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Phenolic Profile and Antioxidant Activities of Rambutan (*Nephelium lappaceum*) and Pulasan (*Nephelium mutabile*) Peels

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Rambutan (*Nephelium lappaceum*) and pulasan (*Nephelium mutabile*) are consumed fresh, canned or processed in human daily life, and the peels are usually discarded as waste. This research is aimed to identify the phenolic compounds and antioxidant activities of the peels of both species. Methanol extract, ethyl acetate and diethyl ether of rambutan and pulasan peels were evaluated for total phenolic content, total flavonoid content and antioxidant activities including α, α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC). The phenolic compounds were identified in the methanol extract of both rambutan and pulasan peels by using UPLC-QTOF/MS. The methanol peel extract for both rambutan and pulasan exhibits the highest total phenolic and total flavonoid content. The methanol extracts of both rambutan and pulasan peels were found to have high antioxidant activities through three different antioxidant assays. The order of antioxidant activity for various extracts was methanol > ethyl acetate > diethyl ether. The abundant phenolic compounds in methanol peel extracts are geraniin, mulberrofuran A, gigantol and arecatannin. The results showed methanol extract of rambutan and pulasan peels with high content of phenolics and the greatest antioxidant properties have the potential to be developed as a functional food.

Keywords: *Nephelium lappaceum L.*; *Nephelium mutabile*; phenolic compounds; antioxidant activities

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Rambutan (*Nephelium lappaceum*) and pulasan (*Nephelium mutabile*) are widely distributed in the area of South East Asia including Thailand, Indonesia and Malaysia. Both fruits belong to the family of Sapindaceae. They are widely grown in warm, humid and low evaporation rate with high-rainfall condition. All parts of both rambutan and pulasan such as leaves, peels, seeds and bark are very useful in daily life. They are widely used in traditional medicine, and also for the manufacturing of soap and oil for cooking [1].

Recently, the *N. lappaceum* and *N. mutabile* are consumed fresh, canned and processed in industry. Undeniably, its consumption had produced a lot of waste, especially from seeds and peels. Based on the study [2], it was found that the use of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) had caused liver damage, carcinogenic effect and high potential of mutagenicity. The phenolic compounds become the determinant of their antioxidant potential as the natural source of antioxidants. According to [3], the antioxidant is described as any substance with low concentration that can be derived naturally and synthetically as well as can delay or inhibit the oxidation reaction significantly. There are some mechanisms for the determination of antioxidant properties such as radical scavenging assay, reducing power, chelating agent and others. The anti-oxidant activities rely on the structural formula of the compounds that bind with hydroxyl groups.

Research by [4] revealed that the *N. lappaceum* peels exhibit higher antioxidant properties than its seeds due to the ellagic acid, corilagin and geraniin components. Based on previous research [5], the major active compound, geraniin was found in the ethanolic extract of rambutan peels compared with grape seed, and the ethanolic extract of rambutan peel also exhibit higher total phenolic content than grape seed. However, the research on the *N. mutabile* has been scarce and there are no reports on its phenolic compounds. Besides, there are also many studies on the antioxidant activities of different extracts of *N. lappaceum* peels. Based on the research, the methanol extract or ethyl acetate extract of peels exhibits higher antioxidant activities [6,4,3]. Therefore, this research is focused on the determination of phenolic compounds and antioxidant activities of *N. lappaceum* and *N. mutabile* peels using DPPH scavenging assay, ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC).

EXPERIMENTAL

Chemicals and Materials

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Peels of *Nephelium lappaceum* and *Nephelium mtabile*, AR grade methanol, diethyl ether, ethyl acetate, distilled water, gallic acid, folin reagent, sodium carbonate, butylated hydroxytoluene (BHT), aluminium chloride, 1,1-diphenyl-2 picryl-hydracyl (DPPH), phosphate buffer (pH 6.6), potassium ferricyanide, trichloroacetic acid, deionized water, ferric chloride, ascorbic acid, copper (II) chloride, neucoproine, ammonium acetate buffer (pH 7).

