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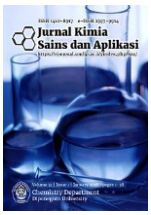

Toxicity and Antioxidant Activities of Indonesian Rambutan (*Nephelium lappaceum* L.) Peel Extracts: A Comparative Analysis Across Varieties Based on Phytochemical Content

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Abstract

Indonesia has long been known as a tropical country rich in various local cultivars of rambutan (*Nephelium lappaceum* L.), including Aceh Lebak (AL), Aceh Pelat (AP), Binjai (B), Gula Batu (GB), and Sikoneng (SK). Due to their phenolic and flavonoid compound, rambutan peel extracts have been reported to exhibit antioxidant and antiproliferative properties. Variation among cultivars suggests potential differences in bioactive compound content and biological activity. The objective of this study was to evaluate the antioxidant and toxicity profiles of five rambutan peel varieties and their correlation with total phenolic compound (TPC) and total flavonoid content (TFC). The antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, and phenolic and flavonoid contents were measured by colorimetric assays. Toxicity was assessed using the Brine Shrimp Lethality Test (BSLT). Bioautography analysis using TLC-DPPH was conducted to identify antioxidant-active compounds. In this study, all rambutan peel extracts indicated the presence of flavonoids, phenolics, saponins, steroids, alkaloids, and triterpenoids. The highest extract yield was observed in Sikoneng, also with the highest TPC and TFC values. The Binjai variety exhibited strong antioxidant activity (IC_{50} = 4.73 ± 0.19 mg/L) and three active antioxidant spots; however, it also exhibited toxicity (LC_{50} = 136.27 ± 6.58 mg/L). The correlation between total phenolic or flavonoid content and the observed biological activity was not statistically significant, indicating that additional metabolites or synergistic interactions may also contribute. These findings underscore the significant impact of biodiversity on differences in compound levels and potential biological activities among rambutan peel varieties.

1. Introduction

Rambutan (*Nephelium lappaceum* L.) is a tropical fruit commonly found in countries like Indonesia, with a seasonal harvest occurring once a year and several known varieties. This fruit has been increasingly popular among the public over the years. According to the Ministry of Agriculture of the Republic of Indonesia [1], in 2023, the average annual per capita consumption of rambutan was 2.55 kg, out of a per capita availability of 1.86 kg. The high consumption rate may lead to considerable peel waste if not properly utilized. Variations in peel and hair color among varieties indicate differences in active compounds. Reported bioactive compounds in rambutan peel include phenolics, especially tannins (geraniin, corilagin, and ellagic acid), flavonoids (quercetin and rutin), hydroxycinnamic acids (p-coumaric acid and caffeic acid), and hydroxybenzoic acids (gallic acid and syringic acid), which contribute to their bioactivity [2]. These compounds may exhibit various biological activities, including antioxidant, antibacterial,

antidiabetic, antiproliferative, anti-inflammatory, and antiviral properties [3].

Among the previously mentioned bioactivities, antioxidant activity is crucial as it can neutralize free radicals involved in oxidative stress, which contributes to various health disorders, including degenerative diseases such as cardiovascular disease, diabetes mellitus, chronic inflammation, and neurodegenerative conditions like Alzheimer's and Parkinson's diseases, as well as premature aging. Therefore, antioxidant compounds are essential for neutralizing free radicals and maintaining the body's redox balance [4]. Antioxidants protect cells by donating electrons or hydrogen atoms, which inhibit oxidation reactions. The antioxidant activity of a compound is often an early indicator of its other potential bioactivities. Due to their role in reducing oxidative stress, antioxidants are associated with various biological activities, including antiproliferation [5].

Antiproliferation is of interest for further research because uncontrolled and improper cell growth, or proliferation, can lead to cancer. The Brine Shrimp Lethality Test (BSLT) is a proven method for initial screening of cancer cell antiproliferation [6]. The toxicity detected in Artemia salina has been shown to correlate with cytotoxicity in certain cancer cell lines. Therefore, extracts that exhibit high toxicity in BSLT may also possess strong antiproliferative potential, making this assay a valuable preliminary tool before more specific studies are conducted [7]. Thus, the exploration of natural sources of local plant species rich in antioxidants and toxic substances continues to identify natural compounds with the potential to be developed as drug candidates.

Methanolic extracts from the Binjai and Aceh varieties exhibited significantly different antioxidant activities [8]. The extract from Var. Rong Rian rambutan peel exhibited toxic properties with active toxicity levels [9]. Similarly, the yellow rambutan peel also demonstrated highly active cytotoxicity [2]. These results indicate that rambutan varieties may differ in both toxicity and antioxidant potential. Since phenolic and flavonoid compounds are key contributors to these biological effects, their levels likely vary among varieties. Hence, this study hypothesizes that differences in total phenolic content (TPC) and total flavonoid content (TFC) directly influence the antioxidant activity and toxicity of rambutan peel.

