

DPPH RADICAL SCAVENGING ACTIVITY AND TOTAL PHENOLIC CONTENT OF RAMBUTAN (*Nephelium lappaceum*) PEEL AND SEED

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Abstract

Rambutan (*Nephelium lappaceum*) is a tropical fruit belongs to the family (Sapindaceae) and it is native to Southeast Asia. Peel and seed are the by-product usually thrown away which may serve as a source of natural antioxidant. The objectives of this research were to determine the total phenolic content (TPC), total flavonoid content (TFC) and DPPH radical scavenging activity from aqueous and ethanolic extracts of rambutan seed and peel. Total phenolic content and total flavonoids content were evaluated using Folin-Ciocalteu reagent and aluminium chloride method, respectively. The antioxidant activity was tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Aqueous extract of peel showed significantly ($p < 0.05$) higher extraction yield of $29.97 \pm 2.69\%$. The results showed ethanolic extract of peel exhibited the highest TPC of 244.00 ± 4.34 mg GAE/g. However, ethanolic extract of rambutan seed recorded highest total flavonoid content of 163.33 ± 1.88 QE/g. Rambutan peel demonstrated the highest free radical scavenging activity with inhibitory concentration (IC_{50}) value of 24.99 ± 2.82 and 144.59 ± 1.36 $\mu\text{g/mL}$ for ethanolic and aqueous extract, respectively. Positive correlations between total phenolic content and DPPH scavenging activity of peel and seed extracts were observed ($r = 0.77$, $p < 0.01$). The present study suggests that rambutan peel can be utilized for the development of functional food.

Keywords: Antioxidant, extracts, yield, correlations, total flavonoid content

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1. INTRODUCTION

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are highly reactive and trigger oxidation reactions in the fatty acids present in biological membranes and foods (Ana et al., 2009). They are the major precursor for many health implications such as cancer, cardiovascular diseases (Köksal, & Gülç, 2008). Antioxidants are used to reduce the risk of disease caused by oxidative stress (Fidrianny, & Sukowati, 2015). They are also widely used in dietary supplements and have been investigated for the prevention and treatment of diseases such as cancer, coronary heart disease (Adedapo et al., 2015). Polyphenolic compounds are found in fruits, vegetables, nuts and grains with antioxidant activity (Arfan et al., 2012). They also functions as free radical scavengers, reducing agents, complexes of pro-oxidant metals,

singlet oxygen quenchers and stimulating the antioxidative defence enzymes activities (Huwaitat et al., 2013). The most effective antioxidants properties of polyphenol is scavenging free radicals and it yield stable products (Samuagam et al., 2013). In addition to the antioxidant, they exhibited antibacterial and antifungal activities (Huwaitat et al., 2013). Rambutan (*Nephelium lappaceum*) is a seasonal fruit belongs to the family of Sapindaceae and are widely planted in Malaysia, Thailand and other southeast countries (Nurhuda et al., 2013). Besides the delicious fruit, various parts of the plant are used to treat many diseases, including diabetes mellitus (Rahayu et al., 2013). Bioactive compounds including ellagic acid, corilagin and geraniin have been isolated in rambutan peel (Thitilertdecha et al., 2008).

Peel and seed are by-products of most fruits and vegetables. Some of these by-products

have been investigated and have proven to be effective sources of phenolic antioxidants. They were also tested in foods that are susceptible to lipid oxidation such as edible oils, fish, meat and poultry products, and have shown high antioxidant activities comparable to that of synthetic antioxidants (Balasundram et al., 2006). Recently researchers are interested in the utilization of underutilized by-product of fruits, vegetables or residual sources from agricultural industries (Suttirak & Manurakchinakorn, 2014). This is due to their abundance in various compounds ranging from hydrophilic to lipophilic, such as flavonoids, phenolic acids, carotenoids, tocopherols, and essential fatty acids that may prevent the effect caused by free radicals (Adnan et al., 2011). Peel and seed of rambutan are discarded after consuming the flesh (Thitilertdech et al., 2008). Previous studies revealed that fruit and vegetable by-products possessed high antioxidant activity. (Shiban et al., 2012) Reported the antioxidant activity of methanolic extract of pomegranate assessed by DPPH and it was found to be stronger than that of α -catechin. Aspects of extraction and yield for sufficient production of natural antioxidants from most of these sources remain to be explored. Therefore, the aim of this research was to evaluate the total phenolic content, total flavonoids content and antioxidant activity of ethanolic and aqueous extracts of rambutan (*Naphelium lappaceum*) peel and seed native to Malaysia.

