Correlation of phytochemical content with antioxidant potential of various sweet potato (*Ipomoea batatas*) in West Java, Indonesia

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**ABSTRACT**

**Objective:** To determine antioxidant activity and phytochemical content from various tubers extracts of sweet potato (*Ipomoea batatas*) and to explore the correlation of phytochemical content with their antioxidant activities. **Methods:** Antioxidant activities were tested using DPPH and FRAP assays. Total phenolic was calculated by Folin-Ciocalteu reagent, flavonoid content by Chang’s method and correlation with their antioxidant activities were analyzed by Pearson’s method. **Results:** PO2 showed highest antioxidant activity, which had the lowest IC$_{50}$ DPPH (10.54 µg/mL) and the lowest EC$_{50}$ FRAP (11.14 µg/mL). PO2 showed the highest total phenolic (11.91 g GAE/100 g) and total flavonoid content (17.83 g QE/100 g). There were significantly negative correlation between total phenolic content and flavonoid content in sample PO with their IC$_{50}$ DPPH and EC$_{50}$ FRAP. IC$_{50}$ DPPH of sample PP and PO showed significantly positive correlation with their EC$_{50}$ FRAP. **Conclusions:** Result of DPPH method shows that all different ethyl acetate and ethanolic tubers extracts of four varieties of sweet potato are classified as strong and very strong antioxidant. Result of DPPH and FRAP methods indicates that phenolic and flavonoid compounds in sample PO contributes together to antioxidant activities. Antioxidant activities of sample PP and PO by DPPH method are linear to FRAP method.

**1. Introduction**

Phenolic and flavonoids compounds are found in many plants which have antioxidant and antibacterial activity[1,2]. Phenolic and flavonoid compounds might have antioxidant capacity[3–6]. They can prevent excessive free radical in oxidative stress that causes many degenerative diseases. Consumption of fruits and vegetables can prevent negative effect of oxidative stress, because they contain phenolic and flavonoid compounds which have antioxidant capacity[7,8]. Previous researches report that antioxidant activities can be influenced by phenolic and flavonoid content[9,10]. Many plants including sweet potato, tea, legumes and guava contain phenolic and flavonoid compounds[5,6,11,12].

2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and 2,2’-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS) methods could be used to determine antioxidant activity in many plants extracts[12]. The previous researches[8,10,12] present that antioxidant activity in fruits, vegetables and food can be evaluated by DPPH, ABTS and FRAP methods. Sweet potato leaves had higher antioxidant activity than sweet potato tubers[13]. Researches by using ORAC, DPPH and ABTS methods show that tubers of sweet potato with varieties colors (white, cream, yellow, orange and purple) has antioxidant activity[14]. Antioxidant activities...
of leaves from six different places of *Ipomoea batatas* (*I. batatas*) in Malaysia and its correlation to total phenolic and flavonoid content are performed by DPPH method\(^\text{[15]}\), but there is no information about color of tubers in this research.

There is no research regarding antioxidant activity of tubers from different varieties of sweet potato (*I. batatas*) which are extracted using increasing polarity solvents (*n*-hexane, ethyl acetate and ethanol) and tested by DPPH and FRAP assays.

The goals of this study are to observe antioxidant potential in various polarity extracts (*n*-hexane, ethyl acetate and ethanol) of tubers from different varieties of sweet potato grown in Tasikmalaya, West Java, Indonesia using DPPH and FRAP assays, and to explore correlations of total phenolic and flavonoid content with their antioxidant activities.

### 2. Materials and methods

#### 2.1. Materials

DPPH, 2,4,6-tripyridyl-S-triazine (TPTZ), gallic acid, quercetin, were purchased from Sigma-Aldrich (MO, USA). All of other reagents were analytical grades.

#### 2.2. Sample preparation

Tubers of four varieties of sweet potato (*I. batatas*) with their peel-flesh color purple-purple were named as PP, purple-orange as PO, yellow-yellow purple as YYP and yellow-yellow orange as YYO. They were collected from Tasikmalaya, West Java, Indonesia, and were washed, sorted, cut, dried and grinded into powder.

#### 2.3. Extraction

Powdered crude drug (300 g) was extracted by reflux. The first extraction used *n*-hexane (three times). Ethyl acetate was used to extract the residue (three times). Then the residue was extracted three times using ethanol. Hence totally there were twelve extracts: *n*-hexane extracts (PP1, PO1, YYP1 and YYO1), ethyl acetate extracts (PP2, PO2, YYP2 and YYO2), and ethanolic extracts (PP3, PO3, YYP3 and YYO3).

