

Antioxidant Capacities of Various Leaves Extract from Five Colors Varieties of Sweet Potatoes Tubers Using ABTS, DPPH Assays and Correlation with Total Flavonoid, Phenolic, Carotenoid Content

Irda Fidrianny, Ari Sri Windyaswari and Komar Ruslan Wirasutisna

School of Pharmacy, Bandung Institute of Technology, Jl. Ganesha 10 Bandung, 40132, Indonesia

Corresponding Author: Irda Fidrianny, School of Pharmacy, Bandung Institute of Technology, Jl. Ganesha 10 Bandung, 40132, Indonesia

ABSTRACT

Antioxidant is a molecule that has ability to inhibit or prevent the other molecules being oxidized. Natural antioxidant is a compound which in limited number can inhibit or prevent the other molecules being oxidized. Sweet potatoes (*Ipomoea batatas* L. Lam) leaves extract had antioxidant activities. The aim of this research, were to determine antioxidant capacity of various leaves extract from five colors varieties of sweet potatoes tubers using two methods of antioxidant testing and its correlation with total flavonoid, phenolic and carotenoid content. The research was started with reflux extraction using gradient polarity solvent (n-hexane, ethyl acetate and ethanol). Antioxidant capacities of each extracts was performed by DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)) diammonium salt. Determination of total flavonoid, phenolic and carotenoid content was done by UV-Visible spectrophotometry. The highest ABTS capacity was given by sample RY3 and the highest DPPH scavenging activity by sample RP3. The total phenolic contents in sample RP was the highest significantly correlated with ABTS and DPPH scavenging capacities. The ABTS scavenging activity in sample Y had positively high correlation with total flavonoid contents. The total carotenoid contents in sample O had high correlation with ABTS scavenging activity and sample Y with DPPH scavenging capacity. There was no significant correlation between ABTS scavenging capacities and DPPH scavenging capacities of various leaves extract from five colors varieties of sweet potatoes tubers.

Key words: Antioxidants, ABTS, DPPH, various extracts, sweet potatoes leaves, total flavonoid, phenolic, carotenoid

INTRODUCTION

Antioxidant activity could be determined by using several assays such as DPPH or ABTS (Pellegrini *et al.*, 2003). Many of plant extracts have shown antioxidant and free radical scavenging capacities were used in treatment of human health problems (Diplock *et al.*, 1998). Fruits and vegetables such as sweet potatoes contain carotenoid, flavonoid and other phenolic compounds (Teow *et al.*, 2007; Islam *et al.*, 2003). The antioxidant capacities and chelating abilities of flavonoid had correlation with their beneficial health effects (Heim *et al.*, 2002).

Free radicals could lead to degenerative diseases such as cancer or hypercholesterolemia which could be scavenged by antioxidant compound. Polyphenol and flavonoid in some plants had significant correlation with their antioxidant capacity (Heim *et al.*, 2002; Islam, 2006). Carotenoid compound also demonstrated their ability to scavenge free radicals (Foote, 1976).

ABTS (2, 2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)) and DPPH (2,2 diphenyl-1 picrylhydrazyl) assays have been widely used to determine the free radical scavenging activity of extracts. Both of ABTS and DPPH procedures are simple to perform (Teow *et al.*, 2007).

Sweet potatoes (*Ipomoea batatas* (L) Lamk.) is a member of Convolvulaceae, has many varieties of tuber colors such as purple, red purple, yellow, red yellow and orange. Anthocyanin was found in purple sweet potatoes, while orange sweet potatoes contained lutein, zeaxanthin and beta carotene that were potential as antioxidant (Islam *et al.*, 2003). Study by Islam (2006) revealed that sweet potatoes leaves had anti cancer and anti mutagenic activities. Antioxidant activity of sweet potatoes tubers with varieties colors by using ORAC, DPPH and ABTS methods had been researched (Teow *et al.*, 2007). Tubers of sweet potatoes had lower antioxidant capacity than it leaves (Cook and Samman, 1996). In other hand, study by Hue *et al.* (2012) revealed study of antioxidant capacity by DPPH method in *Ipomoea batatas* leaves from different places of commercial sweet potatoes farm in Malaysia and determination the influence of total phenolic and flavonoid contents varieties on the antioxidant activities.

The objective of this research was to study antioxidant activity of various extracts (n-hexane, ethyl acetate and ethanol) of sweet potatoes (*Ipomoea batatas*) leaves from five different tubers colors using antioxidant testing ABTS and DPPH assays and correlations of their activity with total flavonoid, phenolic and carotenoid contents in each extracts.

MATERIALS AND METHODS

Chemicals: Ascorbic acid, Trolox[®], gallic acid, quercetin, beta carotene, ABTS (2, 2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)) diammonium salt, DPPH (2,2-diphenyl-1 picrylhydrazyl (3-ethyl-benzothiazoline-6-sulfonic acid)) was purchased from Sigma-Aldrich (MO, USA), potassium persulfate, methanol, ethanol. All other reagents were analytical grades.

