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Antioxidant properties of peels extracts from three varieties of banana (*Musa* sp.) grown in West Java-Indonesia

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Abstract

The aims of this research were to determine antioxidant activity from different polarity peel extracts of three varieties of banana using two methods of antioxidant testing which were DPPH (2,2-diphenyl-1-picrylhydrazyl) and CUPRAC (Cupric Reducing Antioxidant Capacity) and correlation of total phenolic, flavonoid and carotenoid content in different polarity extracts of banana peels with their IC_{50} of DPPH and IC_{50} of CUPRAC antioxidant activities. Extraction was conducted by reflux using different polarity solvents. The extracts were evaporated using rotary evaporator. Antioxidant activities, determination of total phenolic, flavonoid and carotenoid content were performed by UV-visible spectrophotometry and its correlation with IC_{50} of DPPH scavenging activities and EC_{50} of CUPRAC capacities were analyzed by Pearson's method. All of peel extracts banana and nangka banana (except ethanolic peel extract of nangka banana) were categorized as very strong antioxidant, using DPPH assay. Phenolic compounds in tanduk banana and nangka banana peel extracts were the major contributor in their antioxidant activities by DPPH and CUPRAC assays. Peel extracts of nangka banana gave linear results in DPPH and CUPRAC assays.

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Introduction

Phenolic compounds are commonly found in plants, and they have been reported to have multiple biological effects, included antibacterial and antioxidant activity (Mokbel and Hashinaga, 2005; Fawole *et al*. 2012). Previous study (Souri *et al*., 2008; Xu and Chang, 2008; Zielinski *et al*., 2014 Butsat and Siriamornpun, 2016) figured that phenolic and flavonoid content could be correlated to their antioxidant activities. Consumption of antioxidant can prevent oxidative stress which can cause many diseases. Plants include guava, tea, coffee, banana, papaya contain phenolic and flavonoid compounds (Castillo *et al*., 2002; Thaipong *et al*., 2006; Chan *et al*., 2007; Darsini *et al*., 2012; Maisarah *et al*., 2013). Peel of banana contain naringin (flavanone glycoside) and rutin (flavonol glycoside) (Kanazawa and Sakakibara, 2000) and lutein, beta-carotene, alpha-carotene, violaxanthin, auroxanthin, neoxanthin, isolutein, beta-cryptoxanthin and alpha-cryptoxanthin (Subagio *et al*., 1996).

Previous researches (Thaipong *et al*., 2006; Apak *et al*., 2007; Fidrianny *et al*., 2013) expressed that DPPH, CUPRAC, FRAP and ABTS methods could be used to determine antioxidant activity in many plants extracts. Studies by Sulaiman *et al*. (2011) and Darsini *et al*. (2012) stated that banana had antioxidant activities by using DPPH, ABTS and FRAP assays.

The goals of this research were to determine antioxidant activities of various polarity peel extracts (n-hexane, ethyl acetate and ethanol) of three varieties of banana (*Musa* sp.) which grown in West Java-Indonesia using DPPH and CUPRAC assays, and correlations between total phenolic, flavonoid and carotenoid content with their antioxidant activities.

Materials and Methods

Materials

DPPH (2,2-diphenyl-1-picrylhydrazyl) , neocuproine, gallic acid, quercetin, beta carotene were purchased from Sigma-Aldrich (MO, USA), cupric chloride, banana peels. All other reagents were analytical grades.

Preparation of sample

Peels of three varieties of banana (*Musa* sp.) which were: tanduk banana namely as TAN collected from Bandung- West Java, nangka banana as NAN and kepok banana as KEP from Cimahi-West Java, were thoroughly washed with tap water, sorted while wet, cut, dried and grinded into powder.

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Extraction

Three hundred gram of powdered sample was extracted by reflux using various polarity solvents. Extraction using n-hexane was repeated three times. The remaining residue was then extracted three times by using ethyl acetate. Finally the remaining residue was extracted three times using ethanol. Therefore totally nine extracts there were: three n-hexane extracts (namely TAN1, NAN1 and KEP1), three ethyl acetate extracts (TAN2, NAN2 and KEP2) and three ethanolic extracts (TAN3, NAN3 and KEP3).

