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Research Article
Potential Antioxidative Activity of Waste Product of Purple Sweet Potato (*Ipomoea batatas* Lam.)

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Abstract

Background and Objective: Antioxidants are substances that can deactivate free radicals. Phenol and flavonoid are antioxidant compounds widely found in plants, including purple sweet potato (*Ipomoea batatas* L.). This research aimed to investigate three purple sweet potato-based organs' antioxidative activity and flavonoid contents. **Materials and Methods:** Antioxidative activities, total phenolic content and total flavonoid content were performed by UV-visible spectrophotometry. Pearson's method analyzed the correlation of TPC and TFC with antioxidative activities and the correlation between two antioxidative testing methods. **Results:** Antioxidative activity of three organs purple sweet potato using DPPH method showed values varied from 6.572-290.894 mg AAE g^{-1} and using CUPRAC method varied from 25.169-621.254 mg AAE g^{-1}. The highest TPC was found in ethanolic leaves extract (20.885 g GAE 100 g^{-1}), while the highest TFC was found in ethyl acetate leaves extract (10.048 g QE 100 g^{-1}). **Conclusion:** DPPH and CUPRAC tests revealed that purple sweet potato leaves, stem and tuber extracts were potent antioxidants. The potential antioxidative activity was found in the waste product of purple sweet potatoes (leaves and stem). Phenol and flavonoid compounds had contributors to antioxidative activity. DPPH and CUPRAC methods gave linear results for most of the antioxidative activity in three organs of purple sweet potato. Ethanol stem extract contained luteolin 7-O-glucoside, rutin, quercetin, kaempferol and apigenin. Rutin had the highest content, which was 0.399%.

Key words: Antioxidant, sweet potato, organs, DPPH, CUPRAC

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Reactive Oxygen Species (ROS) may cause damage to cell components such as lipids and membranes, nucleic acid and proteins, which are also referred to as oxidative stress[1]. Although there is a cellular antioxidant defence system (endogenous antioxidants) to fight oxidative damage from ROS, oxidative damage accumulates during the life cycle. It contributes to several diseases such as cancer, cardiovascular disease, nervous system dysfunction, neurodegenerative disorders and other chronic diseases[2]. One of the sources of exogenous antioxidants is plants, including purple sweet potato, that can prevent excess free radicals.

Purple sweet potato (*Ipomoea batatas* L.) contains many phenolic and flavonoid compounds: Quercetin, myricetin, kaempferol, luteolin, anthocyanin, hydroxycinnamic acid, hydroxybenzoic acid[3,4]. Several studies showed that phenolic and flavonoid compounds had antioxidant[5], anti-diabetic[6], anti-cancer[7], anti-ulcer[8], antimicrobial[9] and anti-inflammatory activities[10].

The antioxidative activity of purple sweet potato was associated with various bioactive compounds present in purple sweet potato. The antioxidative activity was positively correlated with total phenolic content[11]. The antioxidant synergy among various components resulted in the antioxidative activity of purple sweet potato extract[5]. Although, research discussing the comparison between three organs of purple sweet potato (leaves, stem and tubers) using three solvents with graded polarity has not been reported yet. Purple sweet potato leaves and stems are waste products that might have the same antioxidant activities as the tubers.

This study aimed to analyze the antioxidative activity of three purple sweet potato organs using the DPPH and CUPRAC methods, total phenolic content (TPC) and total flavonoid content (TFC) between three polarities extracts of purple sweet potato leaves, stem and tubers, also to analyze the correlation between antioxidative activity and their chemical content.

MATERIALS AND METHODS

Study area: The study was conducted in 2022 at the Pharmaceutical Biology Laboratory, Bandung Institute of Technology, Indonesia.

Chemicals: Gallic acid, quercetin, ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and neocuproine from Sigma-Aldrich (MO, USA). Analytical grade compounds were also used.

Sample collection: Three parts obtained from the purple sweet potato plant (*Ipomoea batatas* L.) were leaves (LEV), stem (STM) and tubers (TBR). The parts were collected and identified in Research Institute for Various Nuts and Tubers Malang, East Java, Indonesia. The sample was sorted, washed, dried and milled into powder.