The study is designed to test this causal relationship by evaluating whether higher levels of TPC and TFC lead to stronger bioactivities. This study aims to evaluate and compare the phenolic and flavonoid contents, antioxidant activity, and BSLT of five rambutan peel varieties, as well as to identify the interrelationships among these parameters as part of an exploratory approach to the potential of Indonesia's local plant biodiversity. The varieties include Aceh Lebak (AL), Aceh Pelat (AP), Binjai (B), Gula Batu (GB), and Sikoneng (SK), originating from different provinces in Indonesia, namely Banten, Jakarta, Binjai (North Sumatra), Batu (East Java), and Tasikmalaya (West Java). The selection of these five varieties was based on their popularity and recognition as local cultivars in Indonesia. Previous genetic characterization studies have identified Aceh (Lebak (AL) and Pelat (AP)) and Binjai (B) as among the most popular commercial cultivars. Meanwhile, Gula Batu (GB) and Sikoneng (SK) are recognized as distinct accessions in local germplasm collections [10]. Furthermore, preliminary reports of differing antioxidant and cytotoxic activities among rambutan varieties supported their inclusion.

2. Experimental

2.1. Materials

The materials used in this study included rambutan (*Nephelium lappaceum* L.) var. AL, AP, B, GB, and SK (Figure 1) collected from Mekarsari Fruit Garden, Cileungsi, Bogor Regency, West Java Province, Indonesia (6.416°LS 106.984°BT). Samples were collected based on a similar fruiting season, then identified by the Herbarium Bogoriense, National Research and Innovation Agency, to identify their varieties. For each rambutan variety, its peel was separated. The peels were washed and cut into small pieces. All chemicals and reagents, including solvents and general reagents, were analytical grade and purchased from Merck (Darmstadt, Germany). Specifically, Whatman No. 41 and TLC plates G_{60} F_{254} were obtained from Merck as well. Sigma-Aldrich (St. Louis, MO, USA) was the source of ascorbic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH).

2.2. Moisture Content and Extraction

The determination of moisture content was conducted according to the method described by Lestari and Aras [11]. An empty porcelain dish was dried in an oven at 105°C for 30 minutes, followed by a 30-minute cooling period in a desiccator to eliminate moisture. The crucible was weighed, and approximately 2 g of the sample was placed into it. The sample-containing crucible was then dried in an oven at 105°C for 5 hours to dry. Afterward, the crucible was allowed to cool in a desiccator for 30 minutes, and its weight was recorded. Drying and weighing were conducted at 1-hour intervals until a stable weight was obtained. This moisture content was measured in triplicate. The moisture level was determined using Equation (1).

$$\text{Moisture content (\%)} = \frac{(b-c)}{a} \times 100\% \quad (1)$$

Where, a is the initial weight of the sample (g), b is the weight of the dish plus the initial sample (g), and c is the weight of the dish plus the final sample (g).

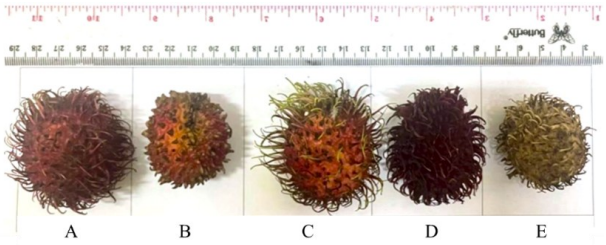

Figure 1. Varieties of Rambutan: A) Aceh lebak (AL), B) Aceh pelat (AP), C) Binjai (B), D) Gula batu (GB), E) Sikoneng (SK)

The extraction procedure was conducted according to the method described by Ilmiawati *et al.* [12]. Rambutan peel powder from each variety (20 g) was macerated with 96% ethanol at a ratio of 1:10 (w/v) for 3 × 24 hours, with the solvent replaced every 24 hours. During maceration, the samples were covered with aluminum foil to prevent light exposure. The resulting mixture was filtered using Whatman No. 41 filter paper, and the filtrate was concentrated using a rotary evaporator at 40°C until a constant weight was obtained. The extraction process was performed in triplicate, and the yield of each extract was calculated using Equation (2).

$$\text{Extraction yield (\%)} = \frac{a}{b} \times (100\% - \%\text{Moisture content}) \quad (2)$$

Where, a is the weight of the extract obtained (g), and b is the weight of the dried sample used for extraction (g).

