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Research Article
Antiradical Activities of Rambutan Peel: Study from Two Cultivars

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Abstract

Background: Rambutan is tropical fruit consumed either in fresh or processed fruit. The direct consumption or processing of rambutan fruit resulted in high amount of waste from peel and seed, therefore, it is necessary to take benefit from rambutan peel to be used as food supplement via antioxidant properties. **Objective:** This study is intended to evaluate the antiradical activities of methanolic extract and its fraction of rambutan peel from two cultivars (Aceh and Binjai) and to correlate the antiradical activities with phenolics and flavonoid contents. **Methodology:** The rambutan peel from two cultivar (Aceh and Binjai) is dried, macerated with methanol, evaporated and added with water. The methanolic extract is fractionated with petroleum ether, chloroform and ethyl acetate to get the corresponding fraction. Methanolic extract and its fraction are then subjected to antiradical activity measurement using synthetic DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical, phenolics determination and flavonoid determination. **Results:** The methanolic extract and its fraction,either cultivar Aceh or Binjai,revealed the high antiradical activities with similar. Ethyl acetate fraction exhibited the strongest antiradical activities among samples evaluated with IC_{50} values of 2.66 μg mL^{-1} (cultivar Aceh) and 2.62 μg mL^{-1} (cultivar Binjai). Ethyl acetate fraction also exhibited the strongest phenolics and flavonoid contents accounting of 37.72±4.52 g Gallic Acid Equivalent (GAE)/100 (Aceh) and 32.40±2.37 g GAE/100 g (Binjai). The correlation between antiradical activities with phenolics and flavonoids contents showed with correlation, each with R^2 value of 0.0271 (phenolics) and 0.1122 (flavonoids). **Conclusion:** The methanolic extract and its fraction of rambutan peel cultivar Aceh and Binjai revealed strong DPPH antiradical activities therefore, rambutan peel can be exploited as natural antioxidant sources and is potential to be used as functional food.

Key words: DPPH radical, phenolics, flavonoids, cultivar Aceh, cultivar Binjai

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nephelium lappaceum L., known as rambutan is belonging to family of Sapindaceae and is widely distributed in South East Asian region such as Malaysia, Indonesia, Thailand and Vietnam. Rambutan fruit has a red or yellow pericarp and is covered with soft spines that vary in coloring from green, yellow and red[1]. There are some cultivars of rambutan with different characteristics, namely Rapiah, Narmada, Sinyonya, Binjai, Garuda, Kapulasan, Lebak Bulus, Si Batuk Ganal, Antalagi, Tangkue Lebak, Simacan, Bahrang and Sibongkok. Two cultivars of rambutan frequently consumed are Aceh and Binjai. In the market, rambutan is sold either in the form of fresh fruit or in processed fruit such as jam, juice, jellies and marmalades[2]. Rambutan consumed in fresh resulted the high amount of waste from seeds and peels. Therefore, it is very challenging to take benefits of rambutan waste as one of food supplement due to the high contents of active components as antioxidant like phenolics compounds. In addition, rambutan fruit cultivar Binjai contain endophytic bacteria. Sequencing analysis results the presence of bacteria coming from genus *Corynebacterium*, *Bacillus*, *Chryseobacterium*, *Staphylococcus* and *Curtobacterium*. These bacteria are suspected play a role as plant growth-promoting bacteria[3].

Rambutan is a tropical plant which grown in warm, humid and low evaporation rates with high rainfall and has some local names such as rambot (Aceh), barangkasa (Maluku), buiuwan (Bali), rambuta (Nusa Tenggara Barat), balatung, boeol, rambusa bolotu and wulangas (Sulawesi), banamon, beliti, bengayu, beriti, kayokan, maliti, puson, rambutan, sagalong, sanggalong, siban (Kalimantan), rambuten (Sumatra) and rambutan (Java, Minangkabau and Madurese)[4]. Some secondary metabolites are contained in rambutan peel namely ellagic acid, corilagin and geraniin[5,6], hederagenin 3-O-(3-O-acetyl-β-D-xylopyranosyl)-(1→3)-α-L-arabinopyranoside along (new compound) with four known compounds of hederagenin, hederagenin 3-O-(4-O-acetyl-α-L-arabinopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside, hederagenin 3-O-α-L-arabinopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside and hederagenin 3-O-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→4)-β-D-xylopyranoside[7]. These compounds are deduced to have antioxidant activity, therefore, in this study, the extracts or fraction of rambutan peel is evaluated for antioxidant activities through radical scavenging mechanism.

