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DPPH Scavenging Activity of Various Extracts of Sweet Potatoes Leaves with Varying Tubers Colors

Irda Fidrianny*, Ari Sri Windyaswari, Komar Ruslan Wirasutisna

School of Pharmacy, Bandung Institute of Technology, Indonesia

ABSTRACT

The antioxidant activities of various extracts of sweet potatoes (*Ipomoea batatas*) leaves with five varying tubers colors were examined by using DPPH (2.2-diphenyl-1-picrylhidrazyl) method. Determination of total flavonoid, phenolic and carotenoid were also measured for each extracts. The antioxidant activity for each extracts was reported as percentage of free radical DPPH scavenging activity. The highest antioxidant activity 97.63 %. was given by A3 (ethanolic extraxt of sample A). Sample B2 (ethyl acetate extract of sample B) contained the highest total flavonoid (59.79 g QE/100 g). Sample B3 (ethanolic extract from sample B) had the highest phenolic contents (19.64 g GAE/100 g), while the highest carotenoid 24.17 g BET/100 g was given by C2 (ethyl acetate extract of sample C). The total phenolic contents was significantly correlated with DPPH scavenging activity in sample A (leaves of red- purple tubers) with r = 0.951 and sample B (leaves of purples tubers) with r = 0.792, but no correlation in sample C, D and E. The DPPH scavenging activity in sample A was negatively correlated with total flavonoid contents (r = - 0.772), while sample B, C, D and E were no correlation. The total carotenoid contents in sample C (leaves of yellow tubers) had correlation with DPPH scavenging activity (r = 0.778) and no correlation in sample A, B, D and E.

KEYWORDS: Antioxidants, sweet potatoes leaves, flavonoid, phenolic, carotenoid, DPPH

***Corresponding author**
Irda Fidrianny
School of Pharmacy, Bandung Institute of Technology
Jl. Ganesha 10 Bandung 40132, Indonesia
Phone/fax: +62-22-2504852
Email adress: irda@fa.itb.ac.id

INTRODUCTION

Many plant extracts used in treatment of human health problems have shown antioxidant and free radical scavenging capacities [1]. Several methods were developed recently for measuring the total antioxidant capacity, these assay differ in their chemistry (generation of different radicals) [2].

Fruits and vegetables such as sweet potatoes are rich source of carotenoid, flavonoid and other phenolic compounds [3,4]. Most of the beneficial health effects of flavonoid were attributed to their antioxidant and chelating abilities [5].

Antioxidant compounds like phenolic acid, polyphenol and flavonoid has ability to scavenge free radicals thus inhibit the oxidative mechanisms that lead to degenerative diseases [5,6]. Study by Foote [7] has indicated that carotenoid has ability to scavenge free radical agent as singlet oxygen quencher.

The main characteristic of an antioxidant is the ability to trap free radicals, because these free radicals may oxidize nucleic acids, proteins, lipids, or DNA and can initiate degenerative disease. A simple method that has been developed to determine the antioxidant activity utilizes the stable 2,2 diphenyl-1 picrylhydrazyl (DPPH) radical.

Another studies regarding antioxidant activity of sweet potatoes tubers with varying flesh colors using ORAC, DPPH and ABTS methods [3] . In other hand, study by Hue [8] 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 simple method of antioxidant testing DPPH assay and correlations of their activity with total flavonoid, phenolic, and carotenoid contents in each extract.

MATERIALS AND METHODS

CHEMICALS

Gallic acid, quercetin, β-carotene, 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.

LEAVES SAMPLES

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 (A), purple tubers (B), yellow tubers (C), red-yellow tubers (D), and orange tubers (E) were analyzed.

PREPARATION OF LEAVES FOR ANALYSIS

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 40ºC. After that, dried samples were grinding into powder using grinder.

EXTRACTION TECHNIQUES

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 A1, B1, C1, D1 and E1), five ethyl acetate extracts (A2, B2, C2, D2 and E2) and five ethanolic extracts (A3, B3, C3, D3 and E3)

MEASUREMENT OF DPPH RADICAL SCAVENGING ACTIVITY

Preparation of DPPH solution

Preparation of DPPH solution were adopted from Blois [9] with minor modification. 2.5 mg DPPH were diluted into 50 mL methanol. The mixture were incubated in the dark room for 30 minutes. Keep the radical stock solution of DPPH in refrigerator (4^{o}C). The radical stock solution of DPPH can stand until 24-48 hours with decreasing absorbance ± 0.2.

