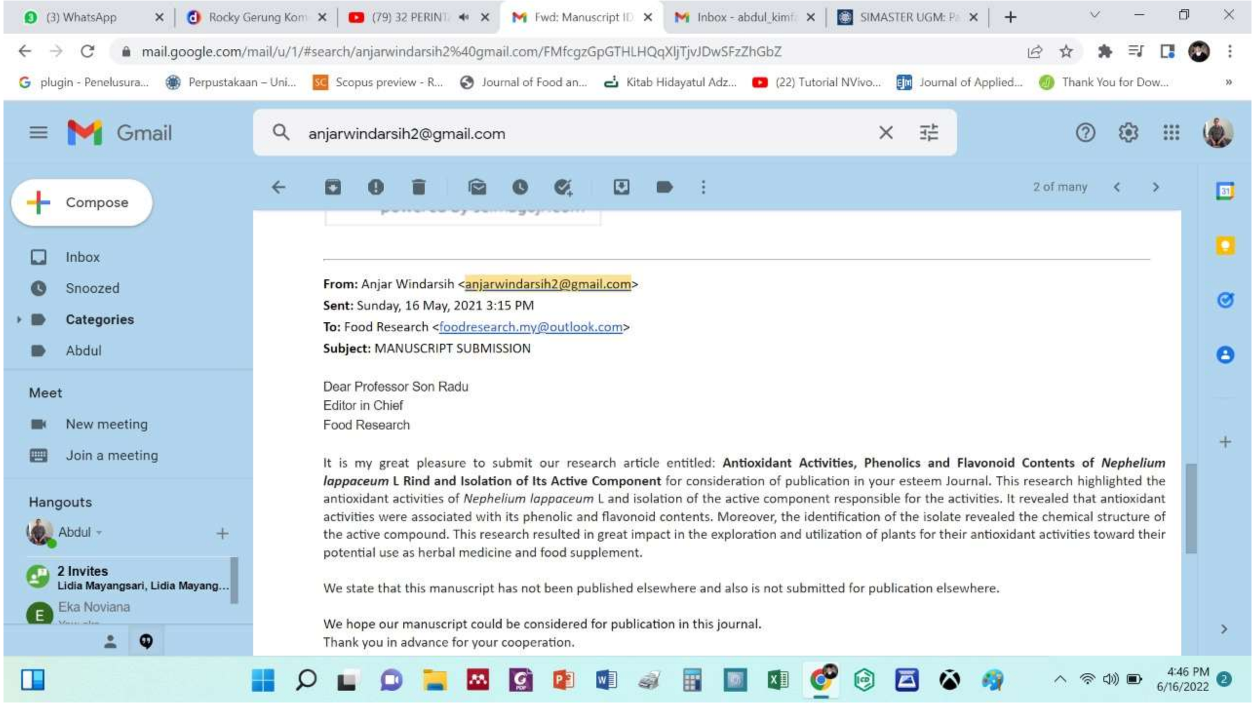

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To: Food Research <foodresearch.my@outlook.com>
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Dear Professor Son Radu
Editor in Chief
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It is my great pleasure to submit our research article entitled: **Antioxidant Activities, Phenolics and Flavonoid Contents of *Nephelium lappaceum* L Rind and Isolation of Its Active Component** for consideration of publication in your esteem Journal. This research highlighted the antioxidant activities of *Nephelium lappaceum* L and isolation of the active component responsible for the activities. It revealed that antioxidant activities were associated with its phenolic and flavonoid contents. Moreover, the identification of the isolate revealed the chemical structure of the active compound. This research resulted in great impact in the exploration and utilization of plants for their antioxidant activities toward their potential use as herbal medicine and food supplement.

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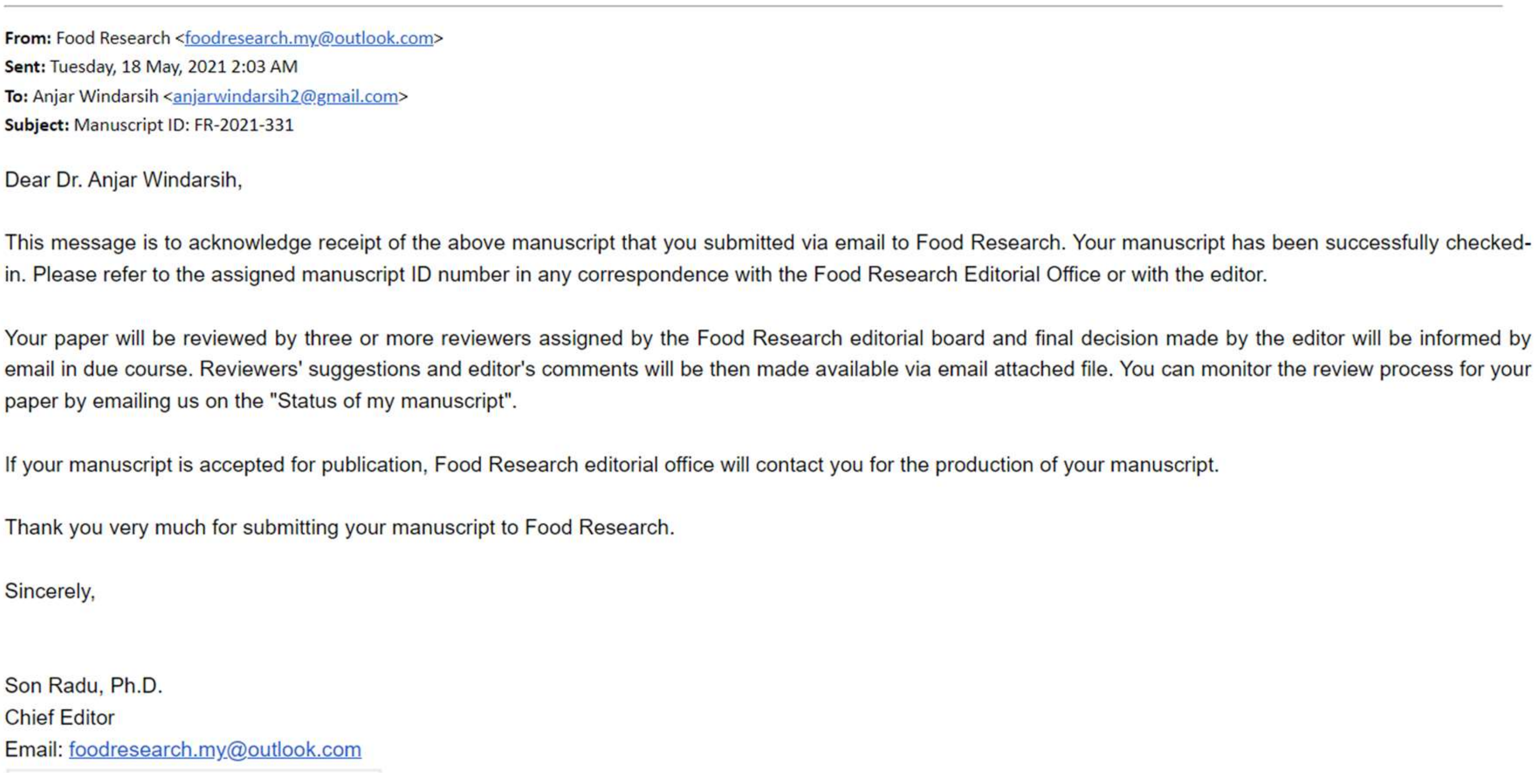

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Dear Dr. Anjar Windarsih,

Manuscript FR-2021-331 entitled " Antioxidant Activities, Phenolic and Flavonoid Contents of Nephelium lappaceum L Rind and Isolation of Its Active Component " which you submitted to Food Research, has been reviewed. The comments of the reviewer(s) are included in the attached file.

The reviewer(s) have recommended publication, but also suggest some revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript. Once the revised manuscript is prepared, please send it back to me for further processing.

