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Research Article
2,2'-diphenil-1-picrylhydrazil (DPPH) Radical Scavenging Activity of Extracts and Fractions of Rambutan (*Nephelium lappaceum* L.) Peel

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Abstract

Objectives: Rambutan, *Nephelium lappaceum* L. is tropical fruit widely distributed in South-East Asia. The consumption of rambutan fruit results in the production of vast amounts of waste from peels of the fruit. **Methodology:** This study is intended to evaluate the radical activity of extracts and fractions of rambutan peel cultivar simacan and lebak bolus toward 2,2'-diphenil-1-picrylhydrazil radical (DPPH) and to correlate its activity with total phenolics and flavonoid contents. Rambutan peel is dried, powdered and macerated using methanol to obtain methanolic extract. The methanolic extract was further subjected to fractionation using petroleum ether and ethyl acetate. The extracts and fractions were evaluated for its radical scavenging activity as well as for determination of phenolics and flavonoids contents. **Results:** Among extracts and fractions evaluated, ethyl acetate fraction of rambutan peel cultivar lebak bolus has the most active antiradical activity with IC_{50} value of 2.732 μg mL^{-1}. Quercetin was used as positive control with IC_{50} of 1.998 μg mL^{-1}. The ethyl acetate fraction of rambutan peel cultivar lebak bolus also has the highest phenolics and flavonoid contents, accounting of 47.71% (wt/wt) gallic acid equivalent and 29.59% (wt/wt) rutin equivalent, respectively. The correlation between IC_{50} and phenolics content revealed R^2 of 0.594, while R^2 for the relationship between IC_{50} and flavonoid content is 0.323, meaning that phenolics and flavonoid contents contributed to 59.4 and 32.30% of antiradical scavenging activity, respectively. **Conclusion:** Extract of rambutan peel is potential to be used as antiradical.

Key words: Rambutan peel, antiradical activity, phenolics content, flavonoid contents, 2,2'-diphenil-1-picrylhydrazil

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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

Free radicals are defined as an atoms, molecules or ions having unpaired electrons that are very highly unstable and very reactive to react with other molecules. They derive from three elements: Oxygen, nitrogen and sulfur, thus creating Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS) and Reactive Sulfur Species (RSS)[1]. Free radicals, ROS, RNS and RSS are able to initiate the reactions that damage organic molecules in the biological systems[2]. These reactions are taken into account to be the cause of some degenerative diseases, such as diabetes and aging[3,4]. Besides, radicals generated during lipid oxidation results in food deterioration, especially in high fat foods. As a consequence, antiradical and other antioxidants are added to inhibit radical reaction.

Antioxidants are compounds capable of delaying or inhibiting the oxidation reactions of lipids, proteins or other molecules by inhibiting the free radical reaction (initiation or propagation)[5]. Antioxidants can be derived from synthetic such as butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, ter-butylhidroquinone and natural antioxidants derived from fruits and vegetables. Synthetic antioxidants are suspected to cause toxic or mutagenic effects[6], therefore, the demand for natural antioxidants have been increased due to the consumer concerns about the safety of synthetic antioxidants[7]. There have been numerous researches on the potential of plants and other bioresources as potential natural antioxidants to replace the synthetic ones[8].

Rambutan, *Nephelium lappaceum* L. is belonging to the Sapindaceae family. Rambutan is an attractive tropical fruit widely distributed in South-East Asia, especially in Indonesia, Malaysia and Thailand[9]. Rambutan fruit is consumed fresh, canned or processed and its consumption yield the vast amount of waste from seeds and peels. Therefore, some researchers intended to use this underutilized part of rambutan fruit as part of food supplement by determining its activity. Rambutan is reported to have antiradical and antioxidant activities, antibacterial activity against five pathogenic bacteria[9,10] due to its phenolic content, anti-herpes simplex contained in rambutan virus type 1[11] and anti-hyperglycemic agent due to geraniin[12].

The ethanolic extract of rambutan peel is reported to contain ellagic acid, corilagin and geraniin[13]. Palanisamy *et al.*[12] also isolated geraniin from *N. lappaceum* rind waste. These compound are responsible for the biological activities of rambutan, including antiradical activity. However, the report concerning the antiradical activities of extracts and fractions of rambutan peel from two cultivars (simacan and lebak bolus) and its correlations with phenolics and flavonoid contents have not yet been published. In this study, determinations on free radical scavenging activity toward 2,2′-diphenyl-1-picrilhydrazil (DPPH) and phenolic contents in two cultivars of rambutan peel were studied.