Measurement

Each extracts with concentration 50 µg/mL was pipetted into DPPH solution concentration 50 µg/mL (1:1) to initiate the reaction. The absorbance was read at wavelength 516 nm after 30 minutes incubation using Hewlett Packard 8435 spectrophotometer UV-Vis. Methanol was used as a blank and DPPH solution 50 µg/mL as standard. Analysis was done in triplicate for standard and each extracts. All measurement procedures were in dark room. Antioxidant activity of each extracts were determined based on the reduction of DPPH absorbance by calculating percentage of antioxidant activity [10].

Total Phenolic Determination

Total phenolic content were measured using the modified Folin-Ciolcalteu method adapted from Pourmorad [11]. Each 0.5 mL extracts was pipetted into 5 mL Folin Ciolcalteu reagent (1:10) and 4 mL sodium carbonate 1 M. The mixtures were incubated for 15 minutes. 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 Flavonoid Determination

Total flavonoid content was measured using adapted method from Chang [12]. Each 0.5 mL extracts was pipetted into 0.1 mL aluminium chloride 10%, 0.1 mL sodium acetate 1M and 2.8 mL aquadest. The mixture were diluted with 1.5 mL ethanol, and incubated for 15 minutes. The absorbance was read at wavelength 415 nm. Analysis was done in triplicate for each extracts. Standard solutions of quercetin with concentration 40-100 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 Carotenoid Determination

Total carotenoid content was measured using the modified carotene method adapted from Thaipong [13]. 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 10-80 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).

RESULT AND DISCUSSION

Tubers of sweet potatoes with varying flesh colors (white, cream, yellow, orange and purple) had antioxidant activity using ORAC, DPPH and ABTS methods [3]. Hue [8] revealed study of antioxidant capacity by DPPH method in *Ipomoea batatas* leaves from six different places of commercial sweet potatoes farm in Malaysia and influence of total phenolic and flavonoid contents varieties on the

antioxidant activities. In this study, leaves was used as sample but threre was no information about color of tubers.

DPPH scavenging activity of various extracts of sweet potatoes leaves

DPPH is a stable free radicals which dissolve in methanol, and their colors show characteristic absorption at wavelength 516 nm. When an antioxidant scavenges the free radicals by hydrogen donation, the colors in the DPPH assay solution become lighter [14]. DPPH assay have been widely used to determine the free radical scavenging activity of extracts.

The antioxidant activity of various extracts of sweet potatoes leaves from five varieties tubers colors that was used in this research were shown in Fig.1. The results indicated that the antioxidant activity varied widely among the various extracts.

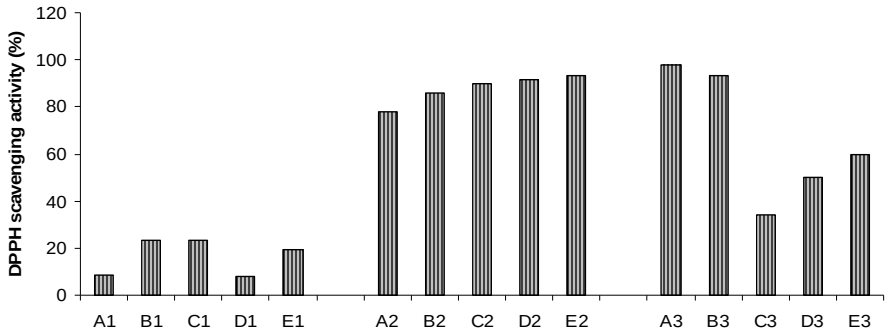

Fig 1: DPPH scavenging activity of various extracts of sweet potatoes leaves

Note: A = leaves of red-purple tubers, B = leaves of purple tubers, C = leaves of yellow tubers, D = leaves of red-yellow tubers, E = leaves of orange tubers, 1 = n-hexane extract, 2 = ethyl acetate extract, 3 = ethanol extract

The DPPH radical scavenging activities of various extracts of *Ipomoea batatas* leaves from five varieties tubers colors ranged from 7.73 to 97.63 %. A3 leaves extract (ethanolic leaves extract of red-purple tubers) had the highest DPPH radical scavenging activity (97.63%), followed by B3 leaves extract (93.34%) while D1 leaves extract (7.73%) had the lowest DPPH antioxidant activity. In previous study showed that methanolic extract of SP-245 tubers that was one genotype of orange tubers colors had the highest antioxidant activity [15].

