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Antioxidant Activity of Ethanolic Extracts of Purple and Orange Sweet Potatoes (*Ipomoea batatas* L.)

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ABSTRACT

Purple and orange sweet potatoes are rich in anthocyanin, beta-carotene and phenolic compounds, an important group of secondary metabolite compounds responsible for the antioxidant activity in fruits and vegetables. This work investigated the potential of ethanol extracts of purple sweet potato (EU) and orange sweet potato (EO) in scavenging free radical species using DPPH and FRAP methods and its correlation to total phenolic and flavonoid contents. The study results showed that EU has potent antioxidant activity with an IC_{50} value of 30.9 µg/mL. In comparison, EO has moderate antioxidant activity with an IC_{50} of 61.5 µg/mL in the DPPH assay. The ability of EU and EO to reduce free radical species using FRAP assay were 215.7 and 177.7 µmol TE/g extract, respectively. Besides that, the flavonoid contents of EU and EO were 34.0 and 22.8 mg QE/g extract, while its phenolic content was 5.97 g and 2.00 g GAE/100 g extract, respectively. These results indicated that ethanolic extracts from purple and orange sweet potatoes have a high potential to be used as a food supplement to protect the human body from diseases caused by free radical species.

Keywords: *Ipomoea batatas, sweet potato, antioxidant, DPPH, FRAP*

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INTRODUCTION

Sweet potato (*Ipomoea batatas* L.) is a source of starch with a high potential to be developed as a functional food. Several studies reported that sweet potato is rich in fibre, minerals, vitamins, and antioxidant compounds such as phenolic acids, anthocyanins, tocopherols, and carotenoids [1, 2]. Sweet potato is considered an excellent source of dietary carotenoids and polysaccharides with antioxidant, anti-inflammatory, hepatoprotective activity, cardiovascular protection, and anti-cancer properties, which are beneficial for health [3]. Besides that, sweet potato also has higher anthocyanin content than purple cabbage, grapes, and *elderberry* [4]. Anthocyanins are essential compounds that prevent or inhibit oxidation by scavenging free radicals (unpaired electrons) and reducing oxidative stress [5]. In this context, sweet potato represents a unique food option and a potential source of functional ingredients for healthy food products, especially to defend against excesses of free radical species produces by cell metabolism.

Paired electrons in the outer shell of a compound and molecules and atoms have an important role because if the electrons are in unpaired conditions, unstable free radicals are formed. If these free radicals accumulate in large numbers and the body's immune system cannot neutralise them, the cells in the human body will slowly experience oxidative damage [6]. The effects of free radicals on human tissue are known to be the triggers for many pathological disorders, including cardiovascular disease, cancer, neurological dysfunction, and problems related to ageing [7]. The reactivity of radical compounds can be reduced and stabilised by antioxidant compounds [8]. In these cases, natural antioxidant compounds contained in plants are known to effectively inhibit oxidative reactions of free radicals to protect the human body from diseases caused by free radicals [9].

In continuing the search for antioxidant compounds from common traditional foods, here we report the antioxidant activity of purple and orange sweet potato tuber extracts (*Ipomoea batatas* L.) using the DPPH and FRAP methods focus on measuring the ability of test extracts to reduce radical compounds.

EXPERIMENTAL

Materials

The purple and orange sweet potato tubers (*Ipomoea batatas* L.) were collected from Karangbangun, Central Java, Indonesia, in September 2021. The following chemicals were used in the analysis, including ethanol, quercetin, Folin-Ciocalteu reagent, $NaCO_3$, Phosphate buffer (pH 6,0), $K_3Fe(CN)_6$, TCA (trichloroacetic), $FeCl_3$, DPPH (1,1-diphenyl-2-picryl-hydrazyl), and FRAP. UV/Visible spectrophotometer and UV-1600PC were used in the determination of samples.