Antioxidants are taken into account as any compounds, either synthetic or natural from plants having the capability to delay or to inhibit the oxidation reactions of lipids, proteins or other molecules by inhibiting the free radical reaction[8]. There are some mechanisms used to evaluate antioxidant activity *in vitro*, namely antiradical, ferric reducing activity, chelating agent, beta-chelating agent and lipid peroxidation method[9,10]. Among these, antiradical mechanism is the most reported ones for antioxidant assay due to its rapidity and the availability of synthetic radical. Fidrianny *et al.*[11] reported the radical scavenging activities of hexane, ethyl acetate and ethanol extracts of rambutan leaves from five cultivars of Rapiah, Rajah, Binjai, Lebak Bulus and non-edible rambutan using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2-azinobis 3-ethylbenzothiazoline 6-sulfonate). Khonkarn *et al.*[12] evaluated the antiradical activities of rind and leaves also revealed the antiradical activity toward ABTS radical with IC_{50} values of 1.7±0.1 and 12.2±0.2 μg mL^{-1}, respectively. Using ABTS radical assay, ethyl acetate extract of rambutan peel showed the highest radical capacity with Trolox Equivalent Antioxidant Capacity (TEAC) of 23.0 mM mg^{-1}. Tachakittirungrod *et al.*[13] reported that ethanolic crude extract of rambutan peel has TEAC of 3.07±0.003 mM mg^{-1}.

Using the literature searching, there is no reports regarding the antiradical activities of extracts and its fractions (hexane, chloroform and ethyl acetate) using DPPH and ABTS radicals. Therefore, the objective of this study is to evaluate the antiradical activities of methanolic extract and its fraction of rambutan peel from two cultivars (Aceh and Binjai) using DPPH and ABTS radicals and to correlate the antiradical activities with phenolics and flavonoid contents.

MATERIALS AND METHODS

Rambutan fruits cultivar of Binjai and Aceh are obtained from several location around Yogyakarta. The 2,2-diphenyl-1-picrylhydrazyl (DPPH), rutin, gallic acid, Folin-Ciocalteu reagent are purchased from Sigma (Aldrich, USA). The other solvents and reagents used were of pro-analytical grade obtained from E. Merck (Darmstat, Germany).

Preparation of methanolic extract and fractions of kepel fruit pulp: Preparation of extract and fractions are performed by Permatasari and Rohman[14]. The rambutan peel is cleaned and cut into small using commercial cutter, blended and

subjected to maceration process using methanol as extracting solvent (1:10) for 3 days. Macerate is filtered and evaporated using vacuum rotary evaporator to obtain methanol extract of kepel fruit pulp. The methanolic extract was added with warm distilled water and is subsequently fractionated using petroleum ether. The residue of methanol extract is then fractionated again using chloroform to obtain chloroform fraction. The residue is finally extracted using ethyl acetate (Fig. 1). The methanolic extracts as well as the fractions of petroleum ether, chloroform, ethyl acetate and water are subjected to antioxidant assay and phenolics and flavonoid contents.