Plant materials: Leaves of sweet potatoes from five varying tubers colors were selected from a commercial sweet potatoes farm in Cihideung, Bandung Barat, Indonesia. The leaves from: Red-Purple tubers (RP), Purple tubers (P), Yellow tubers (Y), Red-Yellow tubers (RY) and Orange tubers (O) were analyzed. Sweet potatoes leaves of five varieties of tubers colors were thoroughly washed with tap water, wet sortation and dried. The ground samples taken three days for drying process at temperature 40°C. After that, dried samples were grinding using grinder into powder.

Extraction: Three hundred grams of powdered samples were extracted with reflux techniques 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 five n-hexane extracts (namely RP1, P1, Y1, RY1 and O1), five ethyl acetate extracts (RP2, P2, Y2, RY2 and O2) and five ethanolic extracts (RP3, P3, Y3, RY3 and O3).

Antioxidant activity in DPPH and ABTS assays

Preparation of ABTS solution and measurement: Preparation of ABTS radical solution were adopted from Li *et al.* (2011) and Pellegrini *et al.* (2003) method with minor modification. ABTS diammonium salt aqueous solution with concentration 2.5 mg/5 mL and potassium persulfate aqueous solution 2.5 mg/5 mL was prepared. Each solutions allowing to stand in the dark room for 12-18 h. A radical stock solution of ABTS was produced by mixing two above solutions and ad to

50 mL with ethanol 95%. ABTS solution would give absorbance 0.71 ± 0.2 at wavelength 734 nm, verified by Hewlett Packard 8435 Diode Array Spectrophotometer (HP, Waldbronn, Germany). Extracts 0.3 mL ($50 \mu\text{g mL}^{-1}$) was pipetted into 0.3 mL ABTS solution $50 \mu\text{g mL}^{-1}$ (1:1) to initiate the reaction. The mixture was diluted with 1.4 mL ethanol. The absorbance was read at wavelength 734 nm without incubation time using Hewlett Packard 8435 spectrophotometer UV-Vis. Ethanol (95%) was used as a blank and ABTS solution $50 \mu\text{g mL}^{-1}$ was used as standard. Analysis was done in triplicate for standard and each extracts. Trolox[®] was used as standard control. All measurement procedures were in dark room. Antioxidant capacity of each extracts were determined based on the reduction of ABTS absorbance by calculating percentage of antioxidant activity (Bedawey *et al.*, 2010).

Preparation of DPPH solution and measurement: Preparation of DPPH solution were adopted from Blois (1958) with minor modification. DPPH 2.5 mg were diluted into 50 mL methanol. The mixture were incubated in the dark room for 30 min. Keep the radical stock solution of DPPH in refrigerator (4°C). The radical stock solution of DPPH can stand until 24-48 h with decreasing absorbance ± 0.2 . Each extracts $50 \mu\text{g mL}^{-1}$ was pipetted into DPPH solution $50 \mu\text{g mL}^{-1}$ (1:1) to initiate the reaction. The absorbance was read at wavelength 516 nm after 30 min incubation using Hewlett Packard 8435 spectrophotometer UV-Vis. Methanol was used as a blank and DPPH solution $50 \mu\text{g mL}^{-1}$ as standard. Analysis was done in triplicate for standard and each extracts. Ascorbic acid was used as standard control. All measurement procedures were in dark room. Antioxidant capacity of each extracts were determined based on the reduction of DPPH absorbance by calculating percentage of antioxidant activity (Bedawey *et al.*, 2010).

Determination of total phenolic content: Total phenolic content were measured using the modified Folin-Ciocalteu method adapted from Pourmorad *et al.* (2006). Each 0.5 mL extracts was pipetted into 5 mL Folin Ciocalteu reagent (1:10) and 4 mL sodium carbonate 1 M. The mixtures were incubated for 15 min. The absorbance was read at wavelength 765 nm. Analysis was done in triplicate for each extracts. Standard solutions of gallic acid with concentration $60\text{-}150 \mu\text{g mL}^{-1}$ were used to obtain a standard curve. The total phenolic content was reported as percentage of total gallic acid equivalents 100 g^{-1} extract (g GAE/100 g).

Determination of total flavonoid: Total flavonoid content was measured using adapted method from Chang *et al.* (2002). Each 0.5 mL extracts was pipetted into 0.1 mL aluminium chloride 10%, 0.1 mL sodium acetate 1 M and 2.8 mL aquadest. The mixture were diluted with 1.5 mL ethanol, and incubated for 15 min. The absorbance was read at wavelength 415 nm. Analysis was done in triplicate for each extracts. Standard solutions of quercetin with concentration $40\text{-}100 \mu\text{g mL}^{-1}$ were used to obtain a standard curve. The total flavonoid content was reported as percentage of total quercetin equivalents 100 g^{-1} extract (g QE/100 g).

