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Research Article

Antioxidant Activities in Various Peel Extracts of Four Varieties Rambutan (*Nephelium lappaceum*) Using DPPH, FRAP Assays

Fidrianny I[1*], Sari PI [1], Wirasutisna KR[1]

[1]*School of Pharmacy, Bandung Institute of Technology, Indonesia*

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ABSTRACT

The objectives of this research were to study antioxidant activities from various extracts of rambutan peels using two methods of antioxidant assays which were DPPH (2-2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power); and correlation of total flavonoid, phenolic, and carotenoid content in various extracts of rambutan peels with DPPH antioxidant activities and FRAP capacities. Extraction was performed by reflux apparatus using different polarity solvents. The extracts were evaporated using rotary evaporator. Antioxidant capacities were tested using DPPH and FRAP assays. Determination of total flavonoid, phenolic, and carotenoid content was performed by spectrophotometer UV-visible and their correlation with DPPH antioxidant activities and FRAP capacities were analyzed by Pearson's method. Ethyl acetate extract of lebak bulus rambutan peels (LB2) had the highest DPPH scavenging activity with IC_{50} 3.5 µg/mL, while ethyl acetate extract of binjai rambutan peels (BJ2) had the highest FRAP capacity with EC_{50} 77.1 µg/mL. N-hexane extract of binjai rambutan peels (BJ1) had the highest total flavonoid (3.46 g QE/100 g), ethyl acetate extract of lebak bulus rambutan peels (LB2) had the highest phenolic content (40.9 g GAE/100 g), and n-hexane extract of rapiah rambutan peels (RP1) had the highest carotenoid content (0.61 g BE/100 g). There was a positively high correlation between total phenolic content with their antioxidant activity using DPPH and FRAP assays. The DPPH scavenging activities in various peel extracts from four varieties rambutan gave linear result with FRAP capacities.

Keywords: Antioxidants, DPPH, FRAP, rambutan peels, flavonoid, phenolic, carotenoid

INTRODUCTION

The risk of many diseases that related to oxidative stress can be reduced by antioxidant. Phenolic compounds are commonly found in plants, and they have multiple biological effects, including antibacterial, anti-inflammatory and antioxidant activity[1-3]. Previous studies[4-7] exhibited that total phenolic content and flavonoid content in plants could be correlated to their antioxidant activities.

Some of antioxidant methods such as DPPH (2-2-diphenyl 1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) were used to determine antioxidant capacity of vegetables, fruits, beverages and food[3,8,9,10]. In previous research[3,11-13] expressed that DPPH and FRAP methods could be used to determine antioxidant activity in many plants extracts. Research by Thitilertdecha[1] and Tachakittirungrod[1] revealed that rambutan (*Nephelium lappaceum*) had antioxidant capacities by using DPPH, ABTS and FRAP assays.

The objective of this research were to study antioxidant activities of different polarity extracts (n-hexane, ethyl acetate and ethanol) of peel form four varieties of rambutan (lebak bulus rambutan, rajah rambutan, rapiah rambutan and binjai rambutan) using antioxidant testing DPPH and FRAP assays and correlations of their antioxidant activities with total flavonoid, phenolic and carotenoid content.

MATERIALS AND METHODS

Materials

TPTZ (2-4-6-tripyridyltriazine), DPPH (2-2-diphenyl-1-picrylhydrazyl), gallic acid, quercetin, beta carotene purchased from Sigma-Aldrich (MO, USA), ferric chloride, peels from four varieties of rambutan. All other reagents were analytical grades.

Preparation of sample

Peels from four varieties of rambutan (*Nephelium lappaceum*) were collected from Purwadadi-Subang (West Java) that were: lebak bulus rambutan as sample LB, rajah rambutan as sample RJ, rapiah rambutan as sample RP, binjai rambutan as sample BJ, were thoroughly washed with tap water, sorted while wet, cut, dried, and grinded into powder.