Statistical analysis of scavenging radical DPPH activity among n- hexane leaves extracts were shown in Table 1. A1 and D1 not significantly different, B1, C1 and E1 not significantly different from each other, while A1 and D1 significantly different with B1, C1 and E1 ($p<0.05$).

Table 1: DPPH scavenging activity in n-hexane leaves extracts

Sample	Scavenging DPPH activity (%)
A1	8.42 ± 10.63 a
B1	23.35 ± 3.32 b
C1	23.18 ± 5.47 b
D1	7.73 ± 2.82 a
E1	19.39 ± 5.07 b
P value	< 0.05

a-b = means within a column with the same letter were not significantly different (p=0.05).

Scavenging radical DPPH activity among ethyl acetate leaves extract (Table 2) indicated that C2, D2 and E2 not significantly different from each other, while A2 significantly different with B2 and both of them significantly different with C2, D2, and E2 ($p< 0.05$).

In ethanolic leaves extract, statistical analysis were shown in Table 3 indicated that A3 and B3 not significantly different, D3 and E3 not significantly different from each other, while C3 significantly different with A3, B3, D3 and E3 ($p<0.05$). In previous study was reported that methanolic extract of purple tubers had the highest DPPH antioxidant activity [3].

Table 2: DPPH scavenging activity in ethyl acetate leaves extracts

Samples	Scavenging DPPH activity (%)
A2	77.96 ± 6.34 a
B2	65.47 ± 7.52 b
C2	90.01 ± 1.77 c
D2	91.47 ± 5.72 c
E2	93.26 ± 0.23 c
P value	< 0.05

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

Table 3: DPPH scavenging activity in ethanolic leaves extracts

Sample	Scavenging DPPH activity (%)
A3	97.63 ± 0.6 a
B3	93.34 ± 1.9 a
C3	34.28 ± 4.96 b
D3	50.04 ± 3.03 c
E3	59.68 ± 12.44 c
P value	< 0.05

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

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 in the different varieties of *Ipomoea batatas* leaves shown different result in the range of 15.43 - 59.79 g QE/100 g. The total flavonoid contents was highest (59.79 g QE/100 g) for B2 leaves extracts, followed by E2 leaves extract (56.52 g QE/100 g), and the lowest (15.43 g QE/100 g) for E3 leaves extract.

Total flavonoid contents among n-hexane extract leaves (Table 4) indicated that C1 and E1 not significantly different from each other. A1, B1, D1 significantly different from each other and all of them significantly different with C1 and E1 (p<0,05).

Statistical analysis for total flavonoid contents among in ethyl acetate leaves extract were shown in Table 5. A2 and C2 not significantly different from each other, B2, D2 and E2 significantly different from each other (p<0.05) and all of them significantly different with A2 and C2 (p< 0.05).

Table 4: Total flavonoid, phenolic and carotenoid in n-hexane leaves extracts

Sample	Total Flavonoid (g QE/ 100 g)	Total Phenolic (g GAE/100 g)	Total carotenoid (g BCE/100 g)
A1	49.99 ± 2.23 a	ND	10.39 ± 0.08 a
B1	42.71 ± 2.73 b	ND	10.94 ± 0.03 b
C1	36.50 ± 1.60 c	ND	12.25 ± 0.13 c
D1	48.52 ± 1.90 d	ND	8.28 ± 0.05 d
E1	37.14 ± 0.33 c	ND	17.32 ± 0.06 e
P value	< 0.05		< 0.05

a-e = means within a column with the same letter are not significantly different (p=0.05). ND = non detectable

Table 5: Total flavonoid, phenolic and carotenoid in ethyl acetate leaves extracts