Extract preparation: Each sample was extracted using the reflux method using different polarity solvents consisting of n-hexane, ethyl acetate and ethanol. Samples (300 g) were extracted three times for each solvent. The liquid extract was then concentrated with a rotatory evaporator to obtain the concentrated extract. There were three n-hexane extracts (LEV1, STM1 and TBR1), three ethyl acetate extracts (LEV2, STM2 and TBR2) and three ethanol extracts (LEV3, STM3, dan TBR3).

***In vitro* antioxidant activities by DPPH assay:** DPPH method was used to examine the antioxidative activity with a slightly modified Celep *et al.*[12]. Ascorbic acid was used as the standard, DPPH 50 μg mL^{-1} as the control and methanol pro analysis as the blank. Samples were prepared in several concentrations. To 12.5 μL sample was added by 112.5 μL of methanol pro analysis and 750 μL of DPPH 50 μg mL^{-1}. The mixture was incubated for 30 min. Then the absorbance was read using a UV-vis spectrophotometer at 517 nm wavelength. Measurements were conducted in six repetitions for each extract. Antioxidative activity can be calculated using the calibration curve of ascorbic acid. The antioxidative activity was reported as mg of ascorbic acid equivalence per g sample (mg AAE/g sample).

***In vitro* antioxidant activities by CUPRAC assay:** Antioxidant assay by CUPRAC method used modified method of Özyürek *et al.*[13]. CUPRAC solution 100 μg mL^{-1} was prepared in ammonium acetate buffer pH 7. Samples were prepared in several concentrations. Ascorbic acid was used as the standard, ammonium acetate buffer as the blank and CUPRAC 100 μg mL^{-1} as the control. To 12.5 μL of the sample, ammonium acetate buffer was added until 250 μL. Then, 750 μL of CUPRAC solution was added. The mixture was incubated for 30 min and then the absorbance was read using a UV-vis spectrophotometer at 450 nm wavelength. Measurements were conducted with six repetitions for each extract. Antioxidative activity can be calculated using the calibration curve of ascorbic acid and reported as mg of ascorbic acid equivalence per g sample (mg AAE/g sample).

Total phenolic content (TPC): Total phenolic content was determined using gallic acid as a standard and Folin-Ciocalteu reagent. Gallic acid was prepared in 60-130 μg mL^{-1} concentration. Gallic acid 0.5 μL was added by 500 μL of 10% Folin-Ciocalteu reagent and 400 μL of 1 M sodium carbonate into a 1.5 mL Eppendorf tube. The mixture was incubated for 15 min and absorbance was read using a UV-vis spectrophotometer at 765 nm wavelength. The same procedure was carried out for the sample. Measurements were conducted with six repetitions for each extract. The total phenol of each extract was calculated using the linear regression equation of the gallic acid calibration curve and reported as g of gallic acid equivalent per 100 g of extract (g GAE 100 g^{-1})[14].

Total flavonoid content (TFC): Determination of total flavonoids was conducted using the Chang method Chang *et al.*[15]. Quercetin was used as the standard in 40-110 μg mL^{-1}. 100 μL quercetin was added by 300 μL of methanol, 560 μL of distilled water, 20 μL of 10% aluminium (III) chloride and 20 μL of 1 M sodium acetate. The mixture was incubated for 30 min before being measured using a UV-vis spectrophotometer at a wavelength of 415 nm. The same procedure was conducted for the sample. Measurements were carried out with six repetitions for each extract. The quercetin calibration curve's linear regression equation was used to quantify the total flavonoid content of each extract. The total flavonoid content expressed as g of quercetin equivalent per 100 g of extract (g QE 100 g^{-1})[15].

Statistical analysis: All the results were averaged, alongside the standard deviation, from at least triplicate experiments. Statistical analysis was done using ANOVA-*post hoc* Tukey ($p<0.05$ and $p<0.01$) by Minitab 20. Pearson's method analyzed the correlation between TPC and TFC in the antioxidant activities and two antioxidant testing methods.