Inhibitory Concentration 50 (IC_{50}) of DPPH scavenging activity

Preparation of DPPH solution was performed using Blois's method (Blois, 1958) with minor modification. Various concentrations of each extract were pipetted into DPPH solution 50 µg/ml (volume 1:1) to initiate the reaction for obtaining a calibration curve. The absorbance was read after 30 minutes incubation at wavelength 515 nm by using UV-Vis spectrophotometer Beckman Coulter DU 720. Methanol was used as a blank, DPPH solution 50 µg/ml as control and ascorbic acid as standard. Analysis was carried out in triplicate for standard and each extract. Antioxidant activity of each extract by DPPH method was determined by calculating percentage of antioxidant activity using reduction of DPPH absorbance (Bedawey, 2010). IC_{50} of DPPH scavenging activity of each extract can be calculated using its calibration curve.

Exhibitory concentration 50 (IC_{50}) of CUPRAC capacity

Preparation of CUPRAC solution was adopted from Apak's method (Apak *et al*., 2007). The CUPRAC solution was prepared in ammonium acetate buffer pH 7. Each extract were prepared in various concentrations and pipetted into CUPRAC 50 µg/ml (1:1) to initiate the reaction for obtaining a calibration curve. After 30 minutes incubation, the absorbance was read at wavelength 450 nm by using UV-Vis spectrophotometer Beckman Coulter DU 720. Ammonium acetate buffer was used as a blank, CUPRAC solution 50 µg/ml as control and ascorbic acid as standard. Analysis was done in triplicate for standard and each extract. Antioxidant capacity of each extract was evaluated based on increasing in Cu (I)-neocuproine absorbance by calculating percentage of antioxidant capacity (Apak *et al*., 2007). EC_{50} of CUPRAC capacity of each extract can be calculated using its calibration curve.

Total flavonoid content (TFC)

Determination of total flavonoid content was conducted using method from Chang *et al*. (2002). The absorbance was read at wavelength 415 nm. Analysis was done in triplicate for each extract. Quercetin standard solution (36-100 µg/ml) was used to obtain a calibration curve. The total flavonoid content was exposed as percentage of total quercetin equivalent per 100 g extract (g QE/100 g).

Total phenolic content (TPC)

Total phenolic content evaluation was performed using Folin-Ciolcalteu reagent (Pourmorad *et al*., 2006) with minor modification. The absorbance was measured at wavelength 765 nm. Analysis was done in triplicate for each extract. Gallic acid standard solution (105-200 µg/ml) was used to obtain a calibration curve. Total phenolic content was presented as percentage of total gallic acid equivalent per 100 g extract (g GAE /100 g).

Total carotenoid content (TCC)

Determination of total carotenoid content was measured using modified method which was adapted from Thaipong *et al*. (2006). Each extract was diluted in n-hexane (Fidrianny *et al*., 2013). The absorbance was read at wavelength 470 nm. Analysis was conducted in triplicate for each extract. Beta carotene standard solution (30-100 µg/ml) was used to obtain a calibration 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 (± standard deviation) of at least three independent experiments. Statistical analysis using ANOVA with a statistical significance level set at $p < 0.05$ and post-hoc Tukey procedure was carried out with SPSS 16 for Windows. Correlation between the total phenolic, flavonoid, carotenoid content and antioxidant activities, and correlation between two antioxidant activity methods were performed using the Pearson's method.

Results and Discussion

Banana had antioxidant activity which was revealed in previous studies by Sulaiman *et al*. (2011) and Darsini *et al*. (2012). There was no research regarding antioxidant activity of various polarity extracts (which were n-hexane, ethyl acetate and ethanol) from peel of three varieties of bananas grown in West Java- Indonesia using DPPH and CUPRAC assays.