Determination of total carotenoid: Total carotenoid content was measured using the modified carotene method adapted from Thaipong *et al.* (2006). Each extracts were diluted into n-hexane solvent. Each 2 mL extracts were measured and the absorbance was read at wavelength 470 nm. Analysis was done in triplicate for each extracts. Standard solutions of beta carotene with concentration $10\text{-}80 \mu\text{g mL}^{-1}$ were used to obtain a standard curve. The total carotenoid content was reported as percentage of total beta carotene equivalents 100 g^{-1} extract (g BET/100 g).

Statistical analysis: The DPPH and ABTS antioxidant activities, total flavonoid, phenolic and carotenoid content of extracts were analyzed by statistically using one way ANOVA-Least Significant Difference. Correlation between total flavonoid, phenolic and carotenoid content of extracts and its antioxidant capacities were analyzed by Pearson's method.

RESULT

Antioxidant capacity of various extracts of sweet potatoes leaves in ABTS and DPPH assays:

The antioxidant capacity of various extracts of sweet potatoes leaves from five varieties tubers colors using ABTS and DPPH assays were shown in Table 1, 2, 3 and Fig. 1. In ABTS method, antioxidant activities of various extracts of *Ipomoea batatas* leaves from five varieties tubers colors in range of 24.51-81.83%. RY3 leaves extract (ethanolic leaves extract of red-yellow tubers) had the highest ABTS scavenging activity (81.83%), while the lowest activity (24.51%) was given by P1 leaves extract.

In the DPPH method, radical scavenging activities of various extracts of *Ipomoea batatas* leaves from five varieties tubers colors ranged from 7.73 to 97.63%. RP3 leaves extract (ethanolic leaves extract of red-purple tubers) had the highest DPPH radical scavenging activity (97.63%), while RY1 leaves extract (7.73%) had the lowest DPPH antioxidant activity.

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

Sample	ABTS scavenging activity (%)	DPPH scavenging activity (%)
RP1	29.42±0.62 ^a	8.42±10.63 ^a
P1	24.58±0.80 ^b	23.35±3.32 ^b
Y1	26.83±0.26 ^c	23.18±5.47 ^b
RY1	46.96±0.34 ^d	7.73±2.82 ^a
O1	43.94±0.46 ^c	19.39±5.07 ^b
p-value	<0.05	<0.05

a-e: Means within a column with the same letter were not significantly different (p = 0.05)

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

Sample	ABTS scavenging activity (%)	DPPH scavenging activity (%)
RP2	50.14±1.30 ^a	77.96±6.34 ^e
P2	43.31±0.40 ^b	65.47±7.52 ^b
Y2	33.29±0.22 ^c	90.01±1.77 ^e
RY2	33.46±0.17 ^c	91.47±5.72 ^e
O2	34.79±1.88 ^c	93.26±0.23 ^e
p-value	<0.05	<0.05

a-c: Means within a column with the same letter were not significantly different (p = 0.05)

Table 3: ABTS and DPPH scavenging activities of ethanolic leaves extracts

Sample	ABTS scavenging activity (%)	DPPH scavenging activity (%)
RP3	57.53±1.20 ^a	97.63±0.60 ^a
P3	64.85±1.65 ^b	93.34±1.90 ^a
Y3	69.96±1.44 ^c	34.28±4.96 ^b
RY3	81.83±2.70 ^d	50.04±3.03 ^c
O3	31.42±0.50 ^e	59.68±12.44 ^c
p-value	<0.05	<0.05

a-e: Means within a column with the same letter were not significantly different (p = 0.05)

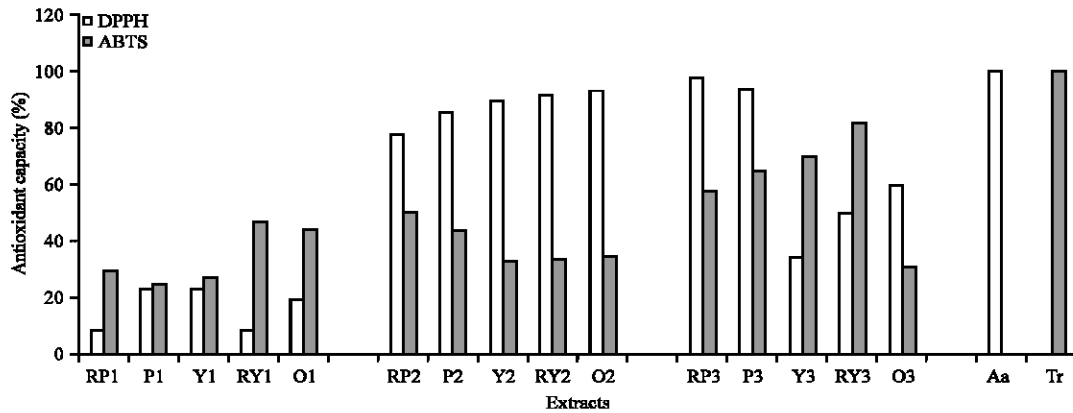


Fig. 1: Antioxidant capacity of various extracts of sweet potatoes leaves in DPPH and ABTS assays. RP: Leaves of red-purple tubers, P: Leaves of purple tubers, Y: Leaves of yellow tubers, RY: Leaves of red-yellow tubers, O: Leaves of orange tubers, 1: n-hexane extract, 2: ethyl acetate extract, 3: ethanol extract, Aa: Ascorbic acid, Tr: Trolox®