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Son Radu, PhD
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Antioxidant Activities, Phenolic and Flavonoid Contents of *Nephelium lappaceum* L. Rind and Isolation of Its Active Component

Abstract

The aim of this study was to explore the antioxidant activities of rambutan rind *in vitro* and to correlate those activities with group of compounds (phenolics and flavonoids). Rambutan rind was cleaned, dried using conventional oven, powdered, and the powder obtained was subjected to maceration. The macerate was fractionated using petroleum ether and chloroform to get chloroform fraction. The sub fractionation was done toward chloroform fraction. The chloroform fraction and its sub fractions were then subjected to antioxidant activity measurement, determination of phenolics and flavonoid contents, and the active compound present in the most active sub fractions was isolated using column chromatography. The isolate was identified using several method including thin layer chromatography, Fourier transform infrared (FTIR) spectroscopy as well as gas chromatography-mass spectrometry (GC-MS). The results showed that chloroform fraction and its sub fractions were correlated with phenolic and flavonoid contents. The phenolics contents were more contributing toward metal chelating activities compared other antioxidant activities with R^2 value of 0.8726, while flavonoid contents more contributed to ABTS radical scavenging activity with R^2 of 0.8916. One of the active compounds present in rambutan rind having antioxidant activity was identified as 1,2-benzenedicarboxylic acid. Thus, the under-utilized part of rambutan rind could be explored as natural antioxidant to be used as food supplement.

Keywords: Rambutan Rind, In Vitro Antioxidant, Phenolic Content, Flavonoid Total, 1,2-benzenedicarboxylic acid.

1. Introduction

The imbalance between reactive oxygen species (ROS) and reactive nitrogen species (RNS) with endogenous antioxidant has led to the formation of oxidative stress. ROS and RNS have been associated with some degenerative diseases including diabetes, cancer, aging, neurodegenerative and cardiovascular diseases (White *et al*., 2014). Some efforts have been driven by group of research to explore exogenous antioxidants either natural or synthetic antioxidants (Palanisamy *et al*., 2008). Antioxidant can be defined as any compounds or materials capable of delaying or inhibiting the oxidation reactions of lipids, proteins and other molecules by terminating free radical reaction (Pavithra and Banu, 2017). Natural antioxidants derived from fruits and vegetables have been explored and commercialized such as grape seed extracts. One of the potential natural antiradicals derived from fruit is Rambutan.

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Rambutan (king of fruits) with scientific name of *Nephelium lappaceum* L. (Family of Sapindaceae) is tropical fruit commonly found in South East Asian region like Indonesia, Thailand Malaysia, and Vietnam *(*Fidrianny *et al.,* 2015). Rambutan existed, and the most commonly consumed is cultivars Aceh, Binjai and Rapiah (~~Abdul~~ Rohman *et al*., 2016). The consumption of Rambutan fruit has resulted vast amount of wastes, including rind and peel. Therefore, it is very interesting to explore of underutilized part of rambutan fruit as natural antioxidant and identifying the active components contributing to antioxidant activities.

Several biological activities of Rambutan fruit including its rind and seed have been reported, namely antiradical due to its phenolic compounds contained (Thitilertdecha and Rakariyatham, 2011), *in vitro* and *in vivo* antioxidant activities using different standardized methods (Pavithra & Banu, 2017),(Iman Kamaludin, Mun, & Sa'adi, 2016),(Mistriyani *et al.*,2020), lipid peroxidation inhibition (Setyawati *et al*., 2015) anti-inflammatory effects (Chingsuwanrote *et al.*, 2016) anti-hypercholesterolemia activities (Muhtadi *et al.*, 2016) antibacterial activities against some pathogenic bacterial strains (Thitilertdecha *et al.*, 2008), inhibitors of alpha-amylase and alpha-glucosidase activities *in* vitro (Thinkratok *et al.*, 2014) hypoglycemic effects (Soeng *et al.*, 2015), anti-hyperglycemic activity (Palanisamy *et al*., 2011)**,** α- and β-glucosidases inhibition (Widowati *et al*., 2015), and anti-diabetic activity on rats induce by alloxan (Muhtadi *et al.*, 2016)

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Some active compounds have been identified in rambutan including geraniin, corilagin and ellagic acid (Hernández *et al*., 2017). A total of 39 compounds were also identified in rambutan rind, including 1 simple phenolic acid, 1 flavone, 5 hydrolyzable tannins, 5 hydroxybenzoic acids, 10 flavonols, 11 flavonols, 6 ellagic acid and conjugates (Zhuang, Ma, Guo, and Sun, 2017). In this study, the chloroform fraction of Rambutan rind was fractionated and evaluated for its antioxidant activities. Furthermore, the active component contributing to antioxidant activity was identified.

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2. Materials and methodse

2.1 Materials

Rambutan fruit was obtained from Bantul, Yogyakarta, Indonesia. The authentication of rambutan samples was performed in Department of Pharmaceutical Biology**,** Faculty of Pharmacy with assistance by Dr. Djoko Santosa. The chemical reagents and solvents used were of pro-analytical grade.

2.2 Prepation of Chloroform E~~k~~xtract o~~O~~f Rambutan

The chloroform fraction was prepared according (~~Abdul~~ Rohman *et al*., 2016). Rambutan rind was cleaned, cut into small pieces using commercial cutter and dried in a conventional oven at 65^{o}C for 2 days and then ground into powder. The powder was subjected to extraction using maceration technique using methanol as extracting solvent and occasionally shaken. The solvent was evaporated using vacuum rotary evaporator to obtain methanolic extract. The extract was then added with aquadest and subjected to partition using petroleum ether, chloroform, and ethyl acetate to get corresponding fractions, namely petroleum ether (PE), chloroform (CH) and ethyl acetate (EA) fractions. CH fraction was then further sub-fractionated to get sub-fractions. The most active sub-fraction as antioxidant was subjected to isolation to identify the compounds responsible for antioxidant activity. The methanol extracts and its fractions (water, PE, CL, and EA) were evaluated by determining antiradical activities using 2,2'-diphenyl-1-picrilhydrazil (DPPH) and 2,2'-azinobis (3-ethylbenzo thiazoline-6-sulphonic acid) diammonium salt (ABTS), reducing power, and metal chelating activity.

2.3 Evaluation of Radical S~~e~~cavenging Activity using DPPH Radical;

Scavenging activity of DPPH radicals was performed according to (Khalil, Khan, Shabbir, & Rahman, 2018) with slight modification. A-50 µL of extract and fraction samples with different concentration was added with 1.0 ml DPPH 0.4 mM and 3.950 mL methanol. The mixture was

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allowed to react at room temperature for 20 min. Then, absorbance was measured at 515 nm with a calibrated UV–visible spectrophotometer. All measurements were done in triplicate.

$$\% \text{ antiradical activity} = \frac{(A_o - A_1)}{A_o} x100$$

Ao is absorbance of control (without samples) and A_1 is absorbance of samples.

2.4 Radical Scavenging Activity using ABTS Technique

The ABTS radical assay of extract and fraction samples was performed according to (Fidrianny *et al*., 2015). The mixture of potassium persulphate 2.45 mM and ABTS 7 mM (1:1) was allowed to stand in the dark for 12–16 h at room temperature to produce ABTS radical cation ($ABTS^+$). This $ABTS^+$ solution was diluted with methanol to obtain absorbance values of 0.600-0.800 at 734 nm. The $ABTS^+$ working solution (3 mL) and 30 µL of blank, standard or sample were mixed and the absorbance was measured at 734 nm after 6 min using a spectrophotometer. The blank was run with methanol.

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2.5 Reducing Power Evaluation

The evaluation of ferric reducing activity power (FRAP) was carriet out according to Mistriyani *et al.* (2018). FRAP reagent was prepared by mixing 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L 2,3,5-triphenyl-1,3,4-triaza-2 -azoniacyclopenta-1,4-diene chloride (TPTZ)(in 40 mmol/L HCl), and 20 mmol/L ferric chloride (10:1:1, v:v:v). To the 4.5 mL of reagent, 150 µL ethanol plant extract was added. The absorbance readings were started after 5 min and performed at wavelength of 593 nm. The blank consisted of FRAP reagent. The final absorbance of each sample was compared with those obtained from the standard curve made from ferric sulphate ($FeSO_4 \times 7H_2O$) (200–1000 µmol/L). Results were expressed in nmol Fe2+/mg dried extract.

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2.6 Metal Chelating Activity

Metal chelating activity of extract and fractions was measured as described by (Wong *et al*., 2014). A certain amount (mL) of samples was added with 0.1 mM $FeSO_4$ (0.2 mL) and 0.25 mM ferrozine (0.4 mL). After incubating at room temperature for 10 min, the absorbance of the mixture was recorded at 562 nm. Chelating activity was calculated using the following formula:

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$$\text{Chelating activity (\%)} = \frac{(A_o - A_1)}{A_o} x100$$

Where Ao is the absorbance of control reaction (without any samples) and A_1 is the absorbance in the presence of extracts or fractions.

