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ANTIOXIDANT ACTIVITIES OF VARIOUS SEED EXTRACTS FROM FOUR VARIETIES OF RAMBUTAN (*NEPHELIUM LAPPACEUM*) USING 2,2-DIPHENYL-1-PICRYLHYDRAZYL AND 2,2'-AZINOBIS (3-ETHYL-BENZOTHIAZOLINE-6-SULFONIC ACID) ASSAYS

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ABSTRACT

Objectives: The objectives of this research were to study antioxidant activities from various seed extracts of four varieties of rambutan using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) methods and correlation of total flavonoid content (TFC), total phenolic content (TPC), and total carotenoid content (TCC) with inhibitory concentration 50 (IC₅₀) of DPPH and IC₅₀ of ABTS.

Methods: Antioxidant activities, TFC, TPC, and TCC of various seed extracts were performed by ultraviolet -visible spectrophotometry and their correlation with IC_{so} of DPPH and IC_{so} of ABTS antioxidant activities were analyzed by Pearson's method.

Results: Ethanolic seed extract of lebak bulus variety had the lowest IC_{50} of DPPH scavenging activity 7 µg/ml, while ethyl acetate seed extract of rajah variety had the lowest IC_{50} of ABTS scavenging activity 7.34 µg/ml. The highest TFC, TPC, and TCC were given by ethyl acetate extract of binjai variety, ethanolic extract of rajah variety, and ethanolic extract of rajah variety, respectively. There were negatively high correlation between TFC, TPC, TCC in seed extracts of rapiah variety with their IC_{50} of DPPH and IC_{50} of ABTS scavenging activities.

Conclusions: All of ethyl acetate and ethanolic seed extracts of four varieties of rambutan were very strong antioxidant by DPPH and ABTS methods. Flavonoid, phenolic, and carotenoid compound in seed extracts of rapiah variety were contributor in their antioxidant activities by DPPH and ABTS methods. The IC_{s0} of DPPH and IC_{s0} of ABTS scavenging activities in seed extracts of lebak bulus, rajah, and rapiah varieties gave linear result.

Keywords: Antioxidant, 2,2-diphenyl-1-picrylhydrazyl, 2,2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid, Seed, Four varieties, Rambutan, Flavonoid, Phenolic, Carotenoid.

INTRODUCTION

The oxidative stress could be correlated with many degenerative diseases. Antioxidant has potency to protect oxidative stress. Phenolic compounds are commonly found in plants, which have multiple biological effects, including antioxidant activity [1-3]. Many studies had reported that phenolic content in plants could be related with their antioxidant activities. Plants containing phenolic and polyphenol compounds can act as antioxidant [4-6].

Some of the antioxidant methods such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid), (ABTS) ferric reducing antioxidant power (FRAP), and cupric ion reducing antioxidant capacity were widely used to predict antioxidant capacity of fresh fruits, beverages, and food [6-9]. Previous studies [4,6,9,10] exposed that DPPH and ABTS methods could be used to measure antioxidant activity in many plants extracts. The previous research [4,11,12] exhibited antioxidant activities of some plants, including rambutan (*Nephelium lappaceum*).

The objectives of this research were to study antioxidant activities of various polarities extracts (n-hexane, ethyl acetate, and ethanol) from four varieties of rambutan (*N. lappaceum*) seeds using DPPH and ABTS assays; and correlation of their antioxidant activities with total phenolic content (TPC), total flavonoid content (TFC), and total carotenoid content (TCC) in each extract.

METHODS

Materials

DPPH, ABTS, gallic acid, quercetin, and beta carotene was purchased from Sigma-Aldrich (MO, USA), seed from four varieties of rambutan. All of other reagents were analytical grades.

Preparation of sample

Seed from four varieties of rambutan (*N. lappaceum*) were collected from Subang - West Java that were: Lebak bulus variety namely sample LE, rajah variety as RJ, rapiah variety as RP, and binjai variety as BI were thoroughly washed with tap water, sorted while wet, cut, dried, and grinded into powder.

Extraction

Three hundred grams of powdered samples were extracted by reflux apparatus using increasing polarity of solvents. The extraction using n-hexane was repeated 3 times. The remaining residue was then extracted 3 times using ethyl acetate. Finally, the remaining residue was extracted 3 times using ethanol. So, totally there were 12 extracts: Four of n-hexane extracts (LE1, RJ1, RP1, and B11), four of ethyl acetate extracts (LE2, RJ2, RP2, and B12), and four of ethanolic extracts (LE3, RJ3, RP3, and B13).

