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Original Article

IN VITRO ANTIOXIDANT ACTIVITIES FROM VARIOUS EXTRACTS OF BANANA PEELS USING ABTS, DPPH ASSAYS AND CORRELATION WITH PHENOLIC, FLAVONOID, CAROTENOID CONTENT

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ABSTRACT

Objectives: The objectives of this research were to study antioxidant capacity from various extracts of banana peels using two methods of antioxidant testing which were ABTS (2.2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) and DPPH (2.2-diphenyl-1-picrylhydrazyl) and correlation of total phenolic, flavonoid and carotenoid content in various extracts of banana peels with ABTS and DPPH antioxidant capacities.

Methods: Extraction was conducted by reflux using various solvents. The extracts were vaporated using rotavapor. Then antioxidant capacities were tested using ABTS and DPPH assays. Determination of total phenolic, flavonoid and carotenoid content were performed by spectrophotometry UV-Vis and its correlation with ABTS and DPPH antioxidant capacities were analyzed by Pearson method.

Results: AL2 (ethyl acetate peels extract of ambon lumut banana) had the highest ABTS scavenging capacity with IC_{50} 1.91 ppm and MU3 had the highest DPPH scavenging activities with EC_{50} 4.39 ppm. MU2 (ethyl acetate peels extract of muli banana) contained the highest total phenolic (3.99 g GAE/100 g), MU2 had highest flavonoid content (6.08 g QE/100 g) and MU2 had also the highest carotenoid 0.34 g BET/100 g. **Conclusions.** There was positively high correlation between total phenolic content in muli banana peels with its antioxidant activity using DPPH assays. ABTS scavenging capacities in muli banana and ambon lumut banana peels had positively high correlation with their DPPH scavenging activities.

Keywords: Antioxidants, ABTS, DPPH, Banana peels, Flavonoid, Phenolic, Carotenoid.

INTRODUCTION

Antioxidant has potency to mobilize protective effects against oxidative stress on account of their high antioxidant activity [1]. Phenolic compounds such as phenolic acid, flavonoid and tannin are commonly found in plants, and they have been reported to have multiple biological effects, including antioxidant activity [1] [2] [3] [4]. Many studies had revealed that antioxidant activities could be correlated with their phenolic content in plants. Plants contained phenolic and polyphenol compounds which have antioxidant activity [1] [5].

Some of antioxidant methods such as DPPH (2,2-diphenyl-1 picrylhydrazyl) and ABTS ((2-2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) were widely used to predict antioxidant capacity of fresh fruits, beverages and food [3]. In previous study [3] [6] [7] [8] exposed that DPPH and ABTS methods could be used to determine antioxidant activity in many plants extracts. The previous study [6] [8] [9] [10] showed antioxidant activities of some plants including banana peels.

The objective of this research were to study antioxidant capacities of various extracts (n-hexane, ethyl acetate and ethanol) from three bananas (raja bulu, muli and ambon lumut) peels using antioxidant testing DPPH and ABTS assays and correlations of their capacities with total flavonoid, phenolic, and carotenoid content in each extracts.

MATERIALS AND METHODS

Materials

ABTS (2-2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt), DPPH (2,2-diphenyl-1 picrylhydrazyl), gallic acid, quercetin, beta carotene were purchased from Sigma-Aldrich (MO,USA), ferric chloride, methanol, ethanol. All other reagents were analytical grades.

Preparation of sample

Fruits peels of three bananas (*Musa sp*) that were: raja bulu banana (RB) collected from Bandung, muli banana (MU) banana and ambon lumut banana (AL) collected from Garut, were thoroughly washed with tap water, wet sortation, cut, dried and grinded into powder.

Extraction

Three hundred grams of powdered samples were extracted by reflux using increasing gradient polarity solvents. The n-hexane extract 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 three n-hexane extracts (namely RB1, MU1, AL1), three ethyl acetate extracts (RB2, MU2, AL2) and three ethanolic extracts (RB3, MU3, AL3).