Extraction

Three hundred grams of powdered sample were extracted by reflux apparatus using increasing polarity of solvents. The extraction using n-hexane was repeated three times. The remaining residue was then extracted three times with ethyl acetate. Finally the remaining residue was extracted three times with ethanol. So there were four n-

**Author for Correspondence*

Table 1: DPPH scavenging activities and FRAP capacities of n-hexane peel extracts

Sample	DPPH scavenging activity (%)	FRAP capacity (%)
LB1	4.77 ± 1.89 [a]	19.11 ± 1.35 [a]
RJ1	2.85 ± 2.32 [a]	2.52 ± 2.29 [b]
RP1	3.47 ± 1.61 [a]	5.04 ± 0.78 [c]
BJ1	4.08 ± 2.24 [a]	1.21 ± 0.52 [b]
Ascorbic acid	94.72 ± 0.15	99.88 ± 0.00
P value	< 0.05	< 0.05

Note: a–e = means within a column with the different letter were significantly different ($p<0.05$)

hexane extracts (LB1, RJ1, RP1, and BJ1), four ethyl acetate extracts (LB2, RJ2, RP2, and BJ2) and four ethanolic extracts (LB3, RJ3, RP3, and BJ3).

DPPH scavenging activity

Preparation of DPPH solution was adopted from Blois[14] with minor modification. Each extract 50 µg/mL was pipetted into DPPH solution 50 µg/mL (1:1) to initiate the reaction. After 30 minutes incubation, the absorbance was read at wavelength 515 nm by using spectrophotometer UV-Vis Hewlett Packard 8435. Ethanol was used as a blank. DPPH solution 50 µg/mL and ethanol (1:1) was used as standard. Analysis was done in triplicate for standard and each extract. Antioxidant activity of each extract was determined based on the reduction of DPPH absorbance by calculating percentage of antioxidant activity[8].

FRAP capacity

Preparation of FRAP solution was adopted from Benzi[15]. The FRAP solution was prepared in acetate buffer pH 3.6. Each extract 50 µg/mL was pipetted into FRAP solution 50 µg/mL (1:1) to initiate the reaction. After 30 minutes incubation, the absorbance was read at wavelength 593 nm by using spectrophotometer UV-Vis Hewlett Packard 8435. Acetate buffer was used as a blank and FRAP solution 50 µg/mL and methanol (1:1) was used as standard. Analysis was done in triplicate for standard and each extract. Antioxidant capacity of each extract was determined based on increasing in Fe (II)-TPTZ absorbance by calculating percentage of antioxidant capacity[15].

Total flavonoid content

Total flavonoid content was measured using adapted method from Chang *et al*[16]. The absorbance was read at wavelength 415 nm. Analysis was done in triplicate for each extract. Standard solutions of quercetin 20-160 µg/mL were used to obtain a standard curve. The total flavonoid content was reported as percentage of total quercetin equivalents per 100 g extract (g QE/100 g).

Total phenolic content

Total phenolic content were measured using the modified Folin-Ciolcalteu method adapted from Pourmorad[17]. The absorbance was read at wavelength 765 nm. Analysis was done in triplicate for each extract. Standard solutions of gallic acid 60-150 µg/mL were used to obtain a standard curve. The total phenolic content was reported as

Table 2: DPPH scavenging activities and FRAP capacities of ethyl acetate peel extracts

Sample	DPPH scavenging activity (%)	FRAP capacity (%)
LB2	93.26 ± 2.51 [a]	43.72 ± 1.11 [a]
RJ2	91.98 ± 1.98 [a]	39.96 ± 0.86 [b]
RP2	92.37 ± 1.63 [a]	27.82 ± 2.03 [c]
BJ2	90.84 ± 2.45 [a]	33.14 ± 0.81 [d]
Ascorbic acid	94.72 ± 0.15	99.88 ± 0.00
P value	< 0.05	< 0.05