2.3. Phytochemical Screening

Each variety was extracted and subjected to phytochemical screening for alkaloids, flavonoids, terpenoids/steroids, saponins, and phenolics [13]. For the alkaloid test, the extract was dissolved in chloroform, followed by the addition of 2 M H_2SO_4, and the mixture was shaken until two distinct layers formed. The acidic layer was divided equally into three test tubes, to which Dragendorff's, Mayer's, and Wagner's reagents were added, respectively. The formation of orange-red, white, or brown precipitates indicated the presence of alkaloids. For the flavonoid test, the extract was diluted with distilled water, boiled for 5 minutes, and filtered. The filtrate was treated with magnesium powder, concentrated HCl (37%), and amyl alcohol (analytical reagent), then vigorously shaken. The appearance of red, yellow, or orange coloration in the amyl alcohol layer confirmed the presence of flavonoids.

For the terpenoid/steroid and saponin tests, the extract was diluted with chloroform (analytical reagent) and filtered into a test tube containing the corresponding reagents. A green or blue coloration indicated the presence of steroids, while a different color change indicated triterpenoids. The residue was then transferred into distilled water, boiled gently for 5 minutes, and shaken vigorously. The persistence of stable foam for about 15 minutes indicated the presence of saponins. For the phenolic test, the extract was diluted with distilled water, filtered, and treated with a few drops of 1% $FeCl_3$ solution. The appearance of green, blue, or purple coloration indicated phenolic compounds.

2.4. Total Phenolic Content (TPC) and Flavonoid Content (TFC)

The determination of total phenolic and flavonoid contents followed the procedure described by Batubara *et al.* [14], with modifications to the sample and standard concentrations. Color analysis for each variety was performed in triplicate. The total phenolic content (TPC) was determined using the Folin-Ciocalteu method with gallic acid as the standard. Both the extracts and gallic acid were dissolved in methanol, and the standard solutions were prepared at five different concentrations. The same treatment was applied to both samples and standards by adding 20 µL of 10% Na_2CO_3, 10 µL of 10% Folin-Ciocalteu reagent, and 160 µL of distilled water. The mixtures were incubated in the dark at room temperature for 30 minutes, and the absorbance was measured using a microplate reader at 750 nm. The TPC was expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g).

The total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method, with quercetin as the standard. Quercetin solutions were prepared at five concentrations in methanol. Subsequently, 60 µL of methanol, 10 µL of 10% $AlCl_3$, 10 µL of 1 M CH_3COOK, and 120 µL of distilled water were added to each tube. The same procedure was applied to the extract samples. After incubation for 30 minutes at room temperature, the absorbance was measured at 415 nm using a microplate reader [14]. The TFC was expressed as milligrams of quercetin equivalent per gram of dry extract (mg QE/g).

2.5. Toxicity Assay by BSLT Method

Referring to the bioassay method described by Arta *et al.* [15], *Artemia salina* larvae were tested at five different extract concentrations, each in triplicate. The test began with the hatching of A. salina eggs in a hatching box filled with seawater and equipped with aeration and continuous illumination for 48 hours. After hatching, ten larvae were transferred into each test solution and incubated for 24 hours. Following incubation, the number of dead larvae was recorded. Larval mortality was compared with that of the solvent control (0 mg/L). The percentage of mortality (MA) was calculated using Equation (3).

$$\text{MA (\%)} = \frac{N_1}{N_2} \times 100\% \quad (3)$$

Where, MA is the observed mortality (%), N_1 is the number of dead larvae after the test, and N_2 is the initial number of larvae. The obtained data were then analyzed using probit analysis to determine the lethal concentration 50% (LC_{50}) in mg/L.

2.6. Antioxidant Activity

The antioxidant activity of the extracts was evaluated using the DPPH method [16]. This assay was carried out in triplicate as a technical replicate. A total of 100 µL of the sample was combined with 100 µL of 125 µM DPPH in methanol in a 96-well microplate. Then incubated at room temperature in the dark for 30 minutes. Following the incubation, the absorbance was recorded at a wavelength of 517 nm with a microplate reader. Results expressed in half-maximal inhibitory concentration (IC_{50}) in mg/L.

2.7. Bioautography Thin Layer Chromatography (TLC)

The bioautography-TLC analysis followed the method described by Praptiwi *et al.* [17]. The sample was spotted onto a silica gel TLC plate (G_{60} F_{254}), which was then developed using the optimal eluent. After development, the plate was sprayed with a 500 mg/L DPPH solution. The appearance of yellow spots against a purple background indicated the presence of antioxidant-active compounds.

2.8. Data Analysis

Data on extract yield, TPC, TFC, toxicity assay, and antioxidant activity were first tested for normality using the Shapiro-Wilk test. Since the data did not meet the assumption of normality, statistical analyses were carried out using the Kruskal-Wallis test in IBM SPSS Statistics version 25. When significant differences were detected, post-hoc comparisons were performed using Dunn's test. The correlations among extract yield, TPC, TFC, toxicity, and antioxidant activity were evaluated using Spearman's rank correlation in RStudio version 4.4.0.