Content of several flavonoids: The content of several flavonoids was determined on the ethanol extract of purple sweet potato stems as the extract with the most significant yield in 1000 μg mL^{-1}. Luteolin-7-O-glucoside, rutin, quercetin, kaempferol and apigenin were used as the standards in 4 μg mL^{-1}. Several flavonoids were identified and determined using the LC-20AD liquid chromatography system with a Shimadzu SPD-20A UV/Vis detector at l 360 nm. The compounds separation was conducted on a column XB-C18 100A (250×4.6 mm id, particle size 5 μm) with methanol (eluent B)-water (eluent A) as a mobile phase through a linear gradient system of 40-60% for 5 min, then the gradient of eluent B was 70% until the 10th min. The gradient of the eluent B was 40% until the 15 min. The analysis was carried out at a 1 mL min^{-1} flow rate, injection volume of 20 μL and column temperature set at 30°C (Shimadzu CTO-2A Oven). The calculation was done using the one-point method by comparing the area under the curve (AUC).

RESULTS

Antioxidant activities of leaves, stems and tubers of purple sweet potato extract were analyzed with the DPPH method. The ascorbic acid calibration curve regression equation in the DPPH method was $y = 12.328x+4.5328$ with $R^2 = 0.997$. The equivalence value with ascorbic acid for DPPH scavenging activity of various purple sweet potato organ extracts showed values ranging from 6.572 to 290.894 mg AAE g^{-1} sample shown in Table 1. The highest antioxidant activity was shown in ethanol leaves extract (290.894±30.739 mg AAE g^{-1} sample).

Antioxidant activities of leaves, stems and tubers of purple sweet potato extract were analyzed with the CUPRAC method. The ascorbic acid calibration curve regression equation in the DPPH method was $y = 8.3923x+12.327$ with $R^2 = 0.998$. The equivalence value with ascorbic acid for CUPRAC scavenging activity of various purple sweet potato organ extracts showed values ranging from 25.492 to 629.048 mg AAE g^{-1} sample shown in Table 2. The highest antioxidant activity was shown in ethanol leaves extract (621.254±15.332 mg AAE g^{-1} sample).

Total phenolic content (TPC) of leaves, stem and tubers extract of purple sweet potato was determined as gallic

Table 1: Antioxidative activities of purple sweet potato organs by DPPH method

	Equivalence with ascorbic acid (mg AAE/g sample)		
Samples	n-Hexane extract	Ethyl acetate extract	Ethanol extract
Leaves	14.712±1.148^{ax}	43.248±3.971^{ay}	290.894±30.739^{az}
Stem	6.572±1.150^{bx}	70.666±5.717^{by}	44.657±3.948^{bz}
Tubers	17.117±0.607^{cx}	23.804±2.480^{cy}	15.818±0.993^{cx}

a-c: Different letter in the same column means significant different ($p<0.05$) and x-y: Different letter in the same row means significant different ($p<0.05$)

Table 2: Antioxidative activities of purple sweet potato organs by CUPRAC method

	Equivalence with ascorbic acid (mg AAE/g sample)		
Samples	n-Hexane extract	Ethyl acetate extract	Ethanol extract
Leaves	37.192±3.498^{ax}	113.026±6.777^{ay}	621.254±15.332^{az}
Stem	25.169±1.058^{bx}	130.184±1.726^{by}	106.293±6.854^{bz}
Tubers	53.449±1.699^{cx}	89.072±3.924^{cy}	26.850±1.386^{cz}

a-c: Different letter in the same column means significant different ($p<0.05$) and x-y: Different letter in the same row means significant different ($p<0.05$)

Table 3: Correlation of TPC and TFC with antioxidative activities

	Pearson's correlation coefficient (r)	
Antioxidative parameters	TPC	TFC
DPPH LEV 1	0.962**	0.975**
DPPH STM 1	0.688*	0.839**
DPPH TBR 1	0.962**	0.639*
DPPH LEV 2	0.679*	0.557^{ns}
DPPH STM 2	0.806**	0.812**
DPPH TBR 2	0.872**	0.995**
DPPH LEV 3	0.624*	0.879**
DPPH STM 3	0.779**	0.976**
DPPH TBR 3	0.928**	0.943**
CUPRAC LEV 1	0.981**	0.968**
CUPRAC STM 1	0.633*	0.879**
CUPRAC TBR 1	0.846**	0.914**
CUPRAC LEV 2	0.828**	0.728*
CUPRAC STM 2	0.923**	0.955**
CUPRAC TBR 2	0.827**	0.911**
CUPRAC LEV 3	0.988**	0.676*
CUPRAC STM 3	0.958**	0.696*
CUPRAC TBR 3	0.871**	0.805**