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Sample preparation and extraction

Samples (1 kg) in the form of fresh purple sweet potato and orange sweet potato (*Ipomoea batatas* L.) were selected and dried. Then the outer skin of the purple sweet potato and orange sweet potato was peeled, and the tubers were sliced thinly to make the drying process faster and more efficient. The sliced purple sweet potato and orange sweet potato samples were dried in an oven at 50ºC for three days. The dried samples were then ground to a fine powder using a blender. Then 500 g of each purple sweet potato powder and orange sweet potato powder was macerated with 3.75 L, 96% ethanol for 3x24 hours while stirring occasionally. The macerates were filtered and remastered, then evaporated using a vacuum rotary evaporator [10].

Determination of total flavonoids

The total flavonoid content in each extract was determined according to Rezaeizadeh et al. [11] with slight modification. A total of 0.5 mL extract with a concentration of 5 mg/mL was mixed with 1.5 mL of ethanol, and then 0.5 mL of 10% $AlCl_3$ was added, followed by 0.5 mL of 1M potassium acetate and 2.0 mL of distilled water. The mixture was incubated at room temperature for 30 minutes. The absorbance was measured using a spectrophotometer at 415 nm in triplicates. The results were expressed in mg QE (quercetin equivalents) per gram of extract. A quercetin standard curve was constructed using five concentrations (15, 20, 25, 30, and 35 µg/mL).

Determination of total phenolics

The total phenolic content in the extracts was determined using the method described by Hue et al. [12]. A total of 50 µL of the extract (5 mg/mL) was added to 250 µL of the Folin-Ciocalteu reagent, and then this mixture was incubated for 5 minutes at room temperature. Then 750 µL sodium carbonate solution was added to the mixture and incubated for 2 hours at room temperature. The absorbance was measured at 765 nm using a UV spectrophotometer in triplicates. A gallic acid standard curve was constructed at varying concentrations (100, 200, 300, 400, and 500 µg/mL). The total phenolic concentration was calculated based on the equation from the gallic acid standard obtained and expressed in g GAE/100 g.

Testing for antioxidant activity by FRAP method

Purple sweet potato tuber (EP) extract (10 mg) and orange sweet potato tuber (EO) extract (10 mg) were each dissolved with 10 mL ethanol (1 mg/mL). An amount of 1 mL of the extract solution was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.0) and 2.5 mL of 1% $K_3Fe(CN)_6$. The mixture was incubated for 20 minutes at 50ºC, followed by adding 2.5 mL of 10% TCA (trichloro-acetate) and then centrifuged for 10 minutes at a speed of 3000 rpm. Then 2.5 mL of the supernatant was diluted with 2.5 mL of distilled water and reacted with 0.5 mL of 0.1% $FeCl_3$. The absorbance of the sample was measured using a UV/vis spectrophotometer at 700 nm. A

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Trolox standard curve was constructed using different Trolox concentrations (60, 80, 100, 120, and 140 µg/mL). The reduced ability of the test sample was expressed in µmol TE/g extract.

Antioxidant activity testing by the DPPH method

The DPPH radical capture activity of the test sample was measured based on the method reported by Jegadeesh et al. [13] with modification. An amount of 10 mg of each purple sweet potato tuber extract (EP) and orange sweet potato tuber (EO) extract was diluted with 10 mL ethanol to obtain a stock solution with a concentration of 1000 µg/mL. Variation in concentrations was made by pipetting 400, 200, 100, 50, and 25 µL from the stock solution to obtain concentrations of 40, 20, 10, 5, and 2.5 µg/mL. A volume of 2 mL from each concentration was then added with 2 mL of 0.01 M DPPH solution in ethanol, mixed thoroughly, and allowed to stand at room temperature for 30 minutes in the dark. The absorbance was measured using a visible spectrophotometer at 516 nm. A Trolox standard curve was constructed based on five series of concentrations (5, 2.5, 1.25, 0.625, and 0.3125 µg/mL). The measurement of the absorbance of the samples was carried out in triplicates.