3. Results and Discussion

3.1. Moisture Content and Extraction

The polarity of the solvent must be compatible with the active compounds present in the raw material to achieve a higher extraction yield, as this ensures optimal dissolution of the desired compounds. Ethanol (96%) is an environmentally friendly polar solvent capable of dissolving both polar and nonpolar compounds, allowing for the effective extraction of phenolic compounds, flavonoids, saponins, alkaloids, steroids, and triterpenoids. Wisdayanti *et al.* [18] reported that 96% ethanol is more effective in extracting polar compounds from plant materials. Therefore, the use of ethanol in this study was based on its high efficiency, safety, and suitability for food and pharmaceutical applications compared to other alternative solvents. Furthermore, ethanol possesses low toxicity, ensuring its safe use in bioactive compound extraction. It is also recognized as Generally Recognized as Safe (GRAS), making it widely accepted in the nutraceutical, pharmaceutical, and food industries due to its renewable origin and biodegradability [19].

The extraction results revealed that the variety of rambutan fruit peel significantly affected the extract yield, which ranged from 32.84% to 42.41% (Table 1). The SK variety produced the highest yield, although it was not significantly different from the AP and AL varieties. In contrast, the GB variety had the lowest yield, but the difference was not statistically significant compared with the B variety ($p < 0.05$). These yields were higher than the 22.62% yield previously reported for rambutan peel extract [20], and also greater than those reported for several Indonesian rambutan varieties, Aceh, Rapiah, Garuda, and Lengkeng, which yielded 14.02%, 14.37%, 15.68%, and 15.29%, respectively [18]. The differences in yield indicate the chemical diversity among rambutan peel varieties, which indirectly reflects the biodiversity potential of each cultivar.

A higher extraction yield suggests that certain varieties contain greater amounts of solvent-soluble compounds; however, this does not necessarily correlate with biological activity, such as antioxidant or toxicity levels. Yield determination is important for quantitatively identifying the most promising varieties and serves as a basis for scaling up production if the extracts are developed for industrial applications. Moreover, yield data contribute to optimizing extraction efficiency in terms of time, cost, and solvent consumption [21].

Table 1. Extraction yield and moisture content of five *N. lappaceum* peel varieties in ethanolic extracts

Rambutan peel variety	Moisture content (%)	Extraction yield (%)
AL	4.61 ± 0.14^{b}	39.55 ± 1.45^{b}
AP	6.09 ± 0.23^{d}	39.95 ± 1.14^{b}
B	3.80 ± 0.09^{a}	32.84 ± 0.45^{a}
GB	3.78 ± 0.15^{a}	31.12 ± 1.48^{a}
SK	5.50 ± 0.11^{c}	42.41 ± 1.48^{b}

Different letters in the same column represent statistically different results at $p < 0.05$

In this study, the moisture content of the five rambutan peel varieties showed significant variation but remained below 10% (Table 1), which is within the maximum acceptable limit [22]. Moisture content plays a crucial role in the stability of bioactive compounds and extraction efficiency. High moisture levels in plant materials can promote enzymatic degradation and microbial growth, leading to a decline in phenolic and flavonoid contents over time. Excess moisture also dilutes solvent interactions, thereby reducing the extraction yield [23]. The low moisture content observed in this study indicates that the powder quality was well-maintained and less susceptible to degradation.

3.2. Phytochemical Screening of Extract Rambutan

All extracts from the different varieties showed significantly positive phytochemical results (Table 2). Phytochemical screening of the five *N. lappaceum* peel varieties revealed the presence of several classes of bioactive compounds, including alkaloids, flavonoids, phenolic compounds, saponins, steroids, and triterpenoids. Varieties AL, AP, and B showed positive results for alkaloids with all three detection reagents (Wagner, Meyer, and Dragendorff), indicating a strong presence of diverse alkaloids. In contrast, varieties GB and SK yielded negative results with the Meyer reagent but reacted positively with the Wagner and Dragendorff reagents. These variations were attributed to differences in the reagents' reaction mechanisms.

Wagner's reagent reacted with the I^- ion in potassium iodide (KI) to produce an I_3^- ion, where the potassium (K^+) ion formed a coordinate covalent bond with the nitrogen atom in the alkaloid [24]. Dragendorff's reagent, on the other hand, reacted with the Bi^{3+} ion and the nitrogen of the alkaloid to yield an orange precipitate. Unlike these two, Meyer's reagent depended on the interaction between the Hg^{2+} ion and the strongly basic nitrogen group [25], forming an insoluble white complex. Therefore, the negative results of GB and SK with Meyer's reagent indicated that their alkaloids were either weaker nitrogen bases or present at lower concentrations, making them less reactive toward Hg^{2+} but still detectable by the more sensitive Wagner and Dragendorff reagents, which can identify a broader range of alkaloid structures.

