

Inhibitory Effects of Different Varieties of Sweet Potato (*Ipomoea batatas* L.) Tubers Extracts on Lipoxxygenase Activity

Sendangratri¹, Rosita Handayani¹, Berna Elya^{1,*}

Sendangratri¹, Rosita Handayani¹, Berna Elya^{1,*}

¹Laboratory of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, Universitas Indonesia, Depok, West Java, INDONESIA.

Correspondence

Berna Elya

Department of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, Universitas Indonesia, Depok, INDONESIA.

E-mail: berna.elya@farmasi.ui.ac.id

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ABSTRACT

Background: Sweet potatoes (*Ipomoea batatas* L.) with purple, orange and white varieties can be differentiated by their skin and flesh tubers' colors. Research on anti-inflammatory activity of this plant is still limited although has been used traditionally. **Objective:** This study aimed to determine IC₅₀ value of sweet potato tubers extracts in inhibiting lipoxxygenase activity. **Methods:** Dried tubers of sweet potato were macerated with ethanol 70%. Each extracts were tested for soybean lipoxxygenase inhibitory activity, phytochemical screening and total flavonoid contents. IC₅₀ value and total flavonoid contents obtained from each extracts were analyzed statistically. **Results:** IC₅₀ value of purple, orange and white sweet potato tubers extracts are 46.09, 52.12 and 63.69 µg/mL respectively. Each extracts contain alkaloids, flavonoids, saponins, tannins and glycosides. Total flavonoid contents in purple, orange and white sweet potato extracts are 8.45±0.41; 7.57±0.03; and 6.12±0.14 mgQE/g extract respectively. **Conclusion:** Total flavonoid contents and IC₅₀ values of each extracts are strongly correlated and inversely proportional with significance value of 0.026 and correlation value of -0.999 which indicate that the higher total flavonoid contents, the stronger inhibitory activity.

Key words: Anti-inflammatory, *Ipomoea batatas* L., Lipoxxygenase, Sweet potato tubers, Total flavonoid contents.

INTRODUCTION

Over the past few decades, researches for medicines derived from plants has been widely carried out. Although most researches are still focused on herbs, vegetables and fruits also have medicinal properties. *Ipomoea batatas* L., commonly known as sweet potato, is root vegetable which is often consumed as food.¹ The value of production and consumption of sweet potato in Indonesia in 2016-2019 is growing every year. Production of sweet potatoes in Indonesia in 2016-2017 experienced growth of 17.88%, while in 2018-2019 predicted growth of 4.08 and 2.39%. Sweet potato consumption in Indonesia in 2016-2017 has increased by 12.46%, while in 2018-2019 is predicted to increase by 5.97 and 3.98%.²

Sweet potato plays an important role as a source of energy and phytochemicals in human nutrition. This plant can have properties such as medicine and various parts of this plant are used in traditional medicine. In Kagawa region of Japan, various sweet potato varieties are consumed directly or raw to treat anemia, hypertension and diabetes.³ Sweet potato tubers are also used to treat type 2 diabetes by tribes in Ghana⁴ and in the treatment of oral inflammation or infection in Brazil.⁵

Inflammation is the body's response which includes blood vessels, body cells, proteins and other mediators to eliminate the initial causes of cell injury, as well as damaged cells and necrotic tissues.⁶ In addition, the respon is also intended to initiate

cell and tissue repair. Inflammation causes local symptoms such as edema (turgor), burning (calor), redness (rubor) and pain (dolor). Inflammatory causes can be in the form of foreign microorganisms, mechanical trauma, chemical mediators, or physical factors. One of the chemical mediators in the body is the leukotrien synthesized from arachidonic acid with the help of 5-Lipoxygenase (5-LOX).⁷ Leukotrien plays a major role in a number of inflammatory and immune responses associated with several diseases such as asthma, atherosclerosis and cancer.⁸ Therefore, inhibition of leukotriene biosynthesis by blocking 5-LOX activity has been recommended for therapeutic use.⁹