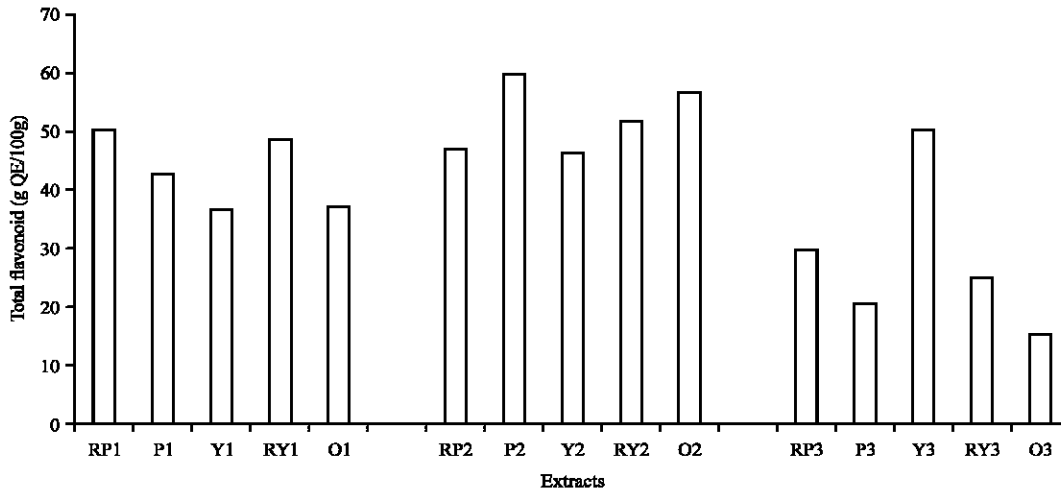


Fig. 2: Total flavonoid of various extracts of sweet potatoes leaves with varying tubers colors

Total flavonoid of various extracts of sweet potatoes leaves: The total flavonoid contents among the different varieties were expressed in term of quercetin equivalent using the standard curve equation $y = 0.005x - 0.008$, $R^2 = 0.994$. The flavonoid contents shown as percentage of total flavonoid extract (g QE/100 g). The total flavonoid contents of various extracts in the different varieties of *Ipomoea batatas* leaves shown different result in the range of 15.43-59.79 g QE/100 g (Fig. 2). P2 leaves extract (ethyl acetate leaves extract of purple tubers) had the highest total flavonoid contents (59.79 g QE/100 g) and the lowest (15.43 g QE/100 g) for O3 leaves extract.

Total phenolic of various extracts of sweet potatoes leaves: The total phenolic contents among the different varieties were expressed in term of gallic acid equivalent using the standard curve equation $y = 0.004x + 0.993$, $R^2 = 0.993$. The phenolic contents shown as percentage of total phenolic extract (g GAE/100 g). The total phenolic contents of various extracts in the different

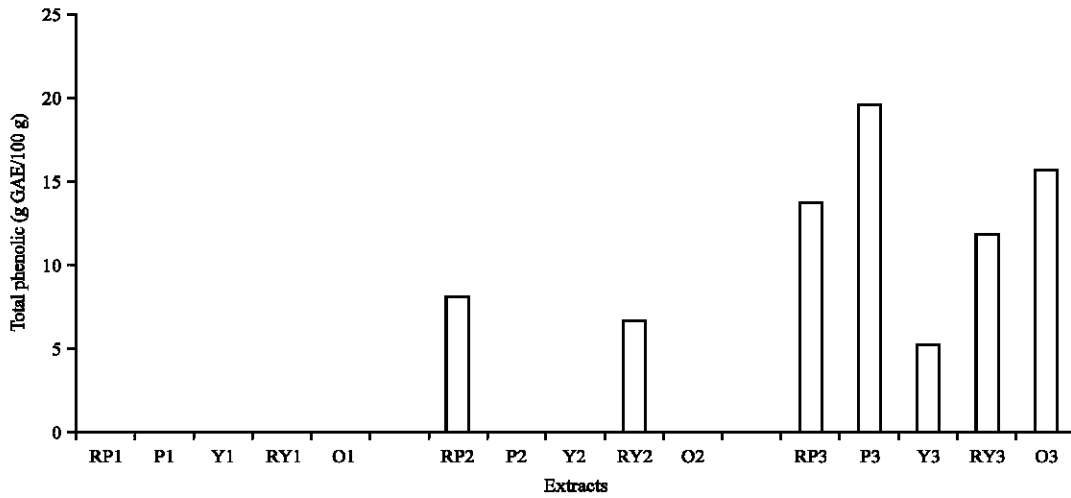


Fig. 3: Total phenolic of various extracts of sweet potatoes leaves with varying tubers colors