MATERIALS AND METHODS

Materials: Rambutan (*Nephelium lappaceum* L.) peel cultivar simacan and lebak bolus was obtained from Mataram, West Nusa Tenggara, Indonesia. The authenticity of rambutan used was performed in Laboratory of Silviculture and Forest Technology, Faculty of Forestry, Mataram University, West Nusa Tenggara.

Chemicals: The 2,2-diphenyl-picrilhydrazil (DPPH), rutin, quercetin and gallic acid were obtained 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 its fraction of rambutan peel: Rambutan peel (two cultivars, simacan and lebak bolus) is cleaned and cut into small using commercial cutter, dried in oven at 65°C for 2 days and powdered. The powder is then subjected to maceration process using methanol as extracting solvent (1:10, powder:solvent) for 3 days. Macerate is filtered and evaporated using vacuum rotary evaporator to obtain methanol extract of rambutan peel. 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 ethyl acetate. The origin methanol extract and the fractions of petroleum ether, ethyl acetate and water are subjected to assay. The fractionation scheme for rambutan peel extract was shown in Fig. 1.

Antiradical activity evaluation: Antiradical activity of samples toward 2,2-diphenyl-1-picrilhydrazil was evaluated using spectrophotometer visible at 517 nm according to Blois method[10]. 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 stand at ambient 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:

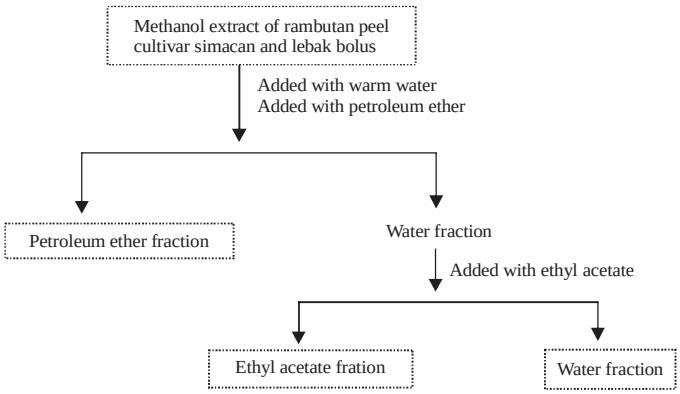

Fig. 1: Fractionation step of methanolic extract of rambutan peel cultivar simacan and lebak bolus

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

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[14].

Determination of total phenolics content: The levels of total phenolics in samples were analysed using Colorimetric Method (Folin-Ciocalteau method) according to Chun *et al.*[15]. A portion of diluted samples were added into 10 mL volumetric flask containing 3 mL aquabidest. A blank using aquabidest instead of sample was also prepared. A-400 μL Folin-Ciocalteu's reagent was added to the mixture and mixed. After 5 min, a-4 mL solution of Na_2CO_3 7% was added and mixed vigorously. The solution was made to volume using aquabidest, allowed to stand for 90 min and the absorbance was measured at 750 nm versus the prepared blank using calibrated spectrophotometer (Genesys 20, Japan) using $K_2C_2O_7$ for photometric accuracy and holmium perchlorate for wavelength check. Total phenolic contents of samples were expressed as gram gallic acid equivalent/100 gram dry samples. The sample was analyzed in triplicate. Gallic acid for preparation of calibration curve was made in the range of 1.0-10.0 mg 100 mL^{-1}.

Analysis of flavonoid content: Flavonoid contents of samples were determined using aluminium chloride colorimetric method according to Zou *et al.*[16]. 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 min. 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 gram rutin equivalent/100 gram dry material. Rutin for preparation of calibration curve was made in the range of 1.0-10.0 mg 100 mL^{-1}.

Data analysis: All data are analysed in triplicate and expressed as Mean±Standard Deviation using Excel (Microsoft Inc., USA). The difference among data was analysed using one way-ANOVA test followed by Duncan New Multi Range Test (DNMRT) using SPSS version 22. The significance level was set at $p<0.05$.