DPPH scavenging capacity

Preparation of DPPH solution were adopted from Blois [11] with minor modification. Each extracts 50 µg/mL was pipetted into DPPH solution concentration 50 µg/mL (1:1) to initiate the reaction. After 30 minutes incubation, the absorbance was read at wavelength 517 nm by using spectrophotometer UV-Vis Hewlett Packard 8435. Methanol was used as a blank and DPPH solution 50 µg/mL as standard. Analysis was done in triplicate for standard and each extracts. Antioxidant activity of each extracts were determined based on the reduction of DPPH absorbance by calculating percentage of antioxidant activity [12].

ABTS scavenging capacity

Preparation of ABTS radical solution were adopted from Li *et al*. [13] and Pellegrini *et al*. [14] method with minor modification. ABTS diammonium salt solution 7.6 mM in ethanol and potassium persulfate solution 2,5 mM in ethanol were prepared. Each solutions allowing to stand in the dark room for 12-18 hours. The two solutions were mixed with 30-60 minutes incubation, then diluted in ethanol. Each extracts 50 µg/mL was pipetted into ABTS solution 50 µg/mL (1:1) to initiate the reaction. The absorbance was read at wavelength 734 nm without incubation time using spectrophotometer UV-Vis Hewlett Packard 8435. Ethanol (95%) was used as a blank and ABTS solution 50 µg/mL was used as standard. Analysis was done in triplicate for standard and each

extracts. Antioxidant capacity of each extracts were determined based on the reduction of ABTS absorbance by calculating percentage of antioxidant activity [12].

Total flavonoid determination

Total flavonoid content was measured using adapted method from Chang *et al* [15]. The absorbance was read at wavelength 415 nm. Analysis was done in triplicate for each extracts. Standard solutions of quercetin with concentration 40-160 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 determination

Total phenolic content were measured using the modified Folin-Ciolcalteu method adapted from Pourmorad [16]. The absorbance was read at wavelength 765 nm. Analysis was done in triplicate for each extracts. Standard solutions of gallic acid with concentration 60-150 µg/mL were used to obtain a standard curve. The total phenolic content was reported as percentage of total gallic acid equivalents per 100 g extract (g GAE /100 g).

Total carotenoid determination

Total carotenoid content was measured using the modified carotene method adapted from Thaipong *et al* [3]. Each extracts were diluted in acetone. The absorbance was read at wavelength 470 nm. Analysis was done in triplicate for each extracts. Standard solutions of beta carotene with concentration 10-40 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 BET/100 g).

Statistic

Each sample analysis 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 $p < 0.05$ with post-hoc Least Significant Difference (LSD) procedure was carried out with SPSS 16.0 for Windows. Correlations between the total phenolic, flavonoid and total carotenoid content and antioxidant capacities were made using the Pearson method ($p < 0.01$).

RESULTS

Antioxidant capacities of various extracts from banana peels using DPPH and ABTS assays

The antioxidant capacities using DPPH and ABTS assays of various peels extracts from banana peels were shown in Table 1, Table 2, Table 3. In DPPH method, antioxidant capacities in the range of 50.11 – 60.24 %. MU3 peels extract (ethanolic extract of muli banana) had the highest DPPH radical scavenging capacity (60.24 %), while the lowest antioxidant capacity (50.11 %) was given by RB1 peels extract.

In the ABTS method, free radical scavenging capacities of various peels extracts from banana peels ranged from 57.33 – 85.35 %. AL2 (ethyl acetate extract of ambon lumut banana) had the highest ABTS capacity (85.35%), while RB1 peels extract (57.33%) had the lowest ABTS capacity.

Table 1: DPPH scavenging capacities and ABTS scavenging activities of n-hexane peels extracts

Sample	DPPH scavenging capacitiy (%)	ABTS capacity (%)
KT1	50.11 ± 0.66 a	57.33 ± 0.91 a
JB1	51.49 ± 0.38 a	48.35 ± 0.83 b
BW1	51.53 ± 0.76 a	63.07 ± 0.55 c
Ascorbic acid	98.49 ± 0.33	99.27 ± 0.03
P value	< 0.05	< 0.05

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

Table 2: DPPH scavenging capacities and ABTS scavenging activities of ethyl acetate peels extracts

Sample	DPPH scavenging capacitiy (%)	ABTS capacity (%)
KT2	51.78 ± 0.17 a	61.12 ± 0.48 a
JB2	58.84 ± 0.38 b	74.17 ± 0.71 b
BW2	56.38 ± 0.40 c	85.35 ± 0.40 c
Ascorbic acid	98.49 ± 0.33	99.27 ± 0.03
P value	< 0.05	< 0.05