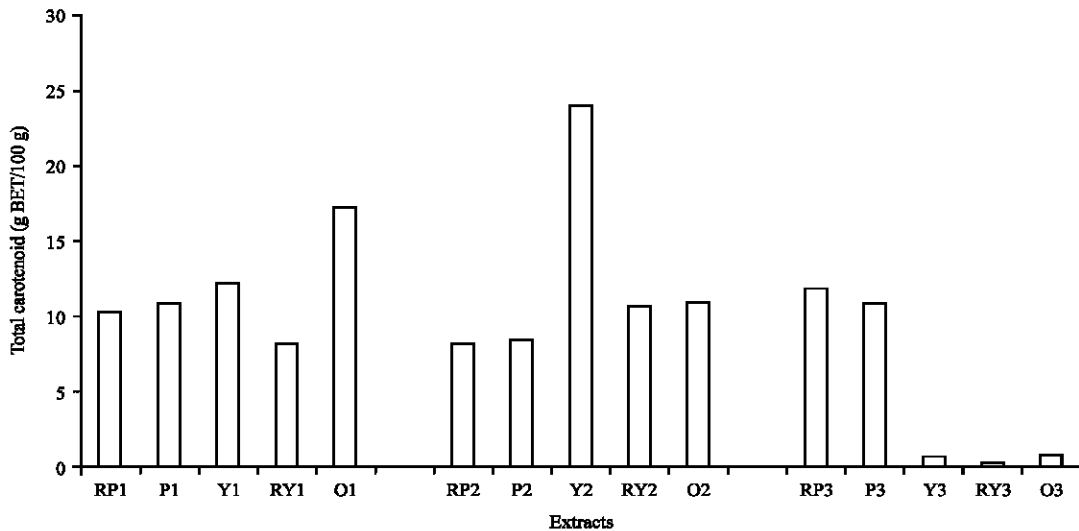


Fig. 4: Total carotenoid of various extracts of sweet potatoes leaves with varying tubers colors

varieties of *Ipomoea batatas* leaves shown different result ranged from 0 to 19.64 g GAE/100 g. P3 leaves extract (ethanolic leaves extract of purple tubers) had the highest phenolic contents (19.64 g GAE/100 g) (Fig. 3).

Total carotenoid of various extracts of sweet potatoes leaves: The total carotenoid contents among the different varieties were expressed in term of beta carotene equivalent using the standard curve equation $y = 0.022x - 0.008$, $R^2 = 0.997$. The carotenoid contents shown as percentage of total carotenoid extract (g BET/100 g). The total carotenoid contents of various extracts in the different varieties of *Ipomoea batatas* leaves shown different result in the range of 0.41-24.17 g BET/100 g (Fig. 4). The highest carotenoid contents (24.17 g BET/100 g) for Y2 leaves extract, while the lowest carotenoid (0.41 g BET/100 g) for RY3 leaves extract.

Table 4: Pearson's correlation coefficient of total flavonoid, total phenolic, total carotenoid of leaves extract from five varieties sweet potatoes tubers and DPPH, ABTS scavenging activity

Sample	Total flavonoid	Total phenolic	Total carotenoid	DPPH AS	ABTS AS
ABTS RP	-0.785 [*]	0.978 ^{**}	0.165 ^{ns}		
ABTS P	-0.596 ^{ns}	0.884 ^{**}	0.4 ^{ns}		
ABTS Y	0.791 [*]	0.907 ^{**}	-0.782 [*]		
ABTS RY	-0.982 ^{**}	0.622 ^{ns}	-0.997 ^{**}		
ABTS O	0.305 ^{ns}	-0.791 [*]	0.911 ^{**}		
ABTS AS	-0.307 [*]	0.534 ^{**}	-0.459 ^{**}	0.303 ^{ns}	
DPPH RP	-0.772 [*]	0.951 ^{**}	0.121 ^{ns}		
DPPH P	-0.453 ^{ns}	0.792 [*]	-0.116 ^{ns}		
DPPH Y	0.3 ^{ns}	-0.307 ^{ns}	0.778 [*]		
DPPH RY	0.107 ^{ns}	0.489 ^{ns}	0.222 ^{ns}		
DPPH O	0.415 ^{ns}	0.048 ^{ns}	-0.416 ^{ns}		
DPPH AS	-0.086 ^{ns}	0.481 ^{**}	0.178 ^{ns}		0.303 ^{ns}

ABTS: ABTS scavenging activity, DPPH: DPPH scavenging activity, RP: Sample RP, P: Sample P, Y: Sample Y, RY: Sample RY, O: Sample O, AS: All of samples, ns: Not significant, *significant at $p < 0.05$, **significant at $p < 0.01$