2.7 Determination of Total Phenolics and Flavonoid Contents

Total phenolics contents of extracts and fractions were determined according to (Kovarovič *et al*., 2019)., while total flavonoids were determined using spectroscopic method according to (~~Abdul~~ Rohman *et al.*, 2010). For phenolics content analysis, Folin Ciocalteu reagent (FCR) was used. Briefly, extracts and fractions in methanol were mixed with 0.4 ml of FCR. The solution was allowed to stand at room temperature for 8-10 min, added with 4 mL Na_2CO_3 7% and made to 10.0 mL with bidistilled water. The mixture was allowed to stand for 2 h and subsequently measured at 725 nm. The phenolics contents were expressed as mg gallic acid equivalents (GAE) per gram of sample (mg/g). For total flavonoid analysis, samples in methanol were added with 4 mL of distilled water and 0.3 mL of $NaNO_2$ 5%. After 5 min, 0.3 mL $AlCl_3$ 10% was added to the mixture. At 6 min, 2 mL NaOH 1 M was added to the mixture. Immediately, the mixture was diluted to volume with the addition of 2.4 mL distilled water and thoroughly mixed and its absorbance was measured at 510 nm versus a blank containing all reagents except samples of extracts or fractions. Total flavonoid content of the extracts and fractions were expressed as mg rutin equivalents (RE) per gram of sample (mg/g).

2.8 Fractionation of Chloroform Extract

Chloroform fraction has good antioxidant activities, therefore, it was further subjected to fractionation using vacum liquid using silica gel G 60 GF_{254} as in (~~Abdul~~ Rohman *et al*., 2010). The chloroform fraction was dissolved in chloroform, then added with anhydrous sodium sulphate previously heated for 2 hour at 110°C, and allowed to stand for one night and then filtered using a filter paper. The solvent was evaporated using vacuum rotary evaporator. Samples were added to column that has been let stand overnight. The chloroform fraction was eluted using the solvents of chloroform, ethyl acetate, and methanol in various compositions.The eluates were collected for each 50 mL, and were evaporated using vacuum rotary evaporator.

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2.9 Fractionation of Active Fraction

The sub-fractions were loaded into TLC using stationary phase of GF254 with suitable eluting solvents. The plate was sprayed with DPPH 0.02% in methanol. Sub-fraction capable of bleaching DPPH color was subjected to further fractionation using gravimetric column. The sub-fraction was loaded into column using silica gel 230-400 mesh (0.040-0.063 mm) and isocratically eluted with n-hexane: acetone (2:2 v/v) and with petroleum ether: ethyl acetate (1:1 v/v) The eluates were collected for each 5 mL.

2.10 The Purity Identification

The purity of isolate was checked using two methods, namely melting point and thin layer chromatography (TLC). The melting point of crystal was checked using Buchi Melting Point B-450. Temperatures were recorded at the time crystal begin to melt and the temperature at which crystal become liquid all. The test was repeated by measuring the temperature of ± 10°C below the melting point obtained, ramped at 1°C/min. For purity test using TLC, the isolate was eluted using three eluent systems with mobile phase having different polarity index, namely acetone: ethyl acetate: chloroform with a ratio of 4: 3: 3 v/v. The compound was considered as pure if it had single spot on TLC system. The spot detection was performed visually using UV$_{254}$ and UV$_{366}$.

2.11 The Identification of Isolate

The chemical structure of isolate was determined using FTIR spectroscopic method (FTIR 100 PERKIN ELMER) and gas chromatography-mass spectrometry (GCMS-QP2010S SHIMADZU, Japan).

3. Results and Discussion

Antioxidant activities of Rambutan rind were evaluated using radical scavenging namely DPPH and ABTS, ferric reducing activity power (FRAP) and metal chelating activity, which can be differentiated as Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET) (Prior *et al.*, 2005; ~~A.~~ Rohman *et al.*, 2020).

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3.1 Antiradical Scavenging Activities

DPPH is a synthetic radical which is stable and soluble in polar organic solvents such as methanol and ethanol. The principle of DPPH radical scavenging test was based on reduction of radical DPPH (violet in color) into non-radical DPPH (colorless), thus, the antiradical compounds could reduce DPPH radical intensity at wavelength 517 nm, with the absorptivity molar decreased from 9660 M^{-1}cm^{-1} (radical DPPH) into 1640 M^{-1}cm^{-1} (non-radical DPPH). When antiradical such as phenolic and flavonoids donate its hydrogen radical into DPPH radical, the color of radical DPPH was decreased. The proposed reaction between antiradical compounds (antioxidants) with DPPH radical could be seen in Figure 1. The parameter used for describing DPPH radical activity was IC$_{50}$, the concentration of the samples necessary to cause 50% scavenging of DPPH radical, calculated from linear regression equation (~~Abdul~~ Rohman *et al.*, 2016). The higher antiradical activities, the lower the IC$_{50}$ values.

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Table 1 compiled the IC$_{50}$ values of methanol extract and its fraction of Rambutan rind using DPPH radical with vitamin C (ascorbic acid) as the positive control. Vitamin C revealed the strongest anti-radical activities (the lowest IC$_{50}$). In addition, ethyl acetate fractions showed antiradical activities among extract and fractions evaluated followed by methanol and chloroform (which are not statistically different or $P > 0.05$) and petroleum ether. Samples with IC$_{50}$ values of 10-50 µg/mL considered have strong antioxidant activity. This can be explained that the presence of compounds capable of donating hydrogen radicals present in EA fraction was effective to decolorize radical DPPH. The antiradical activity of samples measured by ABTS method using trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) as positive control was compiled in Table 2. Among extracts, fractions and positive control evaluated, trolox exhibited the highest antiradical activities using ABTS radical followed by methanol extract, petroleum ether, ethyl acetate and chloroform fractions.

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3.2 Reducing Power Activities

The reducing power of Fe(III) into Fe(II), known as ferric reducing activity power (FRAP), differs from radical scavenging activities because there is no free radicals, but involved the reduction of ferric ion (Fe^{3+}) from kalium ferrycianide into Ferro ion (Fe^{2+}). The ferro ion can be monitored by measuring the intensity of Prussian blue color at wavelength of 700 nm, and the higher the absorbance at 700 nm indicated the higher reduction power. The antioxidant activity based on reducing power of Fe^{3+} into Fe^{2+} was expressed as mg equivalent of vitamin C in one (1) gram samples. The linear equation describing the relationship between concentration of vitamin C (x-axis) and absorbance of Fe^{2+} due to reduction of Fe^{3+} with vitamin C (y-axis) was: $y = 10.771x + 14.878$ ($R^2 = 0.9891$). Table 3 compiled the reducing power of extract and fractions (calculated as mg equivalent vitamin C/g sample). The methanol extract exhibited the strongest reducing power with FRAP value of 14.446 ± 0.161 mg equivalent vitamin C/gram sample followed by ethyl acetate fraction, chloroform fraction, and petroleum ether fraction. This indicated that reducing compounds present in methanol extract were active and may be present in high amount.

3.3 Metal Chelating Activities

Metal chelating activity of extract and fractions was performed in slight acidic medium (pH 6.0). The phenolics compounds can bind to Fe^{2+} and the remaining Fe^{2+} could react with ferrozine to form blue-colored complexes which can be monitored spectrophotometrically at 562 nm. The absorbance of this complex could be reduced by antioxidant such as phenolics compounds, due to its capability to bind metal (Fe^{2+}). Therefore, any compounds capable of reducing the complex Fe^{2+}-ferrozine could be considered as antioxidant through mechanism of metal chelating. As positive control, ethylene diamine tetra acetic (EDTA) was used, as a consequence, the metal chelating activity of extract and fractions were expressed as mg Na. EDTA/gram sample. Table 3 compiled the metal chelating activity of methanol extract and its fractions. The chloroform extract revealed the highest metal activity compared to other fractions and methanol extract.