Data analysis

The measurement of antioxidant activity with the FRAP method was replicated three times. The value of total antioxidant capacity (x) was obtained by entering the absorbance value of the sample (y) in the Trolox standard curve equation (y = bx + a). The total antioxidant capacity of the extract was expressed as the mean ± SD (standard deviation, µmol TE/g extract). The percentage of DPPH radical capture activity was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_0 - A_1/A_0) \times 100] \quad (1)$$

A_0 is the absorbance of the control (absorbance of DPPH without the test sample), and A_1 is the absorbance of the sample test (extract or comparator (Trolox)). Per cent, radical capture was determined using the linear regression equation (y = bx + a) obtained by plotting concentration vs % inhibition. To obtain an IC_{50} value, y = 50 was entered in the linear regression equation, and the value of x was determined. The value of IC_{50} for each combination group was then analysed for statistical differences using the one-way ANOVA (analysis of variance).

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RESULTS AND DISCUSSION

Total flavonoid levels

Quercetin was used as a comparison standard in determining total sample flavonoids. Figure 1 shows the standard curve of the absorbance reading of five series of concentrations with a value of r = 0.9905. The total flavonoid content was measured based on the presence of quercetin in the test extract. $AlCl_3$ reagents form complexes with the hydroxyl group of flavonoid compounds. The more the content of flavonoid compounds, the more concentrated will the yellow colour formed. Purple sweet potato tuber extract (EP) has a total flavonoid content of 34.02 mg QE/g extract, while orange sweet potato tuber extract has a total flavonoid content of 22.82 mg QE/g extract. Insanu et al. [14] reported that the flavonoid content of purple sweet potatoes tubers is lowest compared with their leaves and stem (100.58 mg QE/g and 76.30 mg QE/g, respectively) because the main constituent in the sweet potato tubers is carbohydrate.

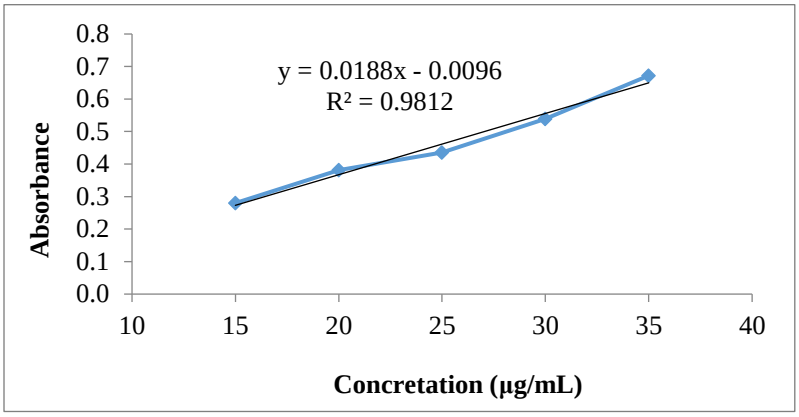

Figure 1: Quercetin standard curve

Total phenolic levels

The total phenolic levels were determined using gallic acid as a comparison standard. Figure 2 shows the gallic acid standard curve. Folin-Ciocalteu reagent is an inorganic reagent that can form complex solutions with phenol compounds. The colour formed can be detected by visible light at 765 nm. Phenol compounds as secondary metabolites have the potential as antioxidants. The hydroxy groups in phenolic compounds function as contributors to hydrogen atoms when reacting with radical compounds through the electron transfer mechanism so that the oxidation process is inhibited.

https://scilett-fsg.uitm.edu.my/
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EP has a total phenolic content of 5.973 ± 0.0235 g GAE/100 g extract, while the total phenolic content of EO is 2.004 ± 0.0292g GAE/100 g extract. These results indicate that the purple colour is an essential factor affecting phenolic content. According to Musilova et al. [15], sweet potato tubers generally contain phenolic compounds such as caffeoylquinic acid, cinnamic acids, and quinic acid derivatives. Besides that, Kongkachuichai et al. (2015) stated that dark-coloured vegetables are known to be good sources of anthocyanins [16], while Chen et al. (2018) reported that the sweet potato with purple leaves contained significantly higher levels of anthocyanins compared to green and yellow leaves [17].