**Significant at $p<0.01$, *Significant at $p<0.05$ and ^{ns}Not significant

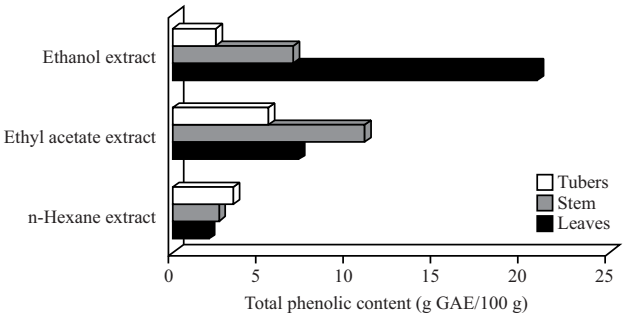

Fig. 1: Total phenolic content in different organs of purple sweet potato

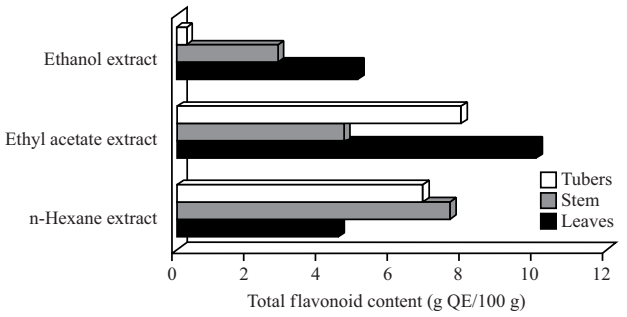

Fig. 2: Total flavonoid content in different organs of purple sweet potato

acid equivalent. The equation for the gallic acid calibration curve regression was $y = 0.0057x-0.0774$ with $R^2 = 0.9916$. TPC values are shown in Fig. 1. LEV3 gave the highest TPC (20,885 g GAE 100 g^{-1}), followed by STM2 (10.992 g GAE 100 g^{-1}) and the lowest level was shown by LEV1 (2.059 g GAE 100 g^{-1}).

Total flavonoid content (TFC) of leaves, stem and tubers extract of purple sweet potato was determined as quercetin equivalent. The equation for the quercetin calibration curve regression was $y = 0.0069x+0.0167$ with $R^2 = 0.9986$. TFC values are shown in Fig. 2. LEV2 gave the highest TFC (10.048 g QE 100 g^{-1}), followed by TBR2 (7.63 g QE 100 g^{-1}) and the lowest level was shown by TBR3 (0.272 g QE 100 g^{-1}).

The correlation analysis between total phenol and flavonoids on antioxidative activity by DPPH and CUPRAC methods on leaves, stems and tubers extracts of purple sweet potato was carried out with Minitab Statistical Software using the pearson correlation method. The test results can be seen in Table 3. The highest correlation coefficient value was found in the correlation between the total flavonoid content in the ethyl acetate tubers extract and the antioxidative activity by the DPPH method ($r = 0.995$). While the lowest correlation coefficient value was found in the correlation between the total flavonoid content in ethyl acetate leaves extract and the antioxidative activity by the DPPH method ($r = 0.557$). Pearson's correlation coefficient (r) value above 0.61 indicates a significant correlation. From the results, it was found that all Pearson's correlation coefficient (r) values were above 0.61 except for the antioxidative activity of ethyl acetate leaves extract (LEV2) by the DPPH method with total flavonoid content (TFC).