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

The IC_{50} of DPPH scavenging activities and EC_{50} of CUPRAC capacities in various extracts of banana peels using DPPH and CUPRAC assays were shown in Figure 1 and Figure 2. IC_{50} of DPPH scavenging activity and EC_{50} of CUPRAC capacity of each extract were compared to IC_{50} and EC_{50} of ascorbic acid as standard. The lowest IC_{50} and EC_{50} value means had the highest antioxidant activity. The lowest IC_{50} means gave the highest antioxidant activity. 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 (Blois, 1958).

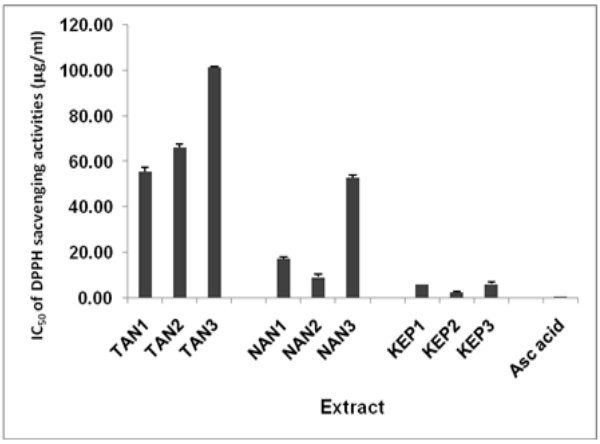

Figure 1. IC_{50} of DPPH scavenging activities in banana peels extracts

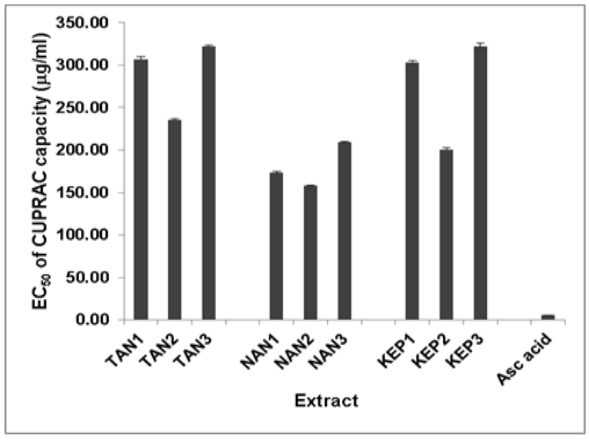

Figure 2. EC_{50} of CUPRAC capacities in banana peels extracts

The DPPH is stable free radicals which dissolve in methanol or ethanol, and its colors show characteristic absorption at wavelength 515-520 nm, respectively. Colors of DPPH would be changed when the free radicals were scavenged by antioxidant (Li *et al*., 2011), from purple color to yellow color. Reagent of CUPRAC is $CuCl_2$ which is combined with neocuproine in ammonium acetate buffer pH 7. Cu (II) will be reduced to Cu (I). Complex Cu (I) – neocuproine gives yellow color and show characteristic absorption at wavelength 450 nm (Apak *et al*., 2007). Intensity of yellow color depends on amount of Cu (II) that is reduced to Cu (I). If a sample reduces Cu (II) to Cu (I), at the same time it will be oxidized, therefore sample can act as antioxidant. Sample will act as antioxidant in CUPRAC assay if sample had reduction potential lower than reduction potential of Cu (II)/Cu (I) which is 0.159 V.