Note: a–e = means within a column with the different letter were significantly different ($p<0.05$)

Table 3: DPPH scavenging activities and FRAP capacities of ethanolic peel extracts

Sample	DPPH scavenging activity (%)	FRAP capacity (%)
LB3	93.71 ± 0.54 [a]	26.55 ± 0.86 [a]
RJ3	89.94 ± 0.96 [b]	28.72 ± 0.32 [b]
RP3	92.80 ± 0.33 [a]	29.94 ± 0.87 [b]
BJ3	91.83 ± 1.85 [a,b]	21.02 ± 0.81 [c]
Ascorbic acid	94.72 ± 0.15	99.88 ± 0.00
P value	< 0.05	< 0.05

Note: a–e = means within a column with the different letter were significantly different ($p<0.05$)

percentage of total gallic acid equivalents per 100 g extract (g GAE/100 g).

Total carotenoid content

Total carotenoid content was measured using the modified carotene method adapted from Thaipong *et al*[3]. Each extract were diluted in n-hexane. The absorbance was read at wavelength 470 nm. Analysis was done in triplicate for each extract. Standard solutions of beta carotene 5-40 µg/mL were used to obtain a standard curve. The total carotenoid content was reported as percentage of total beta carotene equivalents per 100 g extract (g BE/100 g).

Statistical Analysis

Analysis of each sample was performed in triplicate. All results presented were the means (±SD) of at least three independent experiments. Statistical analysis (ANOVA with a statistical significance level set at $p < 0.05$ and post-hoc Tukey procedure) was carried out with SPSS 17.0 for Windows. Correlations between the total flavonoid, phenolic, and carotenoid content with antioxidant capacities were made using the Pearson's method ($p < 0.01$).

RESULTS

Antioxidant capacities of various peel extracts from four varieties of rambutan using DPPH and FRAP assays

The antioxidant activities and capacities using DPPH and FRAP assays of various peel extracts from four varieties of rambutan were shown in Table 1, Table 2, and Table 3. In the DPPH method, free radical scavenging capacities of various peel extracts from four varieties of rambutan

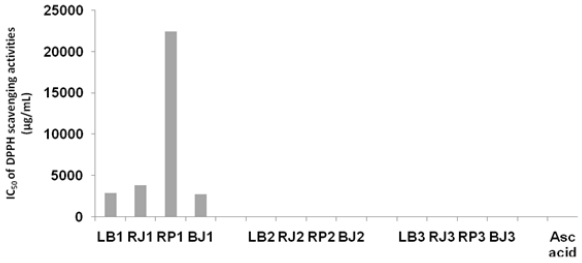

Fig 1: IC_{50} of DPPH scavenging activities in various peel extracts from four varieties of rambutan n=3

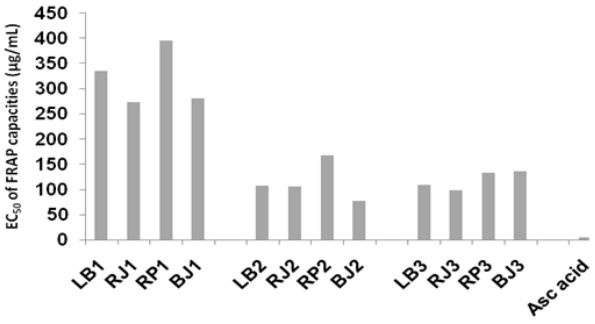

Fig 2: EC_{50} of FRAP capacities in various peel extracts from four varieties of rambutan n=3