Inhibition of 5-LOX activity can be through various ways, one of which is with antioxidant compounds that will reduce ferric iron ions (Fe³⁺) on the active site of the enzyme so that it becomes inactive form.¹⁰ From the results of other studies that have been done explain that 5-LOX inhibitors are found in many classes of phenolic compounds. The most potent phenolic compounds as 5-LOX inhibitors are flavonoids that work by reducing ferric (Fe³⁺) to ferro (Fe²⁺).¹¹ Anthocyanins which belong to flavonoid are found in purple sweet potato tubers. Luteolin is also a flavonoid found in orange and purple sweet potato tubers.¹ Purple, orange and white sweet potato has an IC₅₀ antioxidant of 93.34 ± 1.9; 59.68 ± 12.44 and 34.28 ± 4.96%.¹² Total flavonoid content of purple, orange and white sweet potato tubers is respectively 2.53 ± 0.01; 2.45 ± 0.03; and 0.68 ± 0.02 gQE/100 g extracts.^{13,14}

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Based on the background above, the author intends to conduct research on the inhibition of lipoxygenase activity by sweet potato tubers extract with purple, orange and white varieties in Indonesia. Furthermore, phytochemical screening and determination of total flavonoid contents were also performed to each extracts. In addition, the data obtained are analyzed using SPSS version 22.0 to determine the relationship between IC_{50} value and total flavonoid content.

MATERIALS AND METHODS

Materials

The extract used in this study was produced from the simplicia of sweet potato tuber (*Ipomoea batatas* L.) with varieties of purple, orange and white obtained from Depok, West Java, Indonesia. The standard for lipoxygenase activity inhibition test used apigenin (Sigma Aldrich-10798, USA) and the standard for total flavonoid contents used quercetin (Sigma Aldrich-Q4951, USA). Other materials and reagents were soybean lipoxygenase (Sigma Aldrich-L7395, USA), linoleic acid (Sigma Aldrich-1376, USA), aqua demineralisata (Brataco, Indonesia), 96% ethanol (Brataco, Indonesia), ethanol P (Brataco, Indonesia), DMSO (Brataco, Indonesia) and aqua destilata (Brataco, Indonesia).

Extraction

The extraction was carried out by maceration method. The ratio used between the dried tubers and the solvent is 1:10. The solvent used is 70% ethanol. The immersion process was carried out during the first 6 h with occasional stirring, then allowed to stand for 18 h. Then, the filtration process was carried out to produce filtrate or macerate. This maceration stage was repeated three times. Then, each maserate was evaporated to remove the solvent residue with rotary vacuum evaporator or waterbath until it turned to viscous extracts.

Lipoxygenase activity inhibitory test

Preliminary test consisted of optimization of linoleic acid as substrate with various concentrations (100, 200, 300, 400, 500 μ M). The lipoxygenase activity inhibition test was performed by reacting 50 μ L of apigenin (Sigma-Aldrich) or sample solution (with various concentrations), 1650 μ L of 0.2 M borate buffer (pH 9.0) and 1000 μ L of a linoleic acid substrate (Sigma-Aldrich) (300 μ M). The solution was incubated for 10 min at 25°C. 300 μ L of lipoxygenase solution (1000 units/mL) was added and incubated for 15 min at 25°C. Then, 1000 μ L of methanol as stop solution was added. Finally, the solution's absorbance was measured by UV-Vis spectrophotometer at 234 nm. Analysis was done in triplicate. The procedures were referred to Kemal *et al.* (1987) with few modifications.

Inhibition of lipoxygenase activity calculated using the following equation:

$$\text{Lipoxygenase Inhibitory (\%)} = ((A-B)-(C-D))/((A-B)) \times 100\%$$

A = Absorbance of blank solution with enzyme

B = Absorbance of blank solution without enzyme

C = Absorbance of standard or sample solution with enzyme

D = Absorbance of standard or sample solution without enzyme

The result was expressed as IC_{50} value (μ g/mL) and obtained by Microsoft Office Excel.

Phytochemical screening

The extracts were subjected to phytochemical screening for the detection of following compounds; terpenoids, glycosides, flavonoids, alkaloids, tannins, anthraquinones and saponins. The procedures were referred to Indonesian Herbal Pharmacopoeia (2011).

Determination of total flavonoid contents

Total flavonoid contents was measured with $AlCl_3$ colorimetry method. Prepared samples (0.5 mL) by reacting 1.5 mL of ethanol P (v/v), 0.1 mL of 10% aluminum chloride (w/v), 0.1 mL of 1 M sodium acetate and 2.8 mL of distilled water. The mixture solution were incubated at room temperature for 30 min. The absorbance was measured at wavelength 430 nm. The analysis was done in triplicate. Quercetin was used as a standard with the same treatment as the samples for the calibration curve. The total flavonoid contents was reported as total quercetin equivalent per g extract (mgQE/g extract). The procedures were referred to Indonesian Herbal Pharmacopoeia (2011).