Inhibitory concentration (IC₅₀) of DPPH scavenging activity

Preparation of DPPH solution was adopted from Blois [13] with minor modification. Various concentration of each extract was pipetted into DPPH solution 50 μ g/ml (1:1) to initiate the reaction for obtaining a calibration curve. After 30 minutes incubation, the absorbance was read at wavelength 515 nm by using spectrophotometer ultraviolet-visible (UV-visible) Hewlett Packard 8435. Methanol was used as a blank. DPPH solution 50 μ g/ml was used as control. Ascorbic acid 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 [14]. IC₅₀ of DPPH scavenging activity of each extract can be calculated using its calibration curve.

IC₅₀ of ABTS scavenging activity

Preparation of ABTSradical solution was adopted from Li et al. [15] method with minor modification. ABTS diammonium salt solution 7.6 mM in aquadest and potassium persulfate solution 2.5 mM in aquadest were prepared. Each solution was allowed to stand in the dark room for 12 hrs. The two solutions were mixed with 30 minutes incubation, allowed to stand in refrigerator for 24 hrs, and then diluted in ethanol. Various concentrations of each extract were pipetted into ABTS solution 50 μ g/ml (1:1) to initiate the reaction for obtaining a calibration curve. The absorbance was read at wavelength of 734 nm using spectrophotometer UV-Vis Hewlett Packard 8435. Ethanol (95%) was used as a blank. ABTS solution 50 µg/ml was used as control. Ascorbic acid was used as standard. Analysis was done in triplicate for standard and each extract Antioxidant canacity of each extract was determined based on the reduction of ABTS absorbance by calculating percentage of antioxidant activity [14]. IC₅₀ of ABTS scavenging activity of each extract can be calculated using its calibration curve.

TFC

TFC was measured using adapted method from Chang *et al.* [16]. The absorbance was read at wavelength of 415 nm. Analysis was done in triplicate for each extract. Standard solution of quercetin 20-120 μ g/ml was used to obtain a standard curve. The TFC was reported as percentage of total quercetin equivalent per 100 g extract (g QE/100 g).

трс

TPC were measured using the modified Folin–Ciocalteu method adapted from Pourmorad [3]. The absorbance was read at wavelength of 765 nm. Analysis was done in triplicate for each extract. Standard solution of gallic acid 30-180 μ g/ml was used to obtain a standard curve. The TPC was reported as percentage of total gallic acid equivalent per 100 g extract (g GAE/100 g).

тсс

TCC was measured using the modified carotene method adapted from Thaipong *et al.* [6]. Each extract were diluted in n-hexane. The absorbance was read at wavelength of 470 nm. Analysis was done in triplicate for each extract. Standard solution of beta carotene 5-70 μ g/ml was used to obtain a standard curve. The TCC was reported as percentage of total beta carotene equivalent per 100 g extract (g BE/100 g).

Statistical analysis

Analysis of each sample was performed in triplicate. All results presented were the means (±standard deviation) 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 16.0 for Windows. Correlations between the TFC, TPC, TCC, and antioxidant activities were done using the Pearson's method (p<0.01).

RESULTS

$\mathrm{IC}_{\scriptscriptstyle 50}$ of DPPH scavenging activities and $\mathrm{IC}_{\scriptscriptstyle 50}$ of ABTS scavenging activities

The IC₅₀ of DPPH scavenging activities and IC₅₀ of ABTS scavenging activities in various seed extracts from four varieties of rambutan using DPPH and ABTS assays were shown in Figs. 1 and 2. The IC₅₀ of DPPH scavenging activities and IC₅₀ of ABTS scavenging activities in various seed extracts were compared to IC₅₀ of ascorbic acid standard. The lowest IC₅₀ means had the highest antioxidant activity.

TFC in various seed extracts from four varieties of rambutan

TFC in various extracts were demonstrated in term of quercetin equivalent using the standard curve equation y=0.007x-0.027, $R^2=0.995$. TFC in various rambutan seed extracts showed different result ranged from 0.99 to 5.93 g QE/100 g (Fig. 3). N-hexane rambutan seed extract of rapiah variety (RP1) had the lowest TFC (0.99 g QE/100 g) while the highest (5.93 g QE/100 g) was given by ethyl acetate rambutan seed extract of binjai variety (BI2).