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

Table 3: DPPH scavenging capacities and ABTS scavenging activities of ethanolic peels extracts

Sample	DPPH scavenging capacitiy (%)	ABTS capacity (%)
KT3	51.84 ± 0.30 a	66.17 ± 0.48 a
JB3	60,24 ± 0.29 b	62.56 ± 0.33 b
BW3	54.30 ± 0.71 c	67.88 ± 0.28 c
Ascorbic acid	98.49 ± 0.33	99.27 ± 0.03
P value	< 0.05	< 0.05

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

IC_{50} of DPPH scavenging capacity and IC_{50} of ABTS scavenging capacity

The IC_{50} of DPPH scavenging capacities and IC_{50} of ABTS scavenging activities in various extracts from banana peels using DPPH and ABTS assays were shown in Fig 1 and Fig 2. Both of IC_{50} of DPPH scavenging capacities and IC_{50} of ABTS scavenging activities of each extracts were compared to ascorbic acid as standard. The lowest IC_{50} means had the highest antioxidant capacity.

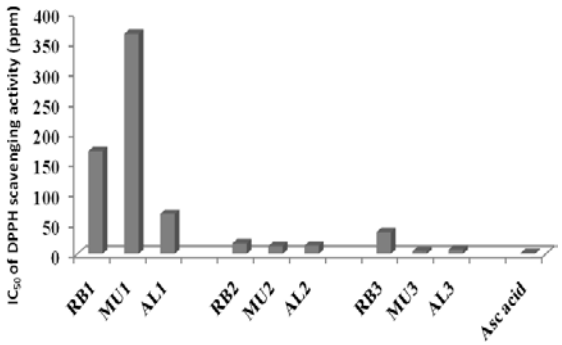

Fig. 1: IC_{50} of DPPH scavenging capacities in various banana peels extracts

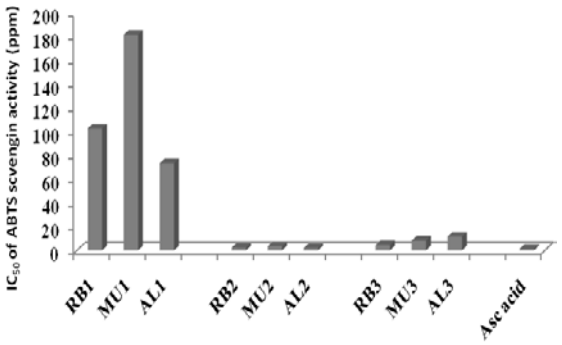

Fig. 2: IC_{50} of ABTS scavenging activities in various banana peels extracts

Total flavonoid in various banana peels extracts

The total flavonoid content among the various extracts were expressed in term of quercetin equivalent using the standard curve equation y = 0.00761355x + 0.00491857, R^2= 0.998. The total flavonoid content in various extracts from banana peels showed different result in the range of 0.55 –10.22 g QE/100 g (Fig 3). RB2 (ethyl acetate peels extract of raja bulu banana) had the highest total flavonoid content (10.22 g QE/100 g) and the lowest (0.55 g QE/100 g) for RB1 peels extract.

Total phenolic in various banana peels extracts

The total phenolic content among the various extracts were expressed in term of gallic acid equivalent using the standard curve equation y = 0.0044 x + 0.031, R^2 = 0.993. The total phenolic content in various extracts from banana peels showed different result ranged from 1.31 to 3.99 g GAE/100 g. MU2 peels extract (ethyl acetate peels extract of muli banana) had the highest phenolic content (3.99 g GAE/100 g) (Fig 4).

Total carotenoid in various banana peels extracts

The total carotenoid content among the various extracts were expressed in term of beta carotene equivalent using the standard curve equation y = 0.02764x - 0.00324857, R^2 = 0.999. The total carotenoid content in various extracts from banana peels showed different result in the range of 0.12 – 0.51 g BET/100 g (Fig 5). The highest carotenoid content (0.51 g BET/100 g) for AL2 peels extract, while the lowest carotenoid (0.12 g BET/100 g) for RB1 peels extract.