Antiradical activity evaluation using DPPH radical: Antiradical activity of samples toward 2,2-diphenyl-1-picrilhydrazil (DPPH) was evaluated using spectrophotometer visible at 517 nm according to Blois method as in Kikuzaki *et al.*[15]. The stable DPPH radical 0.1 mM in methanol was prepared and then 1 mL of this solution was mixed with 3 mL of sample at different concentrations. A control, containing 1 mL of DPPH radical solution and 3 mL of methanol was prepared. The mixture was allowed to room temperature for 20 min and the absorbance was subsequently measured at 517 nm against blank of methanol. The ability of extracts and fractions to scavenge the DPPH radical was calculated using the equation:

$$\text{Radical scavenging}\,(\%) = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100$$

The percentage of antioxidant activity was plotted against the sample concentrations (μg mL^{-1}) to obtain IC_{50}, defined as the concentration of the samples necessary to cause 50% scavenging of DPPH radical, calculated by an equation generated from linear regression[16].

Determination of total phenolics content: The concentration of total phenolics was determined using Folin-Ciocalteau reagent according to Chun *et al.*[17]. Total phenolic contents of samples were expressed as g Gallic Acid Equivalent (GAE)/100 g dry samples. The sample was analysed in triplicate.

Analysis of flavonoid content: Flavonoid contents of samples were determined using aluminium chloride colorimetric method according to Zou *et al.*[18]. An aliquot of diluted sample solution was mixed with 2 mL distilled water and is subsequently added with 0.15 mL $NaNO_2$ 5% and stand for minute. After that, 0.15 mL $AlCl_3$ 10% was added and allowed to stand for 6 min. The mixture was added with 2 mL NaOH 4% solution and immediately is added with distilled water to make final volume of 5.0 mL. The mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus a prepared water blank. Total flavonoid contents of extracts and fractions were expressed as g Rutin Equivalent (RE)/100 g dry material.

Data analysis: All data are analysed in triplicate and expressed as Mean±Standard Deviation using excel (Microsoft Inc., USA).

RESULTS AND DISCUSSION

Antioxidant assay using radical scavenging is the most popular methods reported by researchers in the field of nutrition. Synthetic radical of 2,2-diphenyl-1-picrilhydrazil (DPPH) is used due to its availability as radical without any

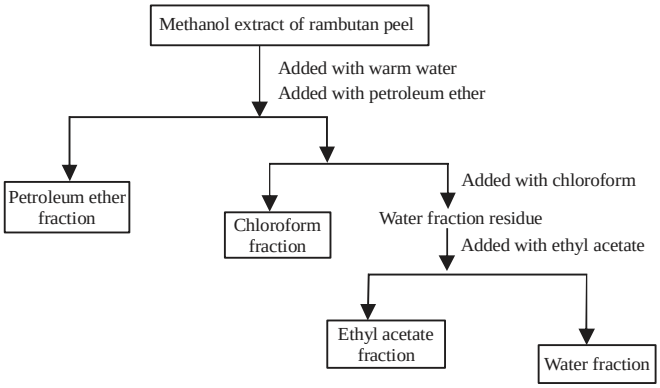

Fig. 1: Extraction and the fractionation step of kepel fruit pulp

addition of chemical reagents and is commercially available. Besides, DPPH radical is also readily soluble. The intensity of DPPH radicals are reduced when added with any compounds or samples having the capability to donate their hydrogen radical such as phenolics and flavonoids. Therefore, these compounds are known as antioxidant. The reduction of colour intensity is expressed with decreasing absorbance values. The solvent used is methanol due to the capability of this solvent to offer the best sensitivity compared with other polar solvents[19].

Table 1 compiled 1 IC_{50} values of methanol extract and its fraction of rambutan peel cultivar Binjai and Aceh along with positive control of vitamin C. The antioxidant activity of rambutan peel using DPPH radical scavenging is very promising due to the low values of IC_{50} values of evaluated samples. The lower the IC_{50} values, the more active of evaluated samples as antiradicals. Both cultivars (Aceh and Binjai) revealed similar antiradical activity, as indicated from the similar IC_{50} values of methanolic extract and its fraction. Among the evaluated samples, ethyl acetate fraction has the strongest antiradical activity (the lowest IC_{50} values). The similar results in which ethyl acetate revealed the most active antioxidant activities are also reported. Among extract and its fraction, ethyl acetate fraction of mengkudu (*Morinda citrifolia* L.) fruit 16, red fruit (*Pandanus conoideus* L.)[20] and stem bark of *Albizia adianthifolia*[21] showed the strongest antiradical activity using DPPH radicals. Ethyl acetate fraction was also reported to more effective than crude acetone extract in all antioxidant assays using scavenging ability against DPPH, beta-carotene bleaching inhibition and reducing power in *Terminalia bellerica* Roxb. fruit[22]. Figure 2 revealed the correlation between radical scavenging activity of ethyl acetate fraction of rambutan peel cultivar Aceh and Binjai using DPPH radical scavenging assay. Ethyl acetate fraction is then subjected to further fractionation to get the active isolate.