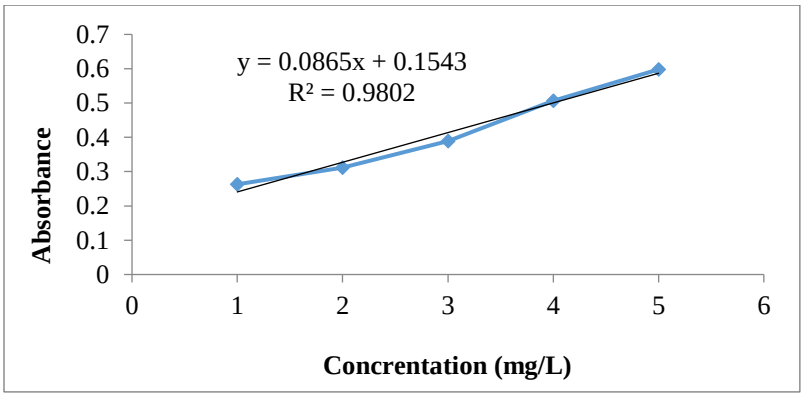

Figure 2: The curve of gallic acid

Antioxidant Activity using FRAP Method

As a comparative compound, Trolox is one of the antioxidants that can capture extracellular free radicals and prevent further oxidative reactions [18]. Trichloro-acetate compounds in this method function as precipitates of potassium ferrocyanide complexes. At the same time, using $FeCl_3$ at the end of the reaction is intended to form a bluish-green complex. The indicator measured in this method is the reducing power of the test compounds which illustrates the ability of antioxidant compounds in the test compounds that can convert Fe^{3+} to Fe^{2+} [18]. The greater the reduction power of a compound, the greater the potential as an antioxidant because the compound can stabilise radicals by donating electrons or hydrogen atoms. The reaction that occurs in the FRAP method is as follows:

$$K_3Fe(CN)_6 \rightarrow K_4Fe(CN)_6 \quad (2)$$

$$Fe^{3+} + e^- \rightarrow Fe^{2+} \quad (3)$$

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Figure 3 shows the Trolox standard curve and the equation obtained, y = 0.0034x + 0.0226, with an r = 0.9706. The FRAP value is expressed in µmol TE/g extract. The results in Table 1 show that the ethanol extract of purple sweet potato tuber (EP) has a greater reduction by 1.2 times compared to the ethanol extract of orange sweet potato tuber (EO), with antioxidant activity of 215.71 ± 12.72 for the EP and 177.67 ± 1.96 for the EO.