Correlations: Pearson's correlation coefficient was positively high if $0.68 \leq r \leq 0.97$ (Thaipong *et al.*, 2006). The highest and positive correlation between total phenolic content and ABTS scavenging capacity ($r = 0.978$, $p < 0.01$) for sample RP, followed by sample Y ($r = 0.907$, $p < 0.01$) and sample P ($r = 0.884$, $p < 0.01$) (Table 4). There was no correlation between total phenolic content and ABTS scavenging activity for sample RY. Negative correlation between total phenolic and ABTS scavenging activity that given by sample O ($r = -0.791$). There was high and positive correlation between total flavonoid and ABTS scavenging activity ($r = 0.791$, $p < 0.05$) for sample Y. Negatively high correlation between total flavonoid and ABTS capacity that given by sample RY ($r = -0.982$, $p < 0.01$), sample RP ($r = -0.785$, $p < 0.05$) and there were no correlation for sample P and O. There were positively high correlation between total carotenoid and ABTS scavenging activity for sample O ($r = 0.911$, $p < 0.05$). Negatively high correlation between total carotenoid and ABTS capacity for sample RY ($r = -0.997$, $p < 0.01$) and sample Y ($r = -0.782$, $p < 0.05$), but there was no correlation for sample RP and P.

The highest and positive correlation between total phenolic content and DPPH scavenging activity ($r = 0.951$, $p < 0.01$) for sample RP, followed by sample P ($r = 0.792$, $p < 0.05$) (Table 4) and no correlation for sample Y, RY and O. Negative correlation between total flavonoid and DPPH scavenging activity that given by sample RP ($r = -0.772$, $p < 0.05$) and there were no correlation for sample P, Y, RY and O. There was good and positive correlation between total carotenoid and DPPH capacity for sample Y and no correlation for sample RP, P, RY and O.

DISCUSSION

Study by Cook and Samman (1996) demonstrated that sweet potatoes leaves had higher antioxidant activity than sweet potatoes tubers. In previous study showed that tubers of sweet potatoes with varieties colors (white, cream, yellow, orange and purple) had antioxidant activity by using ORAC, DPPH and ABTS methods (Teow *et al.*, 2007). Antioxidant activity in *Ipomoea batatas* leaves from six different places of sweet potatoes farm in Malaysia by DPPH method and correlation between total phenolic, flavonoid contents and their capacities (Hue *et al.*, 2012). There was no information about color of tubers in Hue's research.

Both of ABTS and DPPH are stable free radicals which dissolve in methanol or ethanol and their colors show characteristic absorption at wavelength 519 or 734 nm, respectively. When an antioxidant scavenges ABTS and DPPH free radicals by hydrogen donation, the colors of ABTS and DPPH become lighter (Li *et al.*, 2011; Apak *et al.*, 2007). ABTS and DPPH assays have been widely used to determine the free radical scavenging activity of extracts. Both of ABTS and DPPH procedures are simple to perform (Teow *et al.*, 2007).

In this study three different polarities of solvents were chosen to extract different polarities of compounds. N-Hexane was used to get non polar compounds. The remaining of crude drug then was extracted using ethyl acetate solvent for extracting semi polar compounds. Then the remaining of crude drug was extracted by using ethanol, for getting polar compounds.

In ABTS method, antioxidant activities of various extracts of *Ipomoea batatas* leaves from five varieties tubers colors showed that RY3 leaves extract (ethanolic leaves extract of red-yellow tubers) had the highest ABTS scavenging activity, it was similar with the previous study by Everette and Islam (2012) that illustrated that the highest antioxidant activity was found in methanolic extract of SP-129 tubers which was one genotype of orange tubers.

In the DPPH method, radical scavenging activities of various extracts of *Ipomoea batatas* leaves from five varieties tubers colors demonstrated that RP3 leaves extract (ethanolic leaves extract of red-purple tubers) had the highest DPPH radical scavenging activity which was similar with the Everette and Islam (2012) revealed that methanolic extract of SP-245 tubers which was one genotype of orange tubers colors had the highest activity.

Statistical analysis of ABTS scavenging activity among n-hexane leaves extract indicated that all of samples RP1, P1, Y1, RY1 and O1 significantly different from each other ($p < 0.05$). DPPH scavenging radical activity among n-hexane leaves extracts were shown in Table 1. RP1 and RY1 not significantly different ($p > 0.05$), P1, Y1 and O1 not significantly different from each other ($p > 0.05$), while RP1 and RY1 significantly different with P1, Y1 and O1 ($p < 0.05$).

The ABTS and DPPH scavenging capacity among ethyl acetate leaves extract (Table 2) demonstrated that Y2, RY2 and O2 not significantly different from each other ($p > 0.05$), while RP2 significantly different with P2 and both of them significantly different with Y2, RY2, and O2 ($p < 0.05$).

Statistical analysis of DPPH scavenging activity among ethanolic leaves extract showed that RP3 and P3 not significantly different ($p > 0.05$), RY3 and O3 not significantly different from each other ($p > 0.05$), while Y3 significantly different with RP3, P3, RY3 and O3 ($p < 0.05$). In previous study was reported that methanol extract of purple tubers had the highest DPPH antioxidant activity (Teow *et al.*, 2007). The ABTS scavenging capacity in ethanol extract indicated that all of samples RP3, P3, Y3, RY3 and O3 significantly different from each other ($p < 0.05$).

