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Research Article

In-vitro Antioxidant Activities from Three Organs of White Ambon Banana (Musa AAA Group) and Flavonoid, Phenolic, Carotenoid Content

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ABSTRACT

The objectives of this research were to study antioxidant capacity from various organs extracts of white ambon banana using two methods of antioxidant testing which were DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) and correlation of total phenolic, flavonoid and carotenoid content in various organs of white ambon banana with IC_{50} of DPPH and IC_{50} of ABTS antioxidant activities. Extraction was performed by reflux using different polarity solvents. The extracts were evaporated using rotary evaporator. Antioxidant capacities using DPPH and ABTS assays, determination of total phenolic, flavonoid and carotenoid content were performed by spectrophotometry UV-visible and its correlation with IC_{50} of DPPH and IC_{50} of ABTS scavenging capacities were analyzed by Pearson's method. Ethanolic peel extract of white ambon banana (PL3) had the lowest IC_{50} of DPPH scavenging activity 0.39 µg/ml and the lowest IC_{50} of DPPH scavenging activity 0.36 µg/ml. Ethanolic peel extract of white ambon banana (PL3) had the highest total phenolic (29.28 g GAE/100 g), ethyl acetate leaves extract of white ambon banana (LE2) gave the highest flavonoid content (13 g QE/100 g) and ethyl acetate peel extract of white ambon banana (PL2) showed the highest total carotenoid 22.88 g BE/100 g. There were negatively and high correlation between total phenolic content in leaves and peduncle extracts with their IC_{50} of DPPH scavenging activities and IC_{50} of ABTS scavenging activities. All of leaves, peduncle and peel extracts of white ambon banana had linear result in DPPH and ABTS assays.

Keywords: Antioxidant, DPPH, ABTS, organs, white ambon banana, phenolic, flavonoid, carotenoid

INTRODUCTION

Many diseases have correlation with oxidative stress, which can be reduced by antioxidant. Phenolic compounds are commonly found in plants, and they have been expressed to have multiple biological effects, including antioxidant activity[1-3]. Previous research[4-6] demonstrated that phenolic and flavonoid content could be correlated to their antioxidant activities. Plants including banana contained phenolic and polyphenol compounds which can act as antioxidant[7-9]. Some of antioxidant methods such as DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) and FRAP (Ferric Reducing Antioxidant Power) were used to predict antioxidant activity of vegetables, fruits and food[3,9-14]. In previous study[2-3,9] reported that DPPH, ABTS and FRAP methods could be used to determine antioxidant activity in many plants extracts. The previous study[2,15-16] exposed that banana had antioxidant activities by using DPPH, FRAP and ABTS assays. The objective of this research were to study antioxidant activities of different polarities extracts (n-hexane, ethyl acetate and ethanol) of three organs (leaves, peduncle, peel) of white ambon banana (Musa AAA Group) using antioxidant testing DPPH and ABTS assays and correlations of their antioxidant activities with total phenolic, flavonoid and carotenoid content.

MATERIALS AND METHODS

Materials

ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt), DPPH (2,2-diphenyl-1-picrylhydrazyl), gallic acid, quercetin, beta carotene was purchased from Sigma-Aldrich (MO, USA), three organs of white ambon banana, ethanol. All other reagents were analytical grades.

Preparation of sample

Three organs of white ambon banana (Musa AAA group) were: leaves namely as LE, peduncle namely as PD, peel as sample PL were collected from Cipatik-West Bandung, West Java, 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

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extracted three times with ethanol. So totally there were nine extracts: three n-hexane extracts (namely LE1, PD1 and PL1), three ethyl acetate extracts (LE2, PD2 and PL2) and three ethanolic extracts (LE3, PD3 and PL3).