Previous research (Pongoodi *et al*., 2012) reported that the lowest IC_{50} of DPPH scavenging activities (0.9 mg/ml) was given by ethanolic fruit extract of nendran banana compared to other varieties (kadali, karpooravalli, monthan, poovan, pachainadan, rasthali, robusta and sevvazhai), while in ABTS method gave different results which exposed that ethanolic fruit extract of robusta banana had the lowest IC_{50} of ABTS (1 mg/ml), so all of fruit extracts from nine varieties of banana can be categorized as weak antioxidant, because their IC_{50} of DPPH and ABTS greater than 150 µg/ml. Study by Shodehinde *et al*. (2013) figured that aqueous extract of without treatment of unripe plantain banana had IC_{50} of DPPH 33.58 µg/ml which was greater than the roasted treatment (31.77 µg/ml) and boiled treatment 24.76 µg/ml. It was contrary with EC_{50} of FRAP capacity which revealed that EC_{50} FRAP capacity of the boiled treatment (9.37 µg/ml) was higher than roasted treatment (6.88 µg/ml) and without treatment (5.68 µg/ml). Schmidt *et al*. (2015) stated that banana inflorescence (*Musa cavendishii*) which was extracted by stirring using 50% ethanol with temperature 60°C for 30 minutes gave the highest antioxidant activities which showed the lowest IC_{50} of DPPH scavenging activity (0.31 mg/ml) and the lowest EC_{50} of FRAP capacity (29.62 mmol/kg ET) compared to other extractions with different in extraction method, percentage of ethanol solvent, temperature and time. The previous study (Mokbel and Hashinaga, 2005) exposed that percentage of DPPH scavenging activities of chloroform, ethyl acetate and water extracts of Cavendish banana which have green peel were 26.2, 52.1, 75.3%, respectively, which were greater than their yellow peel (8.7, 43.7, 9.8%, respectively). Sulaiman *et al*. (2011) which studied regarding antioxidant activities from 8 cultivars of banana grown in Malaysia demonstrated that antioxidant activity of n-hexane, chloroform and methanol peel extracts of nangka banana were 1.00, 2.01, 0.65 mg TE/g fresh weight and 2.26, 3.99, 3.36 mg TE/g dried weight, respectively by DPPH method

and 2.5, 7.40, 3.54 mg TE/g fresh weight and 3.69, 7.50, 10.32 mg TE/g dried weight, respectively by FRAP method. In the previous research (Fidrianny, Sefiany and Ruslan, 2015) reported that IC_{50} of DPPH of ethanolic leaves, peduncle and peel extracts of white ambon banana were 1.25, 1.57, 0.37 µg/ml, respectively, while the other research (Fidrianny *et al*., 2014) exposed that ethanolic peel extracts of raja bulu banana, muli banana and ambon lumut banana had IC_{50} of DPPH scavenging activities were 36.12, 4.39 and 6.91 µg/ml, respectively. It was contrary with the present study which showed that IC_{50} of DPPH scavenging activities of ethanolic peel extracts of tanduk banana, nangka banana and kepok banana were 101.40, 53.05 and 6.22 µg/ml, respectively. The previous study (Karuppiah and Mustaffa, 2013) stated that methanolic leaves extrac of *M. acuminata*, *M. troglodytarum*, *M. sapientum* and *M. paradisiaca* had antioxidant capacities 50, 20, 30 and 110 mg/g extract respectively. Sahaa *et al*. (2013) expressed that *M. sapientum* var. sylvesteris which was extracted by cold extraction method showed IC_{50} DPPH of methanolic leaves extract 39 µg/ml. It was different from the previous study (Fidrianny, Sefiany and Ruslan, 2015) which exposed that IC_{50} of DPPH scavenging activity of ethanolic leaves extract of white ambon banana which was extracted by reflux method was 1.25 µg/ml. Previous research (Baskar *et al*., 2011) stated that ethanolic peel extract of pachainadan banana variety had total antioxidant activity (5.85 mM AAE/g) higher than kadali, kadali, karpooravalli, monthan, poovan, nendran, rasthali, robusta and sevvazhai varieties. Research by Nagarajaiah and Prakash (2011) demonstrated that methanol peel extract of pachabale banana and yelakkibale banana gave higher percentage of DPPH radical scavenging activity compared to its ethanol extract and aqueous extract. It was contrast with nendranabale banana which showed that its ethanol peel extract gave higher percentage of DPPH radical scavenging activity compared to its methanol extract and aqueous extract. Previous study (Darsini *et al*., 2012) exposed that IC_{50} of DPPH and IC_{50} of ABTS scavenging activity of methanolic fruit extract of awak banana were 65 and 29 µg/ml, respectively.