2. MATERIALS AND METHODS

Sample preparation

The rambutan fruits were purchased from fruit shop in Kampong Raja, Terengganu Malaysia. Upon arrival at the laboratory, the fruits were peeled and the seeds pulp and peel were separated manually and washed with tap water several times to remove mucilage and pulp left. The seeds and peel were air-dried and ground.

Chemicals

Folin-Ciocalteu, sodium carbonate (Na_2CO_3) was purchased from Merck (Germany),

potassium acetate (KA), AlCl_3 , 1,1-diphenyl-2-picryl-hydrazyl (DPPH \cdot) were obtained from sigma Aldrich (Germany). Quercetin, ethanol and other reagents are of analytical grade.

Extraction of phenolic compounds

The method described by (Samuagam et al., 2013) with some modifications was adopted. The powdered sample material (10 g) was extracted with ethanol or water (50 mL). It was allowed for three days with regular shake. The mixture was filtered through a filter paper (Whatman No.1) and concentrated using rotary evaporator at 40°C and the extracts were stored in the -20°C until further analysis.

Determination of total phenolic content

The phenolic content was determined by following the modified Folin-Ciocalteu method (Singleton & Rossi, 1965) using gallic acid as standard. Briefly, 250 μL of the extract at appropriate dilutions were mixed with 1.25 mL of 0.2 M Folin-Ciocalteu reagent and incubated for 5 min at room temperature. Then 1000 μL of 7.5% sodium carbonate was added. The mixture was allowed to stand for 30 min at room temperature. The absorbance of the resulting blue colour was measured at 760 nm (Shimadzu, UV mini 1240, Japan). Phenolic contents were expressed as mg of Gallic acid equivalent (GAE)/g of extract.

Determination of total flavonoids content

The total flavonoid content of both extracts were determined using a method reported by (Oboh & Ademosun, 2012) with slight modification. Briefly 0.5 mL of appropriate dilution was mixed with 0.5 mL methanol, 50 μL 10% AlCl_3 , 50 μL 1 M Potassium acetate and 1.4 mL water, the mixture were allowed to incubate at room temperature for 30 min. The absorbance of the reaction mixture was subsequently measured at 415 nm. The total flavonoid content was expressed as mg of quercetin equivalent QE/g of extract.

Determination of DPPH free radical scavenging activity

Free radical scavenging activity of the peel and seed extracts was determined according to the method of (Shofian et al., 2011) with some

modifications. Briefly, 2.5 mL of methanolic solution of DPPH (25 µg/mL) was added to 0.5 mL extract at different concentrations. The reaction mixture was then kept at room temperature for 30 min. The absorbance was measured at 517 nm using spectrophotometer (Shimadzu, UV mini 1240, Japan). The percentage of DPPH discoloration of the sample was calculated according to the equation as:

$$(\%) = [(A_0 - A_1) / A_0] \times 100$$

Where, A_0 is the absorbance of the control reaction and A_1 is the absorbance of the sample itself. The inhibitory concentration at 50% (IC_{50}) values was determined from the graph of scavenging percentage against the extract concentrations (25, 50, 100 and 250 µg/mL). All determinations were carried out in triplicate.

Statistical analysis

Data was subjected to one way analysis of variance (ANOVA) with statistical level ($p < 0.05$) and the significant difference was determined by Duncan test using the SPSS version 20. All analyses were carried out in triplicate and data were expressed as means \pm SD. while Pearson correlation test was conducted to determine the correlation among variables ($p < 0.01$).

3. RESULTS AND DISCUSSION

Extraction yield

Rambutan peels and seed were extracted with two polar solvents (ethanol and water). This is due to their non-toxicity properties in food and pharmaceutical application. The extract yield of peel and seed ranged from 5.97 to 29.97 %. The maximum extract yield was obtained from

the aqueous peel extract followed by ethanolic peel extract (Table 1). Highest extraction yield (25%) was reported from the water extracts of *P. Macrocarpa* (Easmin et al., 2017). The yield of rambutan peel was in line with the value of 28.23% (Samuagam et al., 2013).