TPC in various seed extracts from four varieties of rambutan

TPC in various extracts were expressed in term of gallic acid equivalent using the standard curve equation y=0.006x-0.055, $R^2=0.998$. TPC in various rambutan seed extracts showed different result ranged from 0.36 to 3.05 g GAE/100 g. Ethanolic rambutan seed extract of rapiah variety (RP3) had the highest phenolic content (3.05 g GAE/100 g) (Fig. 4).

TCC in various seed extracts from four varieties of rambutan

TCC in various extracts were exposed in terms of beta carotene equivalent using the standard curve equation y=0.012x-0.008, $R^2=0.998$. TCC in various rambutan seed extracts showed different result in the range of 0.1-0.32 g BE/100 g (Fig. 5). The highest carotenoid

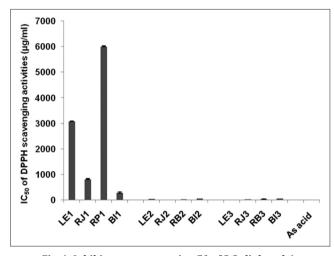


Fig. 1: Inhibitory concentration 50 of 2,2-diphenyl-1picrylhydrazyl scavenging activities in various seed extracts from four varieties of rambutan, n=3

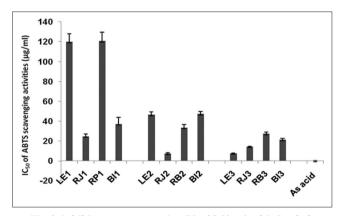


Fig. 2: Inhibitory concentration 50 of 2,2'-azinobis 3-ethylbenzothiazoline-6-sulfonic acid scavenging activities in various seed extracts from four varieties of rambutan, n=3

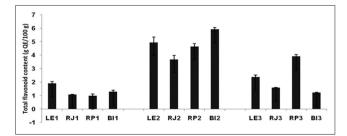


Fig. 3: Total flavonoid content in various seed extracts from four varieties of rambutan, n=3

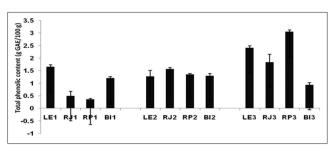


Fig. 4: Total phenolic content in various seed extracts from four varieties of rambutan, n=3

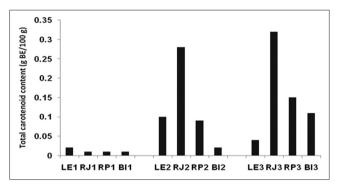


Fig. 5: Total carotenoid content in various seed extracts from four varieties of rambutan, n=3

content (0.32 g BE/100 g) for ethanolic rambutan were given by the seed extract of rajah variety (RJ3), while the lowest carotenoid (0.1 g BE/100 g) were given by the n-hexane rambutan seed extracts of rajah, rapiah, and binjai varieties (RJ1, RP1, and BI1).

Correlations between IC₅₀ of DPPH scavenging activities, IC₅₀ of ABTS scavenging activities and TFC, TPC, and TCC in various seed extracts from four varieties of rambutan

Pearson's correlation coefficient between TFC in various seed extracts from four varieties of rambutan and their antioxidant activities demonstrated that TFC in seed extract of rapiah variety had negatively high correlation with IC_{50} of DPPH (r=-0.978, p<0.01) and IC_{50} of ABTS (r=-0.959, p<0.01). TPC in sample RJ and RP had negative and high correlation with their IC_{50} of DPPH scavenging activities (r=-0.940; r=-0.780, p<0.01, respectively), and only TPC in seed extract of rapiah variety had negative and high correlation with IC_{50} of ABTS scavenging activities (r=-0.802, p<0.01). TCC in sample RJ and RP had negatively high correlation with their IC_{50} of DPPH scavenging activities (r=-0.991, r=-0.916, p<0.01, respectively) and IC_{50} of ABTS scavenging activities (r=-0.850, r=-0.926, p<0.01, respectively).

DISCUSSION

Previous study [4,8,11,12,17] reported that rambutan (*N. lappaceum*) had antioxidant capacity. There were no study regarding antioxidant activity of three different polarities extracts (which were n-hexane, ethyl acetate, and ethanol) of seed from four varieties (lebak bulus, rajah, rapiah, and binjai) of rambutan using DPPH and ABTS assays.

Both of DPPH and ABTS are stable free radicals which dissolve in methanol or ethanol, and their colors show characteristic absorption at wavelength 516 nm or 734 nm, respectively. Colors DPPH and ABTS would be changed when the free radicals were scavenged by antioxidant [18].