TFC, TPC and TCC in banana peels extracts

TFC among the various extracts were expressed in term of quercetin equivalent using the standard curve equation y = 0.007 x + 0.001, R^2 = 0.9991. The TFC in various extracts of banana peels showed different result varied from 0.32 to 5.86 g QE/100 g (Table 1). Ethyl acetate peel extract of nangka banana (NAN2) had the highest total flavonoid content (5.86 g QE/100 g). TPC among the various extracts were revealed in term of gallic acid equivalent using the standard curve equation y = 0.005 x - 0.198, R2 = 0.9971. The TPC in various extracts of banana peels showed different result in the range of 1.46 - 4.63 g GAE/100 g. The highest phenolic content (4.63 g GAE/100 g) was given by ethyl acetate peel extract of nangka banana (NAN2) (Table 1) and the lowest given by ethyl acetate peel extract of tanduk banana (TAN2).

Table 1. Total phenolic, flavonoid and carotenoid content in various banana peels extracts

SAMPLE	TPC (g GAE/100 g)	TFC (g QE/100 g)	TCC (g BE/100 g)
TAN1	1.4959 ± 0.0186 ^{ab}	2.0214 ± 0.0106 ^{a}	2.5961 ± 0.01875 ^{a}
NAN1	1.4593 ± 0.009 ^{a}	1.5802 ± 0.0399 ^{b}	2.6562 ± 0.0138 ^{b}
KEP1	1.5206 ± 0.0151 ^{b}	0.9635 ± 0.004 ^{c}	1.8664 ± 0.0275 ^{c}
TAN2	4.2477 ± 0.0863 ^{a}	3.8681 ± 0.050 ^{a}	2.0707 ± 0.004 ^{a}
NAN2	4.6344 ± 0.0684 ^{b}	5.8646 ± 0.0219 ^{b}	1.5176 ± 0.0204 ^{b}
KEP2	3.5304 ± 0.0771 ^{c}	3.9277 ± 0.0438 ^{a}	1.6077± 0.013 ^{c}
TAN3	3.2945 ± 0.0205 ^{a}	0.3590 ± 0.0086 ^{a}	0.3412 ± 0.002 ^{a}
NAN3	2.4692 ± 0.0144 ^{b}	0.3237 ± 0.002 ^{b}	0.5525 ± 0.0031 ^{b}
KEP3	2.4825 ± 0.0201 ^{b}	0.3435 ± 0.003 ^{c}	0.2854 ± 0.0007 ^{c}

Values are mean ± SD, n = 3

Different letter within the same column figure significant difference ($p<0.05$)

Total phenolic content might be related with antioxidant capacity. Phenolic acid was one of phenolic compound in plant (Ling and Palanisany, 1999). Cinnamic acid showed higher antioxidant activity than benzoic acid and phenyl acetic acid (Heim *et al*., 2002). The present research revealed that the highest of TPC and TFC was given by ethyl acetate peel extract of nangka banana (4.63 g GAE/100 g and 5.86 g QE/100 g, respectively). In the previous study (Fidrianny *et al*., 2014) which studied regarding total phenolic content in peel extracts of muli banana, raja bulu banana and muli banana exposed that ethyl acetate peel extract of muli banana had the highest TPC (3.99 g GAE/100 g) and ethyl acetate peel extract of raja bulu banana gave the highest TFC (10.22 g QE/100 g). Previous study (Nagarajaiah and Prakash, 2011) showed that methanol peel extract of nendranbale banana had the highest total polyphenol content (850 mg tannic