IC_{50} of DPPH scavenging activity

Preparation of DPPH solution was adopted from Blois[17] with minor modification. Various concentration of each extract were pipetted into DPPH solution 50 µg/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 UV-Vis Hewlett Packard 8435. Methanol was used as a blank. DPPH 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 activity of each extract was determined based on the reduction of DPPH absorbance by calculating percentage of antioxidant activity[18]. IC_{50} of DPPH scavenging activity of each extract can be calculated using its calibration curve.

IC_{50} of ABTS scavenging activity

Preparation of ABTS solution was adopted from Li *et al*.[19] 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 allowing to stand in the dark room for 12 hours. The two solutions were mixed with 30 minutes incubation, allowing to stand in refrigerator for 24 hours, then diluted in ethanol. Various concentration of each extract were pipetted into ABTS solution 50 µg/ml (1:1) to initiate the reaction for obtaining a calibration curve. The absorbance was read at wavelength 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 capacity of each extract was determined based on the reduction of ABTS absorbance by calculating percentage of antioxidant activity[18]. IC_{50} of ABTS scavenging activity of each extract can be calculated using its calibration curve.

Determination of total phenolic content (TPC)

Total phenolic content were measured using the modified Folin-Ciolcalteu method adapted from Pourmorad[20]. The absorbance was read at wavelength 765 nm. Analysis was done in triplicate for each extract. Standard solution of gallic acid with concentration 40-165 µg/ml were used to obtain a standard curve. The total phenolic content was reported as percentage of total gallic acid equivalent per 100 g extract (g GAE /100 g).

Determination of total flavonoid content (TFC)

Total flavonoid content was measured using adapted method from Chang *et al* [21]. The absorbance was read at wavelength 415 nm. Analysis was done in triplicate for each extract. Standard solution of quercetin with concentration 36-120 µg/ml were used to obtain a standard curve. The total flavonoid content was reported as percentage of total quercetin equivalent per 100 g extract (g QE/100 g).

Determination of total carotenoid content (TCC)

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 solution of beta carotene with concentration 15-55 µg/ml were used to obtain a standard curve. The total carotenoid content was reported as percentage of total beta carotene equivalent per 100 g extract (g BE/100 g).

Statistical Analysis

Each sample analysis was performed in triplicate. All results presented are means (±SD) of at least three independent experiments. Statistical analysis (ANOVA with a statistical significance level set at $p < 0.05$ with post-hoc Tukey procedure was carried out with SPSS 16 for Windows. Correlations between the total phenolic, flavonoid and total carotenoid content and antioxidant capacities were made using the Pearson procedure ($p < 0.01$).

RESULTS

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

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

TPC in various organs extracts from white ambon banana

TPC among the various organs extracts were exposed in term of gallic acid equivalent using the standard curve equation $y = 0.004 x + 0.0025$, $R^2 = 0.998$. The TPC in various organ extracts from white ambon banana exposed different result ranged from 1.89 to 29.28 g GAE/100 g. Ethanolic peel extract of white ambon banana (PL3) had the highest phenolic content (29.28 g GAE/100 g) (Fig 3) and the lowest was showed by n-hexane peel extract of white ambon banana (PL1).

TFC in various organs extracts of white ambon banana

TFC among the various organs extracts were revealed in term of quercetin equivalent using the standard curve equation $y = 0.006 x - 0.0191$, $R^2 = 0.998$. The TFC in various organ extracts from white ambon banana demonstrated different result in the range of 1.45 – 13 g QE/100 g (Fig 4). Ethyl acetate leaves extract of white ambon banana (LE2) had the highest total flavonoid content (13 g QE/100 g).