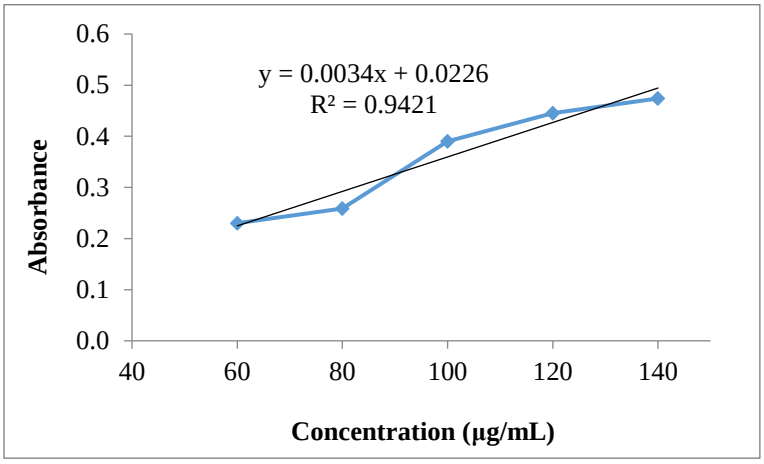

Figure 3: Trolox standard curve

Table 1: Results of antioxidant activity with the FRAP method

Sample	Rep.	Abs	Antioxidant activity (µmol TE/g extract)	Mean ± SD (µmol TE/g extract)
EP	1	0.708	201.59	215.71 ± 12.72
	2	0.792	226.29	
	3	0.768	219.24	
EO	1	0.619	175.41	177.67 ± 1.96
	2	0.631	178.94	
	3	0.630	178.65	

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Antioxidant activity using the DPPH method

The DPPH method is one method of testing the antioxidant power of a compound directly with the mechanism of stabilising the free radical DPPH synthesis by capturing hydrogen atoms from antioxidant compounds contained in a test extract. The donation of hydrogen atoms from antioxidants to DPPH makes these radicals obtain an electron pair, and non-radical *diphenyl-picryl-hydrazyl* is formed [19]. A purple DPPH solution can be detected at a wavelength of 516 nm. The solution then oxidises the compound in the sweet potato tuber extract, which is characterised by the transformation of the colour of the solution from purple to yellow.

Based on Table 2, Trolox, as the standard antioxidant compound, has the smallest IC_{50} of 2.47 µg/mL. EP has IC_{50} of 30.87 µg/mL, and EO has IC_{50} of 61.55 µg/mL. EO at the highest concentration in this study, that is, 40 µg/mL was only able to inhibit DPPH radical by 32.31%, so the IC_{50} value obtained was the result of extrapolation from the linear regression equation (concentration vs % inhibition). A compound is considered to have very strong antioxidant activity if it has an IC_{50}<50 µg/mL, strong if the IC_{50} value is 50-100 µg/mL, moderate if the IC_{50} value is 101-150 µg/mL and is weak if the IC_{50} > 150 µg/mL [20]. Thus, EP is classified as a very strong antioxidant, while EO is categorised as a potent antioxidant.

Table 2: IC_{50} of EP, EO, and Trolox using the DPPH method

Sample	Rep.	IC_{50} (µg/mL)	Mean ± SD (µg/mL)
EP	1	30.928	30.875 ± 0.149
	2	30.706	
	3	30.990	
EO	1	60.601	61.548 ± 1.751
	2	60.474	
	3	63.568	
Trolox	1	2.737	2.468 ± 0.015
	2	2.730	
	3	2.764	

Phenolic and flavonoid compounds contain hydroxy groups essential as hydrogen donors to stabilise DPPH radical compounds. The IC_{50} value of EO, which is greater than the EP, is related to the lower EO flavonoid compound content compared to the EP with a higher flavonoid compound content. When viewed from its chemical structure, the hydroxy groups in the flavonoid compounds in purple sweet potato tubers are more than the hydroxy groups found in the phenolic compounds. This is because, in the EP, there is a large number of anthocyanin compounds,

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whereas, in EO, the flavonoid content is lower due to the absence of anthocyanin. These findings are consistent with the previous reports that the antioxidant activity of highly pigmented vegetables, determined using the DPPH and FRAP assays, was correlated with the total phenolic contents [21, 22].

CONCLUSION

In conclusion, the antioxidant activity of purple sweet potatoes was stronger than orange sweet potatoes as determined by DPPH and FRAP methods. This is in accordance with the higher total flavonoids and total phenolics contents of purple sweet potato compared to the orange sweet potato. Therefore, this result indicated that the daily consumption of purple sweet potato is good for protecting the human body from diseases caused by free radical species.

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AUTHOR'S CONTRIBUTION

Mardi Putri Riyadini carried out the research and wrote the draft of the article. Haryoto and Muhtadi designed the experimental work and supervised the research progress; Agustono Wibowo reviewed and approved the revisions article submission.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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