Table 2. Phytochemical screening of five *N. lappaceum* peel varieties in ethanolic extracts

Rambutan peel varieties	Alkaloid			Flavonoid	Phenolic	Saponin	Steroid	Triterpenoid
	Wagner	Mayer	Dragendorff					
AL	+	+	+	+	+	+	+	+
AP	+	+	+	+	+	+	+	+
B	+	+	+	+	+	+	+	+
GB	+	-	+	+	+	+	+	+
SK	+	-	+	+	+	+	+	+

(+) contains secondary metabolites, (-) does not contain secondary metabolites.

All varieties consistently tested positive for flavonoids, phenolic compounds, saponins, steroids, and triterpenoids, confirming the broad phytochemical richness of rambutan peels. These compounds are widely recognized for their potential biological activities, particularly antioxidant and toxicity-related effects. Previous studies have also reported that rambutan peel contains tannins, terpenes, steroids, flavonoids, and saponins [20, 26]. The results of phytochemical screening thus provide essential insight into the extract's bioactive compound profile, highlighting its pharmacological potential, in which multiple active compounds may act synergistically to produce desirable biological effects.

3.3. Total Phenolics and Flavonoids Content

Phenolic and flavonoid compounds are secondary metabolites commonly found in plants and are known for their important biological functions. The hydroxyl (−OH) groups in their structures can donate hydrogen atoms, enabling these compounds to act as radical scavengers. This mechanism underlies their broad pharmacological properties, including antioxidant and anticancer activities [27, 28].

As shown in Table 3, the total phenolic content (TPC) and total flavonoid content (TFC) of all extracts ranged from 210.78 ± 6.79 to 608.82 ± 11.76 mg GAE/g and from 12.69 ± 0.28 to 15.60 ± 0.16 mg QE/g dry extract, respectively. The SK variety exhibited the highest TPC and TFC values, significantly different from the other varieties, followed by GB and B. Conversely, the AL variety had the lowest TPC and TFC values, which were also significantly different. These findings indicate notable phenolic and flavonoid content variation among the different *N. lappaceum* peel varieties.

Table 3. TPC and TFC of five *N. lappaceum* peel varieties in ethanolic extracts

Rambutan peel variety	TPC (mg GAE/g)	TFC (mg QE/g)
AL	210.78 ± 6.79^{a}	12.69 ± 0.28^{a}
AP	320.59 ± 5.88^{b}	13.40 ± 0.14^{b}
B	444.12 ± 15.56^{c}	$12.82 \pm 0.10^{a,b}$
GB	457.84 ± 13.58^{c}	14.60 ± 0.44^{c}
SK	608.82 ± 11.76^{d}	15.60 ± 0.16^{d}

Different letters in the same column represent statistically different results at $p < 0.05$

The phenolic content of rambutan peel extract was consistent with its flavonoid content, as flavonoids are a subclass of phenolic compounds. Previous studies reported that the ethanolic extract of red rambutan peel contained 340 mg GAE/g of phenolics and 76 ± 2 mg QE/g of flavonoids [26], while the Malwana variety contained 318.59 ± 0.09 mg GAE/g and 245.39 ± 4.83 mg QE/g, respectively [29]. These results suggest that TPC and TFC values are variety-dependent, thereby influencing their biological activity and potential applications. The -OH group in the chemical structures of phenolic and flavonoid compounds can donate hydrogen atoms, enabling radical scavenging activity that contributes to their antioxidant and antiproliferative effects [27, 28].

3.4. Bioactivities of Extract from Rambutan Peel

The antioxidant activity test results showed that all five rambutan peel varieties had IC_{50} values ≤ 50 mg/L (Table 4), indicating very strong antioxidant potential. Based on classification criteria, extracts with IC_{50} ≤ 50 mg/L are categorized as having very strong antioxidant activity; those between 50–100 mg/L as strong; 101–150 mg/L as moderate; and 151–200 mg/L as weak [30]. Among the varieties tested, the SK extract showed the highest antioxidant potential, exceeding even the positive control (ascorbic acid). Conversely, the AP extract had the lowest activity, while the AL, B, and GB varieties still exhibited stronger antioxidant effects than ascorbic acid.

Previous studies have reported comparable results, where the methanolic extracts of the Binjai (B) and Aceh (AL) varieties showed very strong and strong antioxidant activities, with IC_{50} values of 0.76 and 57.94 mg/L, respectively [8]. Similarly, rambutan peel extract has been reported to possess very strong antioxidant activity with an IC_{50} value of 3.69 mg/L [31]. Statistical analysis revealed that the antioxidant activity of SK was not significantly different from that of AL, which had the lowest TPC and TFC values. This suggests that phenolic and flavonoid contents may not be the primary contributors to antioxidant capacity in these extracts.