TCC in various organs extracts of white ambon banana

TCC among the various organs extracts were expressed in term of beta carotene equivalent using the standard curve equation $y = 0.015x + 0.002$, $R^2 = 0.9999$. The TCC in various organ extracts from white ambon banana showed different result in the range of 0.31 – 22.88 g BET/100 g (Fig 5). The highest carotenoid content (22.88 g BE/100 g) was given by ethyl acetate peel extract of white ambon banana (PE2), while the lowest carotenoid (0.31 g

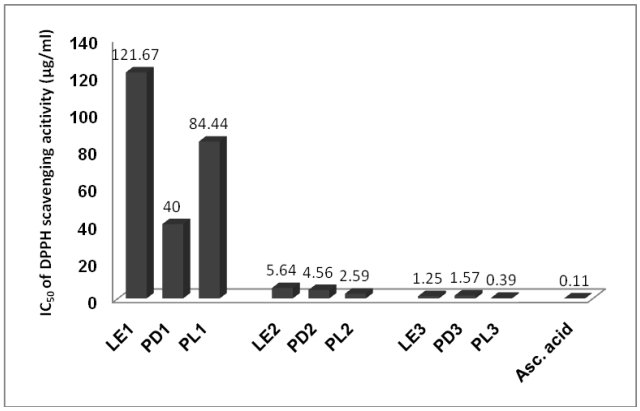

Fig 1: IC_{50} of DPPH scavenging activities in various organs extracts white ambon banana

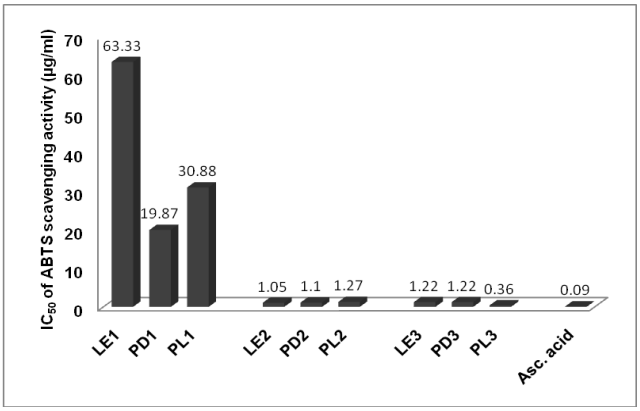

Fig 2: IC_{50} of ABTS scavenging activities in various organs extracts white ambon banana