Preparation of Extract

N. lappaceum and *N. mutabile* peels were cleaned, lyophilized and then powdered. 120 g of the powdered peels were then macerated in 500 mL of methanol for a day. The solution was sonicated for 30 minutes at 55 °C and filtered under a vacuum to obtain the methanol extract. One-third of the methanol extract was left in the fume hood for dryness. The two third of the methanol extract was subjected to a solvent extraction technique to extract diethyl ether and ethyl acetate extract. The methanol extract was added with warm distilled water and extracted using diethyl ether. The residue of the methanol extract was then extracted with ethyl acetate. Both diethyl ether and ethyl acetate were left in the fume hood for dryness.

Total Phenolic Content (TPC)

The total phenolic content was determined by using the Follin Ciocalteau method described by [7] and gallic acid was used as the standard. Total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as milligram gallic acid equivalent (mg gallic acid /g crude extract).

Total Flavonoid Content (TFC)

Total flavonoid content (TFC) was determined by following the procedure of [7] and BHT was used as standard. The content of flavonoids was calculated using the standard graph of BHT and the results were expressed as milligram BHT equivalent (mg BHT /g crude extract).

Antioxidant Activities

DPPH Radical Scavenging Activity

DPPH radical scavenging assay was performed according to the method of [7] with a little modification [6]. A 0.1 mmol/L DPPH solution in methanol was prepared, and then 200 µL of this solution was mixed with 100 µL of extract sample and standard control of BHT at different concentrations respectively. The mixture was incubated at room temperature for 30 min and then the absorbance was measured at 517 nm. The ability to scavenge the DPPH radical was calculated as per cent DPPH scavenging using the following equation:

$$\% \text{ DPPH scavenging} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 was the absorbance of the control and A_1 was the absorbance of the mixture containing extracts. IC_{50} of reference antioxidant compounds, BHT were used for comparison to IC_{50} of the extracts.

Ferric Reducing Antioxidant Power (FRAP)

The reducing power was based on the method described previously by [7]. Results were given as absorbance and compared with ascorbic acid used as standard. The (IC_{50}) was calculated from the graph of absorbance at 700 nm against extract concentration.

Cupric Reducing Antioxidant Capacity (CUPRAC)

The reducing power was based on the method described previously by [8]. Results were given as absorbance and compared with ascorbic acid used as standard. The (IC_{50}) was calculated from the graph of absorbance at 450 nm against extract concentration.

Identification of Phenolic Compounds by UPLC-QTOF/MS

The bioactive compounds profiling of the peel sample was performed on a Waters ACQUITY UPLC I-Class/ Xevo in line with a Waters Xevo G2 Q-TOF mass spectrometer (Milford, MA, USA) based on [9]. The dried extract was redissolved by using 1 mg of extract in 1 mL of methanol and subjected to UPLC-QTOF/MS.

RESULTS AND DISCUSSION

In this study, the total phenolic content, total flavonoid content and antioxidant activities of different solvent extracts of *N. lappaceum* and *N. mutabile* peels were determined. Total phenolic content and total flavonoid content were determined by using the Folin-Ciocalteau method and aluminium chloride in colorimetric method respectively. Table 1 shows the total phenolic content and total flavonoid content of *N. lappaceum* and *N. mutabile* peel extracts. The TPC value of different solvent extracts was directly proportional to the TFC value. The order for TPC value was methanol extract (174.50 mg/g) > ethyl acetate extract (129.12 mg/g) > diethyl ether extract (114.50 mg/g). *N. mutabile*, methanol extract exhibits the highest TPC value with a value of 157.87 mg/g. A high content of phenolic compounds was corresponding to the content of phenolic and flavonoid compounds. Thus, methanol extract in both *N. lappaceum* and *N. mutabile* also exhibit the highest content of flavonoid compounds of 4480 mg/g and 2683.33 mg/g respectively. According to the research of [10], phenolic compounds are more soluble in polar organic solvents because of the presence of the hydroxyl group. Since methanol has the highest polarity among the three solvents, hence there are high phenolic compounds from methanol extracts of *N. lappaceum* and *N. mutabile*. The high TPC and TFC values contribute to high antioxidant properties.

Based on the research of [10], the radical scavenging and metal chelating activity are depending on the structural formula, numbers and the position of hydroxyl groups in the phenolic compounds.

