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Research Article

Comparison of Antioxidant Activities of Ethyl Acetate Extracts of Two Varieties of Sweet Potato Tuber [Ipomoea Batatas (L.)] Using Two Extraction Methods

Hendy Suhendy*, Defri Risviana, Imas Ratnasari

Department of Natural Ingredients Pharmacy, Bachelor of Pharmacy Study Program, STIKes Bakti Tunas Husada, Tasikmalaya
* Corresponding Author's Email: hendysuhendy@stickes-bth.ac.id

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ABSTRACT

The decrease in antioxidant activity can be caused by the destruction of antioxidant compounds due to heating. Several studies stated that there was no significant difference between the antioxidant activity of extracts from cold extraction and hot extraction. This study compares the antioxidant activity of the ethyl acetate extract of two varieties of sweet potato using two different extraction methods. Simplicia was extracted by hot extraction using reflux and cold extraction using maceration. The antioxidant activity of the extracts was tested by the DPPH method using UV-Vis spectrophotometry. There are two types of sweet potato used in this research; firstly, the outer skin of the tuber is purple, the inside is purple (UU), and, secondly, the outer skin is purple, the inside is orange (UO). The IC50 UUR (Purple-Purple Refluxed), UOR (Purple-Orange Refluxed), UUM (Purple-Purple Macerated), UOM (Purple-Orange Macerated) , and ascorbic acid values were 4.583, 4.614, 0.755, 18.142, and 2.680 g/ml; thus, the extraction method and sweet potato varieties affect the antioxidant activity of the extract. Maceration is the best method for UU, while reflux is the best method for UO.

1. INTRODUCTION

The extraction temperature influences the activity of an antioxidant compound. Antioxidant activity decreases with more prolonged heating because antioxidant compounds are damaged

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during the heating process (Hartiati et al., 2009). Another research stated that there was no significant difference between the antioxidant activity of the ethanol extract from the maceration extraction method and the ethanol extract from the reflux extraction method from cherry leaves. (Hasanah et al., 2016).

Research on antioxidant activity of extracts from four varieties of sweet potato tubers only used the hot extraction method. From the investigation, the ethyl acetate extract of purple-purple and purple-orange sweet potato tubers gave the highest antioxidant activity with IC50 values of 13.88 and 10.54 g/mL (Fidrianny et al., 2018). Using different extraction temperatures, further tests on the two varieties showed that heating affected the total flavonoids of sweet potato tubers of purple-purple and purple-orange varieties (Suhendy et al., 2021). it is necessary to research the antioxidant activity of ethyl acetate extract of two types of sweet potato tubers extracted with two extraction methods based on temperature differences to determine the effect of heating treatment on extraction on antioxidant activity.

2. MATERIALS AND METHODS

Materials and Tools

The tools used in this paper are Simplicia drying cabinet, Simplicia grinder, electronic balance, circular flask, electric stove, spatula, measuring cup, test tube, beaker, Erlenmeyer flask, lamp, evaporating dish, rotavapor, the crucible, cuvette, UV-visible spectrophotometer (Hewlett Packard 8435), hairdryer and tools commonly used in laboratories.

Ingredient

Simplicia powder of two varieties of sweet potato tubers Ipomoea batatas, hydrochloric acid, sulfuric acid, magnesium powder, amyl alcohol, acetic anhydride, concentrated nitric acid, chloroform, methanol, ethanol, ethyl acetate, Dragendorff's reagent, Mayer's reagent, aluminum (III) chloride, sodium hydroxide, Liebermann-Burchard reagent, chloroform, methylene chloride, sodium acetate, sodium carbonate, potassium phosphate, citric acid, boric acid, formic acid, FeCl3, H2SO4, DPPH, ascorbic acid, filter paper, parchment paper.

Methods

Materials Collection and Processing

The material in tubers from two varieties of sweet potato Ipomoea batatas, namely the outer skin of the tuber is purple, the inside is purple (UU), and the outer skin is purple, the inside is orange (UO) obtained from Tasikmalaya. Simplicia processing begins with taking fresh tubers, washing, wet

sorting, drying, dry sorting, and milling to store tuber Simplicia dry powder from two varieties of Ipomoea batatas.

Simplicia characterization

Examination of Simplicia characteristics carried out included macroscopic analysis including the characteristics of the shape, color, and texture of tubers as well as shape and color of Simplicia powder and microscopic examination was carried out by placing Simplicia powder on an object glass and giving a few drops of water, then observed under a microscope.

Extraction

Extraction was carried out by reflux and maceration method using ethyl acetate as solvent. The extract obtained was evaporated with a rotary evaporator until a thick extract was obtained. The extract was then weighed, and the thick extract's weight was obtained. Furthermore, the yield of each extract was calculated.