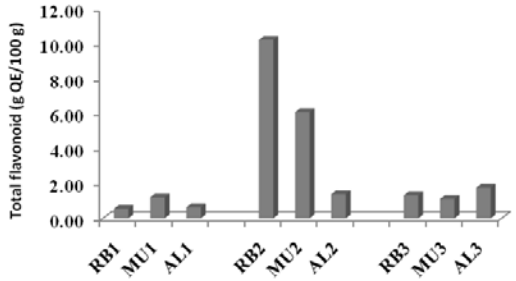

Fig. 3: Total flavonoid content in various banana peels extracts

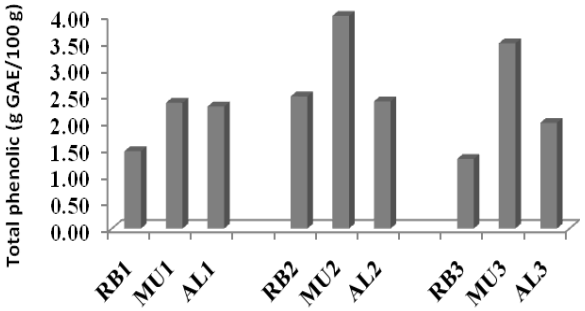

Fig. 4: Total phenolic content in various banana peels extracts

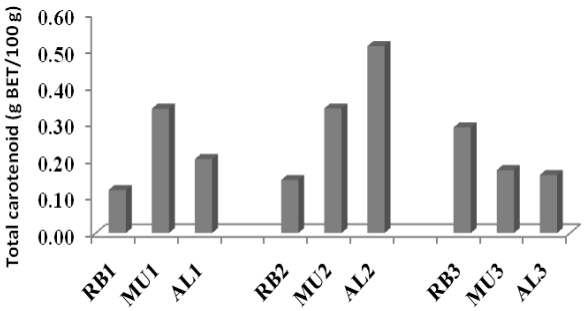

Fig. 5: Total carotenoid content in various banana peels extracts

Correlations between total flavonoid, phenolic, carotenoid content and ABTS, DPPH scavenging activities, in various banana peels extracts

Pearson's correlation coefficient was positively high if $0.68 \leq r \leq 0.97$ [3]. The highly positive correlation between total phenolic content and DPPH scavenging activity (r = 0.740, $p<0.05$) was given by sample MU. The positive and high correlation between carotenoid content and DPPH scavenging activities were given by sample AL (r = 0.716, $p<0.05$) (Table 4).

Table 4: Pearson's correlation coefficient of total flavonoid, phenolic, carotenoid in banana peels extracts and ABTS, DPPH scavenging activities

	Total Flavonoid	Total Phenolic	Total Carotenoid	DPPH RB	DPPH MU	DPPH AL
ABTS RB	-0.150 ns	0.176 ns	0.027 ns	0.108 ns		
ABTS MU	0.1 ns	0.174 ns	0.177 ns		0.808**	
ABTS AL	0.113 ns	0.245 ns	-0.055 ns			0.891**
DPPH RB	0.523 ns	0.063 ns	0.627 ns			
DPPH MU	0.351 ns	0.740*	-0.563 ns			
DPPH AL	0.603ns	0.207 ns	0.716*			

Note: DPPH = DPPH scavenging capacity, ABTS = ABTS scavenging capacity, RB = raja bulu, MU = muli, AL = ambon lumut, ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$

DISCUSSION

Some of tropical plants including banana peels had antioxidant capacity using various antioxidant testing assays [3] [5] [6]. There were no study regarding antioxidant capacity of three various extracts (which were n-hexane, ethyl acetate and ethanol) of banana peels using DPPH and ABTS assays.

Both of ABTS and DPPH are stable free radicals which dissolve in methanol or ethanol, and their colors show characteristic absorption at wavelength 734 nm or 516 nm, respectively. Colors ABTS and DPPH would be changed when the free radicals were scavenged by antioxidant [13] [17].