RESULTS AND DISCUSSION

Among antioxidant mechanisms (namely radical scavengers, lipid peroxidation inhibition, chelating agent, reducing power and synergist), free radical scavenger is the most reported ones for the evaluation of antioxidant capacity of antioxidants derived from plants. The scavenging activity of stable 2,2'-diphenyl-1-picrilhydrazil (DPPH) radical can be employed to measure the antioxidant activities in a relatively short time[17,18]. The DPPH radical is stable and soluble in polar solvents, such as methanol and ethanol. Sharma and Bhat[19] have revisited the use of solvents during DPPH radical scavenging assay and the result showed that methanol and buffered methanol is the choice of reaction medium due to its capability to provide the highest absorbance value, therefore, methanol is used in this study.

The antiradical scavenging activities of extracts and fractions of rambutan peel from two cultivars expressed with

Table 1: Antiradical activities of rambutan peel and quercetin, expressed with IC_{50} value

	IC_{50} (μg mL^{-1})				
Samples	1	2	3	4	$\bar{X}$±SD (μg mL^{-1})
Methanol extra ctcultivar simacan	4.57	5.04	4.28	4.11	4.50±0.41
Methanol extra ctcultivar lebak bolus	4.72	4.69	4.89	5.11	4.85±0.19
Ethyl acetate fraction of cultivar simacan	6.43	6.58	5.90	6.59	6.37±0.33
Ethyl acetate fraction of cultivar lebak bolus	2.81	2.80	2.73	2.59	2.73±0.10
Petroleum ether fraction cultivar simacan	9.67	7.48	11.83	10.28	9.81±1.80
Petroleum ether fraction cultivar lebak bolus	3.54	4.27	4.64	4.93	4.34 ±0.60
Water fraction of cultivar simacan	10.92	16.78	12.45	13.37	13.38±2.48
Water fraction of cultivar lebak bolus	5.55	5.74	7.87	6.85	6.50±1.08
Quercetin	1.81	2.49	1.71	1.98	2.00±0.34

Table 2: Total phenolic contents of extracts and fractions of rambutan peel expressed with Gallic Acid Equivalent (GAE)

	Phenolics contents (% wt/wt GAE)				
Samples	1	2	3	4	$\bar{X}$±SD (% wt/wt GAE)
Methanol extra ctcultivar simacan	20.44	21.15	20.77	19.05	20.35±0.91
Methanol extra ctcultivar lebak bolus	28.54	32.83	34.87	32.03	32.06±2.64
Ethyl acetate fraction of cultivar simacan	31.72	30.80	30.57	31.38	31.12±0.53
Ethyl acetate fraction of cultivar lebak bolus	51.22	50.63	42.09	46.90	47.71±4.21
Petroleum ether fraction cultivar simacan	23.58	24.32	22.12	20.29	22.58±1.78
Petroleum ether fraction cultivar lebak bolus	37.40	36.36	32.96	35.02	35.43±1.92
Water fraction of cultivar simacan	14.28	17.00	12.91	16.92	15.28±2.02
Water fraction of cultivar lebak bolus	32.19	31.32	29.08	30.17	30.69 ±1.36

GAE: Gallic acid equivalent

Table 3: Total flavonoid contents of extracts and fractions of rambutan peel expressed with Rutin Equivalent (RE)

	Flavonoid contents (% wt/wt RE)				
Samples	1	2	3	4	$\bar{X}$±SD (% wt/wt RE)
Methanol extra ctcultivar simacan	12.95	12.57	12.53	12.01	12.52±0.39
Methanol extra ctcultivar lebak bolus	15.37	15.74	17.50	15.78	16.10±0.96
Ethyl acetate fraction of cultivar simacan	26.95	26.32	26.81	27.43	26.88±0.46
Ethyl acetate fraction of cultivar lebak bolus	33.83	29.66	27.23	27.64	29.59±3.02
Petroleum ether fraction cultivar simacan	16.48	15.69	13.51	15.27	15.24±1.26
Petroleum ether fraction cultivar lebak bolus	17.79	17.65	16.58	17.32	17.34±0.54
Water fraction of cultivar simacan	7.98	10.38	9.68	10.30	9.59±1.12
Water fraction of cultivar lebak bolus	13.60	13.70	14.11	14.67	14.02±0.49

RE: Rutin equivalent

IC_{50} value is compiled in Table 1. Among samples evaluated, ethyl acetate fraction of cultivar lebak bolus has the highest antiradical activity with IC_{50} of 2.73±0.10 μg mL^{-1}, but lower than quercetin (2.00±0.34 μg mL^{-1}). The antiradical activity of ethyl acetate fraction of cultivar lebak bolus was significantly different from that of other samples and quercetin based on ANOVA test followed by Duncan New Multi Range Test (DNMRT) at significance level of 0.05. The similar result, in which ethyl acetate fraction has the highest antioxidant activities compared to other fractions was also observed for other plant extracts of *Morinda citrifolia*[14], red fruit (*Pandanus conoideus* Lam)[20] and *Phyllantus urinaria* L.[21].