 $\rm IC_{50}$ of DPPH scavenging activity is the concentration of sample or standard that can inhibit 50% of DPPH scavenging activity. $\rm IC_{50}$ of ABTS scavenging activity is the concentration of sample or standard that can inhibit 50% of ABTS scavenging activity. The lowest $\rm IC_{50}$ means

had the highest antioxidant capacity. The IC₅₀ were used to categorize antioxidant activity of a sample that compared to the standard. Sample that has IC₅₀ <50 µg/ml is very strong antioxidant, 50-100 µg/ml is strong antioxidant, 101-150 µg/ml is medium antioxidant, while IC₅₀ >150 µg/ml is weak antioxidant [13].

The IC₅₀ of DPPH scavenging activities of various seed extracts from four varieties of rambutan in the range of 7-6,000 µg/ml. Ethanolic rambutan seed extract of lebak bulus variety (LE3) had the lowest IC_{E0} of DPPH radical scavenging capacity 7 µg/ml, while ascorbic acid standard gave IC₅₀ of DPPH scavenging capacity 2 μ g/ml. Based on value of IC₅₀ of DPPH scavenging capacity, it can be concluded that the ethyl acetate and ethanolic seed extracts of four varieties of rambutan can be categorized as very strong antioxidant. It was similar with the previous research [17] which was revealed that all of ethyl acetate and ethanolic peel extracts from four varieties of rambutan was classified as very strong antioxidant, and only ethanolic leaves extracts of five varieties of rambutan were categorized as very strong antioxidant [4]. In the previous research [11] exposed that IC_{50} of DPPH scavenging activity of methanol peels extract of rambutan was 4.94 µg/ml which was lower than ascorbic acid as standard, while Samuagam [8] reported that 80% ethanol peels extract which was extracted in 120 minutes at 50°C had IC_{50} of DPPH scavenging activity 8.87 µg/ml.

In the present study, it was exposed that IC_{E0} of ABTS scavenging activities of various seed extracts from four varieties of rambutan ranged from 7.34 to 120.8 µg/ml. Ethyl acetate seed extract of rajah variety had the lowest IC_{50} of ABTS (7.34 µg/ml). Based on the IC_{50} value, it can be concluded that all of seed extracts from four varieties of rambutan (except n-hexane seed extract of lebak bulus and rapiah varieties) were categorized as very strong antioxidant by ABTS method. It was different with the previous study [4] which exhibited that IC_{50} of ABTS scavenging activity of various leaves extracts from five varieties of rambutan in the range of 12.83-259.66 µg/ml. Ethanolic leaves extract of rapiah variety (RPH3) had the lowest IC₅₀ of ABTS scavenging activity (12.83 μ g/ml) while ascorbic acid standard gave IC_{50} of ABTS scavenging capacity 2.69 µg/ml. The previous research [19] revealed that ethanol extract of fruit peels of rambutan (N. lappaceum) had trolox equivalent antioxidant capacity (TEAC) value of 3.07 mM/mg. TEAC assays is the same with ABTS assays. Fruit peels of rambutan can be classified as extremely high antioxidant activity because of its TEAC values above 3.0 mM/mg. Ethyl acetate rambutan peel extract of binjai variety (BI2) had the lowest effective concentration; 50% of FRAP capacity (77.1 µg/ml) compared to other extracts in various peel extracts from four varieties of rambutan [17].

The presence of total phenolic might contributed in antioxidant capacity [12]. The present research reported that the highest TPC was given by ethanolic seed extract of rapiah variety (3.05 g GAE/100 g), while in the previous study it was exposed that the highest TPC was given by ethyl acetate peel extract of lebak bulus variety (40.9 g GAE/100 g) [17] and ethanolic leaves extract of rapiah variety had the highest TPC (29.46 μ g/ml) [4]. Previous research by Thitilertdecha *et al.* [11] demonstrated that total phenolic of methanolic peel extract, ether peel extract, methanol seed extract, ether seed extract, and water seed extract. The previous research [4] exposed total phenolic in methanol peel extract of rambutan of 542 mg/g extract. It was contrast with Samuagam [8] which reported that 80 % ethanolic peel extract had TPC of 53.94 mg GAE/g extract.