In the present study, the highest DPPH scavenging capacity was given by sample MU3 (ethanolic peels extract of muli banana), followed by sample MU2 (ethyl acetate peels extract of muli banana) and AL2 (ethyl acetate peels extract of ambon lumut banana). Ethanolic peels extract of raja bulu banana (RB3), muli banana (MU3) and ambon lumut banana (AL3) had DPPH scavenging capacity 51.84 %, 60.24 % and 54.30 % respectively. The previous research by Karuppiah [18] exposed that methanolic leaves extrac of *Musa acuminata, Musa troglodytarum, Musa sapientum and Musa paradisiaca* had antioxidant capacity 50, 20, 30 qnd 110 mg/g extract respectively. Shodehinde [19] studied regarding various treatment in unripe plantain *Musa paradisiaca* and revealed that aqueous extract of boiled treatment

gave DPPH scavenging ability (80 %) higher than raw (untreatment) 70 % and roasted treatment 65 %.

The highest ABTS scavenging capacitiy was given by AL2 (ethyl acetate peels extract of ambon lumut banana), followed by MU2 (ethyl acetate peels extract of muli banana) and AL3 (ethanolic peels extract of ambon lumut banana).

Concentration of sample that could scavenge 50 % free radical (IC_{50}) was used to determine antioxidant capacity of sample compared to standard. The lowest IC50 means had the highest antioxidant capacity. Sample that had IC_{50} < 50 ppm, it was very strong antioxidant, 50-100 ppm strong antioxidant, 101-150 ppm medium antioxidant, while weak antioxidant with IC_{50} > 150 ppm [11].

MU3 (ethanolic peels extract of muli banana) had the lowest IC_{50} of DPPH scavenging activity (4.39 ppm), while ascorbic acid standard gave IC_{50} of DPPH scavenging capacity 1.45 ppm. All of ethyl acetate extracts and ethanolic extracts of banana peels (raja bulu, muli and ambon lumut) had the IC_{50} of DPPH scavenging capacities in the range of 4.39 – 36.12 ppm. Based on classification of antioxidant potency by Blois [11], it could be classified as very strong antioxidant. In the present study expressed that ethanolic peels extract of RB3 (raja bulu banana), MU3 (muli banana) and AL3 (ambon lumut banana) had IC_{50} of DPPH scavenging capacities was 36.12 ppm, 4.39 ppm and 6.91 ppm. The previous study [19] demonstrated that aqueous extract of unripe plantain banana with boiled treatment had IC_{50} of DPPH scavenging capacity 24.76 ppm which was lower than IC_{50} of roasted treatment (31.77 ppm) and raw (untreatment) 33.58 ppm. It was different with EC_{50} of FRAP capacity which showed raw (untreatment) gave EC_{50} FRAP capacity (5.68 ppm) lower than roasted treatment (6.88 ppm) and boiled treatment (9.37 ppm) [19].

Various extracts from banana peels had IC_{50} of ABTS scavenging activities ranged from 1.91 to 182.15 ppm, which all of ethyl acetate extracts and ethanolic extracts of banana peels (raja bulu, muli and ambon lumut) had the IC_{50} of ABTS scavenging capacities ranged from 1.91 to 11.63 ppm, so it could be classified as very strong antioxidant. AL2 (ethyl acetate peels extract of ambon lumut banana) had the lowest IC_{50} of ABTS capacity 1.91 ppm, while ascorbic acid standard gave IC_{50} of ABTS scavenging capacity 1.27 ppm and its exposed that antioxidant capacity of AL2 around a half of potency of ascorbic acid using ABTS method.

The presence of total phenolic might contribute to antioxidant activity [5]. Phenolic acid might contributed in antioxidant activity. Phenyl acetic acid and benzoic acid had lower antioxidant capacity than cinnamic acid [20]. In present study total phenolic of ethanolic peels extract of raja bulu banana, muli banana and ambon lumut banana were 1.31 g GAE/100 g, 3.48 g GAE/100 g and 1.98 g GAE/100 g, respectively. It was similar with research by Karuppiah [18] which exposed that total phenolic in methanolic leaves extract of *Musa acuminata, Musa troglodytarum, Musa sapientum and Musa paradisiaca* were 45 mg GAE/g, 20 mg GAE/g, 30 mg GAE/g and 100 mg GAE/g respectively. The result of the present study were different with previous study [19] which showed that total phenolic in aqueous extract of unripe plantain banana with raw (untreatment), roasted treatment and boiled treatment were 0.94 mg/g, 0.89 mg/g and 0.93 mg/g respectively

Total flavonoid of ethanolic extract in the present study exposed that ambon lumut banana peels had the highest total flavonoid (1.75 g QE/100 g) compared to raja bulu banana (1.31 g QE/100 g) and muli banana (1.11 g QE/100 g). It was different with previous study [19] revealed that total flavonoid in aqueous extract of unripe plantain banana with boiled treatment, roasted treatment and raw (untreatment) were 0.61 mg/g, 0.48 mg/g, 0.71 mg/g respectively.