The presence of total phenolic might contribute to antioxidant activity in *Ipomoea batatas* leaves (Hue *et al.*, 2012). P3 leaves extract (ethanolic leaves extract of purple tubers) had the highest phenolic contents (19.64 g GAE/100g) which was similar with previous study from sweet potatoes tubers that the methanolic extract of purple tubers had the highest total phenolic content (Teow *et al.*, 2007).

The highest carotenoid contents (24.17 g BET/100 g) for Y2 leaves extract (ethyl acetate leaves extract of yellow tubers). In the previous study by Teow *et al.* (2007) demonstrated that n-hexane extract of orange tubers had the highest total carotenoid.

In generally, total flavonoid, total phenolic, total carotenoid contributed in antioxidant activity, but not all flavonoid or phenolic or carotenoid compound had contribution in antioxidant activity. Flavonoid not always be phenolic compounds its depending on position of OH in flavonoid. Phenolic compound included tannins, flavonoid, phenolic acid and other compounds that had phenolic structure. Flavonoid that had OH in A ring and or B ring would be called as phenolic groups. Phenolic acid had the lower antioxidant activity than flavonoid (Heim *et al.*, 2002). Flavonoid would give antioxidant activity which has OH in ortho C3',4', OH in C3, oxo function in C 4, double bond at C2 and C3. The OH with ortho position in C3'-C4' had the highest influence to antioxidant activity of flavonoid. Flavonoid had OH in C3 and double bond at C2-C3 gave higher antioxidant activity than flavonoid had OH in C3 only. The flavonoid aglycones would give higher antioxidant activity than flavonoid glycosides (Heim *et al.*, 2002).

Sample RP (leaves of sweet potatoes with red-purple tubers) and sample P (leaves of sweet potatoes with purple tubers) had positively high correlation between total phenolic content and ABTS, DPPH scavenging capacities. This data indicated that higher total phenolic content in sample RP and sample P would give higher scavenging activity in both of ABTS and DPPH assays. It can be concluded that ABTS and DPPH scavenging activities of sample RP and sample P can be predicted indirectly by using their total phenolic content. There were negatively high correlation between total flavonoid and ABTS, DPPH scavenging activities. It illustrated that higher total flavonoid in sample RP would give lower ABTS and DPPH scavenging capacities. Based on the data it was predicted that flavonoid in sample RP has no OH in ortho C3',4', OH in C3, oxo function in C4, double bond at C2 and C3 which would influence scavenging capacity. While total phenolic and total flavonoid in sample Y had positively high correlation with ABTS scavenging capacity. There was demonstrated that ABTS scavenging activity of sample Y can be estimated indirectly by using its total phenolic and flavonoid content.

Carotenoid had antioxidant activity by scavenging free radical. More double bonds in carotenoid would give higher scavenging free radical activity (Foote, 1976). Carotenoid that consisted of maximum 7 double bonds gave lower scavenging radical free capacity than more double bonds (Beutner *et al.*, 2000). In previous study by Kobayashi and Sakamoto (1999) stated 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 (Charles, 2013).

The total carotenoid of sample O that was leaves of sweet potatoes with orange tubers had positively high correlation with ABTS scavenging activity. Sample Y (leaves of sweet potatoes with yellow tubers) had positively high correlation with scavenging DPPH activity which contained the highest total carotenoid from 3 extracts (Y1, Y2 and Y3). The total carotenoid of leaves extract from sample Y might have correlations with yellow color of tubers. The above data showed that carotenoid which reacted in scavenging radical ABTS and DPPH were different.

ABTS and DPPH methods had the same mechanism reaction that was electron transfer assays (Huang *et al.*, 2005), but the results of the present study showed that ABTS scavenging capacity not always linier with DPPH scavenging activity. The Pearson's correlation coefficient of various extract of sweet potatoes leaves with varying tubers colors also indicated that there was no correlation between ABTS capacities and DPPH capacities. It was predicted there was other different mechanism in ABTS and DPPH assays besides electron transfer.

CONCLUSION

The highest ABTS scavenging capacity was given by sample RY3 (ethanolic extract from leaves of sweet potatoes with red-yellow tubers) while sample RP3 that was ethanolic extract from leaves of sweet potatoes with red-purple tubers had the highest DPPH scavenging activity.

The positively high correlation between total phenolic content and ABTS scavenging capacity in sample RP, followed by sample Y and P, negative correlation in sample O, no correlation in sample RY. There was positively high correlation between total flavonoid and ABTS scavenging activity that given by sample Y, negative correlation in sample RY and RP, no correlation in sample P and O. The positively high correlation between total carotenoid and ABTS scavenging activity in sample O, negative correlation in sample RY and Y, no correlation in sample RP and P.

