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Research Article

Determination of Phenolic Total, Flavonoid Total, Antioxidant Assay and Inhibition of Xanthine Oxidase Enzyme Activity Kemloko Fruits Extract (*Phyllanthus Embilica. L*)

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ABSTRACT

People use Kemloko Fruits as sweets and some also use it as a herbal medicine to reduce uric acid. The sour-tasting Kemloko fruit contains a lot of ascorbic acid. Ascorbic acid and ingredients contained in it have the effect of preventing or reducing uric acid levels. This research intends to determine to secondary metabolite, antioxidant and inhibitory of the xanthine oxidase enzyme Kemloko Fruit extract, as well as determine the phenolic total and flavonoid total levels. The method used to make Kemloko fruit extract uses maceration with ethanol 96% solvent. The phytochemical screening method uses an in-tube reaction for flavonoid content using the Shinoda test, alkaloids using the Dregendorf reagent, saponins using the foam test and terpenoids/steroids using the Liebermann Burchard reagent. Meanwhile, the methods for determining phenolics and flavonoids total are the colorimetric used spectrophotometry UV-Vis, the DPPH methods for antioxidant assay, and measurenment of inhibiting xanthin oxidase activity using a microplate at a wavelength of 375 nm. Qualitative data produced by the ethanol extract of Kemloko fruit contains phenols, tannins, flavonoids, alkaloids, terpenoids, saponins and glycosides. Meanwhile, Quantitative data showed that the phenolic and flavonoid total levels were 1465.63±0.003 mgGAE/g extract and 9.52±0.001 µg/mL, respectively, as well as the IC50 for xanthin oxidase enzyme inhibition of 20.65 µg/mL and the IC50 antioxidant power of

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9.49 ±0.0007 µg/mL. Kemloko fruit ethanol extract contains tannins, flavonoids, alkaloids, terpenoids, saponins and glycosides has inhibitor activity against xanthine oxidase and antioxidant is a strong.

1. Introduction

People use Kemloko Fruits as sweets and some also use it as a herbal medicine to reduce uric acid (Cesari et al., 2015; Khoiriyah et al., 2015). The sour-tasting Kemloko fruit contains a lot of ascorbic acid. Ascorbic acid and ingredients contained in it have the effect of preventing or reducing uric acid levels (Asmilia et al., 2020). The aim of this research was to treat the antioxidant effect and inhibitory of the xanthine oxidase enzyme Kemloko Fruit extract, as well as determine the phenolic total and flavonoid total levels (Huang et al., 2006).

The Ingredient compound of the kemloko plant are phenolic compounds and flavonoid (Asmilia et al., 2020). Kemloko extract ethanol has antioxidant activity so it can lower blood sugar levels or act as antidiabetic (Cahyaningrum et al., 2019).

A plant that contains phenolic compound may also function as an antioxidant because the phenolic group capture free radicals. The greater the total phenolic content of a simplicia, the greater its free radical scavenging activity (antioxidant power). Another reason is that natural sources of antioxidants mostly in plants and the phenolic compounds contained in them

Xanthine oxidase (XO) is an enzyme responsible for the catabolism of purines in the body and converting them into uric acid (Ea, 2011). XO is a target enzyme for the treatment of hyperuresemia and gout (Luna et al., 2019). This research aims to determine the levels of flavonoids and phenols, antioxidant power and inhibition of the xanthine oxidase enzyme.

2. Materials And Methods

Extraction

500 grams of simplicia powder is macerated with 3L of ethanol 96% for 7 days. After that, the solution was filtered using filter paper to separate the dregs and filtrate. The filtrate was evaporated using a rotary evaporator at a temperature of 60^{o}C. The evaporated liquid extract is then evaporated again using an oven at 60^{o}C to obtain a thick extract.

Phytochemical Screening

Phenolic test: Extract a certain amount of simplicia powder with 20 mL of ethanol 96%. Two drops of 5% $FeCl_3$ in methanol are added to 1 mL of sample to form a green or blue-green color (Slavova et al., 2022).

Flavonoid test: A quantity of simplicia powder was macerated with 4 mL of alcohol, and reacted with 0.1 mg of Mg powder and 0.4 mL of amyl alcohol. The reaction results form a yellow to orange color on the amyl alcohol layer (Slavova et al., 2022).