3.4 Phenolics and Flavonoid Contents

Due to its capability to provide hydrogen radicals, to reduce Fe^{3+} and to bind metals catalyzing oxidation reactions, phenolic and flavonoids contents were correlated with these antioxidants. Total phenolic contents were determined using Folin-Ciocalteau (F-C) reagent and gallic acid was used as standard, therefore phenolic contents were expressed as mg gallic acid equivalent/gram sample (mg GAE/g). The linear regression describing for the relationship between gallic acid (*x-axis*) and its absorbance after reaction with F-C reagent (*y-axis*) was expressed as $y = 1.338x – 0.0068$ ($r^2 = 0.998$). In addition, flavonoid contents were determined after being reacted with NaNO$_2$, AlCl$_3$ and NaOH to form red-colored complex which can be measured spectrophotometrically at 510 nm. Rutin was used as standard during quantitative analysis of flavonoid, therefore, the flavonoid contents were expressed as mg rutin equivalent/g sample (mg RE/g). The linear regression describing the correlation

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between rutin (*x-axis*) and its absorbance (*y-axis*) was expressed as $y = 0.1438x - 0.0365$ ($r^2 = 0.995$). Table 4 compiled the phenolic and flavonoid contents of methanol extract and its fractions. Methanol extract has the highest phenolics contents accounting for 32.39 ± 2.37% compared to other methanol fractions, while the highest flavonoid contents was found in ethyl acetate fraction accounting for 78.51 ± 0.579 mg RE/g.

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The phenolics and flavonoid contents were then correlated with antiradical activities using DPPH and ABTS radicals, ferric reducing activity power, and metal chelating activity. The correlation between antioxidant activities with phenolics and flavonoid contents was expressed as linear regression with certain coefficient determination (R^2) values, as compiled in Table 5. The R^2 value indicated the quantitative contribution of one variable (phenolics and flavonoids) toward antioxidant activities. Based on R^2 values, phenolics contents were more contributing toward metal chelating activities compared to other antioxidant activities with R^2 value of 0.8726. This value indicated that 87.26% of metal chelating activity came from phenolics contents. In addition, flavonoid contents more contributed to ABTS radical scavenging activity with R^2 of 0.8916 which indicated that 89.16% ABTS radical activity was coming from flavonoid contents.

3.5 Isolation and Identification of Active Compound

Chloroform fraction was then fractionated to get sub-fractions to obtain isolates with good antiradical activities. Isolate 1 has been isolated from chloroform fraction and subjected to purity test using TLC and melting point test. In addition, structure identification was performed using infrared spectroscopy and mass spectrometry. Purity test performed by TLC using three different solvent systems indicated that isolate 1 was TLC pure because only one spot was observed. Melting point analysis showed that isolate 1 had sharp melting point of 62-64°C. Based on TLC and melting point results, isolate 1 can be considered as pure and can be continued to be identified. Identification of isolate 1 using FTIR spectroscopy resulted IR spectra with several peaks. Peak at 2925 cm^{-1} corresponded to stretching vibration of C-H. Peak at 1741 cm^{-1} originated from stretching vibration of carbonyl group of C=O, while peak at 1458 cm^{-1} was corresponding to stretching vibration of C=C (benzene), while peaks at 989cm^{-1} and 722 cm^{-1} came from C-H bending vibration. Using mass spectrometry, the molecular ion (M^+) appeared at m/z of 390 amu with base peak at m/z 279 amu. The fragment ions appeared at m/z of 279 ($M-CHCH_3C_2H_5O(C_2H_5)_2$ $(CH_2)_3)OCH_3$), 167, 149($M-CH_2CH_3(CH_2)_2C(O)CHCH_3CHCH$), 132, 113, 93,83,71,57,43. Based on IR and mass spectra, the compound was tentatively identified as 1,2-benzenedicarboxylic acid, *bis*(2-ethylhexyl) ester (CAS) bis(2-ethylhexyl) phthalate (Figure 2).

Conclusion:

Rambutan rind showed good antioxidant activities determined using either DPPH or ABTS method. Moreover, it also demonstrated good reducing power and metal chelating capacity. The content of phenolic and flavonoid compounds was correlated to the antioxidant and metal chelating activities. Identification from the most active fraction found that compound of 1,2-benzenedicarboxylic acid is predicted as the active compound responsible for antioxidant activities. This result could be further explored the potential use of rambutan rind compound as food supplement having antioxidant activities.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

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Table 1: IC$_{50}$ values of methanol extract and its fraction of rambutan rind using DPPH radical

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Extract or fraction	**IC$_{50}$ values (μg/mL)**			**Average ± SD**
	Replicate 1	**Replicate 2**	**Replicate 3**	**(μg/mL)**
Vitamin C	3.43	3.29	3.26	3.33 ± 0.09
Petroleum ether	49.31	47.35	49.25	48.64 ± 1.12
Methanol	49.37	49.15	48.90	49.14 ± 0.23
Chloroform	49.53	49.82	49.96	49.77 ± 0.22
Ethyl acetate	45.65	45.64	45.87	45.72 ± 0.13

Table 2: IC_{50} values of methanol extract and its fraction of rambutan rind using ABTS radical

Extract or fraction	IC_{50} values (µg/mL)			Average ± SD (µg/mL)
	Replicate 1	**Replicate 2**	**Replicate 3**	
Trolox	3.43	3.29	3.26	3.33 ± 0.09
Petroleum ether	34.591	34.609	34.515	34.57 ± 0.05
Methanol	27.57	27.17	27.17	27.39 ± 0.17
Chloroform	45.06	45.48	45.55	45.36 ± 0.27
Ethyl acetate	38.18	37.95	37.74	39.93 ± 3.61

Table 3. The reducing power of fe^{3+} into fe^{2+} by extract and fractions of rambutan rind (calculated as mg equivalent vitamin c/g sample)

Samples	**Reducing power (Mean ± SD, as mg vitamin C equivalent/g sample)**	**Metal chelating activity (Mean ± SD, as mg Na EDTA equivalent/g sample)**
Methanol extract	14.446 ± 0.161	250.463 ± 1.062
Petroleum ether fraction	18.796 ± 0.161	284.075±0.0024
Chloroform fraction	34.478 ± 0.245	332.753±0.695
Ethyl acetate fraction	47.636 ± 0.161	200.692±0.0034

Table 4: The phenolic and flavonoid contents of methanol extract and its fractions of rambutan rind

Samples	**Phenolics contents (Mean ± SD, mg gallic acid equivalent/g)**	**Flavonoid contents (Mean ± SD, as mg rutin equivalent/g)**
Methanol extract	21.36 ± 2.25	96.36 ± 0.894
Petroleum ether fraction	27.11 ± 1.23	93.06 ± 0.579
Chloroform fraction	32.32 ± 0.79	55.05 ± 3.900
Ethyl acetate fraction	32.39 ± 2.37	78.51 ± 0.579

Table 5: The correlation between antioxidant activities with phenolics and flavonoid contents of methanol extract and its fraction of rambutan rind

Antioxidant activity tests (y-axis)	Its correlation with phenolics contents (x-axis)		Its correlation with flavonoid contents	
	Equation	**R^2**	**Equation**	**R^2**
DPPH radical scavenging	y = 0.056x + 46.732	0.0269	y = -0.0139x + 49.437	0.0211
ABTS radical scavenging	y = 0.6245x + 5.7764	0.7794	y = -0.3722x + 66.109	0.8916
Ferric reducing activity power	y = 0.3048x + 19.511	0.7813	y = -0.505x + 69.6	0.3899
Metal chelating activity	y = 9.9119x – 13.464	0.8726	y = -0.1712x + 126.46	0.2567

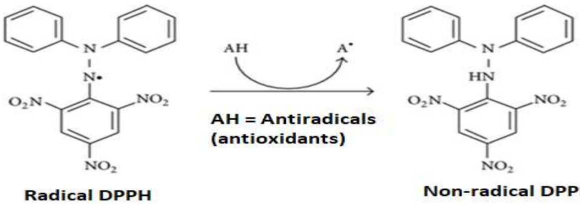

Figure 1 Reaction Between Radical 2,2’-Diphenyl-1-Picrilhydrazil (DPPH) with Antioxidants (AH) into Non-Radical DPPH Causing t~~T~~he Discoloration of DPPH (Alam *et al.*, 2013)

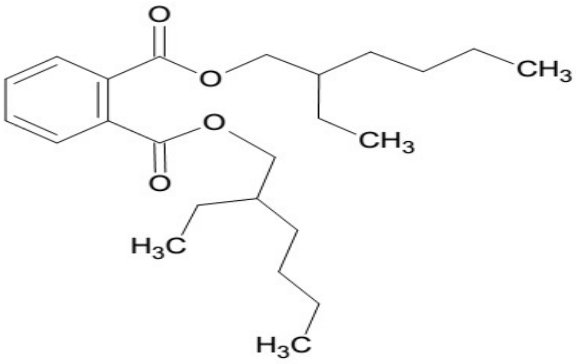

Figure 2. The Chemical Structure of Bis(2-Ethylhexyl) Ester (CAS) *Bis* (2-Ethylhexyl) Phthalate