The antioxidant activities for each extract of *N. lappaceum* and *N. mutabile* peels were determined by using different assays which were DPPH scavenging activity, ferric-reducing antioxidant power (FRAP), and cupric reducing antioxidant capacity. The antioxidant activities were calculated as IC_{50}. Table 2 showed the IC_{50} values of DPPH, FRAP and CUPRAC assay of *N. lappaceum* and *N. mutabile* peel extracts. The free radical scavenging capacity assay is based on the ability of the antioxidants to reduce DPPH. The decolorization of the purple colour of DPPH radical to yellow colour occurred when the radical accepts electron from the antioxidants. The cupric reducing antioxidant capacity (CUPRAC) assay is based on the reduction of Cu^{2+} to Cu^{+}. In this reaction, the colour change from pale blue to yellow colour. In the FRAP assay, there is a colour change from yellow of Fe^{3+} to green or blue of Fe^{2+} solution depending on the reduction capacity of extracts

Table 1. Total phenolic content and total flavonoid content of *N. lappaceum* and *N. mutabile* peel extracts.

Samples	Total Phenolic Content (mg/g crude extract)		Total Flavonoid Content (mg/g crude extract)	
	N. lappaceum	*N. mutabile*	*N. lappaceum*	*N. mutabile*
Methanol extract	174.50±0.22	157.87±1.68	4480.00±5.25	2683.33±2.75
Diethyl ether extract	114.50±0.87	25.74±0.80	3860.00±3.12	2291.67±3.49
Ethyl acetate extract	129.12±2.76	96.23±0.61	3158.33±1.10	2630.00±4.21
*Gallic acid	1115.42±0.30	1115.42±0.30	-	-
*BHT			750.00±0.14	750.00±0.14

The values are expressed as means ± standard deviation
*Standard control

Table 2. IC_{50} values of DPPH, FRAP and CUPRAC assay of *N. lappaceum* and *N. mutabile* peel extracts

Samples	IC_{50}		
	DPPH	FRAP	CUPRAC
N. lappaceum			
Methanol	2.51 ± 0.018	285.10 ± 0.041	219.31 ± 0.050
Diethyl ether	3.08 ± 0.013	379.33 ± 0.048	287.26 ± 0.043
Ethyl acetate	2.79 ± 0.020	603.34 ± 0.069	351.30 ± 0.025
N. mutabile			
Methanol	3.67 ± 0.049	483.35 ± 0.025	330.96 ± 0.015
Diethyl ether	9.36 ± 0.018	718.52 ± 0.016	889.50 ± 0.010
Ethyl acetate	5.47 ± 0.013	474.94 ± 0.040	497.32 ± 0.026
Standard Control			
BHT	13.89 ± 0.016	359.19 ± 0.050	-
Ascorbic Acid	-	-	282.03 ± 0.070