Extract characterization

The characterization carried out was only a phytochemical screening of secondary metabolites of the phenol and flavonoid groups.

Quantitative Test of Extract's Antioxidant Activity

Each sample was made in several concentrations, then 1 mL of sample solution was taken at all concentrations and added with 1 mL of 50 g/mL DPPH solution (volume ratio 1:1). The mixture was then incubated for 30 minutes, and the absorbance was measured atλ515 nm. Methanol was used as a blank, 50 g/mL DPPH solution as control, and ascorbic acid as a comparison.

The linear regression equation of the calibration curve was used to determine the IC50 value. The IC50 value is calculated by entering the 50% value into the regression equation as the y-value, then the x-value is calculated as the IC50 concentration.

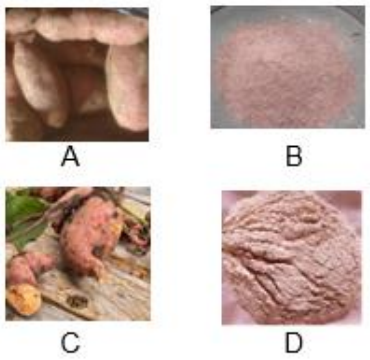
Data Analysis

The test results were analyzed using SPSS version 16.00 and expressed as mean ± standard deviation using one-way analysis of variance (ANOVA) and post hoc turkey method (p-value <0.05).

3. RESULTS AND DISCUSSIONS

Preparation of Test Materials

Simplicia is processed by taking fresh tubers and then washing, wet sorting, drying, dry sorting, and grinding to store dry Simplicia coarse powder. Wet simplicia and dry simplicia powder of two varieties of sweet potato tubers can be seen in **Figure 1**.



Description :
A = Wet simplicia of purple-purple sweet potato tubers
B = Dried simplicia powder from purple-purple sweet potato tubers
C = Wet simplicia of purple-orange sweet potato tubers
D = Dried simplicia powder of purple-orange sweet potato tubers

Simplified characterization

The physical characterization of simplicia carried out included macroscopic and microscopic examination of simplicia powder. Observations showed that the sample used was sweet potato tubers, as shown in **Table 1**.

Table 1. Macroscopic and microscopic test results of two varieties of sweet potato tubers

Sample	Shape	Flavor	Color	Smell	Fragment identifier
Purples	Powder	chelate	purple-brown	No smell	Starch Grains
purple-orange	Powder	chelate	Yellow	No smell	Starch Grains

Extraction

Extraction was carried out using two different methods on two varieties of sweet potato tubers. Reflux was chosen as the hot extraction method, while maceration was selected as the cold

extraction method. The extraction results were converted into the yield percentage, as shown in **Table 2**. The difference in extraction temperature affected the extract yield in both varieties of sweet potato tubers. Maceration is the best extraction method for extracting secondary metabolites for purple-purple and purple-orange sweet potato tubers.

Table 2. Yield of thick tuber ethyl acetate extract of two varieties of sweet potato

Method	Sample	yield
Maceration	Purples	5.38%
	Purple-Orange	2.53%
reflux	Purples	0.7%
	Purple-Orange	1.5%

In addition to the extraction temperature, several factors must be considered to obtain high yield extract, one of which is the replacement of the solvent. The replacement of the solvent will affect the saturation of the extraction process. The saturation of the solvent can result in the compound not being completely dissolved (Harborne, 1987).

Extract characterization

After being extracted using two different methods, phytochemical characterization was carried out on the extracts to determine the stability of phenol and flavonoid compounds as the main contributors to antioxidant activity. The results of observations in **Table 3** show that the four sections still contain phenol and flavonoid groups so that qualitatively the two extraction methods used do not affect the presence of these two groups of compounds.

Table 3. Phytochemical Screening of Tuber Extracts of Two Sweet Potato Varieties

Sample	Flavonoids	Phenol
UUR	+	+
UUM	+	+
UOR	+	+
UOM	+	+

Description :

UUR = Purple-purple tuber ethyl acetate extract with reflux

UOR = Purple-orange tuber ethyl acetate extract with reflux

UUM = extract of purple-purple tuber ethyl acetate by maceration

UOM = Purple-orange tuber ethyl acetate extract by maceration

(+) detected (-) not detected

Quantitative Test of Extract's Antioxidant Activity

Measurement of antioxidant activity using the DPPH free radical scavenging method aims to measure the total antioxidant capacity of each extract, namely UUR (purple-purple ethyl acetate extract with reflux), UOR (purple-orange ethyl acetate extract with reflux), UUM (purple-purple ethyl acetate extract with maceration) and UOM (purple-orange ethyl acetate extract with maceration) maceration) by UV-visible spectrophotometry. A sample is declared to have potent antioxidant activity if it has an IC50 or EC50 value of <50 g/mL, is declared strong if the IC50 value is 50-100 g/mL, is declared moderate if the IC50 value is 100-150 g/mL and is declared weak if it has an IC50 150-200 g/mL (Molyneux, 2004).