Sample	Total Flavonoid (g QE/ 100 g)	Total Phenolic (g GAE/100 g)	Total carotenoid (g BCE/100 g)
A2	46,87± 1,32 a	8,14 ± 0,37 a	8,25 ± 0,01 a
B2	59,79 ± 0,50 b	ND	8,528 ± 0,02 a
C2	46,28 ± 0,83 a	ND	24,17 ± 0,08 b
D2	51,88 ± 0,11 c	6,66 ± 2,88 a	10,74 ± 0,04 c
E2	56,52 ± 0,45 d	ND	11,03 ± 0,32 d
P value	< 0.05	< 0.05	< 0.05

a-d = means within a column with the same letter are not significantly different (p=0.05). ND = non detectable

Table 6: Total flavonoid, phenolic and carotenoid in ethanol leaves extracts

Sample	Total Flavonoid (g QE/ 100 g)	Total Phenolic (g GAE/100 g)	Total carotenoid (g BCE/100 g)
A3	29,72 ± 0,12 a	13,8 ± 1,50 a	11,95 ± 0,07 a
B3	20,45 ± 0,14 b	19,64 ± 0,92 b	10,94 ± 0,003 b
C3	49,94 ± 0,27 c	5,25 ± 2,10 c	0,685 ± 0.00 c
D3	24,79 ± 0,20 d	11,85 ± 2,73 a	0,414 ± 0.00 d
E3	15,43 ± 0,26 e	15,74 ± 1,30 a	0,79 ± 0,016 e
P value	< 0.05	< 0.05	< 0.05

a-e = means within a column with the same letter are not significantly different (p=0.05).

The total flavonoid contents in ethanolic leaves extract (Table 6) indicated that all of samples A3, B3, C3, D3 and E3 significantly different from each other ($p<0.05$).

Total phenolic of various extracts of sweet potatoes leaves

The presence of total phenolic might contribute to antioxidant activity in *Ipomoea batatas* leaves [8]. 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 in the different varieties of *Ipomoea batatas* leaves shown different result ranged from 0 to 19.64 g GAE/100 g.

B3 leaves extract (ethanolic leaves extract of purple tubers) had the highest phenolic contents (19.64 g GAE/100g), followed by E3 leaves extract (15.74 g GAE/100 g). The methanolic extract of purple tubers had the highest total phenolic content and the lowest for methanolic extract of white tubers [3].

Statistical analysis for total phenolic contents among in ethyl acetate leaves extract were shown in Table 5. A2 and D2 not significantly different from each other. The total phenolic contents in ethanol leaves extract (Table 6) indicated that A3, D3 and E3 not significantly different from each other. B3 and C3 significantly different from each other and both of them significantly different with A3, D3 and E3 ($p<0.05$).

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 in the different varieties of *Ipomoea batatas* leaves shown different result in the range of 0.41-24.17 g BET/100 g.

The highest carotenoid contents (24.17 g BET/100 g) for C2 leaves extract, followed by E1 leaves extract (17.32 g BET/100 g), while the lowest carotenoid (0.41 g BET/100 g) for D3 leaves extract. In the previous study n-hexane extract of orange tubers had the highest total carotenoid and the lowest for methanolic extract of white tubers [3].

Statistical analysis for carotenoid contents in n-hexane leaves extract were shown in Table 4. All of samples (A1, B1, C1, D1 and E1) significantly different ($p<0.05$) from each other. Total carotenoid contents in ethyl acetate leaves extract indicated that A2 and B2 not significantly different. C2, D2 and E2 significantly different from each other ($p<0.05$) and all of them significantly different with A2 and B2 ($p<0.05$). In ethanolic leaves extract, the total carotenoid contents for all of samples (A3, B3, C3, D3 and E3) significantly different from each other ($p<0.05$).

Correlations between total phenolic, flavonoid, carotenoid contents and DPPH scavenging activity

Pearson's correlation coefficient was positively high if $0.61 \leq r \leq 0.97$ [13]. The highest correlation between total phenolic content and DPPH scavenging activity ($r = 0.951$, $p<0.01$) for sample A, followed by sample B ($r = 0.792$, $p<0.05$) (Table 7). There was no correlation between total phenolic content and DPPH scavenging activity for sampel C, D and E. Negative correlation between total flavonoid and DPPH scavenging activity that given by sample A ($r = -0.772$, $p< 0.05$) and there were no correlation between total flavonoid and DPPH scavenging activity for sample B, C, D and E.