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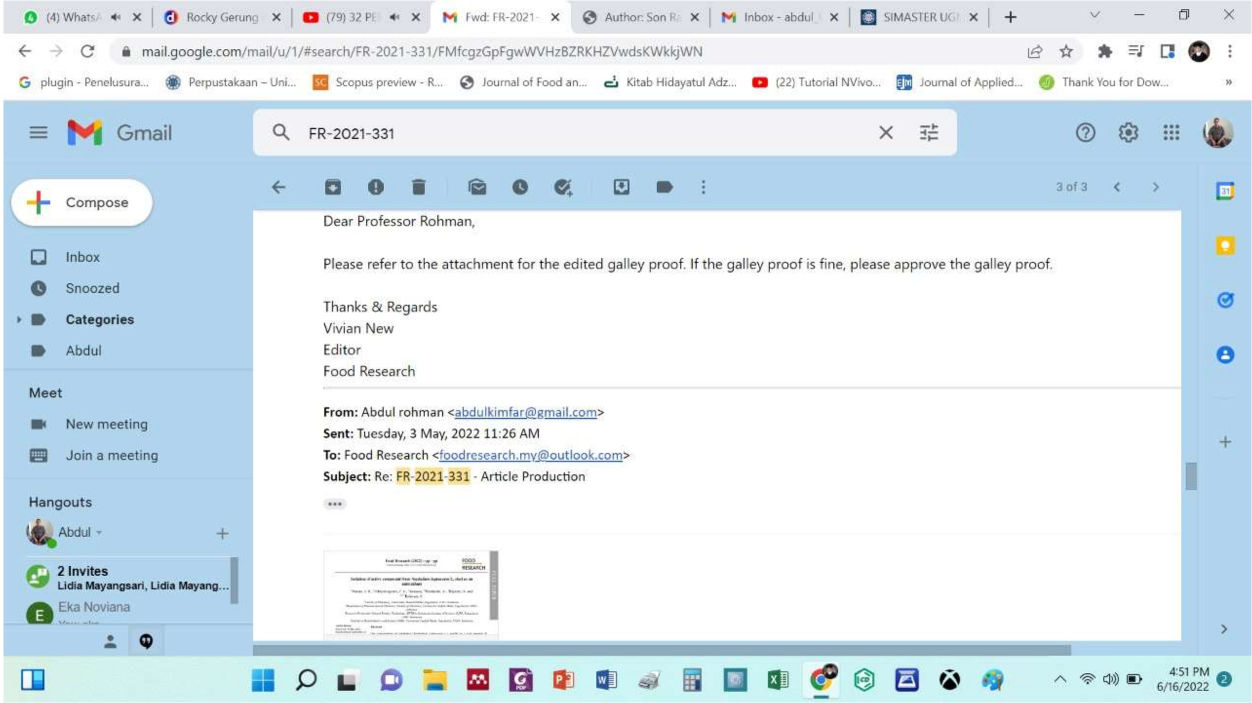

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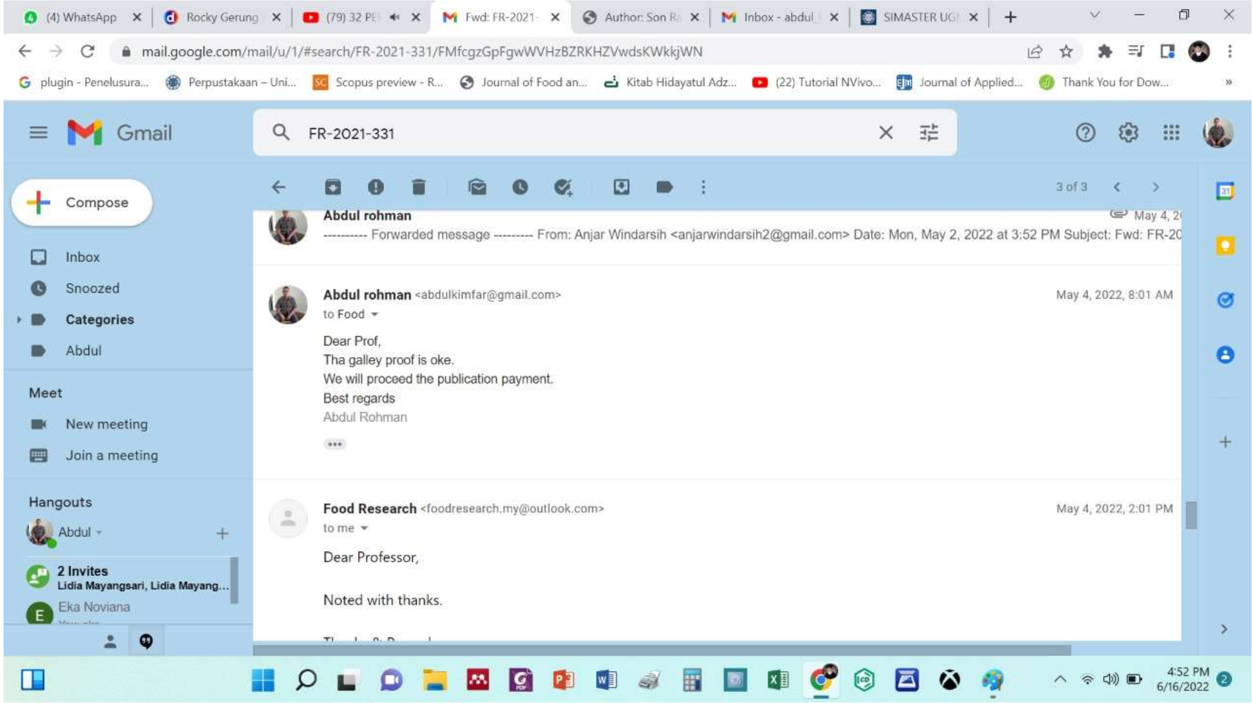

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Isolation of active compound from *Nephelium lappaceum* L. rind as an antioxidant

[1]Nurani, L.H., [1]Edityaningrum, C.A., [2,3]Suhaera, [4]Windarsih, A., [2]Riyanto, S. and [2,5,*]Rohman, A.

[1]*Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta 55164, Indonesia*
[2]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia*
[3]*Study Program of Pharmacy, Health Institute of Mitra Bunda, Batam, 29444, Riau Island, Indonesia*
[4]*Research Center for Food Technology and Processing (PRTPP), National Research and Innovation Agency (BRIN), Yogyakarta, 55861, Indonesia*
[5]*Institute of Halal Industry and Systems (IHIS), Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia*

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Abstract

The consumption of rambutan (*Nephelium lappaceum* L.) results in a vast amount of rambutan rind. It is very interesting to explore rambutan rind as natural antioxidants. This study aimed to explore the antioxidant activities of rambutan rind *in vitro* and to correlate those activities with a group of compounds (phenolics and flavonoids). Rambutan rind was cleaned, dried using a conventional oven, powdered, and the powder obtained was subjected to maceration. The macerate was fractionated using petroleum ether and chloroform to get chloroform fraction. The sub fractionation was done toward chloroform fraction. The chloroform fraction and its subfractions were then subjected to antioxidant activity measurement, determination of phenolics and flavonoid contents, and the active compound present in the most active subfractions was isolated using column chromatography. The isolate was identified using several methods including thin-layer chromatography (TLC), Fourier transform infrared (FTIR) spectroscopy as well as gas chromatography-mass spectrometry (GC-MS). The results showed that the chloroform fraction and its subfractions were correlated with the phenolic and flavonoid contents found in rambutan rinds. The phenolic and flavonoid content of chloroform fraction was 32.32±0.79 and 55.05±3.900, respectively, whereas the IC_{50} of antioxidant activity was 49.77±0.22 for DPPH and 45.36±0.27 for ABTS. The reducing power was 34.478±0.245 (mg vitamin C equivalent/g sample) and the metal chelating activity was 332.753±0.695 (mg Na EDTA equivalent/g sample). The phenolic contents contributed towards the metal chelating activities compared to other antioxidant activities with an R^2 value of 0.8726, while flavonoid contents contributed more to ABTS radical scavenging activity with an R^2 value of 0.8916. One of the active compounds present in rambutan rind having antioxidant activity was identified as 1,2-benzenedicarboxylic acid. Thus, the under-utilized part of rambutan rind could be explored as a natural antioxidant to be used as a food supplement.