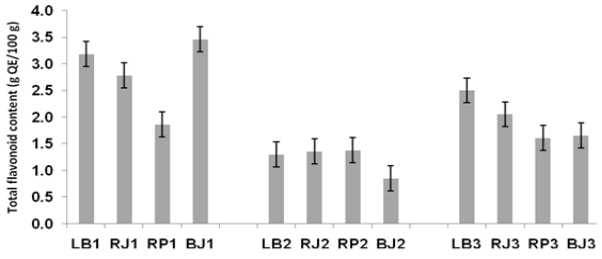

Fig 3: Total flavonoid content in various rambutan peel extracts n=3

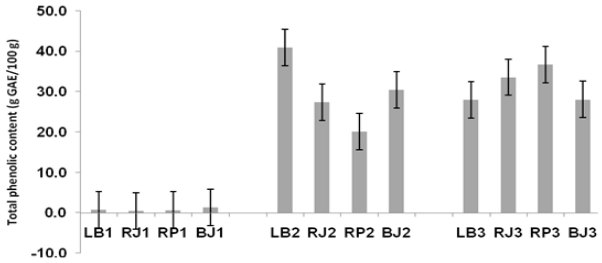

Fig 4: Total phenolic content in various rambutan peel extracts n=3

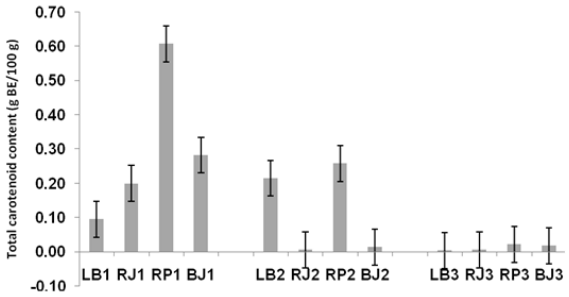

Fig 5: Total carotenoid content in various rambutan peel extracts n=3

ranged from 2.85 to 93.71%. Ethanolic extract of lebak bulus rambutan peels (LB3) had the highest DPPH radical scavenging activity (93.71%), while n-hexane extract of rajah rambutan peels (RJ1) had the lowest DPPH antioxidant capacity (2.85%). Using FRAP method, antioxidant capacities in the range of 1.21 to 43.72%. Ethyl acetate extract of lebak bulus rambutan peels (LB2) had the highest FRAP capacity (43.72%), while the lowest capacity (1.21%) was given by n-hexane extract of binjai rambutan peels (BJ1

IC_{50} of DPPH scavenging activity and EC_{50} of FRAP capacity

The IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities in various extract from four varieties of rambutan peels using DPPH and FRAP assays were shown in Fig 1 and Fig 2. The half maximum inhibitory concentration (IC_{50}) of DPPH scavenging activities was compared to IC_{50} ascorbic acid standard, while EC_{50} of FRAP capacities of each extracts was compared to EC_{50} ascorbic acid standard. The lowest EC_{50} or IC_{50} means had the highest antioxidant capacity.

Total flavonoid in various rambutan peel extracts

The total flavonoid content among the various extracts was expressed in term of quercetin equivalent using the standard curve equation y = 0.0072x - 0.0196, R^2 = 0.9996. The total flavonoid content in various peel extracts from four varieties of rambutan showed different results within the range of 0.85 - 3.46 g QE/100 g (Fig 3). N-hexane extract of binjai rambutan peels (BJ1) had the highest total flavonoid content (3.46 g QE/100 g) and ethyl acetate extract of binjai rambutan peels (BJ2) had the lowest (0.85 g QE/100 g).

Total phenolic in various rambutan peel extracts

The total phenolic content among the various extracts was expressed in term of gallic acid equivalent using the standard curve equation y = 0.0058x - 0.0394, R^2 = 0.9986. The total phenolic content in various peel extracts from four varieties of rambutan showed different result ranged from 0.45 to 40.9 g GAE/100 g. Ethyl acetate extract of lebak bulus rambutan peels (LB2) had the highest phenolic content (40.9 g GAE/100 g), while the lowest phenolic content was given by n-hexane extract of rajah rambutan peels (RJ1) 0.45 g GAE/100 g (Fig 4).