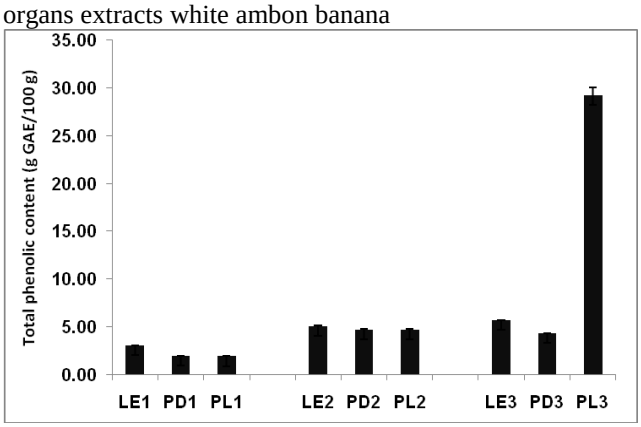

Fig 3: Total phenolic content in various organs extracts of white ambon banana

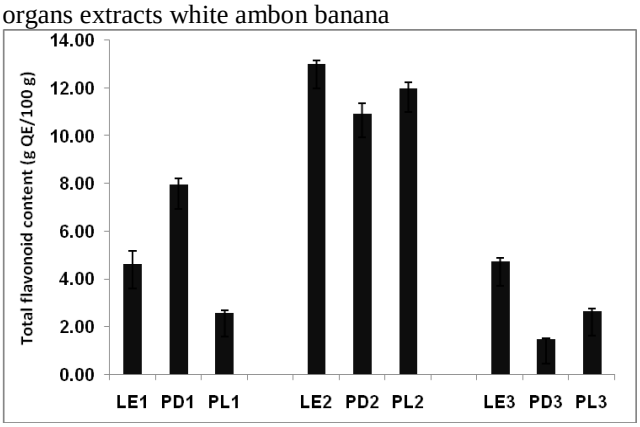

Fig 4: Total flavonoid content in various organs extracts of white ambon banana

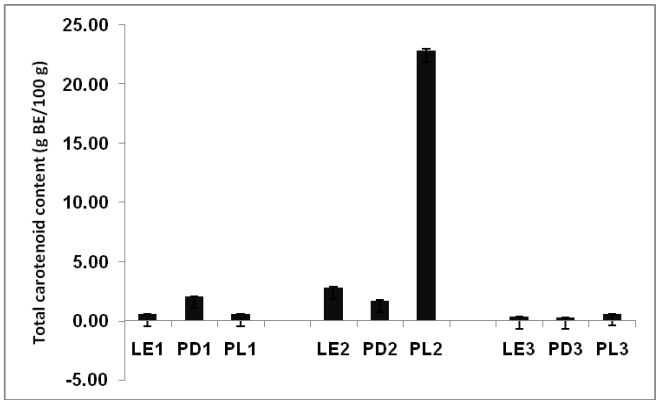

Fig 5: Total carotenoid content in various organs extracts of white ambon banana

BE/100 g) for ethanolic peduncle extract of white ambon banana (PD3).

Correlations between IC_{50} of DPPH scavenging activities, IC_{50} of ABTS scavenging activities, total phenolic, flavonoid and carotenoid content in various organs extracts of white ambon banana

Pearson's correlation coefficient between TPC in various organs extracts of white ambon banana and their antioxidant activities exhibited that TPC in leaves and peduncle extracts had negative and high significant correlation with IC_{50} of DPPH scavenging activities (r = -0.975; r = -0.979, $p<0.01$, respectively) and IC_{50} of ABTS scavenging activities (r = -0.966; r = -0.985, $p<0.01$, respectively). TFC in all of leaves, peduncle and peel extracts had no significant correlation with their IC_{50} of DPPH scavenging activities and IC_{50} of ABTS scavenging activities (Table 1).

DISCUSSION

The previous research[1-2,16,22-24] reported that banana had antioxidant capacity. There were no study regarding antioxidant activity of various extracts (which were n-hexane, ethyl acetate and ethanol) of different parts of white ambon banana using DPPH and ABTS assays.

DPPH and ABTS free radicals which dissolve in methanol or ethanol, and their colors have characteristic absorption at wavelength 516 nm or 734 nm, respectively. Colors of DPPH and ABTS would be changed when the free radicals were scavenged by antioxidant[19].

The IC_{50} of DPPH scavenging activities and IC_{50} of ABTS scavenging activities in various organs extracts of

Table 1. Pearson's correlation coefficient of IC_{50} of DPPH scavenging activities, IC_{50} of ABTS scavenging activities and total phenolic, flavonoid, carotenoid content in various organs extracts of white ambon banana

	TPC	TFC	TCC	IC_{50} DPPH LE	IC_{50} DPPH PD	IC_{50} DPPH PL
IC_{50} DPPH LE	-0.975**	-0.478	-0.411			
IC_{50} DPPH PD	-0.979**	0.278	0.701*			
IC_{50} DPPH PL	-0.596	-0.485	-0.482			
IC_{50} ABTS LE	-0.966**	-0,507	-0.442	0.999**		
IC_{50} ABTS PD	-0.985**	0.199	0.643		0.988**	
IC_{50} ABTS PL	-0.597	-0.48	-0.477			0.998**

Note: IC_{50} DPPH = IC_{50} DPPH scavenging capacity, IC_{50} ABTS = IC_{50} ABTS scavenging capacity, LE = leaves extract, PD = peduncle extract, PL = peel extract, ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$

white ambon banana using DPPH and ABTS assays were shown in Fig 1 and Fig 2. The IC_{50} of DPPH scavenging activities and IC_{50} of ABTS scavenging activities in various organs extracts compared to IC_{50} of ascorbic acid standard. The lowest IC_{50} means had the highest antioxidant capacity. IC_{50} were used to determine antioxidant capacity of sample was compared to standard. Sample which had IC_{50} or EC_{50} lower than 50 µg/ml was a very strong antioxidant, 50-100 µg/ml was a strong antioxidant, 101-150 µg/ml was a medium antioxidant, while a weak antioxidant with IC_{50} or EC_{50} greater than 150 µg/ml [17].