Total phenolic content

Plants contain significant amount of phenolic compounds comprise of two major groups of flavonoids and phenolic acid (Yunusa et al., 2015). They are the major contributors to the overall antioxidant activities of plant foods (Xu & Chang, 2007). Total phenolic contents (TPC) of the extracts from peel and seed are shown in Table 1. Results are expressed in terms of gallic acid equivalent using the standard curve (Figure 1A). Seed and peel extracts from aqueous and ethanolic differed significantly ($p < 0.05$). The TPC of peel and seed extracted using aqueous and ethanol ranged from 7.93 to 244.00 mg GAE/g. The TPC of both peel and seed were affected with the solvent used with larger variation. The TPC were in the order: ethanolic peel > aqueous peel > ethanolic seed > aqueous seed. These results suggest that ethanol is the best solvents for extracting phenolic compound from both peel and seed. The highest phenolic content was found in ethanolic extract of peel. This is in line with the work of (Shiban et al., 2012) who reported highest total phenolic content in the methanolic extract of pomegranate peel (274 ± 17 mg GAE/g). The phenolic content methanolic extract of rambutan peel was reported to be higher than the value obtained in the present study (542.2 mg/g catechin/g) (Thitilertdecha et al., 2008).

Table 1: Extraction yield, total phenolic and total flavonoids content

Solvent	fruit part	Yield (%)	TPC (mg GAE/g)	TFC (mg QE/g)
Ethanolic	Peel	22.1 \pm 1.89 ^b	244.00 \pm 4.34 ^a	69.36 \pm 7.17 ^d
	Seed	5.97 \pm 1.76 ^c	27.10 \pm 1.09 ^c	163.33 \pm 1.88 ^a
Aqueous	Peel	29.97 \pm 2.69 ^a	49.92 \pm 2.41 ^b	101.40 \pm 2.28 ^b
	Seed	7.50 \pm 0.74 ^c	7.93 \pm 1.07 ^d	85.17 \pm 1.73 ^c

Values are mean (n=3) \pm standard deviation. Values with the same superscript letter within each column are not significant different ($p > 0.05$).

This shows that methanol has the highest affinity to the phenolic compounds than ethanol. However, the phenolic content reported by present study was higher than that of several fruits such as *H. polyrhizus* (15.92 mg GAE/100g) and *H. Undatus* (20.14 mg GAE/100g) (Choo & Yong, 2011). The differences in total phenolic content among the extracts could be attributed to many factors such as solvent (Shiban et al., 2012). Higher phenolic content in the peel may be due to the fact that the secondary metabolites are predominantly concentrated at outer part to protect the internal tissue from environmental hazard. Peel of both aqueous and ethanolic extract was found to contain higher phenolic content than rambutan seed, this was agreed with (Thitilertdecha et al., 2008) who also observed lower TPC in the seed of rambutan compared to the peel part in ether, methanolic and aqueous extracts.

Total flavonoids content

Flavonoids are diversely found in different plant materials and contribute largely to the antioxidant activity of different food of plant origin (Shiban et al., 2012). It comprises of flavonol, flavanol flavanone, flavone and anthocyanin. The antioxidant activity of the individual flavonoid depends on the degree and position of the hydroxyl group. The total flavonoids of rambutan peel and seed are presented in Table 1. Results are expressed in

terms of quercetin equivalent using the standard curve (Figure 1B). The TFC of ethanolic and aqueous extracts of peel and seed varied from 69.36 ± 7.17 to 163.33 ± 1.88 mg QE/g. The ethanolic seed extract recorded the highest ($p < 0.05$) value, while ethanolic extract of peel part contained the lowest TFC. In this study, aqueous extract of peel part demonstrated highest TFC compared to its ethanolic counterpart. Similar trend was also reported in the water extract of young leaves of *C. caudatus* (Noriham et al., 2015).

DPPH scavenging activity

Antioxidant activity of rambutan (*Naphelium lappacium*) peel and seed was evaluated using DPPH. DPPH was found to be the most widely used assay because of its stability, simplicity, and required short time for analysis (Adnan et al., 2011). The decrease in absorbance at maximum wavelength of 517nm indicates reduction of DPPH radicals (Huwaitat et al., 2013). The potency of free radical scavenging activity of the extract is accessed with IC_{50} value which is defined as the concentration of the extract capable of 50% radical inhibition. Therefore, lower IC_{50} value is associated with a stronger DPPH scavenging capacity (Fidrianny et al., 2015).

The DPPH values of aqueous and ethanolic extracts of peel and seed are presented in Table 2.