#### 2.4. Antioxidant activity by DPPH assay

Antioxidant activity by DPPH assay was determined by Blois’s method\(^\text{[16]}\) with minor modifications. Each extract was prepared in various concentrations. Two mL of each concentration was added into 2 mL DPPH 50 µg/mL. After 30 min incubation, the absorbance was observed at λ 515 nm by UV-Vis spectrophotometer Hewlett Packard 8435. DPPH 50 µg/mL was used as control, methanol as a blank and ascorbic acid as standard. Analysis was performed in triplicate for each extract and standard. Antioxidant activity was determined by calculating percentage of reduction of DPPH absorbance. Based on its calibration curve the inhibitory concentration 50% (IC\(_{50}\)) of DPPH scavenging activity of each extract could be calculated.

#### 2.5. Antioxidant capacity by FRAP assay

FRAP solution was prepared using Benzi’s method\(^\text{[17]}\), in acetate buffer with pH 3.6. Two mL of variation concentration of each extract was added into 50 µg/mL FRAP. After 30 min incubation, the absorbance was measured at λ 593 nm. Fifty µg/mL FRAP was used as control, acetate buffer as a blank, and ascorbic acid as standard. Analysis was carried out in triplicate for each extract and standard. Antioxidant capacity was determined based on increase in Fe (\([\text{III}]\)) -TPTZ absorbance by calculating percentage of antioxidant capacity. Exhibitory concentration 50% (EC\(_{50}\)) of FRAP capacity of each extract could be determined using its calibration curve.

#### 2.6. Total phenolic content (TPC)

Total phenolic content was determined using Folin-Ciocalteu reagent\(^\text{[18]}\). The absorbance was observed at wavelength of 765 nm. Analysis was conducted in triplicate for each extract. Standard solution of gallic acid (80-200 µg/mL) was used to obtain a calibration curve. Total phenolic content was reported as gram gallic acid equivalent per 100 g extract (g GAE/100 g).

#### 2.7. Total flavonoid content (TFC)

Total flavonoid content was measured using Chang’s method\(^\text{[19]}\) with minor modifications. The absorbance was read at λ 415 nm. Three replications were performed for each extract. Quercetin solution (40-120 µg/mL) was used to obtain a calibration curve. Total flavonoid content was expressed as gram quercetin equivalent per 100 g extract (g QE/100 g).

#### 2.8. Statistical analysis

Three replications were conducted for each sample. All of presented results were presented as means±standard deviation. Statistical analysis was performed by SPSS 16 for Windows using ANOVA and followed by post-hoc Tukey. Correlation between the total phenolic, flavonoid, content and antioxidant activity, and correlation between two antioxidant testing methods were conducted using the Pearson’s method.

### 3. Results

#### 3.1. Antioxidant activity by DPPH and FRAP assays

IC\(_{50}\) of DPPH scavenging activities and EC\(_{50}\) of FRAP capacities
were determined in various extracts of sweet potato tubers with ascorbic acid as standard. The highest antioxidant activity expresses the lowest IC_{50} or EC_{50}. PO2 showed the lowest IC_{50}, DPPH (Table 1) and the lowest EC_{50}, FRAP (Table 2).

Table 1

| Sample | n-hexane extract | Ethyl acetate extract | Ethanol extract |
|--------|------------------|-----------------------|----------------|
| PP     | 152.73±0.1270 g QE/100 g | 13.88±0.0820 g QE/100 g | 81.66±0.0120 g QE/100 g |
| PO     | 105.99±0.2510 g QE/100 g | 10.54±0.0660 g QE/100 g | 76.02±0.1490 g QE/100 g |
| YYP    | 117.80±0.1410 g QE/100 g | 66.27±0.1680 g QE/100 g | 98.18±0.6500 g QE/100 g |
| YYO    | 161.55±0.3550 g QE/100 g | 75.22±0.8280 g QE/100 g | 45.05±0.4300 g QE/100 g |
| Ascorbic acid | 2.87±0.0013 g QE/100 g | 2.87±0.0016 g QE/100 g | 2.67±0.0013 g QE/100 g |

Different letter in the same column showed significant difference (P<0.05).

3.2. TPC in sweet potato extracts

The linear regression equation of gallic acid (y=0.004 x-0.051, R²=0.998) was used to calculate TPC in various extracts of sweet potato tubers, hence the TPC was expressed as gallic acid equivalent. TPC in sweet potato tubers extracts showed different results in the range of 1.05-11.91 g GAE/100 g (Table 3). PO2 had the highest TPC in sweet potato tubers extracts showed different results in the range of 1.05-11.91 g GAE/100 g (Table 3). PO2 had the highest TPC (11.91 g GAE/100 g), followed by PP2 (9.44 g GAE/100 g), and the lowest TPC (0.60 g GAE/100 g) by PP3 (Table 4).