Statistical analysis

Statistical analysis was performed to determine the correlation of IC_{50} values with total flavonoid contents of each extracts. Analysis was done with SPSS version 22.0 through two types of tests. The first test was normality test to get the distribution of data in a group. The amount of data in this study were less than 30, so the result of the analysis chosen was by Shapiro-Wilk method. Then, the correlation test with Pearson method was carried out if the results showed normal distribution.

RESULTS AND DISCUSSIONS

Extraction

Each simplicia is extracted by maceration method. The solvent used for maceration is 70% ethanol. According to the Indonesian Herbal Pharmacopoeia (2011), 70% ethanol is a solvent commonly used for the extraction of simplicia if not stated otherwise. Extraction was carried out as many as 3 repetitions with a comparison of simplicia-solvent 1:10. The filtrate or macerate was evaporated with waterbath or rotary evaporator to remove the remaining solvent until a dried extract was obtained. The dried extract was then weighed and the results were obtained as shown in Table 1.

Lipoxygenase activity inhibitory test

The inhibition of lipoxygenase activity aimed to compare IC_{50} values between samples and standards. Apigenin was used as a standard or positive control because it belongs to the class of flavonoid compounds which are potent inhibitors of lipoxygenase activity and has free hydroxyl (OH) group which can reduce Fe^{3+} on the active side of the enzyme, so that the substrate will not oxidized to form products.¹⁰ In addition, based on previous study, IC_{50} value of apigenin was 7.51 μ M (2.03 μ g/mL)^{15,16} which showed that apigenin could inhibit lipoxygenase activity. The IC_{50} apigenin value in this study was 7.69 μ M (2.08 μ g/mL) which showed similarities with the results of previous study.

Inhibition of lipoxygenase activity by extracts was carried out using an optimized linoleic acid substrate of 300 μ M and a lipoxygenase solution of 1000 U/mL. Inhibition of lipoxygenase activity by purple, orange and white sweet potato tubers extracts were done at concentrations of 2000, 4000, 6000, 8000 and 10.000 μ g/mL (final concentrations of 25, 50, 75, 100, 125 μ g/mL). The selection of this concentration varieties was based on experiments that have been conducted several times by researchers.

Based on the results obtained, purple sweet potato extract had the lowest IC_{50} value of 46.09 μ g/mL, while orange and white sweet potato extracts were 52.12 and 63.69 μ g/mL respectively. The percentage of inhibition of lipoxygenase activity was directly proportional to the concentration of the extract tested, so the higher the concentration of the extract, the stronger the inhibitory activity. When compared with apigenin as standard (Figure 1), the IC_{50} value of apigenin was lower than the three extracts. This showed that apigenin as a nature compound had better activity than the three extracts in inhibiting lipoxygenase activity.

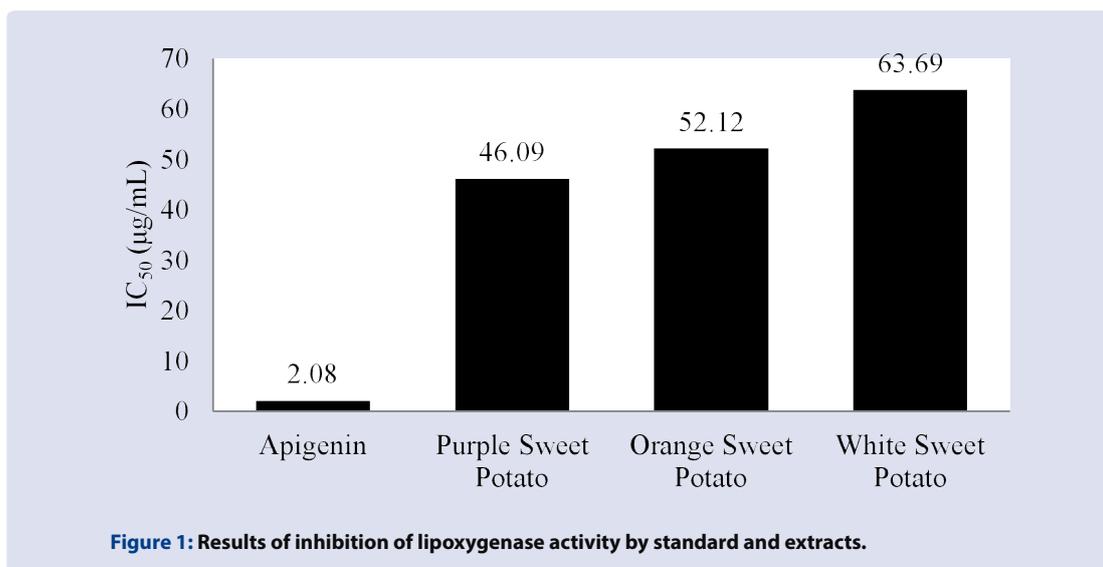


Figure 1: Results of inhibition of lipoygenase activity by standard and extracts.