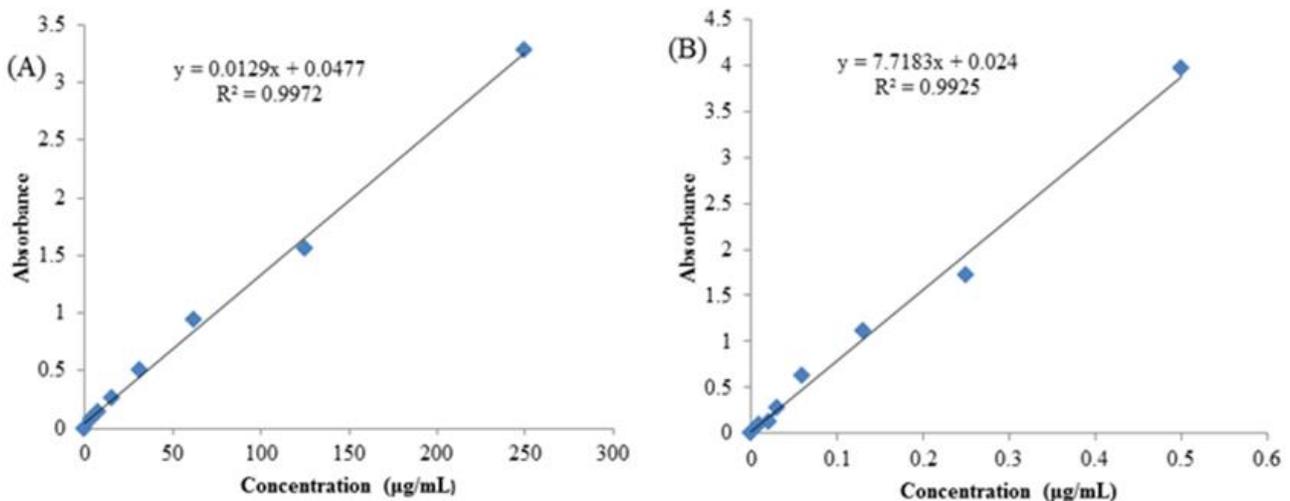


Figure 1. Standard curve for Gallic acid (for TPC) (A) and quercetin (for TFC) (B)

Table 2: DPPH scavenging activity of aqueous and ethanolic extract of rambutan peel and seed

Solvent	Fruit part	Inhibition (%)	IC ₅₀ (µg/mL)
Ethanol	Peel	95.73 ± 1.63 ^a	24.99 ± 2.82 ^b
	Seed	1.67 ± 0.98 ^c	ND
Water	Peel	80.25 ± 3.15 ^b	144.59 ± 1.36 ^c
	Seed	1.90 ± 0.98 ^c	ND
	Quercetin	94.54 ± 0.25 ^a	11.91 ± 0.41 ^a

Values are mean (n=3) ± standard deviation. Values with the same superscript letter within each column are not significant different ($p>0.05$).

The percentage inhibition varied from 1.67 to 95.73 %. The highest inhibition was found in the peel of both extracts. The DPPH value greatly affected by the extracting solvents and followed: ethanolic peel extract > aqueous peel extract > aqueous seed extract > ethanolic seed extract. Strong free radical scavenging activity of DPPH was observed for peel part with IC₅₀ value of 24.99 and 144.59 µg/mL for ethanolic and aqueous extract, respectively. It is consistently agreed with work of Fidrianny et al. (2015) who found that the ethanolic extract of extract of binjai rambutan leaves had the highest DPPH radical scavenging capacity (94.63%) with corresponding lower IC₅₀ of 14.666 µg/mL. It is also in line with reported value of Huwaita et al., (2013) who reported a lower IC₅₀ (26.436) in *Iris Nigricans* leaves. In a similar vein, (Chemah et al., 2010) found higher DPPH scavenging activity in the ethanolic extract of pitaya seed than that of the water extract.

Pearson correlation between phenolic content and DPPH radical scavenging activity

Correlation between polyphenolic contents and antioxidant has been studied intensively. Hypothesis revealed that phenolic compounds contribute significantly to the total antioxidant capacity of plants species (Piluzza & Bullitta, 2011), several literatures were found to be consistent with hypothesis. However, several literatures contradicted with hypothesis. For instance (Li et al., 2013) reported a very weak correlation between FRAP value and TPC in infusions from 223 medicinal plants. Also (Dian-Nashiela et al., 2015) observed a negative correlation between TPC antioxidant

activities of *C. caudatus* herbal tea. According to (Piluzza & Bullitta, 2011), beside phenolic compounds, there are other phytochemical compounds which contribute to the antioxidant activity of plants. Correlation analyses between phenolic contents and DPPH among all samples were performed. The extracts from both water and ethanol exhibited significant ($p<0.01$) linear correlations between TPC and DPPH ($r = 0.77$). Our coefficient was similar to 70% ethanol extracts ($r = 0.77$) of legumes (Xu & Chang, 2007).

4. CONCLUSION

In conclusion, our study demonstrated that total phenolic content and DPPH radical scavenging activity vary considerably among rambutan by-products; peel had the highest phenolic content and free radical scavenging activity. The peel part has a high potential to be utilized as natural antioxidant in food or pharmaceutical industry.

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