Table 2

| Sample | n-hexane extract | Ethyl acetate extract | Ethanol extract |
|--------|------------------|-----------------------|----------------|
| PP     | 153.40±0.8400 g GAE/100 g | 33.96±0.1300 g GAE/100 g | 64.33±0.1100 g GAE/100 g |
| PO     | 75.24±0.3600 g GAE/100 g | 11.14±0.0900 g GAE/100 g | 141.47±0.6300 g GAE/100 g |
| YYP    | 147.77±0.4800 g GAE/100 g | 49.94±0.8800 g GAE/100 g | 192.41±0.1200 g GAE/100 g |
| YYO    | 127.11±0.2500 g GAE/100 g | 70.06±0.5200 g GAE/100 g | 210.98±0.7200 g GAE/100 g |
| Ascorbic acid | 2.48±0.0009 g QE/100 g | 2.48±0.0009 g QE/100 g | 2.48±0.0009 g QE/100 g |

Different letter in the same column showed significant difference (P<0.05).

3.3. TFC in sweet potato extracts

Quercetin was used as standard. TFC among various extracts of sweet potato was expressed as quercetin equivalent, using the linear regression equation of quercetin (y=0.009 x-0.155, R²=0.998). TFC in sweet potato tubers extracts varied from 0.60 to 17.83 g QE/100 g. The highest TFC (17.83 g QE/100 g) was presented by PO2, followed by YYP2 (13.72 g QE/100 g), and the lowest TFC (0.60 g QE/100 g) by PP3 (Table 4).

Table 3

| Sample | n-hexane extract | Ethyl acetate extract | Ethanol extract |
|--------|------------------|-----------------------|----------------|
| PP     | 12.79±0.0900 g QE/100 g | 4.96±0.0500 g QE/100 g | 0.60±0.0100 g QE/100 g |
| PO     | 10.07±0.0900 g QE/100 g | 17.83±0.0020 g QE/100 g | 0.68±0.0020 g QE/100 g |
| YYP    | 12.80±0.1000 g QE/100 g | 13.72±0.0100 g QE/100 g | 2.45±0.0030 g QE/100 g |
| YYO    | 10.11±0.0200 g QE/100 g | 10.81±0.0000 g QE/100 g | 2.53±0.0100 g QE/100 g |

Different letter in the same column showed significant difference (P<0.05).

3.4. Correlations between total phenolic, flavonoid content in sweet potato tubers extracts with their antioxidant activities

TPC in various tubers extracts of sample PO, YYP and YYO had significantly negative correlation with their EC_{50} FRAP capacities (r=-0.974, r=-0.849, r=-1.000, P<0.01, respectively). TFC in tubers extracts of sample PO, YYP and YYO also showed negative and significant correlation with their EC_{50} FRAP capacities (r=-0.999, r=-0.789, r=-0.943, P<0.01, respectively) (Table 5).

4. Discussion

The phytochemical content and antioxidant activities among the extracts can be compared if the density of the extracts are similar. Therefore all extracts in the present study should be prepared in similar density. DPPH free radicals dissolve in methanol and show absorption at
of each extract. The percent of DPPH scavenging activity could not present the real antioxidant activities. Antioxidant activity can be expressed in percentage of DPPH scavenging activity, which is conducted normally by adding extract with concentration of 50 µg/mL only to 50 µg/mL DPPH solution (volume 1: 1). If the 50 µg/mL extract can scavenge 42% of the 50 µg/mL DPPH, it does not mean 60 µg/mL extract will always scavenge more than 42% DPPH. The 60 µg/mL extract may present the percentage of DPPH greater than 42% or lower than 42%. It’s due to extract consisted of many compounds and not all compounds in extract have antioxidant activities, some of which may act as antagonist of antioxidant. In 50 µg/mL extract, the compounds which can act as antagonist antioxidant have not achieved yet its effective concentration; therefore the antioxidant components can scavenge 42% DPPH. In 60 µg/mL extract, the antagonist of antioxidant components might have achieved its effective concentration, it will reduce the ability of antioxidant components, therefore 60 µg/mL extract will give percentage of DPPH lower than 42%.

Antioxidant activity can be stated as IC50. Many concentrations of extract should be used for calculating IC50, which show linear decreasing in absorbance of DPPH. Based on the results, the linear regression equation of calibration curve of each extract can be calculated. After determining linear regression equation, value of IC50 DPPH can be observed. Based on the explanation above, it can be seen that the percentage of DPPH scavenging activities will not represent the real antioxidant activities though the real of antioxidant activities will be exposed by IC50 DPPH value. Antioxidant activity can also be stated by using µmol or mmol trolox equivalent per g sample. Trolox is a standard that be used in antioxidant activity. The largest µmol or mmol trolox equivalent per g sample showed the highest antioxidant activity.