Table 1: Results of extract's yield.

Extract	Simplicia Weight (g)	Solvent Total Volume (L)	Extract Weight (g)	Yield (%)
Purple	800.0	3 × 8.0	198.96	24.87
Orange	620.0	3 × 6.2	174.87	28.20
White	760.0	3 × 7.6	164.46	21.63

Table 2: Phytochemical screening of each extracts.

Extract	Results						
	Alkaloids	Flavonoids	Terpenes	Tannins	Saponins	Glycosides	Antraquinons
Purple	+	+	-	+	+	+	-
Orange	+	+	-	+	+	+	-
White	+	+	-	+	+	+	-

Phytochemical screening

Phytochemical screening showed presence of alkaloids, flavonoids, tannins, saponins glycosides and negative to terpenes and antraquinons. The results shown in Table 2.

Determination of total flavonoid contents

Quercetin levels were calculated as total flavonoid contents in the sample. From the calculation of quercetin standard curve, there was a linear correlation between absorbance and concentration with linear regression equation of $y = 0.0945x + 0.1245$ and relation coefficient value of $(r) = 0.9999$. Based on the results of this test showed that in 1 g of purple sweet potato extract contained 8.45 ± 0.41 mgQE. Whereas in 1 g of orange and white sweet potato extract contained 7.57 ± 0.03 and 6.12 ± 0.14 mgQE respectively. From these results, purple sweet potato tuber extract had the highest total flavonoid content followed by orange and white sweet potato. Thus, it can be expected that compounds that play a role in inhibiting lipoygenase activity by purple, orange and white sweet potato extract are flavonoids because they have the highest to the lowest order which equals to the IC₅₀ value.

Statistical analysis

In the results of the normality test (Table 3), the significance value indicated by Sig. on the three variables showed $p > 0.05$ which means that H₀ is accepted or the data distribution is normal.

From the results of the above normality test, the correlation test can be done with Pearson because it meets the requirements if the data

distribution is normal. Correlation tests were conducted to determine the degree of closeness and direction of the relationship between variables. The test results (Table 4) showed a significance value of 0.026 which means <0.05 , H₁ is accepted or there is a relationship between the variables of total flavonoid levels and IC₅₀, Pearson correlation value showed -0.999 which means that the relationship is strong and inversely proportional because it is close to 1.000 and there is a "-" sign. From the test results above, it can be concluded that there is a strong relationship and inversely proportional between the total flavonoid levels and IC₅₀, or it can be said that the higher the total flavonoid contents, the lower the IC₅₀ or the stronger the inhibitory activity.

CONCLUSION

Based on the results of research conducted on the three extracts of *Ipomoea batatas* L., it can be concluded that IC₅₀ values of sweet potato tuber extracts of purple, orange and white varieties against inhibition of lipoygenase activity were 46.09; 52.12; and 63.69 µg/mL respectively. The group of compounds contained in sweet potato, purple and white varieties of sweet potato tuber extracts are alkaloids, flavonoids, tannins, saponins and glycosides. Total flavonoid levels of sweet potato, purple, orange and white sweet potato tuber extracts are 8.45 ± 0.41 ; 7.57 ± 0.03 ; and 6.12 ± 0.14 mgQE/g extract respectively. Total flavonoid levels and IC₅₀ values of the three extracts are strongly correlated and inversely proportional to the significance value of 0.026 and the correlation of -0.999 which indicates that the higher the total flavonoid contents, the stronger the inhibitory activity.

Table 3: Normality test results with Shapiro-Wilk.

Variable	Sig.
Total Flavonoid Contents	0.732
IC ₅₀	0.656
Extract	1.000

Table 4: Correlation test results with pearson.

		TFC	IC ₅₀
Total Flavonoid Contents	Pearson Correlation	1	-0.999
	Sig. (2-tailed)		0.026
IC ₅₀	Pearson Correlation	-0.999	1
	Sig. (2-tailed)	0.026	-

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CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

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