The values are expressed as means ± standard deviation

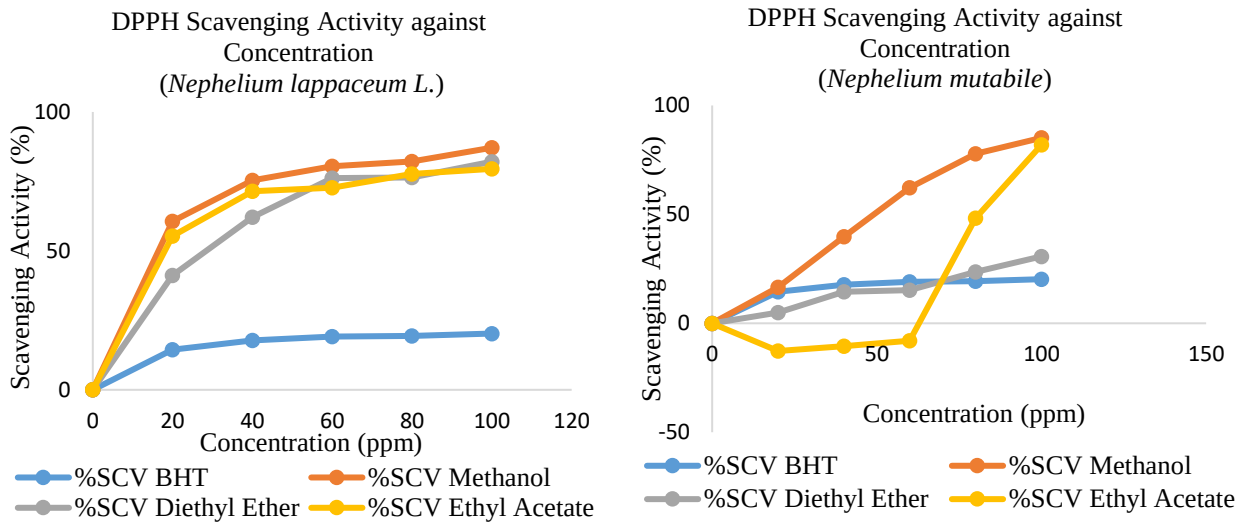

Figure 1. DPPH Scavenging Activity of (a) *N. lappaceum* (b) *N. mutabile.*

The DPPH scavenging assay measured the ability of antioxidant compounds to scavenge the free radical. Figures 1(a) and 1(b) show the DPPH scavenging activities of both rambutan and pulasan peel extracts. Figure 1. (a), the methanol extract of *N. lappaceum* shows the highest radical scavenging activity with IC_{50} value of 2.510 µg/mL. The order for the radical scavenging activities was methanol extract followed by ethyl acetate extract (2.789 µg/mL) and diethyl ether (3.081 µg/mL). The methanol extract of *N. mutabile* peels revealed the highest scavenging ability with IC_{50} value of 3.671 µg/mL. The lower IC_{50} value indicates that the compounds exhibit better antioxidant activities. It was found that the standard, butylated hydroxytoluene (BHT) has the lowest anti-oxidant activities compared with both *N. lappaceum* and *N. mutabile* peel extracts. The results were in agreement with the results of previous research conducted Fadhli et al. (2018) reported that the methanol extract of Pulasan peels was categorized as a strong antioxidant with IC_{50} 57.389 µg/mL [11]

The reducing power of various peel extracts was determined by using FRAP and CUPRAC assay. The reducing ability indicated its antioxidant activities. The reducing power increased with the concentration of extracts. Figures 2(a) and 2(b) show the FRAP-reducing power of *N. lappaceum* and *N. mutabile* peel extracts. From Figure 2. (a), methanol extract of *N. lappaceum* peels show a powerful reducing power with IC_{50} values of 285.102 µg/mL followed by diethyl ether extract (379.332 µg/mL) and ethyl acetate extract (603.337 µg/mL). However, there were some differences with the reducing power of *N. mutabile*. Figure 2. (b) shows ethyl acetate extract (474.939 µg/mL), exhibits the highest reducing ability than methanol extract (483.353 µg/mL) followed by diethyl ether extract (718.522 µg/mL). The difference might be contributed by the different polarity of the compound present in the extract.