1. Introduction

The imbalance between reactive oxygen species (ROS) and reactive nitrogen species (RNS) with endogenous antioxidants has led to the formation of oxidative stress. ROS and RNS have been associated with some degenerative diseases including diabetes, cancer, ageing, neurodegenerative and cardiovascular diseases (White *et al*., 2014). Some efforts have been driven by a group of researchers to explore exogenous antioxidants either natural or synthetic antioxidants (Palanisamy *et al*., 2008). The antioxidant can be defined as any compounds or materials capable of delaying or inhibiting the oxidation reactions of lipids, proteins and other molecules by terminating free radical reaction (Pavithra and Banu, 2017). Natural antioxidants derived from fruits and vegetables have been explored and commercialized such as grape seed extracts. One of the potential natural antiradical derivatives is from Rambutan (*Nephelium lappaceum* L.).

Rambutan (king of fruits) with the scientific name of *Nephelium lappaceum* L. (Family of Sapindaceae) is a tropical fruit commonly found in South East Asian

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*Corresponding author.
Email: *abdulkimfar@gmail.com*

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regions like Indonesia, Thailand Malaysia, and Vietnam (Fidrianny *et al.,* 2015). Rambutan is most commonly consumed in cultivars Aceh, Binjai and Rapiah (Rohman *et al.*, 2016). The consumption of Rambutan fruit has resulted in a vast amount of waste, including rind and peel. Therefore, it is very interesting to explore of underutilized part of rambutan fruit as a natural antioxidant and identify the active components contributing to antioxidant activities.

Several biological activities of *Nephelium lappaceum* L. fruit including its rind and seed have been reported, namely antiradical due to its phenolic compounds (Thitilertdecha and Rakariyatham, 2011), *in vitro* and *in vivo* antioxidant activities using different standardized methods (Pavithra and Banu, 2017; Iman Kamaludin *et al.*, 2016; Mistriyani *et al.*, 2018), lipid peroxidation inhibition (Setyawati *et al.*, 2015) anti-inflammatory effects (Chingsuwanrote *et al.*, 2016) anti-hypercholesterolemia activities (Muhtadi *et al.*, 2016) antibacterial activities against some pathogenic bacterial strains (Thitilertdecha *et al.*, 2008), inhibitors of alpha-amylase and alpha-glucosidase activities *in vitro* (Thinkratok *et al.*, 2014) hypoglycemic effects (Soeng *et al.*, 2015), anti-hyperglycemic activity (Palanisamy *et al.*, 2011)**,** α- and β-glucosidases inhibition (Widowati *et al.*, 2015), and anti-diabetic activity on rats induced by alloxan (Muhtadi *et al.*, 2016)

Some active compounds have been identified in rambutan including geraniin, corilagin and ellagic acid (Hernández *et al.*, 2017). A total of 39 compounds were also identified in rambutan rind, including 1 simple phenolic acid, 1 flavone, 5 hydrolyzable tannins, 5 hydroxybenzoic acids, 10 flavonols, 11 flavonols, 6 ellagic acid and conjugates (Zhuang *et al.*, 2017). However, the study on the antioxidant activities of *Nephelium lappaceum* L. rind fraction is still limited. To the best of our knowledge, the isolation of active antioxidant components from *Nephelium lappaceum* L. rind chloroform fraction has not been reported. Mistriyani *et al*. (2021) have carried out the isolation of antioxidant components in the ethyl acetate fraction of Rambutan peel. In this study, the chloroform fraction of Rambutan rind was chosen for further fractionation to investigate the active antioxidant compound because the chloroform fraction and subfraction showed good antioxidant activities. Furthermore, the active component contributing to antioxidant activity was identified.

2. Materials and methods

2.1 Materials

Rambutan fruit was obtained from Bantul, Yogyakarta, Indonesia. The authentication of rambutan samples was performed in the Department of Pharmaceutical Biology, Faculty of Pharmacy with assistance from Dr Djoko Santosa. The chemical reagents and solvents used were of pro-analytical grade.

2.2 Preparation of chloroform extract of rambutan

The chloroform fraction was prepared according to Rohman *et al*. (2016). Rambutan rind was cleaned, cut into small pieces using a commercial cutter and dried in a conventional oven at 65°C for 2 days then grounded into a powder. The powder was subjected to extraction using the maceration technique using methanol as extracting solvent and occasionally shaken. The solvent was evaporated using a vacuum rotary evaporator to obtain the methanolic extract. The extract was then added with aquadest and subjected to partition using petroleum ether, chloroform, and ethyl acetate to get corresponding fractions, namely petroleum ether (PE), chloroform (CH) and ethyl acetate (EA) fractions. CH fraction was then further sub-fractionated to get sub-fractions. The most active sub-fraction as an antioxidant was subjected to isolation to identify the compounds responsible for antioxidant activity. The methanol extracts and their fractions (water, PE, CL, and EA) were evaluated by determining antiradical activities using 2 2'-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis (3-ethylbenzo thiazoline-6-sulphonic acid) diammonium salt (ABTS), reducing power, and metal chelating activity.

2.3 Evaluation of radical scavenging activity using DPPH radical

Scavenging activity of DPPH radicals was performed according to (Khalil *et al.*, 2018) with slight modification. A-50 µL of extract and fraction samples with different concentrations was added to 1.0 mL DPPH 0.4 mM and 3.950 mL methanol. The mixture was allowed to react at room temperature for 20 mins. Then, the absorbance was measured at 515 nm with a calibrated UV–visible spectrophotometer. All measurements were done in triplicate.

$$\%\ \text{Antiradical activty} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_o is the absorbance of the control (without samples) and A_1 is the absorbance of samples.

2.4 Radical scavenging activity using ABTS technique

The ABTS radical assay of extract and fraction samples was performed according to Fidrianny *et al.* (2015). The mixture of potassium persulphate 2.45 mM and ABTS 7 mM (1:1) was allowed to stand in the dark for 12–16 hrs at room temperature to produce ABTS radical cation ($ABTS^+$). This $ABTS^+$ solution was diluted with methanol to obtain absorbance values of 0.600-0.800 at 734 nm. The ABTS$^+$ working solution (3 mL) and 30 µL of blank (methanol), standard or sample were mixed and the absorbance was measured at 734 nm after 6 mins using a spectrophotometer.

2.5 Reducing power evaluation

The evaluation of ferric reducing activity power (FRAP) was carried out according to Mistriyani *et al.* (2018). FRAP reagent was prepared by mixing 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4-diene chloride (TPTZ)(in 40 mmol/L HCl), and 20 mmol/L ferric chloride (10:1:1, v:v:v). To the 4.5 mL of reagent, 150 µL ethanol plant extract was added. The absorbance readings were analysed after 5 mins and performed at a wavelength of 593 nm. The blank consisted of FRAP reagent. The final absorbance of each sample was compared with those obtained from the standard curve made from ferric sulphate ($FeSO_4.7H_2O$) (200–1000 µmol/L). Results were expressed in nmol Fe^{2+}/mg dried extract.

2.6 Metal chelating activity

Metal chelating activity of extract and fractions was measured as described by (Wong *et al*., 2014). A certain amount (mL) of samples was added with 0.1 mM $FeSO_4$ (0.2 mL) and 0.25 mM ferrozine (0.4 mL). After incubating at room temperature for 10 min, the absorbance of the mixture was recorded at 562 nm. The chelating activity was calculated using the following formula:

$$\text{Chelating Activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_o is the absorbance of the control reaction (without any samples) and A_1 is the absorbance in the presence of extracts or fractions.