Saponins test: 500 mg of sample powder were put into a glass test tube, 10 mL of hot water was added, cooled and then shaken vigorously for 10 seconds.Produce a lot of foam for at least 10 minutes as high as 1 t 10 cm. The presence of saponin is indicated by the addition of HCl, The foam is not lost. (Susanti et al., 2015).

Alkaloids test: 500 mg of simplicia powder was added with 1 mL of 2N HCl and 9 mL of distilled water, heated over a water bath for 2 minutes, then cooled and filtered. The filtrate obstained was used for alkaloid testing.

Take 2 test tubes, then add 0.5 mL of filtrate to each. In each test tube, 2 drops of Mayer's and Dragendorff's reagents were added. Alkaloids are positive if sediment or turbidity occurs (Wirasti, 2019).

Tannins test: 500 mg of simplicia powder was boiled for 3 minutes in 10 mL of distilled water then cooled and filtered. The filtrate is diluted until it is almost colorless, then 1-2 drops of $FeCl_3$ reagent are added, the result is positive if a blackish blue or blackish green (Wirasti, 2019).

Triterpenoid/Steroids test: A total of 2 mL of the sample was evaporated. The residue was reacted with 0.5 mL of chloroform, 0.5 mL of anhydrous acetic acid and 2 mL of concentrated sulfuric acid. If a brownish or violet ring form, it indicates triterpenoids, if it is bluish green ring it contains steroids. (Susanti et al., 2015).

Glycoside test: The test simplicia powder was dissolved in the solvent 90% ethanol, evaporates on water soaking. The residue was added with 5 mL of anhydrous acetic acid P, and 10 drops of sulfuric acid P were formed blue or green color formed indicates the presence of glycosides (Susanti et al., 2015).

Determination of Phenolic total levels

100 mg of extract was added to 5 mL of 96% ethanol, stir until dissolved, then distilled water was added 10 mL so that the concentration was 10 mg/mL. Take 1 mL of extract, diluteand dilute with distilled water until the extract, dilute and dilute with distilled water until the extract levels is 1 mg/mL. Take 0.2 mL of extract, add 15.8 mL of distilled water and 1 mL of Folin-ciocalteu reagent then shake. Leave it for 8 minuts then add 3 mL of Na_2CO_3 to the mixture. Leave the solution for 2 hours at room temperature. Measure absorbance with UV-Vis spectrophotometer at a maximum absortion wavelength of 768 nm. The result of the phenol content were expressedin mg gallix acid equivalent sample (GAE). (Sawant et al., 2010)

Determination of Flavonoids Total Levels

A total of 20 mg of sample was weighed and 10 mL of ethanol was added until it dissolved, so that it became 2000 µg/mL. Test sample 0.5 mL plus 1.5 mL methanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M sodium acetate, and 2.8 mL distilled water were added. Incubate at 25^oC for 30 minutes, absorbance was measured using a UV-Vis Spectrophotometer at the maximum wavelength (Wirasti, 2019)

Antioxidant test

A total of 25 mg of extract was added with 25 mL of methanol until dissolved, bringing the concentration to 1000 µg/mL. The solution was diluted so that the levels were 25, 50, 75 and 100 µg/mL. 1 mL of each level was taken, reacted with 1 mL of 100 µg/mL DPPH and diluted with 2 mL of methanol then shaken until homogeneous, incubated at 37 ^oC for 30 mins. Next, it is measured at the maximum wavelength (Kamunde et al., 2019).

Xanthin Oxidase Inhibition Test

In a 96 well plate, 85 µL each well, add 74 µL Buffer, 5 µL Xanthine, 5 µL WST-8, 1 µL enhancer. Add 20 µL of sample was mixed at room temperature 25^oC and left for 30 mins, then the solution is measured at the wavelength from 450 nm.

Calculation ΔA tes = Δtest - Δblank

Standard curve, $y = 574.46x + 8.2497$, $R^2 = 0.9996$

The calculation of XO activity in the sample is :

$$\text{XO activity} = y \times V \text{ sample} + \left(\frac{W \times V \text{ sample}}{V \text{ sample total}}\right) \times n = \frac{y}{W \, x \, n}$$

3. Results and Discussions

Kemloko fruit (*Phyllanthus embilica L*) was obtained in Watu Ireng Village, Kandang Serang District, Pekalongan Regency. The result of phytochemical screening showed that all tests produced colors in accordance with literature, namely one positive. However, it shows that kemloko fruit extract contained phenols, tannins, flavonoids, alkaloids, terpenoids, saponins and glycosides, according to the result displayed from isolation (Cesari et al., 2015). The test result are displayed in **Table 1**.