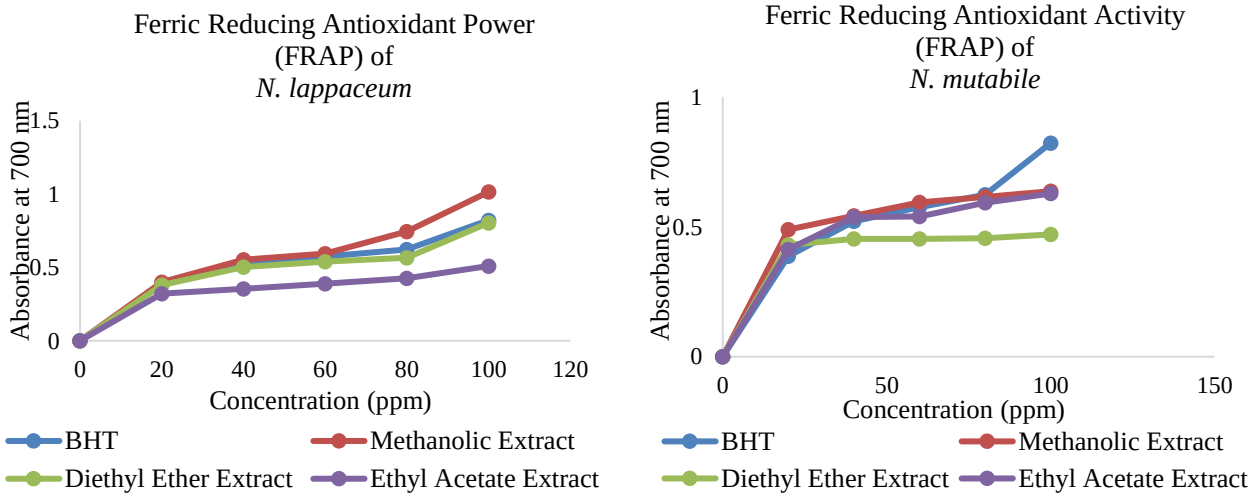

Figure 2. FRAP of (a) *N. lappaceum* (b) *N. mutabile.*

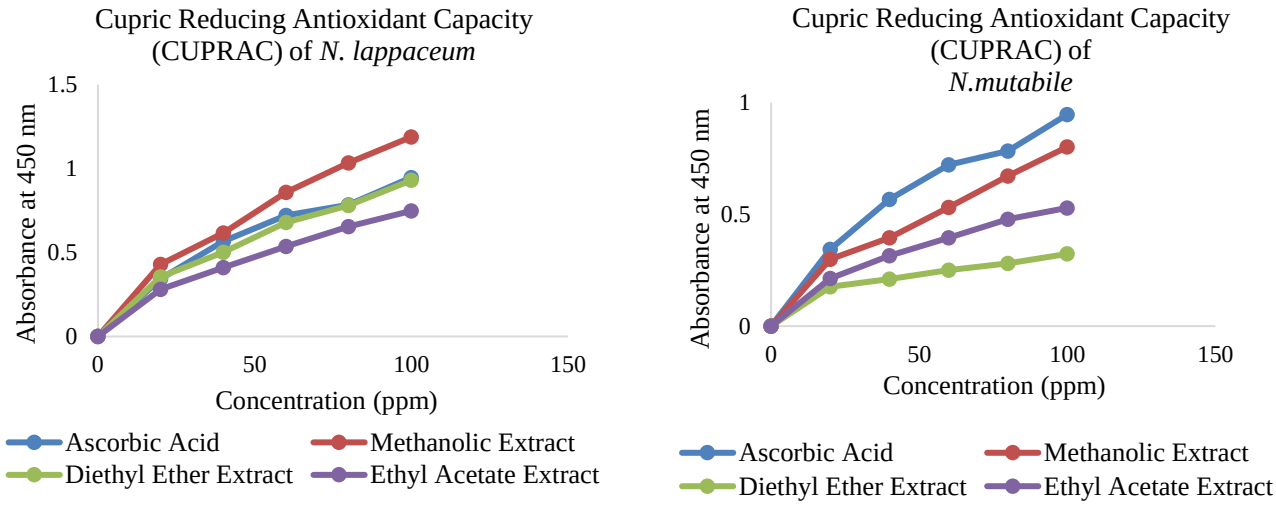

Figure 3. CUPRAC of (a) *N. lappaceum* (b) *N. mutabile.*

For CUPRAC assay, the reducing power of both *N. lappaceum*. and *N. mutabile* peel extracts were different compared with the FRAP assay. Figures 3(a) and 3(b) show the CUPRAC of *N. lappaceum* and *N. mutabile* peel extracts. Based on the graph, both methanol extract of rambutan and pulasan peels exhibit the highest reducing power with IC_{50} values of 219.309 µg/mL and 330.955 µg/mL, respectively. The order of antioxidant activities for *N. lappaceum* peel extracts was methanol extract > diethyl ether fraction > ethyl acetate fraction. However, the order of antioxidant activities for *N. mutabile* peel extracts was methanol extract > ethyl acetate fraction > diethyl ether fraction.

According to [10], copper is better in the detection of every class of antioxidants with very little interference from free radicals than using iron in reducing power assay. Besides, the kinetic reaction of using copper is faster compared with iron. From the three mechanisms of antioxidant activities including DPPH scavenging assay, FRAP assay and CUPRAC assay, the methanol extract of both *N. lappaceum* and *N. mutabile* peel extracts exhibit the highest antioxidant activities due to the high phenolic compounds present.