2.7 Determination of total phenolics and flavonoid contents

Total phenolics contents of extracts and fractions were determined according to (Kovarovič *et al*., 2019)., while total flavonoids were determined using the spectroscopic method according to Rohman *et al.* (2010). For phenolics content analysis, Folin Ciocalteu reagent (FCR) was used. Briefly, extracts and fractions in methanol were mixed with 0.4 mL of FCR. The solution was allowed to stand at room temperature for 8-10 mins, added with 4 mL Na_2CO_3 7% and made to 10.0 mL with bidistilled water. The mixture was allowed to stand for 2 hrs and subsequently measured at 725 nm. The phenolic contents were expressed as mg gallic acid equivalents (GAE) per gram of sample (mg/g). For total flavonoid analysis, samples in methanol were added with 4 mL of distilled water and 0.3 mL of $NaNO_2$ 5%. After 5 mins, 0.3 mL $AlCl_3$ 10% was added to the mixture. At 6 mins, 2 mL NaOH 1 M was added to the mixture. Immediately, the mixture was diluted to a volume of 2.4 mL distilled water and thoroughly mixed and its absorbance was measured at 510 nm versus a blank containing all reagents except samples of extracts or fractions. The total flavonoid content of the extracts and fractions were expressed as mg rutin equivalents (RE) per gram of sample (mg/g).

2.8 Fractionation of chloroform extract

Chloroform fraction has good antioxidant activities, therefore, it was further subjected to fractionation using vacuum liquid using silica gel G 60 GF_{254} as in (Rohman *et al*., 2010). The chloroform fraction was dissolved in chloroform, then anhydrous sodium sulphate was added that was previously heated for 2 hrs at 110°C, and allowed to stand for one night and filtered using a filter paper. The solvent was concentrated using a vacuum rotary evaporator. Samples were added to a column that has been let stand overnight. The chloroform fraction was eluted using the solvents of chloroform, ethyl acetate, and methanol in various compositions. A volume of 50 mL of eluates each was collected for each and evaporated using a vacuum rotary evaporator.

2.9 Fractionation of active fraction

The sub-fractions were loaded into TLC using the stationary phase of GF254 with suitable eluting solvents. The plate was sprayed with DPPH 0.02% in methanol. Sub-fraction capable of bleaching the DPPH colour was subjected to further fractionation using a gravimetric column. The sub-fraction was loaded into a column using silica gel 230-400 mesh (0.040-0.063 mm) and isocratically eluted with n-hexane: acetone (2:2 v/v) and with petroleum ether: ethyl acetate (1:1 v/v), 5 mL of each elute was then collected.

2.10 Isolate purity identification

The purity of the isolate was identified using two methods, melting point and thin-layer chromatography (TLC). The melting point of the crystal was identified using the Buchi Melting Point B-450. Temperatures were recorded at the time the crystal began to melt and the temperature at which the crystal turns into liquid. The test was repeated by measuring the temperature of ±10°C below the melting point obtained, ramped at 1°C/min. For the purity test using TLC, the isolate was eluted using three eluent systems with a mobile phase with different polarity index, acetone: ethyl acetate: chloroform with a ratio of 4: 3: 3 v/v. The compound was considered pure if it had a single spot on the TLC system. The spot detection was performed visually using UV$_{254}$ and UV$_{366}$.

2.11 Isolate identification

The chemical structure of the isolate was determined using FTIR spectroscopic method (FTIR 100 PERKIN ELMER) and gas chromatography-mass spectrometry (GCMS-QP2010S SHIMADZU, Japan).

3. Results and discussion

Antioxidant activities of *Nephelium lappaceum* L. rind were evaluated using radical scavenging DPPH and ABTS, ferric reducing activity power (FRAP) and metal chelating activity, which can be differentiated as Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET) (Prior *et al*., 2005; Rohman *et al*., 2020).

3.1 Antiradical scavenging activities

DPPH is a synthetic radical which is stable and soluble in polar organic solvents such as methanol and ethanol. The principle of the DPPH radical scavenging test was based on the reduction of radical DPPH (violet in colour) into non-radical DPPH (colourless). Thus, the antiradical compounds were able to reduce DPPH radical intensity at wavelength 517 nm, with the absorptivity molar decreased from 9660 M^{-1}cm^{-1} (radical DPPH) into 1640 M^{-1}cm^{-1} (non-radical DPPH). When antiradical such as phenolics and flavonoids donate their hydrogen radical into DPPH radical, the colour of radical DPPH decreases. The proposed reaction between antiradical compounds (antioxidants) with DPPH radical could be seen in Figure 1. The parameter used for describing DPPH radical activity was IC$_{50}$, the concentration of the samples necessary to cause 50% scavenging of DPPH radical, calculated from a linear regression equation (Rohman *et al*., 2016). The higher the antiradical activities, the lower the IC$_{50}$ values.

Table 1 compile the IC$_{50}$ values of methanol extract and its fraction of *Nephelium lappaceum* L. rind using DPPH radical with vitamin C (ascorbic acid) as the positive control as it has the strongest anti-radical activities (the lowest IC$_{50}$). In addition, ethyl acetate fractions showed antiradical activities among extract and fractions evaluated followed by methanol and chloroform (which are not statistically different or $P > 0.05$) and petroleum ether. It was in accordance with the study conducted by Mistriyani *et al*. (2021). The antioxidant activity of the ethyl acetate fraction of *Nephelium lappaceum* L. peel was the lowest compared to other fractions (22.6 μg/mL). Samples with IC$_{50}$ values of 10-50 μg/mL were considered to have strong antioxidant activity. This can be explained that the presence of compounds capable of donating hydrogen radicals present in the EA (ethyl acetate) fraction was effective to decolourize radical DPPH. The antiradical activity of samples measured by the ABTS method using Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) as a positive control was compiled in Table 2. Among extracts, fractions and positive control evaluated, Trolox exhibited the highest antiradical activities using ABTS radical followed by methanol extract, petroleum ether, ethyl acetate and chloroform fractions.

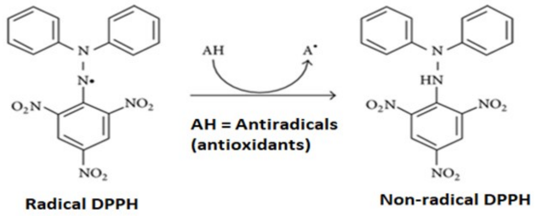

Figure 1. Reaction Between Radical 2,2'-Diphenyl-1-Picrilhydrazil (DPPH) with Antioxidants (AH) into Non-Radical DPPH Causing the Discoloration of DPPH (Alam *et al*., 2013)

Table 1. IC$_{50}$ values of methanol extract and its fraction of rambutan rind using DPPH radical

Extract or fraction	IC$_{50}$ values (μg/mL)			Average±SD (μg/mL)
	Replicate 1	Replicate 2	Replicate 3	
Vitamin C	3.43	3.29	3.26	3.33±0.09
Petroleum ether	49.31	47.35	49.25	48.64±1.12
Methanol	49.37	49.15	48.9	49.14±0.23
Chloroform	49.53	49.82	49.96	49.77±0.22
Ethyl acetate	45.65	45.64	45.87	45.72±0.13

Table 2. IC$_{50}$ values of methanol extract and its fraction of rambutan rind using ABTS radical

Extract or fraction	IC$_{50}$ values (μg/mL)			Average±SD (μg/mL)
	Replicate 1	Replicate 2	Replicate 3	
Trolox	3.43	3.29	3.26	3.33±0.09
Petroleum ether	34.591	34.609	34.515	34.57±0.05
Methanol	27.57	27.17	27.17	27.39±0.17
Chloroform	45.06	45.48	45.55	45.36±0.27
Ethyl acetate	38.18	37.95	37.74	39.93±3.61

3.2 Reducing power activities

The reducing power of Fe(III) into Fe(II), known as ferric reducing activity power (FRAP), differs from radical scavenging activities because there are no free radicals, but involved the reduction of ferric ion (Fe^{3+}) from potassium ferricyanide into Ferro ion (Fe^{2+}). The Ferro ion can be monitored by measuring the intensity of Prussian blue colour at a wavelength of 700 nm, and the higher the absorbance at 700 nm indicated the higher the reduction power. The antioxidant activity based on the reducing power of Fe^{3+} into Fe^{2+} was expressed as mg equivalent of vitamin C in one (1) gram sample. The linear equation describing the relationship between concentration of vitamin C (x-axis) and absorbance of Fe^{2+} due to reduction of Fe^{3+} with vitamin C (y-axis) was: $y = 10.771x + 14.878$ ($R^2 = 0.9891$). Table 3 compiled the reducing power of extract and fractions (calculated as mg equivalent vitamin C/g sample). The methanol extract exhibited the strongest reducing power with a FRAP value of 14.446±0.161 mg equivalent vitamin C/gram sample followed by ethyl acetate fraction, chloroform fraction, and petroleum ether fraction. This indicated that reducing compounds present in methanol extract were active and may be present in a high amount.