Total carotenoid in various rambutan peel extracts

The total carotenoid content among the various extracts was expressed in term of beta carotene equivalent using the standard curve equation y = 0.0175 x - 0.0045, R^2 = 0.9992. The total carotenoid content in various peel extracts from four varieties of rambutan showed different result in the range of 0 to 0.61 g BE/100 g (Fig 5). N-

Table 4: Pearson's correlation coefficient of total flavonoid, phenolic, carotenoid in rambutan peel extracts and DPPH scavenging activities, FRAP capacities

	Total Flavonoid	Total Phenolic	Total Carotenoid	FRAP LB	FRAP RJ	FRAP RP	FRAP BJ
DPPH LB	-0.750*	0.945**	0.067ns	0.728*			
DPPH RJ	-0.862**	0.980**	-0.997**		0.958**		
DPPH RP	-0.811**	0.889**	-0.918**			0.991**	
DPPH BJ	-0.938**	0.996**	0.998**				0.922**
FRAP LB	-0.955**	0.900**	0.729*				
FRAP RJ	-0.936**	0.887**	-0.944**				
FRAP RP	-0.755*	0.915**	-0.940**				
FRAP BJ	-0.981**	0.951**	-0.937**				

Note: FRAP = FRAP capacity, DPPH = DPPH scavenging activity, LB = sample LB, RJ = sample RJ, RP = sample RP, BJ = sample BJ, ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$

hexane extract of rapiah rambutan peels (RP1) had the highest carotenoid content (0.61 g BE/100 g).

Correlations between total flavonoid, phenolic, carotenoid content with DPPH scavenging activities, and FRAP capacities in various rambutan peel extracts

Pearson's correlation coefficient was positively high if $0.68 \leq r \leq 0.97$[3]. The highest and positive correlation between total phenolic content and DPPH scavenging activity ($r = 0.996$, $p<0.01$) was given by sample BJ. The highest and positive correlation between total phenolic content and FRAP capacity was given by sample BJ also ($r = 0.951$, $p<0.01$), followed by sample RP ($r = 0.915$, $p<0.01$) (Table 4). The correlation between total flavonoid and their antioxidant capacities exposed that all of rambutan peel extracts sample had negative correlation with DPPH scavenging activities and FRAP capacities. Pearson's correlation coefficient between total carotenoid form various rambutan peel extracts and their antioxidant capacities revealed that only BJ sample had positive correlation with DPPH scavenging activities ($r = 0.998$, $p<0.01$) and sample LB with FRAP capacities ($r = 0.729$, $p<0.05$).

DISCUSSION

Previous study revealed that rambutan had antioxidant capacity[1,4,11]. There were no study regarding antioxidant activity of three different polarities extracts (which were n-hexane, ethyl acetate and ethanol) of peel form four varieties of rambutan using DPPH and FRAP assays. The DPPH is stable free radicals which dissolve in methanol or ethanol, and its colors show characteristic absorption at wavelength 515-520 nm. Colors of DPPH would be changed when the free radicals were scavenged by antioxidant[18,19]. Reagent of FRAP is $FeCl_3$ that combined with TPTZ in acetate buffer pH 3.6. Fe (III) will be reduced to Fe (II). Complex Fe (II) - TPTZ gives blue color and show characteristic absorption at wavelength 593 nm. Intensity of blue color depends on amount of Fe (III) that is reduced to Fe (II). If a sample reduces Fe (III) to Fe (II), at the same time it will be oxidized, so that sample can act as antioxidant. Sample will act as antioxidant in FRAP assays if sample had reduction potential lower than reduction potential of Fe (III)/Fe (II) which was 0.77 V, so the sample had the reducing power to reduce Fe (III) to Fe (II) and this sample will be oxidized.