The data in Table 4 exposed that there were positively high correlation between total phenolic content in muli banana peels sample and antioxidant capacities using DPPH assays. Total phenolic content in muli banana had high and positive correlation with DPPH scavenging capacity that were $r = 0.740$, $p<0.05$. Based on this data it could be concluded that antioxidant capacities in muli banana peels sample by DPPH methods might be estimated indirectly by determining their total phenolic content.

Phenolic acid had the lower antioxidant capacity than flavonoid [20]. Flavonoid would give higher antioxidant capacity if flavonoid 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 glycosides would give lower antioxidant capacity than flavonoid aglycones [20]. Fig 3 showed that total flavonoid in MU2 (ethyl acetate peels extracts of muli banana) was higher (6.08 g QE/100 g) than the MU3 extracts (1.11 g QE/100 g), but DPPH scavenging capacities of MU2 (58.84 %) was lower than MU3 extracts (60.24 %). Based on this data it can predicted that many flavonoids in ethyl acetate peels extract of muli banana were flavonoid that had no OH in ortho C3',4', OH in C3, oxo function in C4, double bond at C2 and C3. In contrast it can demonstrated that MU3 extract contained many flavonoids which had high antioxidant effect.

Total carotenoid content in ambon lumut banana had high and positive correlation with DPPH scavenging activities ($r = 0.716$, $p<0.05$). Based on this data it could be concluded that antioxidant capacities in ambon lumut banana peels sample by DPPH methods might be estimated indirectly by determining their total carotenoid content.

Carotenoid with more double bonds would give higher scavenging free radical capacity [21]. Carotenoid that consisted of maximum 7 double bonds gave lower scavenging radical free capacity than more double bonds [22]. In previous study by Kobayashi and Sakamoto [23] revealed that increasing in lipophilicity of carotenoid would increase scavenging radical capacity. Beta carotene was used as standard because of it had conjugation double bonds due to its ability to scavenge free radicals [24] [25]. Fig 3 revealed that total carotenoid in AL2 peels extract was higher than the other extracts. It was similar with its ABTS scavenging activity, which was higher than the other extracts. Based on the above data, it could be seen that many carotenoid in ethyl acetate peels extract of muli banana were higher than 7 double bonds, which had high antioxidant capacity.

ABTS and DPPH methods had the same mechanism reaction that was electron transfer assays [26], but the results of the present study showed that ABTS scavenging capacity not always linear with DPPH scavenging activity. The Pearson's correlation coefficient of various extracts from banana peels sample indicated that DPPH scavenging capacities of muli banana and ambon lumut banana had positive and high correlation with their ABTS scavenging activities. It could be seen that antioxidant capacities in muli and ambon lumut banana peels sample by DPPH assays were linear with ABTS assays.

CONCLUSION

To assess the antioxidant capacity of sample, variety of methods must be used in parallel, because different methods often give different results. The ethyl acetate peel extracts and ethanol extracts of all of banana samples can be classified as very strong antioxidant. Total phenolic content in muli banana peels sample had positively high correlation with DPPH scavenging activities. Antioxidant capacity by DPPH assays in muli banana peels sample might be estimated indirectly by using total phenolic content. Phenolic compounds were the major contributor in antioxidant capacity in muli banana peels sample. Muli and ambon lumut banana peels showed linear correlation between DPPH and ABTS scavenging activities. Ethyl acetate and ethanolic peels extract of raja bulu banana, muli banana and ambon lumut banana may be exploited as natural antioxidant in food applications as well as for health supplements to alleviate oxidative stress.

CONFLICT OF INTERESTS

Declared None

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