3.3 Metal chelating activities

The metal chelating activity of extract and fractions was performed in a slightly acidic medium (pH 6.0). The phenolic compounds can bind to Fe^{2+} and the remaining Fe^{2+} could react with ferrozine to form blue-coloured complexes which can be monitored spectrophotometrically at 562 nm. The absorbance of this complex could be reduced by antioxidants such as phenolic compounds, due to its capability to bind to metal (Fe^{2+}). Therefore, any compounds capable of reducing the complex Fe^{2+}-ferrozine could be considered an antioxidant through the mechanism of metal chelating. As a positive control, ethylene diamine tetra-acetic (EDTA) was used, as a consequence, the metal chelating activity of extract and fractions were expressed as mg Na. EDTA/gram sample. Table 3 displays the metal chelating activity of methanol extract and its fractions. The chloroform extract revealed the highest metal activity compared to other fractions and methanol extract. Previous research also reported high metal chelating activity of chloroform fraction of *Nephelium lappaceum* L. peel, however, the highest metal chelating activity was found in the water fraction (Mistriyani *et al.*, 2021).

Table 3. The reducing power of Fe^{3+} into Fe^{2+} by extract and fractions of rambutan rind (calculated as mg equivalent vitamin C/g sample)

Samples	Reducing power (Mean±SD, as mg vitamin C equivalent/g sample)	Metal chelating activity (Mean±SD, as mg Na EDTA equivalent/g sample)
Methanol extract	14.446±0.161	250.463±1.062
Petroleum ether fraction	18.796±0.161	284.075±0.0024
Chloroform fraction	34.478±0.245	332.753±0.695
Ethyl acetate fraction	47.636±0.161	200.692±0.0034

3.4 Phenolics and flavonoid contents

Due to its capability to provide hydrogen radicals, reducing Fe^{3+} and binding metals catalyzing oxidation reactions, phenolic and flavonoids contents were correlated with these antioxidants. Total phenolic contents were determined using Folin-Ciocalteau (F-C) reagent and gallic acid was used as standard, therefore phenolic contents were expressed as mg gallic acid equivalent/gram sample (mg GAE/g). The linear regression describing the relationship between gallic acid (x-axis) and its absorbance after reaction with F-C reagent (y-axis) was expressed as $y = 1.338x – 0.0068$ ($R^2 = 0.998$). In addition, flavonoid contents were determined after being reacted with $NaNO_2$, $AlCl_3$ and NaOH to form a red-coloured complex which can be measured spectrophotometrically at 510 nm. Rutin was used as standard during quantitative analysis of flavonoids, therefore, the flavonoid contents were expressed as mg rutin equivalent/g sample (mg RE/g). The linear regression describing the correlation between rutin (x-axis) and its absorbance (y-axis) was expressed as $y = 0.1438x – 0.0365$ ($R^2 = 0.995$). Table 4 compiled the phenolic and flavonoid contents of methanol extract and its fractions. Methanol extract has the highest phenolic contents accounting for 32.39±2.37% compared to other methanol fractions, while the highest flavonoid contents were found in ethyl acetate fraction accounting for 78.51±0.579 mg RE/g.

Table 4. The phenolic and flavonoid contents of methanol extract and its fractions of rambutan rind

Samples	Phenolics contents (Mean±SD, mg gallic acid equivalent/g)	Flavonoid contents (Mean±SD, as mg rutin equivalent/g)
Methanol extract	21.36±2.25	96.36±0.894
Petroleum ether fraction	27.11±1.23	93.06±0.579
Chloroform fraction	32.32±0.79	55.05±3.900
Ethyl acetate fraction	32.39±2.37	78.51±0.579

The phenolics and flavonoid contents were then correlated with antiradical activities using DPPH and

Table 5. The correlation between antioxidant activities with phenolics and flavonoid contents of methanol extract and its fraction of rambutan rind

Antioxidant activity tests (y-axis)	Its correlation with phenolics contents (x-axis)		Its correlation with flavonoid contents	
	Equation	R^2	Equation	R^2
DPPH radical scavenging	y = 0.056x + 46.732	0.0269	y = -0.0139x + 49.437	0.0211
ABTS radical scavenging	y = 0.6245x + 5.7764	0.7794	y = -0.3722x + 66.109	0.8916
Ferric reducing activity power	y = 0.3048x + 19.511	0.7813	y = -0.505x + 69.6	0.3899
Metal chelating activity	y = 9.9119x – 13.464	0.8726	y = -0.1712x + 126.46	0.2567

ABTS radicals, ferric reducing activity power, and metal chelating activity. The correlation between antioxidant activities with phenolics and flavonoid contents was expressed as linear regression with certain coefficient determination (R^2) values, as compiled in Table 5. The R^2 value indicated the quantitative contribution of one variable (phenolics and flavonoids) toward antioxidant activities. Based on R^2 values, phenolic contents contributed toward metal chelating activities compared to other antioxidant activities with an R^2 value of 0.8726. This value indicated that 87.26% of metal chelating activity came from phenolic contents. In addition, flavonoid contents contributed to ABTS radical scavenging activity with an R^2 value of 0.8916 which indicated that 89.16% of ABTS radical activity was coming from flavonoid contents.

3.5 Isolation and identification of active compound

The chloroform fraction was then fractionated to sub-fractions to obtain isolates with good antiradical activities. Isolate 1 has been isolated from chloroform fraction and subjected to a purity test using TLC and melting point test. In addition, structure identification was performed using infrared spectroscopy and mass spectrometry. The purity test performed by TLC using three different solvent systems indicated that isolate 1 was TLC pure because only one spot was observed. Melting point analysis showed that isolate 1 had a sharp melting point of 62-64°C. Based on TLC and melting point results, isolate 1 can be considered pure and can be continued to be identified. Identification of isolate 1 using FTIR spectroscopy resulted in IR spectra with several peaks. The peak at 2925 cm^{-1} corresponded to the stretching vibration of C-H. The peak at 1741 cm^{-1} originated from the stretching vibration of the carbonyl group of C=O, while the peak at 1458 cm^{-1} was corresponding to the stretching vibration of C=C (benzene), while peaks at 989cm^{-1} and 722 cm^{-1} came from C-H bending vibration. Using mass spectrometry, the molecular ion (M^+) appeared at m/z of 390 amu with a base peak at m/z 279 amu. The fragment ions appeared at m/z of 279 ($M\text{-}CHCH_3C_2H_5O(C_2H_5)_2\ (CH_2)_3)OCH_3$), 167, 149($M\text{-}CH_2CH_3(CH_2)_2C(O)CHCH_3CHCH$), 132, 113, 93,83,71,57,43. Based on IR and mass spectra, the compound was tentatively identified as 1,2-benzenedicarboxylic acid, *bis*(2-ethylhexyl) ester (CAS) bis(2-ethylhexyl) phthalate (Figure 2). This compound can be found in some plant extracts such as *Podophyllum hexandrum* rhizome, the stem of *Hugonia mystax* L., and endophytic fungi and it has been reported to have strong antioxidant activity (Li *et al*., 2012; Vimalavady and Kadavul, 2013; Govindappa *et al*., 2014).

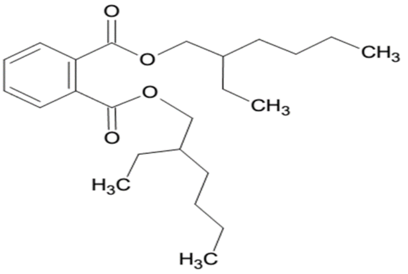

Figure 2. The Chemical Structure of Bis(2-Ethylhexyl) Ester (CAS) *Bis* (2-Ethylhexyl) Phthalate

4. Conclusion

Nephelium lappaceum L. (rambutan) rind showed good antioxidant activities determined using either DPPH or ABTS method. The chloroform fraction demonstrated IC50 of 49.77±0.22 for DPPH and 45.36±0.27 for ABTS. Moreover, it also demonstrated good reducing power (34.478±0.245 as mg vitamin C equivalent/g sample) and metal chelating capacity (332.753±0.695 as mg Na EDTA equivalent/g sample). The content of phenolic and flavonoid compounds was correlated to the antioxidant and metal chelating activities. Identification from the most active fraction found that the compound of 1,2-benzenedicarboxylic acid is the active compound responsible for antioxidant activities. This result could be further explored the potential use of rambutan rind compound as a food supplement of antioxidant activities.

Conflict of interest

The authors declare no conflict of interest.

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