The antioxidant activity was determined using the DPPH radical scavenging assay. In this method, antioxidant compounds donate electrons or hydrogen atoms to the stable violet DPPH radical, reducing it to the yellow-colored non-radical form, DPPH-H. The decrease in absorbance intensity corresponds to the radical scavenging ability of the extract. The hydroxyl groups present in the compounds of rambutan peel are particularly effective in this reaction, acting as hydrogen

donors. Therefore, the IC_{50} values obtained from the DPPH assay serve as reliable indicators of the free radical–neutralizing capacity of rambutan peel extracts [32].

TLC bioautography analysis using DPPH was conducted to identify antioxidant-active compounds in the rambutan peel extracts. The appearance of yellow bands at R_f values of approximately 0.06 and 0.2 (Figure 2D) after DPPH spraying indicated the presence of polar antioxidant compounds. As shown in Figure 2A, all five extracts displayed a black band at the same R_f value, suggesting the presence of phenolic compounds [33]. These results imply that polar phenolic constituents are the primary contributors to the antioxidant activity of rambutan peel extracts.

Under UV light at 366 nm (Figure 2B), the bands at $R_f \approx 0.2$ did not fluoresce, indicating that the phenolic compounds possess short conjugated systems that do not emit visible fluorescence. Conversely, the bands at $R_f \approx 0.06$ fluoresced under 366 nm UV light, signifying the presence of polar compounds with extended conjugated chromophore groups. After derivatization with H_2SO_4 (Figure 2C), multiple spots were observed, with the B variety exhibiting the most distinct profile (19 spots in total) compared to the other varieties.

Variety B also displayed a characteristic spot at $R_f \approx 0.96$, distinguishing it from the others. This spot appeared black under 254 nm UV and red under 366 nm UV, and turned yellow after DPPH spraying, suggesting it corresponds to a nonpolar compound with an extended conjugated chromophore system. Based on the DPPH bioautography results, the Binjai variety exhibited three active antioxidant spots, while the other four varieties showed only two. This finding suggests that the Binjai variety contains a greater number of antioxidant-active compounds. The R_f values of the identified bands observed under UV (254 and 366 nm) and after 10% H_2SO_4 derivatization are summarized in Table 5.

Further evaluation is necessary to determine other biological potentials of the compounds after assessing their antioxidant activity. Toxicity testing was conducted to identify potential cytotoxic effects, with the BSLT employed as a preliminary and cost-effective cytotoxicity screening method. This assay has been widely reported, as mortality in *Artemia salina* has been correlated with cytotoxic responses in certain mammalian and cancer cell lines. However, BSLT has inherent limitations, particularly its lack of specificity toward human cells and its inability to fully replicate the complex biological responses of mammalian systems. Therefore, the results of the BSLT in this study were interpreted as an initial indication of cytotoxic potential, requiring further validation through more specific assays involving mammalian or cancer cell models to confirm pharmacological relevance [7].

The results of the toxicity evaluation revealed significant differences in LC_{50} values among the rambutan peel varieties (Table 4). The LC_{50} value represents the concentration required to kill 50% of *Artemia salina* larvae. Varieties AL, B, GB, and SK exhibited LC_{50} values between 100 and 250 mg/L, indicating moderate toxicity, while AP showed a higher LC_{50} value (251–1000 mg/L), classified as weak toxicity [15]. Among the tested varieties, B had the lowest LC_{50} value, suggesting the highest level of toxicity, whereas AP displayed the lowest toxicity, although not significantly different from GB, as indicated by the same letter notation (c). Interestingly, despite SK having higher antioxidant levels than B, its LC_{50} value was lower and not significantly different from AL. This suggests that each extract contains distinct combinations of compounds influencing toxicity levels.

Extracts showing moderate toxicity indicate the presence of bioactive compounds with potential antiproliferative activity, which is relevant for pharmaceutical development, particularly in cancer research. Overall, B demonstrated strong antioxidant activity (IC_{50} = 4.73 ± 0.19 mg/L) and exhibited three active antioxidant spots in the TLC bioautography with DPPH, implying the presence of multiple synergistic compounds. Although Binjai did not record the highest IC_{50}, TPC, or TFC values, its antioxidant activity remained within the "very strong" category. Furthermore, its relatively high toxicity (LC_{50} = 136.27 ± 6.58 mg/L) suggests a richness and diversity of bioactive compounds contributing to its overall biological activity. Therefore, B is considered the most promising variety, not solely because of its phenolic or flavonoid content, but due to the complex interplay of compounds that enhance its pharmacological potential.