The correlation analysis between antioxidative activity by DPPH and CUPRAC methods on leaves, stems and tubers extracts of purple sweet potato was carried out with Minitab Statistical Software using the Pearson's correlation method. The test results can be seen in Table 4. The highest correlation coefficient value was found in the antioxidative activity of the n-hexane leaves extract ($r = 0.929$), while the lowest correlation coefficient was found in the antioxidative activity of ethanol leaves extract ($r = 0.555$). Pearson's correlation coefficient (r) value above 0.61 indicates a significant

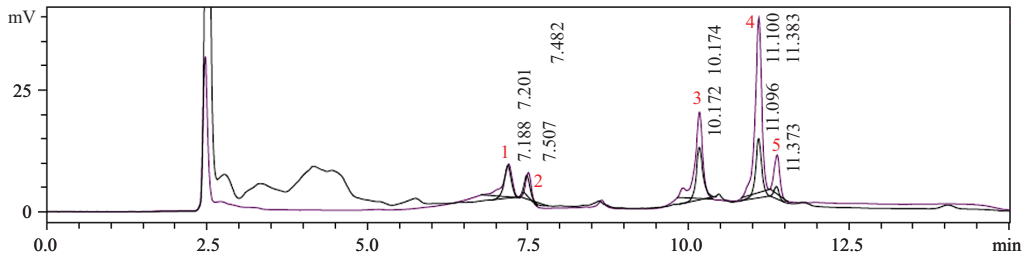

Fig. 3: Chromatogram pattern of 5 standard flavonoids and ethanol stem extract
1: Luteolin 7-O-glucoside, 2: Rutin, 3: Quercetin, 4: Kaempferol, 5: Apigenin, purple line chromatogram = Standard and black line chromatogram = Sample

Table 4: Correlation of DPPH and CUPRAC methods

Antioxidative parameters	Pearson's correlation coefficient (r)								
	DPPH LEV 1	DPPH STM 1	DPPH TBR 1	DPPH LEV 2	DPPH STM 2	DPPH TBR 2	DPPH LEV 3	DPPH STM 3	DPPH TBR 3
CUPRAC LEV 1	0.929**								
CUPRAC STM 1		0.724*							
CUPRAC TBR 1			0.772**						
CUPRAC LEV 2				0.748*					
CUPRAC STM 2					0.923**				
CUPRAC TBR 2						0.925**			
CUPRAC LEV 3							0.555^{ns}		
CUPRAC STM 3								0.674*	
CUPRAC TBR 3									0.749*

**Significant at p<0.01, *Significant at p<0.05 and ns: Not significant

Table 5: Content of several flavonoids in ethanol stem extract

Flavonoids	Retention time (min)		AUC		Content (%)
	Sample	Standard	Sample	Standard	
Luteolin 7-O-glucoside	7.188	7.201	66999	52459	0.313
Rutin	7.482	7.507	27353	27292	0.399
Quercetin	10.172	10.174	154501	81346	0.211
Kaempferol	11.096	11.100	259685	83376	0.128
Apigenin	11.373	11.383	47214	13532	0.115

correlation. From the results, it was exposed that all Pearson's correlation coefficient (r) values were above 0.61 except for the antioxidative activity of ethanol leaves extract (LEV3).

Determination of several flavonoids content in the ethanol stem extract of purple sweet potato was carried out using HPLC by comparing the retention times of five standard flavonoids (luteolin 7-O-glucoside, rutin, quercetin, kaempferol, apigenin) and ethanol stem extract using liquid chromatography system with UV-vis detector at l 360 nm. The results obtained can be seen in Fig. 3 and Table 5.

From the results of calculations using the one-point method based on the AUC sample and standard, it was revealed that rutin had the highest concentration among the test compound group with a concentration of 0.399%, while the lowest level was apigenin with a concentration of 0.115%.

DISCUSSION

Antioxidant activity of leaves, stem and tubers extract of purple sweet potato were performed by DPPH and CUPRAC methods. In this study, the density of each extract showed values in the range of 0.775-1.047 g mL^{-1}, which were the insignificant difference in density. Based on the present study of antioxidative testing using the DPPH and CUPRAC methods, it was found that the ethanol leaves extract of purple sweet potato had the highest antioxidative activity in both methods (290.894 mg AAE g^{-1} sample by DPPH and 621.254 mg AAE g^{-1} sample by CUPRAC) compared to its stem and tubers extracts. The DPPH and CUPRAC methods gave a significant correlation except for ethanolic leaf extract. It is possible to happen because these two methods had different testing principles.