It is widely known that a group of compounds responsible for antiradical scavenging activities are phenolics and flavonoid, due to the capability of phenolics and flavonoids to donor hydrogen radical into DPPH radicals, therefore some antioxidant activities from natural sources are correlated with the total contents of phenolics and flavonoids[23,24]. The phenolics content is expressed as g GAE/100 g samples due to the reference standard used is gallic acid, while the contents of flavonoid are expressed as g RE/100 g samples[25]. Table 2 showed the total contents of phenolics and flavonoids in extracts and fractions of rambutan peel from both cultivar. The extract and fractions of both cultivars of rambutan peel revealed similar phenolics and flavonoid contents. Among methanolic extract and its fraction, ethyl acetate fraction revealed the highest phenolics and flavonoid contents, accounting of 37.72±4.52 g GAE/100 g sample and 32.25±0.35 g RE/100 g samples, respectively for cultivar Aceh. Ethyl acetate fraction of rambutan peel cultivar Binjai also exhibited the highest phenolic content (32.40±2.37 g GAE/100 g) and flavonoid content (39.26±2.77 g RE/100 g).

Figure 3 exhibited the correlation between IC_{50} values of radical radical scavenging with phenolics (Fig. 3a) and flavonoid contents (Fig. 3b) of extracts and fractions of

Table 1: Antiradical activity of methanol extract and its fraction using DPPH radical assay

Samples	IC_{50} (μg mL^{-1})
Vitamin C	3.55
Methanol extract cultivar Aceh	3.13
Petroleum ether fraction cultivar Aceh	3.189
Chloroform fraction cultivar Aceh	3.05
Ethyl acetate fraction cultivar Aceh	2.66
Water fraction cultivar Aceh	4.08
Methanol extract cultivar Binjai	3.42
Petroleum ether fraction cultivar Binjai	3.54
Chloroform fraction cultivar Binjai	4.26
Ethyl acetate fraction cultivar Binjai	2.62
Water fraction cultivar Binjai	3.20

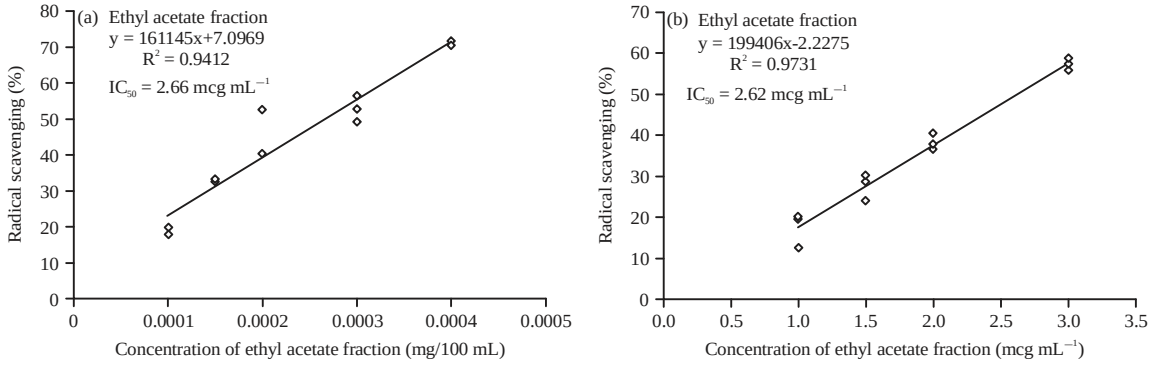

Fig. 2(a-b): Relationship between concentration of methanolic extract and its fraction of rambutan peel cultivar (a) Aceh and (b) Binjai with its radical scavenging activity (%)