Antioxidant capacities by FRAP assay are expressed by EC50 of FRAP capacity. EC50 is exibitory concentration 50%, concentration of antioxidant sample that can increase FRAP capacity 50% and expressed by increasing absorbance of complex of Fe (III)-TPTZ after adding antioxidant sample. Linear regression equation of calibration curve of each extract can be used to calculate EC50 FRAP.

The present research shows that all of ethyl acetate tubers extracts of sweet potato (I. batatas) have EC50 FRAP capacity in the range of 11.14-70.06 µg/mL and it means their antioxidant can be classified as very strong antioxidant (< than 50 µg/mL) by FRAP assay, except YYO2 (70.06 µg/mL) as strong antioxidant. PO2 has EC50 FRAP of 11.14 µg/mL, while ascorbic acid standard has EC50 FRAP of 2.49 µg/mL. It means antioxidant potential of ascorbic acid is around five times antioxidant potential of PO2 by FRAP assay. Reducing power of methanolic leaves extract of I. batatas var. Indon (with purple flesh color) had higher antioxidant activity than the other flesh color varieties[15]. Previous research[14] stated that methanolic fraction (hydrophilic fraction) of dark purple flesh sweet potato clones (NC415 and 13-18) had higher ORAC values (27 and 23 µmol TE/g fresh weight, respectively) compared to the other clones. It was
similar to their antioxidant activity by ABTS method which showed NC 415 gave the highest antioxidant activity (1.6 mmol TE/g fresh weight).

Teow et al. reported that TPC of methanolic tubers extract of different flesh sweet potato varied from 0.003 mg chlorogenic acid equivalent (CAE)/g fresh weight to 0.949 mg CAE/g fresh weight. The highest TPC (0.949 mg CAE/g fresh weight) was given by sample 13-18 (with purple flesh color) and the lowest (0.003 mg CAE/g fresh weight) showed by sample Xushu 18 (with white flesh color). Anthocyanin, one of the phenolic compounds in plant, was also determined in this research. Sample 13-18 gave the highest total anthocyanin (0.531 mg/g fresh weight) compared to the other sample[14]. Previous research revealed that methanolic leaves extract of different varieties of sweet potato presented that TPC of \( I. batatas \) var. Indon (with purple flesh color) showed the highest TPC (5.35 g GAE/100 g) compared to the other varieties[15]. It was similar to the present study which denoted that PP3 had higher TPC (1.59 g GAE/100 g) than PO3 (1.05 g GAE/100 g). The result was also similar to the previous research[21] which showed that ethanolic leaves extract of purple sweet potato gave the highest TPC (19.64 g GAE/100 g). TFC of methanolic leaves extract of \( I. batatas \) var. Batu Bisa 263.5 µg catechin equivalent/g was higher than the other varieties[15]. It was similar to the present study which exhibited that TFC of PO (0.68 g QE/100 g) was higher than TFC of PP3. Previous research also figured that ethanolic leaves extract of yellow flesh sweet potato had higher TFC than the other ethanolic leaves extract[21].

The glycoside of flavonoid demonstrates lower antioxidant activity than its aglycone. Flavonoid which had ortho di OH at C-3’-C4’, OH at C-3, C-4 o xo, double bond at C-2 and C-3 showed high antioxidant activity[22]. The most significant influence of di OH C-3’-C-4’. TFC in YY2 (2.45 g QE/100 g) was similar to TFC in YY3 (2.53 g QE/100 g), but antioxidant activity of YY3 by DPPH assay was higher than YY3. YY3 with IC\(_{50}\) DPPH value of 45.05 µg/mL could be categorized as very strong antioxidant (\( \leq 50 \) µg/mL) and YY3 with IC\(_{50}\) DPPH value of 98.18 µg/mL as strong antioxidant (50-100 µg/mL). Based on this result it can be predicted that many flavonoids in YY3 have high antioxidant activity, with ortho di OH at C-3’-C4’, OH at C-3, oxo function at C-4, double bond at C-2 and C-3, while many flavonoids in YY2 with other position have low antioxidant activity. TPC in YY2 (6.89 g GAE/100 g) was higher than TPC in YY3 (2.49 g GAE/100 g), but antioxidant activity of YY3 by DPPH assay expressed by IC\(_{50}\) DPPH value denoted that IC\(_{50}\) DPPH of YY3 45.05 µg/mL (very strong antioxidant) was lower than IC\(_{50}\) DPPH of YY2 75.22 µg/mL (strong antioxidant). The result figured that YY3 contained most phenolic compounds with high antioxidant activity, while in YY2 with low antioxidant activity. PP3 contained higher phenolic content with 1.59 g GAE/100 g than PO3 (1.05 g GAE/100 g). Anthocyanin compound was predicted in sample PP (purple peel and purple flesh color). Anthocyanin is phenolic group which is also included in flavonoid compound and is correlated with antioxidant activities and categorized as flavonoid glycoside. In the above statement, it can be seen that flavonoid aglycone has higher antioxidant activities than flavonoid glycoside. In addition, only flavonoid which has certain OH position will give high antioxidant activities. Based on result of the present study, antioxidant activity of PP3 by DPPH method was lower than antioxidant activity of PO3. It suggests that many anthocyanin compounds in PP3 do not have ortho di OH at C3’-C4’ which have high antioxidant activity.