The highest phenolic content was shown by ethanol leaves extract (20.885 g GAE 100 g^{-1}), while the highest

flavonoid content was found in ethyl acetate leaves extract (10.048 g QE 100 g^{-1}). This result indicated that the highest TPC and TFC didn't always give the higher antioxidative activities. But TPC can be correlated with the antioxidative activity of the sample because the antioxidative activity of ethanol and ethyl acetate extracts was higher than n-hexane extract in this study. The positive and significant correlation indicated a correlation between TPC and TFC on antioxidative activity, which means that in general the higher levels of content of phenols and flavonoids in the samples had the higher antioxidative activity. The present study also identified flavonoid compounds that showed the ethanol stem extract contained luteolin 7-O-glucoside, rutin, quercetin, kaempferol and apigenin, which the highest level being given by rutin (0.399%).

Research by Makori *et al.*[16] conducted the antioxidative assay by the DPPH method on four edible parts of purple sweet potato in the various cultivars (Simon 1, Yuzi 7, Shangshu 19 and Pushu 32) that showed the sequence of antioxidative activity: Leaves>stalk>stem>skin>flesh. The results showed similarities with the present study (Table 1), in which the highest antioxidative activity was found in leaves, followed by stem and tubers. It was also found that TPC and TFC in sweet potato leaves were higher than in other edible parts. These results were in close agreement with the current research. Ghasemzadeh *et al.*[17] investigated the antioxidative activity of six various *Ipomoea batatas* leave extracts in methanol extracts (Vardaman, Bush Porto Rico, Beauregard, Centennial, Jewell, Georgia). The result showed that the leaves extract had a high concentration of radical scavenging compounds. Hue *et al.*[18] demonstrated the radical scavenging activity in leaves of six varieties of *Ipomoea batatas* (Batu Kelantan, Batu Biasa and Biru Putih Oren, Vitato, Indon) by DPPH assay. Except for Biru Putih, all types had stronger radical scavenging activity than the ascorbic acid standard. As a result, *Ipomoea batatas* leaves could be used as a natural antioxidant source.

Research by Kim *et al.*[19] presented the effect of heat treatment conditions of sweet potato cultivars on the DPPH method. Sweet potato cultivars which were treated with heat and pre ethanol A had the strongest DPPH radical scavenging capabilities and the highest TPC and TFC. It showed that thermal processing affected the extraction of phenolic and flavonoid compounds[20]. Im *et al.*[21] reported that the outer layer had the richest source of antioxidants and higher TPC values than the inner layer. This result indicated the correlation between TPC and antioxidative activity. Zengin *et al.*[22] presented antioxidative activity from *Ipomoea batatas* leave extract from several types of extraction, including Soxhlet, decoction and microwave extraction. The decoction extract exhibited the best results in antioxidant activity and the highest phenol content. There was the correlation between phenol concentration with antioxidative activity. Research by Fu *et al.*[23] stated that the extraction solvent had a substantial impact on the recovery of phenolics and flavonoids. The highest TPC was found in 50% acetone extract. TFC levels were greatest in 70% ethanol and 90% acetone extracts.

The results showed that the extracts of leaves, stems and tubers of purple sweet potato had antioxidative activities that can be used as a source of potent antioxidants. The content of phenols and flavonoids contributed to the antioxidative activity. Further research can be carried out to isolate antioxidant compounds from extracts of leaves, stems and tubers of purple sweet potato.

CONCLUSION

Aside from the edible part (tubers), purple sweet potato waste products (leaves and stem) also had antioxidative activity. In general, extracts of leaves, stems and tubers had strong antioxidative activity from tests using DPPH and CUPRAC methods. Higher TPC and TFC didn't always indicate a higher antioxidant capacity. Phenolic compounds in purple sweet potato leaves, stem and tubers extracts were the main contributors to their antioxidant activities by DPPH and CUPRAC methods. Only leaves ethanolic extract gave a non-linear result within two antioxidant assay methods. Ethanol stem extract contained luteolin 7-O-glucoside, rutin, quercetin, kaempferol and apigenin, which gave the highest level of 0.399%.

SIGNIFICANCE STATEMENT

This study discovered that the leaves, stem and tubers of purple sweet potato (*Ipomoea batatas* L.) had antioxidant activities and the potential to be used as a source of further natural antioxidants.

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