The parameter used as an interpretation of the results of the DPPH method is IC50 which can be defined as the concentration value of antioxidant compounds that can reduce DPPH free radical activity by 50%, the smaller the IC50 value, the greater the antioxidant activity. (Molyneux, 2004).

Based on the results of testing the antioxidant activity in table 4 with the DPPH free radical reduction method, it was found that the smallest IC50 value was owned by UUM (0.755 g/ml), even lower than the IC50 of ascorbic acid, this indicates that the purple-purple sweet potato tuber ethyl acetate extract has enormous potential to be developed as a source of antioxidants. The high

antioxidant activity of UUM extract is in line with the research of Suhendy (2021), which stated that the most extensive total phenol value was found in purple-purple sweet potato tubers extracted by the maceration method.

Table 4. IC50 Value of Tuber Extract of Two Sweet Potato Varieties

Sample	Average IC50 (µg/ml) ± SD
UUR	4.583 ± 0.007a
UOR	4.614 ± 0.05b
UUM	0.755 ± 0.02c
UOM	18,142 ± 0.003d
Ascorbic acid	2.680 ± 0.010e

Description :

ae = different letters in one column indicate there is a significant difference ($p<0.05$)

UUR = Purple-purple tuber ethyl acetate extract with reflux

UOR = Purple-orange tuber ethyl acetate extract with reflux

UUM = extract of purple-purple tuber ethyl acetate by maceration

UOM = Purple-orange tuber ethyl acetate extract by maceration

Data processing was carried out statistically using one-way ANOVA – Tukey. The IC50 value of each extract showed a significant difference ($p<0.05$) in that the extraction method and sweet potato tuber variety affected the antioxidant activity of the extract. Although there were statistically significant differences, all extracts had an IC50 DPPH value of less than 50 g/mL, meaning that all extracts had potent antioxidant activity.

IC50 UUM showed a smaller value and was significantly different from UUR ($p<0.05$). In other words, the cold extraction method provided better antioxidant activity than the hot extraction method. Several studies also give the same result (Manggala et al., 2017; Nurlaela et al., 2016; Risnadewi et al., 2019; Sulastri et al., 2020). Increasing temperature can result in molecular movement that accelerates dissolution, increasing flavonoid levels. However, increasing the temperature too high will cause denaturation of thermo-sensitive antioxidants, which may be more

stable at lower temperatures. (Jing et al., 2015). Therefore, the increase in flavonoid levels is not necessarily linear with its antioxidant activity. Likewise, phenol levels do not necessarily affect antioxidant activity as in the research carried out where no results were obtained that corresponded to the total phenolic content with the antioxidant activity of each extract (Verawati et al., 2017). This may be because that which provides antioxidant activity is compounds of the phenolic group and combinations of other groups such as essential oils, steroids, and terpenoids. Antioxidant activity is not only offered by anthocyanins but by other secondary metabolite compounds such as alkaloids, flavonoid polyphenols and tannins (Pu et al., 2013; Yin et al., 2010).

Purple-orange sweet potatoes with the reflux method showed a smaller IC50 value than the maceration method, meaning that the hot extraction method provided better antioxidant activity than cold extraction, following previous studies. (Dewi et al., 2020; Fadlilaturrahmah et al., 2020; Nurhasnawati & Handayani, 2017; Saptarini & Wardati, 2020; Verawati et al., 2017). The extraction temperature of the required antioxidant components can be perfectly extracted so that the more dissolved components, the greater the antioxidant activity (Setyowati & Damayanti, 2014). The temperature in the Soxhlet extraction process affects the extracted phenolic compounds. The higher the extraction temperature, the higher the solubility of phenolic compounds (Mokoginta et al., 2013).

4. CONCLUSIONS

Ethyl acetate extract from maceration and reflux of tubers of two sweet potato varieties is a powerful antioxidant using the DPPH method. The IC50 values for UUR, UOR, UUM and UOM are 4.583; 4.614; 0.755 and 18.142 g/ml, respectively. The extraction method and varieties of sweet potato tubers affected the antioxidant activity of the ethyl acetate extract of two varieties of sweet potato tubers. Maceration is the best method for UU, while reflux is the best method for UO.

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