Study by Fidrianny *et al.* [4] showed that the highest TFC was given by n-hexane leaves extract of binjai variety (3.5 g QE/100 g). It was different with the present study which revealed that the highest TFC was given by ethyl acetate seed extract of binjai variety, but it was similar with the previous research which demonstrated that n-hexane peel extract of binjai variety had the highest TFC [17].

The present study reported that TCC in ethanolic seed extract of rajah variety (0.32 g BE/100 g) was the highest, it was contrast with the

previous study which exhibited that n-hexane extract of peel and leaves of rapiah variety rambutan gave the highest TCC 0.61 g BE/100 g and 6.41 g BE/100 g, respectively [4,17].

Pearson's correlation coefficient was positively high if $0.61 \le r \le 0.97$ [6] and negatively high if $-0.61 \le r \le -0.97$. Sample which had the lowest IC₅₀ of DPPH scavenging activity or IC₅₀ of ABTS scavenging activity gave the highest antioxidant activity. So, the good correlation among TPC, TFC, and TCC with IC₅₀ DPPH and or IC₅₀ ABTS will be given in negatively and high correlation [20]. It means increase in TFC, TPC, and TCC caused increase in antioxidant activities, which was expressed by lower IC₅₀ of DPPH scavenging activity and or IC₅₀ of ABTS scavenging activity [20].

The data in Table 1 demonstrated that there were negative and high correlation among TFC, TPC, and TCC contents in various seed extracts of rapiah variety with IC_{50} of DPPH (r=-0.979, r=-0.779, r=-0.915, p<0.01, respectively) and with IC_{50} of ABTS (r=-0.959, r=-0.802, r=-0.926, p<0.01, respectively). Based on this data, it can be concluded that flavonoid, phenolic, and carotenoid compounds in various seed extracts of rapiah variety were contributors in antioxidant activities by DPPH and ABTS methods. In the previous research it was exposed that TPC in various peel extracts of four varieties of rambutan (rajah, rapiah, lebak bulus, and binjai) had positive and high correlation with percentage of DPPH scavenging activities and percentage of FRAP capacities [17]. The same result which exposed that TPC in various leaves extracts of five varieties of rambutan (lebak bulus, binjai, rajah, rapiah, and non-consumption) had positive and high correlation with percentage of DPPH and ABTS scavenging activities [4].

Flavonoid, phenolic acid, tannins were included in phenolic compounds. Flavonoid which has -OH in A ring and/or B ring will be included in phenolic groups. Flavonoid had higher antioxidant capacity than phenolic acid [21]. The higher antioxidant capacity of flavonoid was depended on position of -OH in C-3'-C-4', -OH in C-3, oxo function in C-4, double bond in C-2 and C-3. Ortho position of hydroxyl group in C-3'-C-4' had the highest influence in antioxidant capacity of flavonoid. The flavonoid aglycones would give higher antioxidant activity than flavonoid glycosides [21].

Fig. 3 revealed that TFC in ethanolic rambutan seed extract of binjai variety (BI3) 1.22 g QE/100 g was similar with TFC in n-hexane rambutan seed extract of binjai variety (BI1) 1.28 g QE/100 g, but IC_{50} of DPPH scavenging activity of BI3 46.5 µg/ml (very strong antioxidant) was lower than IC_{50} of DPPH scavenging activity of BI1 270 µg/ml, which was categorized as weak antioxidant. Based on this data, it can be predicted that many flavonoid compounds in BI3 had -OH in C-3'-C-4', -OH in C-3, oxo function in C-4, double bond at C-2 and C-3 which had high influence in antioxidant activity, while almost all of flavonoid compounds in BI1 had –OH in other position which had low antioxidant activity.

It could be seen in Fig. 4, TPC in ethyl acetate rambutan seed extract of rajah variety (RJ2) 1.57 g GAE/100 g was similar with TPC in n-hexane rambutan seed extract of lebak bulus variety (LE1) 1.66 g GAE/100 g, but RJ2 had IC₅₀ DPPH scavenging activity of 7.8 µg/ml and IC₅₀ of ABTS scavenging activity of 7.34 µg/ml (very strong antioxidant) were lower than IC₅₀ of DPPH and IC₅₀ of ABTS of LE1 (3071 µg/ml and 120 µg/ml, respectively). Based on the above data it can be supposed that many phenolic compounds in n-hexane rambutan seed extract of lebak bulus variety had lower antioxidant activity. It was contrast in ethyl acetate rambutan seed extract of rajah variety that contained many phenolic compounds which had high antioxidant activity.