Table 4. Antioxidant and toxicity activity of five *N. lappaceum* peel varieties in ethanolic extracts

Rambutan peel varieties	DPPH IC_{50} (mg/L)	BSLT LC_{50} (mg/L)
AL	$4.32 \pm 0.21^{a.b}$	228.08 ± 2.10^{b}
AP	8.00 ± 0.21^{e}	256.16 ± 9.63^{c}
B	$4.73 \pm 0.19^{b.c}$	136.27 ± 6.58^{a}
GB	4.81 ± 0.08^{c}	245.45 ± 3.08^{c}
SK	3.87 ± 0.10^{a}	218.30 ± 1.07^{b}
Ascorbic acid*	5.76 ± 0.19^{d}	-

Different letters in the same column represent statistically different results at $p < 0.05$.
*positive control

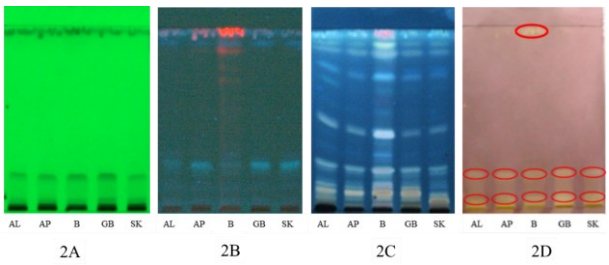

Figure 2. Bioautography chromatogram profiles of rambutan peel extracts from each variety eluted with dichloromethane-ethanol (9:1). (A) Observed under UV light at 254 nm, (B) observed under UV light at 366 nm, (C) after spraying with 10% sulfuric acid and visualized under UV light at 366 nm, and (D) bioautography after DPPH spraying

Table 5. Rf value of five *N. lappaceum* peel varieties in ethanolic extracts

Rambutan peel varieties	Spot	R_f value	Color			Compound class
			254 nm	366 nm	10% H_2SO_4 (366 nm)	
Aceh Lebak (AL)	1	0.04	Black	Blue	-	Phenolic
	2	0.06	Black	-	Orange	Flavonoid
	3	0.11	-	-	Orange	Flavonoid
	4	0.14	-	Blue	Blue	Steroid
	5	0.2	Black	-	-	Phenolic
	6	0.25	-	Light blue	Green	Steroid
	7	0.36	-	-	Blue	Terpenoid
	8	0.49	-	-	Green	Steroid
	9	0.61	-	-	Blue	Terpenoid
	10	0.70	-	-	Green	Steroid
	11	0.79	-	-	Blue	Terpenoid
	12	0.86	-	-	Green	Steroid
	13	0.91	-	Blue	Green	Steroid
	13	0.96	Black	Red	Green	Terpenoid
Aceh Pelat (AP)	1	0.03	Black	Blue	Orange	Flavonoid
	2	0.06	Black	-	Blue	Phenolic
	3	0.10	-	-	Yellow	Flavonoid
	4	0.13	-	Blue	Blue	Steroid
	5	0.19	Black	-	-	Phenolic
	6	0.23	-	Light blue	Green	Steroid
	7	0.30	-	-	Blue	Terpenoid
	8	0.44	-	-	Green	Steroid
	9	0.74	-	-	Blue	Terpenoid
	10	0.88	-	Red	Green	Phenolic
	11	0.91	-	Blue	Green	Steroid
	12	0.96	Black	Red	Green	Terpenoid
Binjai (B)	1	0.04	Black	Blue	Pink	Terpenoid
	2	0.06	Black	-	Yellow	Flavonoid
	3	0.10	-	-	Orange	Flavonoid
	4	0.15	-	Blue	Violet	Terpenoid
	5	0.20	Black	-	-	Phenolic
	6	0.21	-	Blue	-	Terpenoid
	7	0.23	-	Blue	Orange	Flavonoid
	8	0.28	-	Blue	-	Terpenoid
	9	0.30	-	-	Blue	Terpenoid
	10	0.43	-	-	Green	Steroid
	11	0.55	-	-	Blue	Terpenoid
	12	0.61	-	-	Red	Terpenoid
	13	0.66	-	-	Green	Steroid
	14	0.71	-	Red	-	Phenolic
	15	0.74	-	-	Blue	Terpenoid
	16	0.78	-	Red	-	Phenolic
	17	0.85	-	Red	Green	Phenolic
	18	0.91	-	Blue	Green	Steroid
	19	0.96	Black	Red	Red	Terpenoid
Gula Batu (GB)	1	0.03	Black	Blue	Orange	Flavonoid
	2	0.06	Black	-	Yellow	Flavonoid