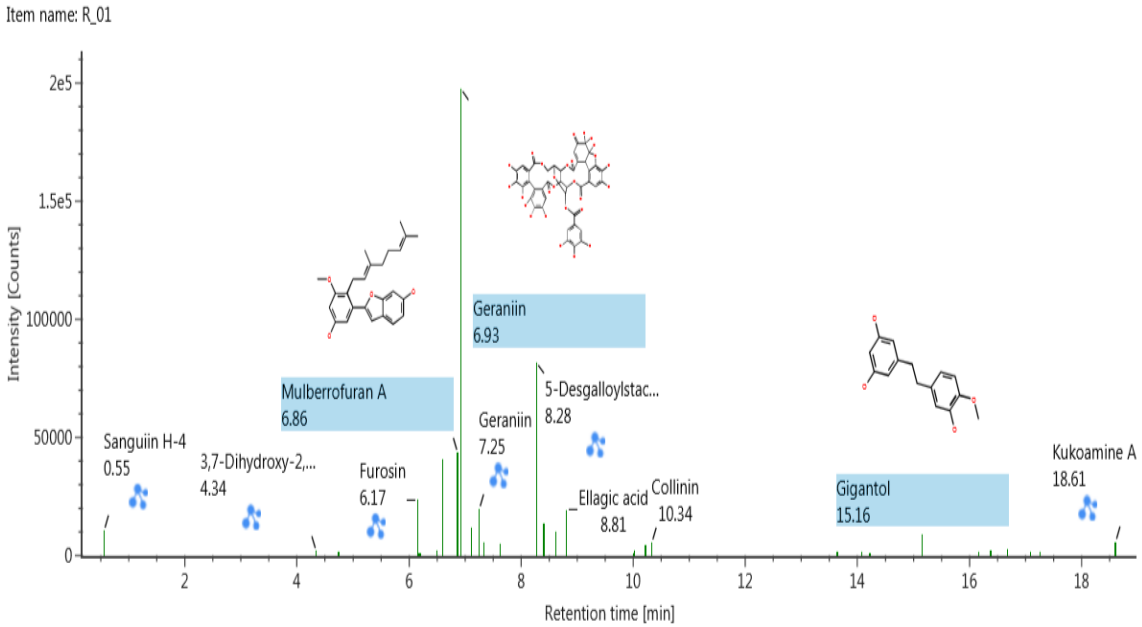

Figure 4. Good match phenolic compounds in methanol extract of *N. lappaceum* peels.

The methanol extract of *N. lappaceum* and *N. mutabile* peels are selected for further identification of phenolic compounds by UPLC-QTOF/MS. The three most abundant phenolic compounds in the *N. lappaceum* include geraniin (RT 6.93 min) detected at m/z 975.0709, mulberrofuran A (RT 6.86 min) detected at m/z 393.2099, and gigantol (RT 15.16 min) detected at m/z 261.1103 (Figure 4). However, the three most abundant phenolic compounds detected in the *N. mutabile* were arecatannin B1 (RT 6.19 min) detected at m/z 867.2126, mulberrofuran A (RT 6.86 min), detected at m/z 393.2099, and gigantol (RT 15.15 min) detected at m/z 261.1099 (Figure 5). The most abundant phenolic compounds were based on the higher ion response with low mass error.

According to the research of [1], the identification of the major phenolic compounds from the methanol extract of *N. lappaceum* peels has been carried out by using HPLC and NMR. There are three major compounds detected which are ellagic acid, corilagin, and geraniin. For 1g of methanol extract, it was found that there is the highest amount of geraniin. Another research carried out by [4] also found that geraniin is the major active compound in ethanolic. In this study, geraniin has been found as the most abundant identified phenolic compound. However, ellagic acid and corilagin obtained are related lower compared to the research of [1]. The ellagic acid was detected at a retention time between 8.41 min while the corilagin was detected at a retention time min and 6.61 min. Based on the research, the low amount of ellagic acid and corilagin (Table 3) in methanol extract may due to the hydrolysis of geraniin during the extraction process of *N\. lappaceum*.