acid/100 g) and the highest TFC (1035 mg QE/100 g). In previous research by Saha *et al*. (2013) figured 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 from the previous study (Fidrianny, Sefiany and Ruslan, 2015) which revealed that ethanolic leaves extract of white ambon banana had TPC 5.68 g GAE/100 g and TFC 4.72 g QE/100 g, respectively. Study by Baskar *et al*. (2011) found that ethanolic peel extract of rauthali banana gave the highest TPC (0.06 g CE/100 g), and ethanolic peel extract of poovan banana showed the highest TFC (2.2 g RE/100 g), while the previous researches expressed 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 (Fidrianny, Sefiany and Ruslan, 2015), fresh peel extract and dried peel extract of raja banana had the highest TPC (0.61 mg GAE/g FW and 12.27 mg GAE/g DW, respectively) (Sulaiman *et al*., 2011), 50% ethanol extract of *Musa cavendishii* which was extracted by stirring 30 minutes and temperature 60oC gave the highest TPC 16.90 g GAE/kg and TFC 3.55 g RE/kg (Schmidt *et al*., 2015). Karupiah *et al*. (2013) demonstrated that 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 previous study (Shodehinde and Oboh, 2013) exhibited that TFC and TPC in aqueous extract of boiled treatment of unripe plantain banana, roasted treatment and without treatment were 61, 71, 48 mg QE/100 g, respectively and 93, 94, 89 mg GAE/100 g, respectively. Darsini *et al*. (2012) exposed that methanolic fruit extract of awak banana gave TPC 0.12 g GAE/100 g and TFC 0.44 g/100 g.

TCC among the various extracts were reported in term of beta carotene equivalent using the standard curve equation y = 0.007x - 0.002, R2 = 0.9979. The TCC in various extracts of banana peels gave different result ranged from 0.29 to 2.66 g BE/100 g (Table 1). The highest carotenoid content (2.66 g BE/100 g) was given by ethyl acetate peel extract of tanduk banana (TAN2), while the lowest carotenoid (0.29 g BE/100 g) for ethanolic peel extract of kepok banana (KEP3).

Correlations between total phenolic, flavonoid, carotenoid content in various banana peels extracts and IC_{50} of DPPH scavenging activities, EC_{50} of CUPRAC capacities

Pearson's correlation coefficient was significantly negative if $-0.61 \leq r \leq -0.97$ and significantly positive if $0.61 \leq r \leq 0.97$ (Thaipong *et al*., 2006). Sample which had the highest antioxidant activity would have the lowest IC_{50} of DPPH scavenging activity and EC_{50} of CUPRAC capacity. It means increasing in TFC, TPC and TCC which caused increasing in antioxidant activities, will be expressed by lower IC_{50} of DPPH scavenging activity and or EC_{50} of CUPRAC capacity. Therefore the good correlation between TPC, TFC and TCC with IC_{50} of DPPH or EC_{50} of CUPRAC will be given by negative and significant correlation (Fidrianny, Johan and Sukrasno, 2015).

Data in Table 2 expressed that TPC in peel extracts of tanduk banana and nangka banana showed significantly negative correlation with IC_{50} of DPPH scavenging activities ($r = -0.845$, $p<0.01$; $r = -0.677$, $p<0.05$, respectively) and TPC in all of peel extracts of tanduk banana, nangka banana and kepok banana had significant and negative correlation with their EC_{50} of CUPRAC capacities ($r = -0.781$; $r = -0.988$; $r = -0.998$, $p<0.01$, respectively). It was contrary with the previous study which stated that TPC in peel extract of white ambon banana had no correlation with their IC_{50} of DPPH and IC_{50} of ABTS scavenging activities (Fidrianny, Sefiany and Ruslan, 2015), while its peduncle extract had negative and significant correlation with their IC_{50} of DPPH and IC_{50} of ABTS scavenging activities ($r = -0.979$; $r = -0.985$, $p<0.01$). It was different from the previous research (Fidrianny *et al*., 2014) which analyzed correlation between TPC,

Table 2. Pearson's correlation coefficient between total phenolic, flavonoid, carotenoid content in various banana peels extracts with their IC_{50} of DPPH scavenging activities and EC_{50} of CUPRAC capacities

Antioxidant activities	Coefficient correlation Pearson (r)					
	TFC	TPC	TCC	EC_{50} CUPRAC TAN	EC_{50} CUPRAC NAN	EC_{50} CUPRAC KEP
IC_{50} DPPH TAN	0.182 ns	-0.845**	0.820**	0.153ns		
IC_{50} DPPH NAN	-0.208 ns	-0.677*	0.928**		0.778**	
IC_{50} DPPH KEP	0.153 ns	0.486 ns	0.929**			0.012ns
EC_{50} CUPRAC TAN	-0.868**	-0.781*	-0.270 ns			
EC_{50} CUPRAC NAN	-0.774*	-0.988**	0.491 ns			
EC_{50} CUPRAC KEP	-0.808**	-0.998**	0.096 ns			