Rambutan peel varieties	Spot	R_f value	Color			Compound class
			254 nm	366 nm	10% H_2SO_4 (366 nm)	
	3	0.11	-	-	Orange	Flavonoid
	4	0.13	-	Blue	Yellow	Flavonoid
	5	0.20	Black	-	-	Phenolic
	6	0.23	-	Blue	Green	Steroid
	7	0.30	-	-	Blue	Terpenoid
	8	0.43	-	-	Green	Steroid
	9	0.68	-	-	Green	Steroid
	10	0.74	-	-	Blue	Terpenoid
	11	0.76	-	-	Blue	Terpenoid
	12	0.86	-	-	Green	Steroid
	13	0.91	-	Blue	Green	Terpenoid
	14	0.96	Black	Red	Green	Terpenoid
Sikoneng (SK)	1	0.01	Black	Blue	-	Phenolic
	2	0.05	Black	-	Orange	Flavonoid
	3	0.09	-	-	Yellow	Flavonoid
	4	0.13	-	Blue	Blue	Steroid
	5	0.15	-	-	Green	Steroid
	6	0.19	Black	-	-	Phenolic
	7	0.20	-	-	Green	Steroid
	8	0.23	-	Blue	Green	Steroid
	9	0.31	-	-	Blue	Terpenoid
	10	0.45	-	-	Green	Steroid
	11	0.69	-	-	Green	Steroid
	12	0.74	-	-	Blue	Terpenoid
	13	0.76	-	-	Blue	Terpenoid
	14	0.88	-	-	Green	Steroid
	15	0.91	-	Blue	Green	Steroid
	16	0.96	Black	Red	Green	Terpenoid

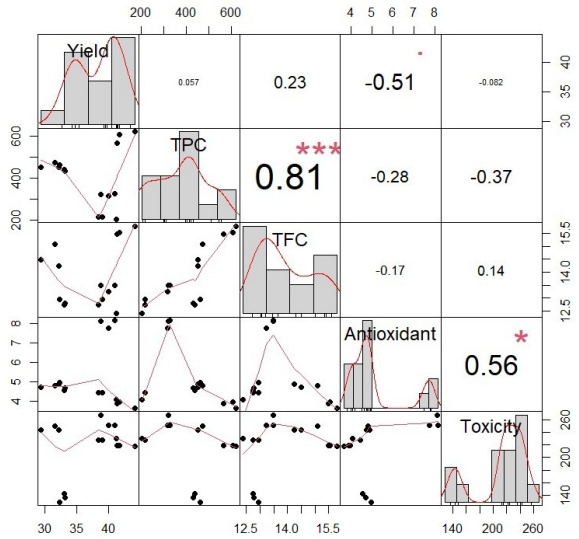

Figure 3. Correlation matrix between extract yield, TPC, TFC, DPPH, and BSLT of five *N. lappaceum* peel varieties in ethanolic extracts. The upper panels display the correlation coefficients, while the lower panels present the scatter plots. The symbols ***, *, and · indicate significance levels of $p < 0.001$, $p < 0.05$, and $p < 0.1$, respectively

3.5. Correlation Matrix

The correlation matrix analysis was performed to evaluate the relationships among extraction yield, TPC, TFC, antioxidant activity, and toxicity of the five rambutan peel varieties. As shown in Figure 3, TPC exhibited the strongest and most significant correlation with TFC, with an r value of 0.81. Other correlation values were generally low to moderate and mostly not significant. A negative correlation was observed between antioxidant activity and both total phenolic and flavonoid contents, although this relationship was not statistically significant. This could be attributed to the structural diversity of the bioactive compounds and the influence of other metabolites such as alkaloids, terpenoids, and saponins, which may have acted synergistically. Additionally, the presence of interfering substances, such as reducing agents that could react with the Folin-Ciocalteu or $AlCl_3$ reagents used for TPC and TFC determination, may have affected the accuracy of these colorimetric assays [34].

The contribution of non-phenolic antioxidants or degradation of active compounds might also have played a significant role in radical scavenging activity, yet these

factors were not captured by TPC and TFC measurements. Thus, the antioxidant activity of rambutan peel extracts likely resulted from complex interactions among various phytochemicals rather than the contribution of phenolics and flavonoids alone. Similarly, the weak correlation between phenolic and flavonoid contents with toxicity suggests that their quantities do not directly determine cytotoxic potential. Interestingly, antioxidant activity showed a significant positive correlation with toxicity, indicating that higher antioxidant potential was associated with greater toxic effects.

4. Conclusion

Based on antioxidant activity, TLC bioautography with DPPH, and toxicity assays, the Binjai variety demonstrated the greatest potential among the five rambutan peel varieties. Although the correlations between phenolic and flavonoid compounds with antioxidant activity and toxicity were not significant, this may be attributed to the presence of other metabolites such as alkaloids, terpenoids, and saponins, as well as interference from reducing agents in colorimetric assays. These findings suggest that the antioxidant activity arises from complex interactions among multiple phytochemicals. Overall, the Binjai rambutan peel exhibits strong potential as a natural source of bioactive compounds, with prospective applications as a natural antioxidant in nutraceutical and food industries, and as a preliminary candidate for plant-based therapeutic development in the pharmaceutical field.

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