In the present study demonstrated that IC_{50} of DPPH scavenging activities of various organs extracts from white ambon banana ranged from 0.39 to 121.67 µg/ml and ethanolic peel extract of white ambon banana (PL3) had the lowest IC_{50} of DPPH scavenging capacity 0.39 µg/ml and ascorbic acid standard 0.11 µg/ml. Based on value of IC_{50} of DPPH scavenging capacity it can be concluded that all of ethyl acetate and ethanolic organs extracts of white ambon banana (leaves, peduncle and peel) can be classified as very strong antioxidant. It revealed that potency of ascorbic acid was around three times potency of ethanolic peel extract of white ambon banana (PL3) using DPPH method. Ethanolic peel extract of white ambon banana (PL3) had the lowest IC_{50} of ABTS scavenging activities (0.36 µg/ml) while ascorbic acid standard showed IC_{50} of ABTS scavenging activity 0.09 µg/ml. It expressed that potency of ascorbic acid was around forty times of potency of PL3 using ABTS assay.

In the present study revealed that ethanolic leaves, peduncle and peel extract of white ambon banana were 1.25, 1.57, 0.37 µg/ml, respectively, while in the previous research[16] reported that ethanolic peel extracts of raja bulu banana, muli banana and ambon lumut banana had IC_{50} of DPPH scavenging capacities were 36.12, 4.39 and 6.91 µg/ml, respectively. Study by Pongoodi[23] expressed that ethanolic fruit extract of nendran variety had the lowest IC_{50} of DPPH scavenging activities (0.9 mg/ml) compared to other varieties (kadali, karpooravalli, monthan, poovan, pachainadan, rasthali, robusta and sevvazhai), while in ABTS method gave different result which exposed that ethanolic fruit extract of robusta variety had the lowest IC_{50} of DPPH (1 mg/ml). Based on the value of IC_{50} of DPPH and ABTS, all of fruit extracts sample can be categorized as weak antioxidant because of their value of IC_{50} of DPPH and ABTS greater than 150 µg/ml. Sahaa[15] stated that methanolic leaves extract of *M. sapientum* var. sylvesteris which was extracted by cold extraction method gave IC_{50} of DPPH scavenging activity 39 µg/ml. It was contrast with the present study which reported that IC_{50} of DPPH scavenging activity of ethanolic leaves extract of white ambon banana was 1.25 µg/ml. The previous research by Karuppiah[25] exposed that methanolic leaves extrac of *M. acuminata*, *M. troglodytarum*, *M. sapientum* and *M. paradisiaca* had antioxidant capacity 50, 20, 30 qnd 110 mg/g extract respectively. Study by Shodehinde[22] expressed that aqueous extract of unripe plantain banana with raw (untreatment) had IC_{50} of DPPH 33.58 µg/ml was greater than IC_{50} of roasted treatment (31.77 µg/ml) and boiled treatment 24.76 µg/ml. It was different with EC_{50} of FRAP capacity which showed that raw (untreatment) gave EC_{50} FRAP capacity (5.68 µg/ml) lower than roasted treatment (6.88 µg/ml) and boiled treatment (9.37 µg/ml)[22]. Previous study by Baskar[1] showed that total antioxidant activity of ethanolic peel extract of pachainadan variety (5.85 mM AAE/g) was higher than kadali, kadali, karpooravalli, monthan, poovan, nendran, rasthali, robusta and sevvazhai varieties. Darsini' research[2] exposed that methanolic fruit extract of awak banana had IC_{50} of DPPH 65 µg/ml and IC_{50} of ABTS scavenging activity 29 µg/ml. The previous research[24] demonstrated that percentage of DPPH scavenging activities of chloroform, ethyl acetate and water extract of green peel of Cavendish banana were 26.2, 52.1, 75.3 %, respectively, which were greater than their yellow peel (8.7, 43.7, 9.8 %, respectively).