IC_{50} DPPH = IC_{50} of DPPH scavenging activity, EC_{50} CUPRAC = EC_{50} of CUPRAC capacity, TAN = tanduk banana, NAN = nangka banana, KEP = kepok banana, ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$

TFC and TCC with their percentage of DPPH and ABTS scavenging activities. In this study the good correlation if increasing in TPC, TFC or TCC could give increasing in percentage of DPPH and ABTS scavenging activities. The results stated that only TPC in peel extracts muli banana had positive and significant correlation with their percentage of DPPH and ABTS scavenging activities. It was similar to the previous study which showed that total polyphenol and TFC in pachabale banana, yelakkibale banana and nendrabale banana gave positive and significant correlation with their percentage of DPPH scavenging activities (Nagarajaiah and Prakash, 2011).

The present research reported that TCC in peel extracts of tanduk banana, nangka banana and kepok banana gave no correlation with their IC_{50} of DPPH and EC_{50} of CUPRAC. It was similar to the previous study (Fidrianny, Sefiany and Ruslan, 2015) which revealed 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 contrary with the previous study (Darsini *et al*., 2012) 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. This result similar to the other study (Fidrianny *et al*., 2014) which stated that TCC in peel extracts of ambon lumut had significant and positive correlation with their percentage of DPPH scavenging activities.

Flavonoid, phenolic acid and tannins were included in phenolic groups. Flavonoid which had ortho di OH in C 3'-4', OH in C-3, oxo function in C-4, double bond at C-2 and C-3 have high antioxidant activity. The ortho di OH with position in C-3'-C-4' had the highest influence to antioxidant activity of flavonoid. Flavonoid had greater antioxidant activity than phenolic acid (Heim *et al*., 2002). In Table 1 it could be found that TFC in ethyl acetate peel extract of kepok banana (KEP2) 0.32 g QE/100 g was similar to TFC in ethanolic peel extract of kepok banana (KEP3) 0.34 g QE/100 g), but IC_{50} of DPPH of KEP2 (2.48 µg/ml) was lower than IC_{50} of DPPH of KEP3 (6.22 µg/ml). The flavonoid aglycones would give higher antioxidant activity than flavonoid glycosides (Heim *et al*., 2002). Peel of banana contained naringin which was flavanone glycoside and rutin (flavonol glycoside) (Kanazawa and Sakakibara, 2000). Based on the result it can be supposed that many flavonoid compounds in KEP2 was flavonoid aglycones which soluble in ethyl acetate, while many flavonoid compound in KEP3 was flavonoid glycosides which soluble in ethanol. TPC in n-hexane peel extract of kepok banana (KEP1) 3.29 g GAE/100 was higher than TPC in ethanolic peel extract of kepok banana (KEP3) 2.48 g GAE/100 g, but IC_{50} of DPPH of KEP1 (6.23 µg/ml) was similar to IC_{50} of DPPH of KEP3 (6.22 µg/ml). It can be estimated that many phenolic compounds in KEP1 had low antioxidant activity, while phenolic compounds in KEP3 had high antioxidant activity. TPC in ethyl acetate peel extract of tanduk banana (TAN2) 1.46 g GAE/100 g was similar to TPC in ethanolic peel extract of tanduk banana (TAN3) 1.52 g GAE/100 g, but EC_{50} of CUPRAC capacity of TAN3 (322.21 µg/ml) was higher than EC_{50} of CUPRAC capacity of TAN2 (235.64 µg/ml). The reaction of CUPRAC assay was depended on reduction of Cu (II) to Cu (I). Sample which contained many antioxidant with reduction potential lower than reduction potential of Cu(II)/ Cu(I) 0.159 V, will reduce Cu(II) to Cu(I) and at the same time the sample will be oxidized. Based on the result it can be predicted that many phenolic compounds in TAN2 has reduction potential lower than 0.159 V while many phenolic compounds in TAN3 has reduction potential higher than 0.159 V. In the present study reagent of DPPH and CUPRAC were prepared in the same concentration, so the result of this study was to compare reaction of DPPH and CUPRAC method in concentration of reagent 50 µg/ ml. DPPH with concentration of 50 µg/ml is enough for completing reaction, it showed by low value of IC_{50} of DPPH. In CUPRAC it should be prepared enough reagent of cupric (II) chloride or excessive of reagent cupric (II) chloride, hence sample which contain antioxidant will reduce Cu (II) to Cu (I), and then Cu (I) will react with neocuproine and gives yellow color. If sample contain many antioxidant and only a little amount of reagent cupric (II) chloride, so all of Cu (II) reduce to Cu (I) and still many antioxidant in sample will be oxidized again Cu (I) to Cu (II). This reaction will be repeated in many times form Cu (II) to Cu (I) and Cu(I) to Cu(II). Therefore the result EC_{50} of CUPRAC will be seen in high value.