Table 7: Pearson's correlation coefficient of total phenolic, total carotenoid, total flavonoid and DPPH scavenging activity of leaves extract from five varieties sweet potatoes tubers

Trait	TPH	TBC	TFL
TBC	-0.419**		
TFL	-0.761**	0.242^{ns}	
DPPH A	0.951**	0.121^{ns}	-0.772*
DPPH B	0.792 *	-0.116^{ns}	-0.453^{ns}
DPPH C	-0.307^{ns}	0.778*	0.3^{ns}
DPPH D	0.489^{ns}	0.222^{ns}	0.107^{ns}
DPPH E	0.048^{ns}	-0.416^{ns}	0.415^{ns}
DPPH AS	0.481**	0.178^{ns}	-0.086^{ns}

TPH = total phenolic, TBC = total carotenoid, TFL= total flavonoid, DPPH = DPPH scavenging activity, A= sample A, B = sample B, C = sample C, D = sample D, E = sample E, AS = all of samples, ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$

The result of Pearson's correlation coefficient indicated that DPPH scavenging activity of sample A had correlation with total phenolic and total flavonoid contents. Sample A was the leaves of sweet potatoes with red-purple tubers, contain the highest total phenolic and total flavonoid from 3 extracts (n-hexane extract A1, ethyl acetate extract A2 and ethanolic extract A3).

Phenolic compound included tannins, flavonoid, phenolic acid and other compounds that had phenolic structure. Flavonoid not always be phenolic compounds its depending on position of OH in flavonoid. Only flavonoid that had OH in A ring and or B ring would be said as phenolic groups. Phenolic acid had the lower antioxidant activity than flavonoid [5]. Cinnamic acid had antioxidant activity higher than phenyl acetic acid and benzoic acid. Flavonoid would give antioxidant activity which has OH in ortho C 3',4', OH in C 3, oxo function in C 4, double bond at C 2 and C 3. The OH with ortho position in

C3'-C4' had the highest influence to antioxidant activity of flavonoid. Flavonoid had OH in C 3 and double bond at C2 - C3 gave higher antioxidant activity than flavonoid had only OH in C 3. The flavonoid aglycones would give higher antioxidant activity than flavonoid glycosides [5].

The positively high correlation between total carotenoid and scavenging DPPH activity ($r = 0.778$, $p < 0.05$) for sample C that was leaves of sweet potatoes with yellow tubers, that contain the highest total carotenoid from 3 extracts (C1, C2 and C3). The total carotenoid of leaves extract from sampel C might have correlations with yellow color of tubers. There were no correlation between total carotenoid and DPPH scavenging activity for sample A, B, D and E.

For all of samples (A, B, C, D and E) the result of Pearson's correlation coefficient indicated that there were negative correlation between total phenolic and total flavonoid ($r = -0.761$, $p < 0.01$) and between total phenolic and total carotenoid ($r = - 0.419$, $p < 0.01$).

CONCLUSIONS

In the present study, antioxidant activities varied widely among the various extracts of sweet potatoes leaves from five varieties tubers colors. The highest DPPH scavenging activity was given by sample A3 that was ethanolic extract from leaves of sweet potatoes with red-purple tubers.

The highest correlation between total phenolic content and scavenging radical DPPH activity in sample A, followed by sample B and no correlation in sampel C, D and E. There was negative correlation between total flavonoid and DPPH scavenging activity that given by sample A and no correlation in sample B, C, D and E. The positively high correlation between total carotenoid and DPPH scavenging activity in sample C and no correlation in sample A, B, D and E.

In conclusion, the n-hexane, ethyl acetate and ethanol extract of sweet potatoes leaves from five varieties tubers colors of *Ipomoea batatas* exhibited potent in *in vitro* antioxidant activity. The plants merits further investigation in preclinical test using animal models to confirm the antioxidants activity, and to isolate the active constituent from *Ipomoea batatas* leaves extract.

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