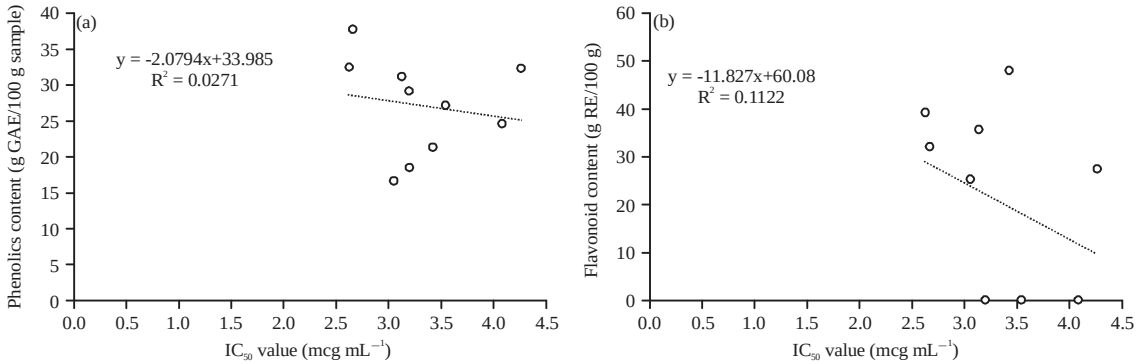

Fig. 3(a-b): Correlation between IC_{50} values (a) Phenolic contents and (b) Flavonoid contents as well as IC_{50} values

Table 2: Contents of total phenolics and total flavonoid of methanolic extract and fractions of rambutan peel cultivar Aceh and Binjai

Extract or fraction	Phenolic contents (Mean±SD)*	Flavonoid contents (Mean±SD)**
Methanolic extract cultivar Aceh	31.13±1.73	35.71±6.12
Petroleum ether fraction cultivar Aceh	29.15±1.08	0.10±0.01
Chloroform fraction cultivar Aceh	16.60±0.55	25.41± 0.51
Ethyl acetate cultivar Aceh	37.72±4.52	32.25±0.35
Water fraction cultivar Aceh	24.69±2.31	0.10±0.01
Methanolic extract cultivar Binjai	21.36±2.25	48.18±0.45
Petroleum ether fraction cultivar Binjai	27.11±1.23	0.10±0.01
Chloroform fraction cultivar Binjai	32.32±0.80	27.53±1.95
Ethyl acetate cultivar Binjai	32.40±2.37	39.26±2.77
Water fraction cultivar Binjai	18.44±2.13	0.10±0.01

*Expressed as g GAE/100 g sample, **Expressed as g RE/100 g sample

rambutan peel of cultivar Aceh and Binjai. The coefficient determination (R^2) value is used as parameter describing the contribution of phenolics and flavonoid contents toward radical scavenging activity[26]. The R^2 values obtained are 0.0271 and 0.1122 for correlation between IC_{50} values with phenolics and flavonoid contents, indicating that phenolics and flavonoids contents contributed to 2.71 and 11.22% toward antiradical scavenging activities, respectively. The weak correlation between antiradical activity with phenolics and flavonoid contents indicated that individual compounds are weakly associated with antiradical parameters, suggesting that antiradical activities are not simply attributed to specific constituents[27]. This can be deduced that phenolics and flavonoids do not contribute significantly toward radical scavenging activities.

CONCLUSION

The methanolic extract and its fraction of rambutan peel cultivar Aceh and Binjai revealed strong antiradical activities toward DPPH radicals. Among samples evaluated, ethyl acetate fraction showed the highest antiradical activity, phenolics content and flavonoid contents. Rambutan peel can be exploited as natural antioxidant sources and is potential to be used as functional food.

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