Previous research (Foote, 1976) exposed that carotenoid have antioxidant capacity by scavenging free radical. Carotenoid consist of more than 7 double bonds will show higher scavenging radical activity (Beutner *et al*., 2000). Charles (2013) stated that beta carotene was used as standard because it had conjugation double bonds which had ability to scavenge free radicals. The previous study (Kobayashi and Sakamoto, 1999) revealed that increasing in lipophilicity of carotenoid would increase scavenging radical activity and will give the lower IC_{50} radical scavenging activity. Peel of banana contained carotenoid compounds likes lutein, beta-

carotene, alpha-carotene, violaxanthin, auroxanthin, neoxanthin, isolutein, beta-cryptoxanthin and alpha-cryptoxanthin, which soluble in n-hexane and have antioxidant activity (Subagio *et al*., 1996). TCC in n-hexane peel extract of nangka banana (NAN1) 2.07 g BE/100 g which was higher than TCC in ethanolic peel extract of nangka banana 1.61 g BE/100 g (NAN3), but IC_{50} of DPPH scavenging activity of NAN1 17.24 µg/ml which was categorized as very strong antioxidant and smaller than IC_{50} of DPPH of NAN3 53.06 µg/ml as strong antioxidant. It can be predicted that NAN1 contained many carotenoid compounds above which have high antioxidant activity likes lutein, beta-carotene and beta-cryptoxanthin, which have more than seven double bonds, while many carotenoid compounds in NAN3 which have maximum seven double bonds and low antioxidant activity.

DPPH and CUPRAC had different mechanism reaction. Mechanism of CUPRAC was redox assay (Apak *et al*., 2007) while DPPH that was electron transfer assay (Huang *et al*., 2005). In the previous research (Fidrianny *et al*., 2014) exposed that IC_{50} of DPPH scavenging activities in all of leaves, peduncle and peel extracts of white ambon banana were linear with their IC_{50} of ABTS scavenging activities. The present study demonstrated that Pearson's correlation coefficient between IC_{50} of DPPH scavenging activities peel extracts of nangka banana had significantly positive correlation with their EC_{50} of CUPRAC capacities ($r = 0.778$, $p<0.01$). It could be seen that antioxidant activities of peel extracts of nangka banana by DPPH and CUPRAC assays gave linear result.

Conclusion

Different results could be given by various antioxidant methods, therefore antioxidant activity of sample should be measured by different methods in parallel. All of ethyl acetate and ethanolic peel extracts of tanduk banana, nangka banana and kepok banana (except ethanolic extract of nangka banana) were very strong antioxidant, using DPPH assays. Phenolic compounds in peel extracts of tanduk banana and nangka banana were the major contributor in their antioxidant activity by DPPH and CUPRAC methods. There was linear correlation between IC_{50} of DPPH scavenging activities and EC_{50} of CUPRAC capacities of peel extract of nangka banana. Peel of tanduk banana, nangka banana and kepok banana may be exploited as sources of natural antioxidant.

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