In the present study, 50 µg/mL of ethanolic peel extracts from four varieties of rambutan (lebak bulus rambutan, rajah rambutan, rapiah rambutan and binjai rambutan) which were reacted with 50 µg/mL DPPH solution gave DPPH scavenging capacity 93.71 %, 89.94 %, 92.80 %, 91.83%, respectively.

The half maximum inhibitory concentration (IC_{50}) of DPPH scavenging activity is the concentration of sample or standard that can inhibit 50% of DPPH scavenging activity, while EC_{50} of FRAP capacity is the concentration of sample or standard that can exhibit 50% of FRAP capacity. The lowest IC_{50} or EC_{50} means had the highest antioxidant capacity. The IC_{50} or EC_{50} were used to determine antioxidant capacity of a sample that compared to standard. Sample that has IC_{50} or EC_{50} less than 50 µg/mL is a very strong antioxidant, 50-100 µg/mL is a strong antioxidant, 101-150 µg/mL is a medium antioxidant, while IC_{50} or EC_{50} greater than 150 µg/mL is a weak antioxidant[14].

In the DPPH method, IC_{50} of various peel extracts from four varieties of rambutan ranged from 3.5 to 3803.3 µg/mL. LB2 (ethyl acetate peel extract of lebak bulus rambutan) had the lowest IC_{50} of DPPH radical scavenging capacity 3.5 µg/mL, while ascorbic acid standard gave IC_{50} of DPPH scavenging capacity 4.7 µg/mL. Its showed that potency antioxidant activity of LB2 was similar with ascorbic acid. Previous study[1] revealed that IC_{50} of DPPH scavenging activity of methanol peel extract of rambutan was 4.94 µg/mL which was similar with IC_{50} of DPPH scavenging activity of ethanolic peel extracts of rambutan in the present study. Based on value of IC_{50} of DPPH scavenging activities in the range of 3.5-8.6 µg/mL for ethyl acetate extracts and 6.5-9.4 µg/mL for ethanolic extracts, it can be concluded that ethyl acetate extracts and ethanol extracts of all of sample can be categorized as very strong antioxidant. Ethanol 80 % peel extract which was extracted in 120 minutes at 50°C had IC_{50} of DPPH scavenging activity 8.87 µg/mL[5], while Thitilertechda[4] demonstrated that methanolic peel extract of rambutan had strong antioxidant activity

EC_{50} of FRAP capacity of various peel extracts from four varieties of rambutan in the range from 77.1 to 334.7 µg/mL. Ethyl acetate peel extract of binjai rambutan (BJ2) had the lowest EC_{50} of FRAP capacity (77.1

μg/mL) while ascorbic acid standard gave EC_{50} of FRAP capacity 5 μg/mL. Its exposed that potency of ascorbic acid was around fifteen times of potency of BJ2 using FRAP assays. Previous research by Tachakittirungrod[11] exhibited that ethanol extract of fruit peel of rambutan (*N. lappaceum*) had TEAC (Trolox Equivalent Antioxidant Capacity) values was 3.07 mM/mg. TEAC assays is the same with ABTS assays. Based on that research fruit peel of rambutan can be classified as extremely high antioxidant activity because of its TEAC values above 3.0 mM/mg.

The presence of total phenolic might contributed in antioxidant capacity[9]. The present study exposed that total phenolic in ethanolic peel extracts of lebak bulus rambutan, rajah rambutan, rapiah rambutan and binjai rambutan were 28.01, 33.57, 36.71, 28.07 g GAE/100 g, respectively. Previous research by Thitilertdecha[1] expressed that total phenolic of methanolic peel extract of rambutan (542.2 mg catechin/g) was higher than water peel extract, ether peel extract, methanol seed extract, ether seed extract and water seed extract. Study by Samuagam[5] demonstrated that ethanolic 80 % peel extract had total phenolic content 53.94 mg GAE/g extract. It was in contrast with previous research[4] which exposed total phenolic in methanol peel extract of rambutan was 542 mg/g extract.