Table 1. Screening Phytochemical result of Kemloko Fruits Extract

Phytochemical Test	Reagent	Positive Test	Result Test	Literature
Phenols	$FeCl_3$ 3%	Green or blue green	Blue green	(Slavova et al., 2022)
Tannins	$FeCl_3$ 1%	Blue dark, blackish Blue, greenish Black	blackish Blue (+)	(Wirasti, 2019)
Flavonoid	Mg powder + concentrated HCl	Orange/ red	Orange (+)	(Slavova et al., 2022)
Alkaloids	Meyer reagent Dragendroff reagent	White precipitate Orange precipitate	White precipitate (+) Orange precipitate (+)	(Wirasti, 2019)
Triterpenoids/Steroids	Cloroform + an hydrous acetic acid + concentrated sulfuric acid	Reddish Brown Ring	Reddish Brown Ring (+)	(Susanti et al., 2015)
Saponins	Hot Aquades+ Chloric acid 2N	Stable Foam is formed (foam does not disappear)	Stable Foam is formed (foam does not disappear) (+)	(Wirasti, 2019)
Glycosides	Ethanol 96 % Evaporated + an hydrous acetic acid + concentrated sulfuric acid	Blue/green	Green (+)	(Susanti et al., 2015)

Various polyphenolic compound in foods have been found to exhibit inhibitory effect on Xanthin Oxidase (XO), where XO mediates diseases originating from oxidative stress due to its ability to produce reactive oxygen species (ROS) including superoxide anion radicals (SOD) and hydrogen per of measuring phenolic total and flavonoids total (Özyürek et al., 2009).

The content of polyphenol compound which function as free radical stabilizers, is generally expressed in terms of phenolic total content and flavonoids total content. **Table 2** is a graph of the result. Phenolic total and Flavonoid total levels are generally related to the ability of an extract as an antioxidant. Both compouds contain many hydroxyl groups (OH-) which can function as proton donors (Free radical stabilizers)(Abdelhamid et al., 2018; Naspiah et al., 2021).

The results of antioxidant testing using 2,2 diphenyl picryl hydrazyl (DPPH) in kemloko fruit extract were 9.496±0,0007 µg/mL. This value indicates that kemloko fruit extract has strong antioxidant power (< 50 µg/mL). In Accordance with the theory that IC50 below 50 µg/mL declared very strong (Junopia et al., 2020). Antioxidant power is associated with the inhibition of various enzyme activities that damage cells. One of the excessive activities of an enzyme that is detrimental to the body is the xanthine oxidation enzyme (Naspiah, Pratama and Sukardiman, 2021). This enzyme stimulates purine catabolism and converts it into uric acid. One disease that is often associated with excess uric acid levels is hyperuresemia (Luna, Dolzhenko and Mancera. 2019; Mehmood et al., 2019). Kemloko fruit extract has been proven to inhibit the activity of the xanthin oxidase enzyme by 20.65 µg/mL.

The result of the research that the ethanol extract of kemloko fruit (*Phyllanthus embilica L*) contained tanninns, flavonoids, alkaloids, terpenoids, saponins and glycocide. The result of this research are in accordance with previous research (Chularojmontri et al., 2013). The result of phenolic total and flvonoid total levels of 1465.62 mgGAE/g Extract and 9.52±0.0007 µg/mL respctively. The value of IC_{50} for inhibition of the xanthin oxidase enzyme is 20.65 µg/mL, and the IC_{50} for antioxidant power is 9.49 ± 0.0007 µg/mL.

Table 2. Phenol Total and Flavonoid Total Result Test

Test Type	Result
Phenolic Total	1465,625±0 mg GAE/g Extract
Flavonoid Total	9,52 ±0,002 mg/L

The suggestion from the reseachers is that is necessary to carry out fractionation and isolation guided by the xanthine oxidase enzyme.

4. Conclusions

Kemloko fruit ethanol extract contains phenols, tannins, flavonoids, alkaloids, terpenoids, saponins and glycosides has inhibitory activity against xanthine oxidase and antioxidant with strong activity.

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