Antioxidant capacity might be related with the presence of total phenolic content, included phenolic acid[8]. Benzoic acid and phenyl acetic acid had lower antioxidant activity than cinnamic acid[26]. In previous research[15] stated that TPC and TFC in methanolic leaves extract of *M. sapientum* var. sylvesteris were 0.092 g GAE/100 g and 28.75 g RE/100 g. It was different with the present study which revealed that TPC and TFC in ethanolic leaves extract of white ambon banana were 5.68 g GAE/100 g and 4.72 g QE/100 g respectively. The previous study[2] found that TPC and TFC in methanolic fruit extract of awak banana were 0.12 g GAE/100 g and 0.44 g/100 g, respectively, and Bashkar[1] exposed that TPC in ethanolic peel extract of rauthali variety (0.06 g CE/100 g) was higher than other varieties, and TFC in ethanolic peel extract of poovan variety (2.2 g RE/100 g)

was higher than other varieties. It was contrast with the present study which demonstrated that TPC and TFC in ethanolic peel extract of white ambon banana were 29.28 g GAE/100 g and 2.63 g QE/100 g, respectively. In the previous study[16] revealed that ethyl acetate peel extract of muli variety had the highest TPC (3.99 g GAE/100 g) compared to n-hexane and ethanolic peel extract of three varieties of banana, and ethyl acetate peel extract of raja bulu variety gave the highest TFC (10.22 g QE/100 g) compared to n-hexane and ethanolic extract of three varieties. Previous research[25] reported that total TPC in methanolic leaves extract of *M. troglodytarum, M. acuminata, M. paradisiaca* and *M. sapientum* were 2, 4.5, 10 and 3 g GAE/100 g respectively, while Shodehinde[22] stated that TPC and TFC in aqueous extract of unripe plantain banana with raw (untreatment), roasted treatment and boiled treatment were 94, 89, 93 mg GAE/100 g, respectively and 71, 48, 61 mg QE/100 g, repectively.

Pearson's correlation coefficient was positively high if $0.61 \leq r \leq 0.97$[3] and negatively high if $-0.61 \leq r \leq -0.97$. Sample which had the lowest IC_{50} of DPPH and IC_{50} of ABTS scavenging activity had the highest antioxidant activity. So the good correlation between IC_{50} DPPH or IC_{50} of ABTS with TPC, TFC and TCC will be given in negatively and high correlation. It means increasing in TFC, TPC and TCC caused increasing in antioxidant activities, which was expressed by lower IC_{50} of DPPH scavenging activity and or IC_{50} of ABTS scavenging activity.