The data in Table 4 revealed that there were positive and high correlation between total phenolic content in all of sample (LB, RJ, RP, BJ) with DPPH scavenging activity and FRAP capacity. Based on this result it can be concluded that DPPH scavenging activity and FRAP capacity in peel extract of four varieties of rambutan can be predicted indirectly by determining total phenolic content.

Flavonoid, phenolic acid tannins, qoumarine and quinone were included. Phenolic acid had lower antioxidant activity than flavonoid[20]. Flavonoid would give higher antioxidant activity if had OH in ortho C 3',4', OH in C3, oxo function in C4, double bond at C2 and C3. The OH with ortho position in C3'-C4' had the highest influence to antioxidant capacity of flavonoid. The flavonoid aglycones would give higher antioxidant activity than flavonoid glycosides[20].

Total flavonoid content in BJ1 (n-hexane peel extract of binjai rambutan) was higher (3.46 g QE/100 g) than total flavonoid in BJ2 (0.85 g QE/100 g), but antioxidant activity of BJ2 was higher than BJ1, which was IC_{50} DPPH scavenging activity of BJ2 (6.7 μg/mL) was lower than BJ1 (2707.3 μg/mL). Based on the data above it can predicted that almost all of flavonoids in BJ1 were flavonoid that had OH in position which not influence antioxidant activities, while many flavonoid in BJ2 had OH in ortho C 3',4', OH in C3, oxo function in C4, double bond at C2 and C3 position that had high influence antioxidant activities.

In the present study demonstrated that only total carotenoid in BJ rambutan with their DPPH scavenging activity and total carotenoid in LB rambutan with FRAP capacity had positively and high correlation. Carotenoid had antioxidant capacity by scavenging free radical. More double bonds in carotenoid would give higher scavenging free radical capacity[21]. Carotenoid that consisted of more than 7 double bonds would give higher scavenging radical free capacity than carotenoid which had less and 7 double bonds[22]. Increasing in lipophilicity of carotenoid would increase scavenging radical capacity[23]. Beta carotene was used as standard in total carotenoid content because of it had conjugation double bonds which has ability to scavenge free radicals[24]. RP1 peel extract had higher carotenoid (0.61 g BE/100 g) than RP2 (0.26 g BE/100 g), but antioxidant activity of RP2 was higher than RP1, which was IC_{50} of DPPH scavenging activity of RP2 (8.6 μg/mL) was lower than RP1 (22430 μg/mL). Based on this data, it could be seen that many carotenoids in RP2 had more than 7 double bonds, that had high antioxidant capacity. It was in contrast, many carotenoid in RP1 had less than 7 double bonds.

The FRAP and DPPH methods had different mechanisms reaction. Mechanism of DPPH that was electron transfer assays[25] and FRAP was redox assays. So the results of the two methods not always linear. The Pearson's correlation coefficient of peel extracts from four varieties of rambutan indicated that all of sample (LB, RJ, RP and BJ) had positively high correlation between DPPH scavenging activities and FRAP capacities. The results of this study showed that DPPH scavenging activities in all of extracts sample were linear with their FRAP capacities.

CONCLUSION

Antioxidant capacity of sample should perform using variety of methods in parallel, because different methods could give different results. Ethanolic extracts and ethyl acetate extracts of rambutan peels had IC_{50} of DPPH scavenging capacities less than 50 μg/mL that were very strong antioxidant. The positively and high correlation between total phenolic content with DPPH scavenging activities and FRAP capacities were given by all of peel extracts sample. Phenolic compounds were the major contributor in DPPH scavenging activities and FRAP capacities in peel extracts of four varieties of rambutan. There were liner correlation between DPPH and FRAP result in all of peel extracts sample. Peels of lebak bulus rambutan, rajah rambutan, rapiah rambutan, and binjai rambutan may be exploited as a source of beneficial compounds for human health to alleviate oxidative stress.

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