825.68 ppm (Table 2). Based on Meyer's toxicity classification, both values indicate low toxicity (> 500 ppm), suggesting that the methanol extract and ethyl acetate fraction are relatively safe for further pharmacological exploration, particulary as antioxidant agent (Daniel et al., 2023; Pohan et al., 2023). These results are consistent with the phytochemical screening, which identified the presence of flavanoids, tannins, and phenolic-compouds known for their antioxidant potential and relatively low cytotoxicity at moderate concentration (Dahlia et al., 2021).

The mechanism of larvae death is attributed to the presence of these compounds, which are act as stomach poison or gastric toxins when ingest by the larvae. They disrupted the digestive system and interfere with the sensory receptors located in the mouth region. This disruption hampers the larvae's ability to detect and recognize food stimuli, resulting in starvation and eventual death (Pohan et al., 2023). Moreover, the ethyl acetate fraction, which showed the strongest antioxidant activity, also demonstrated slightly lower toxicity, reinforcing the correlation between selective bioactivity and compound polarity.

CONCLUSION

This study demonstrated that both methanolic extract and ethyl acetate fraction of rambutan (*Nephelium lappaceum* L.) seeds contain key secondary metabolites, including flavonoids, phenols, and tannins. The ethyl acetate fraction exhibited notably stronger antioxidant activity, with an IC_{50} value of 32.78 ppm, while toxicity testing confirmed that both extracts were non-toxic. These results indicate that the ethyl acetate fraction, in particular, holds strong potential as a natural antioxidant with a favourable safety profile. The novelty of this study lies in its comparative evaluation of methanolic and ethyl acetate extracts of rambutan seeds, combining antioxidant assessment with toxicity screening an approach that remains limited in current literature. By providing new insights into solvent-specific extraction outcomes and safety considerations, this research contributes to the broader understanding of plant-based antioxidant sources. Moreover, it highlights the potential for utilizing rambutan seeds, an often-discarded by-product, as a valuable raw material in the development of pharmaceutical and nutraceutical products, supporting both scientific innovation and sustainable waste reduction.

RECOMMENDATIONS

Based on these findings, further studies are recommended to isolate and characterize the active antioxidant compounds present in *Nephelium lappaceum L.* seeds extract, as well as to conduct more comprehensive in vitro and in vivo toxicity evaluation to confirm their safety and explore broader pharmaloggical applications.

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