The data in Table 1 exhibited that TPC in peduncle extract had the highest and negatively correlation with IC_{50} of DPPH scavenging activities ($r = -0.979$, $p<0.01$) and IC_{50} of ABTS scavenging activities ($r = -0.985$, $p<0.01$), while TPC in peel extract had no correlation with IC_{50} of DPPH and IC_{50} of ABTS scavenging activities. It was similar with the previous study[16] which reported that peel extracts of three varieties banana had no correlation with their percentage of DPPH and ABTS scavenging activities (except muli variety). Research by Darsini[2] exposed different result which showed that TPC and TFC in methanolic fruit extract of awak banana had positive and high correlation with their percentage of DPPH and ABTS scavenging activities. In the present study exposed that TCC in leaves and peel extracts of white ambon banana had no correlation with their IC_{50} of DPPH and IC_{50} of ABTS scavenging activities, but the previous study[16] revealed that TCC in peel extracts of ambon lumut had positive and high correlation with their percentage of DPPH scavenging activities. Flavonoid, phenolic acid, tannins, qoumarine and quinone were included in phenolic compound. Flavonoid which have OH in A ring and or B ring will be included in phenolic groups. Flavonoid had greater antioxidant activity than phenolic acid[26]. Flavonoid which had OH in ortho C 3',4', OH in C3, oxo function in C4, double bond at C2 and C3 would give higher antioxidant activity. The OH with ortho position in C3'-C4' had the highest influence to antioxidant activity of flavonoid. The flavonoid aglycones would give higher antioxidant activity than flavonoid glycosides[26]. It could be seen in Fig 4 that TFC in n-hexane leaves extract of white ambon banana (LE1) 4.61 g QE/100 g was similar with TFC in ethanolic leaves extract of white ambon banana (LE3) 4.72 g QE/100 g, but IC_{50} of DPPH scavenging activity of LE3 (1.25 µg/ml) was not similar with LE1 (121.67 µg/ml). Based on this data it can be supposed that many flavonoids in LE1 had OH in in C5, C7, or C3' only, or C4' only, or C3 only without oxo function in C4, that had no and low influence in antioxidant activities. In contrast, almost all of flavonoid in LE3 were flavonoid that had OH in position which can influence high antioxidant capacities. TPC in n-hexane peduncle extract (PD1) 1.90 g GAE/100 g was similar with n-hexane peel extracts (PE1) 1.89 g GAE/100 g, but IC_{50} of DPPH scavenging activity of PD1 was 40 µg/ml and classified as very strong antioxidant activity, while IC_{50} of DPPH scavenging activity of PL1 was 121.67 µg/ml medium antioxidant. It might be predicted that many phenolic compounds in PD1 which had influence high antioxidant capacities, on other hand in PL1 had low antioxidant activities.

Carotenoid had antioxidant capacity by scavenging free radical. Higher scavenging free radical capacity will be given by more double bonds in carotenoid[27]. Carotenoid which contained more than 7 double bonds gave higher scavenging radical capacity[28]. Beta carotene was used as standard because of it had conjugation double bonds which had ability to scavenge free radicals[29]. In previous study[30] exposed that decreasing in lipophilicity of carotenoid would decrease scavenging radical capacity, it means give the greater IC_{50} of DPPH scavenging capacity. TCC in n-hexane leaves extract of white ambon banana (LE1) 0.53 g BE/100 g was similar with TCC in ethanolic peel extract of white ambon banana (PL3) 0.6 g BE/100 g, but IC_{50} of DPPH and IC_{50} of ABTS scavenging activities of PL3 (0.39 and 0.36 µg/ml, respectively) were lower than IC_{50} of DPPH and IC_{50} of ABTS of LE1 (121.67 and 63.33 µg/ml, respectively). It can be supposed that many carotenoid in LE1 contained maximum 7 double bonds and many carotenoid in PL3 consisted of more than 7 double bonds which had high antioxidant activities.

ABTS and DPPH methods had the same mechanism reactions, that was electron transfer assays[31]. All of leaves, peduncle and peel extracts of white ambon banana expressed positively high correlation between IC_{50} of DPPH scavenging activities and IC_{50} of ABTS scavenging activities. So the results of this study showed that IC_{50} of DPPH scavenging activities in all of extracts sample were linear with their IC_{50} of ABTS scavenging activities.

CONCLUSION

Antioxidant capacity of sample should be determined by different methods in parallel, because variety of methods could give different results. Ethyl acetate and ethanolic extracts of leaves, peduncle and peel of white ambon banana were very strong antioxidant, using DPPH and ABTS assays. The negatively and high correlation between TPC and IC_{50} of DPPH, IC_{50} of ABTS scavenging activities were given by leaves and peduncle

extracts of white ambon banana. Phenolic compounds in leaves and peduncle extracts were the major contributor in IC_{50} of DPPH scavenging activity and IC_{50} of ABTS scavenging activity. There were linear correlation between IC_{50} of DPPH scavenging activities and IC_{50} of ABTS scavenging activities of all of extracts sample. Leaves, peduncle and peel extracts of white ambon banana may be exploited as natural antioxidant sources to alleviate oxidative stress.

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