TPC in PP3 (1.59 g GAE/100 g) was similar to TPC in YY2 (1.73 g GAE/100 g), but EC\(_{50}\) FRAP of YY3 (192.41 µg/mL) was higher than EC\(_{50}\) FRAP of PP3 (64.53 µg/mL). The result showed that antioxidant activity of PP3 was higher than YY3 by FRAP assay. It suggests that majority phenolic compounds in PP3 have lower reduction potential than Fe ([Fe(II)]/Fe ([III]) 0.77 V, meanwhile many phenolic compounds in YY3 have reduction potential greater than 0.77 V. Sample will be oxidized. At the same time Fe ([II]) reduces to Fe ([I]) and then the Fe ([I]) reacts with TPTZ, producing blue color complex.

The lowest IC\(_{50}\) DPPH scavenging activity and EC\(_{50}\) FRAP capacity will reveal the highest antioxidant activity. Coefficient of Pearson correlation was significantly negative if 0.61 \( \leq r \leq -0.97 \) and significantly positive if 0.61 \( \leq r \leq 0.97 \)[12]. It means increase in TFC and TPC causes increase in antioxidant activities, which is stated by lower IC\(_{50}\) DPPH and/or EC\(_{50}\) FRAP. Therefore the good correlation between TPC and TFC with IC\(_{50}\) DPPH or EC\(_{50}\) FRAP was significant and negative[23]. The present study showed that TPC in sample PP had significant and negative correlation with their IC\(_{50}\) DPPH (\( r = -0.744, P < 0.05 \)). It can be predicted that phenolic compounds are the main contributor in antioxidant activities of sample PP by DPPH method. TPC and TFC in sample PO had significantly negative correlation with their antioxidant activities by DPPH method (\( r = -0.814, P < 0.01; r = 0.629, P < 0.05 \), respectively). Antioxidant activity had similar result by FRAP method, the TPC and TFC in sample PO showed negative and significant correlation with their EC\(_{50}\) FRAP (\( r = -0.974 ; r = 0.999, P < 0.01 \), respectively). It suggests that phenolic and flavonoid compounds are the major contributors in their antioxidant activities by FRAP method.

The present study also revealed that IC\(_{50}\) DPH of sample PP and PO had significantly positive correlation with their EC\(_{50}\) FRAP (\( r = 0.966, P < 0.01 ; r = 0.664, P < 0.05 \)). It means antioxidant activity of sample PP and PO are linearly correlated in DPPH and FRAP methods. The previous research studied the correlation between two antioxidant testing methods by determining linear regression of calibration curve, while in the present study Pearson’s coefficient correlation was used. Antioxidant activities of nineteen sweet potato with varying flesh colors by DPPH method were linearly correlated to ABTS method (\( R^2 = 0.822 \)). The linear correlations were also given by hydrophilic ORAC and DPPH method (\( R^2 = 0.859 \)), as well as hydrophilic ORAC and DPPH method (\( R^2 = 0.761 \))[14].

Determination of antioxidant activities by various methods may
lead to different results. DPPH assay result shows that all different ethyl acetate and ethanolic tubers extracts of four sweet potato varieties vary from strong to very strong antioxidant activities. DPPH and FRAP assays indicate that phenolic and flavonoid compounds in sample PP and PO contribute together to their antioxidant activities. DPPH and FRAP methods gave linear result in antioxidant activities of sweet potato PP and PO. Tubers of four sweet potato varieties (PP, PO, YYP, and YYO) have many benefits to prevent oxidative stress and potential as sources of natural antioxidant for further exploitation.

Conflict of interest statement

We declare that we have no conflict of interest.

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