The positively high correlation between total phenolic content and scavenging radical DPPH activity in sample RP, followed by sample P and no correlation in sample Y, RY and O. There was negative correlation between total flavonoid and DPPH capacity that given by sample RP and no correlation in sample P, Y, RY and O. The positively high correlation between total carotenoid and DPPH scavenging activity in sample Y and no correlation in sample RP, P, RY and O.

In conclusion, the n-hexane, ethyl acetate and ethanol extracts of sweet potatoes leaves from five varieties tubers colors of *Ipomoea batatas* had shown antioxidant activity by ABTS and DPPH methods. The Pearson's correlation coefficient showed that there was no correlation between ABTS capacities and DPPH capacities of various extract of sweet potatoes leaves with varying tubers colors and it was predicted that other different mechanism in ABTS and DPPH assays besides electron transfer.

ACKNOWLEDGMENT

The authors are thankful to Biological Pharmacy Research Group Fund 2012 School of Pharmacy, Bandung Institute of Technology for financial support.

REFERENCES

- Apak, R., K. Guclu, B. Demirata, M. Ozyurek and S.E. Celik *et al.*, 2007. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. *Molecules*, 12: 1496-1547.
- Bedaway, A., E. Mansour, M. Zaky and A. Hassan, 2010. Characteristics of antioxidant isolated from some plant sources. *Food Nutr. Sci.*, 1: 5-12.
- Beutner, S., B. Bloedorn, T. Hoffmann and H.D. Martin, 2000. Synthetic singlet oxygen quenchers. *Methods Enzymol.*, 319: 226-241.
- Blois, M.S., 1958. Antioxidant determination by the use of stable free radicals. *Nature*, 181: 1199-1200.
- Chang, C.C., M.H. Yang, H.M. Wen and J.C. Chern, 2002. Estimation of total flavonoids content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.*, 10: 178-182.
- Charles, D.J., 2013. *Antioxidant Properties of Spices Herbs and Other*. John Willey, England.
- Cook, N.C. and S. Samman, 1996. Flavonoids-chemistry, metabolism, cardioprotective effects and dietary sources. *J. Nutr. Biochem.*, 7: 66-76.
- Diplock, A.T., J.L. Charleux, G. Crozier-Willi, F.J. Kok and C. Rice-Evans *et al.*, 1998. Functional food science and defence against reactive oxidative species. *British J. Nutr.*, 80: S77-S112.
- Everette, J.D. and S. Islam, 2012. Effect of extraction procedures, genotypes and screening methods to measure the antioxidant potential and phenolic content of orange-fleshed sweetpotatoes (*Ipomoea batatas* L.). *Am. J. Food Technol.*, 7: 50-61.

- Foote, C.S., 1976. Free Radicals in Biology. 3rd Edn., Academic Press, New York.
- Heim, K.E., A.R. Tagliaferro and D.J. Bobilya, 2002. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. *J. Nutr. Biochem.*, 13: 572-584.
- Huang, D., B. Ou and R.L. Prior, 2005. The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.*, 53: 1841-1856.
- Hue, S.M., A.N. Boyce and C. Somasundram, 2012. Antioxidant activity, phenolic and flavonoid contents in the leaves of different varieties of sweet potato (*Ipomoea batatas*). *Aust. J. Crop Sci.*, 6: 375-380.
- Islam, M.S., M. Yoshimoto, K. Ishigure, S. Okuno and O. Yamakawa, 2003. Effect of artificial shading and temperature on radical scavenging activity and polyphenolic composition in sweetpotato (*Ipomoea batatas* L.) leaves. *J. Am. Soc. Horticult. Sci.*, 128: 182-187.
- Islam, S., 2006. Sweetpotato leaf: Its potential effect on human health and nutrition. *J. Food Sci.*, 71: R13-R21.
- Kobayashi, M. and Y. Sakamoto, 1999. Singlet oxygen quenching ability of astaxanthin esters from the green alga *Haematococcus pluvialis*. *Biotech. Lett.*, 21: 265-269.
- Li, X.C., X.Z. Wang, D.F. Chen and S.Z. Chen, 2011. Antioxidant activity and mechanism of protochatechuic acid *in vitro*. *J. Funct. Food Health Dis.*, 7: 232-244.
- Pellegrini, N., M. Serafini, B. Colombi, D. Del Rio and S. Salvatore *et al.*, 2003. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different *In vitro* assays. *J. Nut.*, 133: 2812-2819.
- Pourmorad, F., S.J. Hosseinimehr and N. Shahabimajd, 2006. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *Afr. J. Biotechnol.*, 5: 1142-1145.
- Teow, C.C., V.D. Truong, R.F. McFeeters, R.L. Thompson, K.V. Pecota and G.C. Yencho, 2007. Antioxidant activities, phenolic and β -carotene contents of sweet potato genotypes with varying flesh colours. *Food Chem.*, 103: 829-838.
- Thaipong, K., U. Boonprakob, K. Crosby, L. Cisneros-Zevallos and D.H. Byrne, 2006. Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J. Food Comp. Anal.*, 19: 669-675.