The identified phenolic compounds of the methanol extract of *N. mutabile* are illustrated in Figure 5 and listed in Table 4. Three most abundant phenolic compounds in the *N. mutabile* such as mulberrofuran A (RT 6.86 min) detected at m/z 393.2099, and gigantol (RT 15.15 min) detected at m/z 261.1099. As a result, a high amount of mulberrofuran A can be found in the methanol extract of *N. mutabile* peels. Currently, there is a lack of studies investigating the identification of phenolic compounds of *N. mutabile* peels. However, by comparing both phenolic compounds of *N. lappaceum* and *N. mutabile* in methanol peel extract, some of the phenolic compounds can be found in both peels. For example, the major compound in both peels, mulberrofuran A and gigantol. Geraniin cannot be found in the methanol extract of *N. mutabile*. It also found that the phenolic compounds obtained in the methanol extract of *N. lappaceum* by UPLC-QTOF/MS are more than the *N. mutabile*. This results of the TPC and TFC value were correlated with the phenolic compound identified by using UPLC-QTOF/MS.

Table 3. Good match phenolic compounds of methanol extract of *N. lappaceum* peels.

No.	Phenolic Compounds	Observed (m/z)	Mass error (mDa)	Observed RT (min)	Response
1	Ellagic acid	303.0139	0.3	8.41	13380
2	Furosin	673.0643	-0.5	6.17	23647
3	Terchebin	977.0861	-0.6	8.28	31148
4	Corilagin	657.0698	0.0	6.61	40638
5	Mulberrofuran A	393.2099	3.9	6.86	43368
6	5-Desgalloylstachyurin	785.0833	0.1	8.28	81541
7	Pedunculagin_1	785.0833	0.1	8.28	81541
8	Geraniin	975.0709	-0.1	6.93	197406
9	Gigantol	261.1103	-1.9	15.16	9111

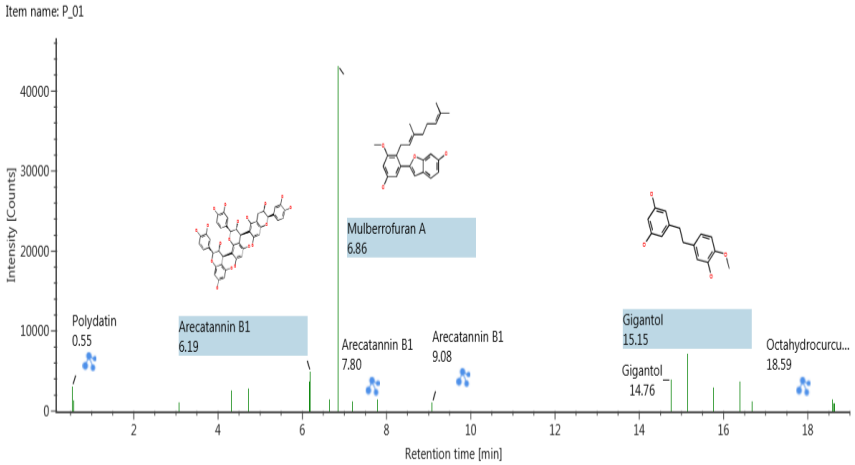

Figure 5. Good match phenolic compounds in methanol extract of *N. mutabile* peels.

Table 4. Good match phenolic compounds in methanol extract of *N. mutabile* peels.

No.	Phenolic Compounds	Observed (m/z)	Mass error (mDa)	Observed RT (min)	Response
1	Polydatin	413.1239	3.2	0.55	3071
2	Dendrocandin B	483.2058	4.5	16.39	3642
3	Moracin M-3′-O-β-D-glucopyranoside	427.1026	2.6	6.18	3648
4	Gigantol	261.1103	-1.8	14.76	3909
5	Arecatannin B1	867.2126	-0.5	6.19	4908
6	Gigantol	261.1099	-2.2	15.15	7181
7	Mulberrofuran A	393.2099	3.9	6.86	43075

CONCLUSION

This research evaluated the phenolic compounds and antioxidant activities of rambutan (*N. lappaceum).* and pulasan (*N. mutabile*) peels. Methanolic extract and both fractions of diethyl ether and ethyl acetate have been used to identify the biological activities of the *N. lappaceum* and *N. mutabile* peels. Higher potential antioxidant activities were found in the methanolic extract of both rambutan and pulasan peels. The order of the antioxidant activities was methanolic extract > ethyl acetate fraction > diethyl ether fraction. Based on the result, methanol solvent shows a higher capability to extract phenolic compounds and the antioxidant results show the possibility of rambutan and pulasan peels being used as natural antioxidants.

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