Phenolic compounds has the capability to donor radical hydrogen, therefore, in this study, the levels of phenolics and flavonoids contents in all extracts and fractions of rambutan peel are determined. Furthermore, the levels of phenolics and flavonoid are correlated with IC_{50} values of evaluated samples in order to seek the contribution of phenolics and flavonoids toward radical scavenging activities. Table 2 and 3 compiled the contents of phenolics and flavonoids in extracts and fractions of rambutan peel. Ethyl acetate fraction of rambutan peel cultivar lebak bolus revealed the highest contents of phenolics and flavonoids of 47.71±4.21% (wt/wt) gallic acid equivalent and 29.59±3.02 rutin equivalent, respectively.

Figure 2 and 3 exhibited the correlation between phenolics and flavonoid contents (x-axis) with antiradical activities (IC_{50} values) (y-axis) of extracts and fractions evaluated. The equations describing such correlation are y = -0.264x+14.34 (phenolics) and y = -0.282x+11.54 (flavonoid), respectively. The coefficient of determination as a

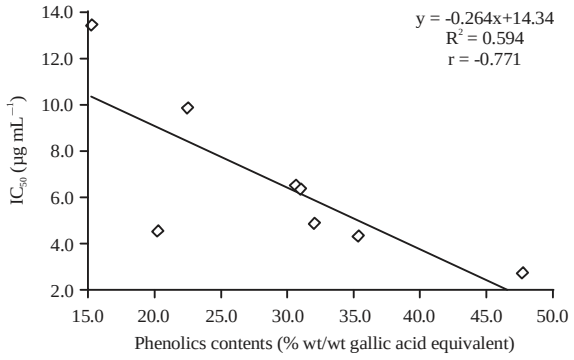

Fig. 2: Correlation between phenolic contents (x-axis) and IC_{50} values (y-axis) of extracts and fractions of rambutan peel

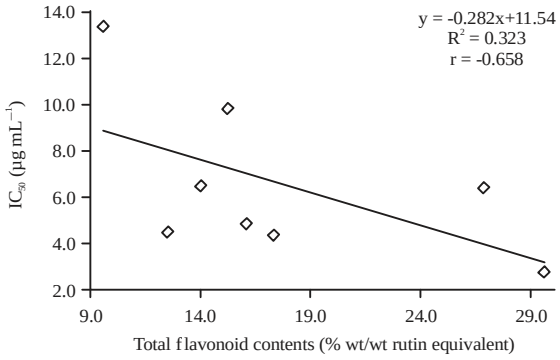

Fig. 3: Correlation between flavonoid contents (x-axis) and antiradical activities (IC_{50} values) (y-axis) of extracts and fractions of rambutan peel

means for relationship evaluation are 0.594 and 0.323, for phenolics and flavonoids, respectively. This finding indicated that phenolics and flavonoids contents contributed to 59.1 and 32.3%, for antiradical activities, respectively. Besides, it can be also deduced that antiradical activity of extracts and fractions of rambutan peel is not limited to phenolics and flavonoids. Antiradical activity may also come from the presence of other antiradical components such as alkaloids, vitamins and others[22].

CONCLUSION

Among extracts and fractions of rambutan peel evaluated, ethyl acetate fraction of cultivar lebak bolus has the highest antiradical activity, phenolis content and flavonoid contents. The IC_{50} value of ethyl acetate fraction is of 2.732 µg mL^{-1}, comparable to IC_{50} of quercetin (1.998 µg mL^{-1}). There is a correlation between IC_{50} values and phenolics as well flavonoid contents with R^2 values of 0.594 and 0.323, respectively. This finding indicates that ethyl acetate fraction of rambutan peel can be used as natural antioxidant components.

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