Carotenoid had antioxidant capacity by scavenging free radical. More double bonds in carotenoid would give higher free radical scavenging capacity [22]. Carotenoid which contained more than seven double bonds would give higher free radical scavenging activity [23]. Decrease in lipophilicity of carotenoid would decrease free radical scavenging capacity [24]. Beta carotene was used as standard because it had conjugation double bonds due to its ability to scavenge free radicals [25,26]. In Fig. 5 it could be seen that TCC in ethyl acetate seed extract of binjai variety (BI2) 0.02 g BE/100 g was similar with TCC in n-hexane seed extract of lebak bulus variety (LE1) 0.02 g BE/100 g. BI2 had IC_{50} of DPPH scavenging activities (44.4 µg/ml) and IC_{50} of ABTS scavenging activity (47.25 μ g/ml) (very strong antioxidant) which were lower than IC₁₀ of DPPH (3071 µg/ml) and IC₁₀ of ABTS (120 µg/ml) for LE1. Based on the data, it can be predicted that almost all of carotenoid in BI2 had more than seven double bonds which have high antioxidant activity and only a little of carotenoid in LE1 had more than seven double bonds.

The DPPH and ABTS methods had the same mechanism reaction. Mechanism of DPPH and ABTS that was electron transfer assays [27], but the results of the two methods not always linear, because of not all of compound which can react with free radical of DPPH also react with free radical of ABTS. The Pearson's correlation coefficient indicated that IC_{50} of DPPH scavenging activities in seed extracts of three varieties of rambutan (lebak bulus, rajah, and rapiah) had positively high correlation with their IC_{50} of ABTS scavenging activities in seed extracts of lebak bulus, rajah, and rapiah varieties.

CONCLUSION

Different results could be given by different methods. Variety of methods must be used in parallel to assess the antioxidant capacity of sample. All of ethyl acetate and ethanolic seed extracts of four varieties of rambutan had IC_{50} of DPPH and IC_{50} of ABTS scavenging activities <50 µg/ml that means were very strong antioxidant. There were negatively high correlation between TFC, TPC, and TCC in seed extracts of rapiah variety with their IC_{50} of DPPH and IC_{50} of ABTS scavenging activities. Flavonoid, phenolic, and carotenoid compounds in seed

 Table 1: Pearson's correlation coefficient of IC₅₀ of DPPH scavenging activities, IC₅₀ of ABTS scavenging activities and TFC, TPC, TCC in various seed extracts from four varieties of rambutan

Antioxidant activities	Pearson's correlation coefficient (r)						
	TFC	TPC	TCC	IC ₅₀ ABTS LE	IC ₅₀ ABTS RJ	IC ₅₀ ABTS RP	IC ₅₀ ABTS BI
IC ₅₀ DPPH LE	-0.607*	-0.184 ^{ns}	-0.641*	0.940**			
IC ₅₀ DPPH RJ	-0.646*	-0.940**	-0.991**		0.907**		
IC ₅₀ DPPH RP	-0.978**	-0.780**	-0.916**			0.994**	
IC ₅₀ DPPH BI	-0.487 ^{ns}	0.246 ^{ns}	-0.586 ^{ns}				0.081 ^{ns}
IC ₅₀ ABTS LE	-0.320 ^{ns}	-0.488 ^{ns}	-0.378 ^{ns}				
IC ₅₀ ABTS RJ	-0.889**	0.769**	-0.850**				
IC ₅₀ ABTS RP	-0.959**	-0.802**	-0.926**				
IC ₅₀ ABTS BI	0.755**	0.889**	-0.826**				

DPPH: DPPH scavenging activity, ABTS: ABTS scavenging activity, TPC: Total phenolic content, TFC: Total phenolic content, TCC: Total carotenoid content, LE: Sample LE, RJ: Sample RJ, RP: Sample RJ, BI: Sample BI, ns: Not significant, *Significant at p<0.05, **Significant at p<0.01, DPPH: 2,2-diphenyl-1-picrylhydrazyl ABTS: 2,2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid, IC₅₀: Inhibitory concentration 50

extracts of rapiah variety were contributor in antioxidant activity using DPPH and ABTS assays. Antioxidant activities of various seed extracts from three varieties of rambutan (lebak bulus, rajah, and rapiah) gave linear result using DPPH and ABTS assays. Seed extracts of four varieties (lebak bulus, rajah, rapiah, and binjai) of rambutan (*N. lappaceum*) may be exploited as natural antioxidant sources.

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