ANTIOXIDANT ACTIVITY OF ETHANOL EXTRACTS AND FRACTIONS KETEPENG CINA LEAVES(Cassia alata) WITH ABTS Assay

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ABSTRACT

Antioxidants are compounds that have a molecular structure that can donate electrons to free radical molecules and can break the chain reaction of free radicals. This research was conducted to analyze the flavonoid content in the ethanol extract of ketepeng cina leaves and to determine the antioxidant activity of the ethanol extract and the fraction of the ketepeng cina leaves. This research is an experimental descriptive study. Ketepeng cina leaves were extracted using 96% ethanol, then partitioned using the liquid-liquid partition method. Flavonoid levels were determined using UV-VIS spectrophotometry at a wavelength of 435 nm. Antioxidant activity test using the ABTS method at a wavelength of 740 nm. The results showed that the flavonoid content of the ethanol extract of the ketepeng cina leaves was 11,054 ± 0.228 QE. The results of the antioxidant activity test of the ethanol extract, n-hexane fraction, ethyl acetate fraction, and water fraction with IC_{50} values were 23,709 ppm, 106,598 ppm, 14,534 ppm, and 49,844 ppm, respectively. The highest antioxidant activity was indicated by the ethyl acetate fraction with an IC_{50} value of 14,534 ppm with very strong activity.

KEYWORDS: Ketepeng cina leaves, Flavonoid, Fraction, Antioxidant, ABTS

INTRODUCTION

Free radicals are reactive oxygen compounds that are known to be compounds that have free or unpaired electrons. Sources of free radicals Free radicals can come from within (the rest of the body's metabolism) or from outside the body (Khaira, 2016). Free radicals will look for new partners so that they will easily bind to other substances such as proteins, fats, and DNA in the body, causing cell damage and causing various degenerative diseases (Sayuti, 2015). Degenerative diseases caused by free radicals include cancer, coronary heart disease, rheumatism, respiratory disease, cataracts, liver, and aging. To prevent this, antioxidants are needed to protect the body from free radical attacks and reduce their negative effects (Winarti, 2010).

Antioxidants are substances needed by the body to neutralize free radicals and prevent the damage that free radicals can cause to normal cells, proteins, fats, and antioxidants have the ability to donate electrons to stabilize free radicals. Antioxidants can be produced inside or outside the body. Many natural sources of antioxidants come from fruits, vegetables, or other plants that contain vitamins A, C, anthocyanins, phenolic compounds, and flavonoids (Kainuma et al., 2015).

One of the plants that contain one or more active ingredients that can be used in traditional medicine is the ketepeng cina leaves (*Cassia alata*). The content of ketepeng cina leaves are alkaloids, saponins, flavonoids, tannins, and anthraquinones (Karima, 2017). Flavonoids ketepeng cina leaves have anti-inflammatory, anti-allergic, antibacterial effects and are effective for several groups of fungi (Hidayat et al., 2015). Rahmawati et al, 2015 stated that the ethyl acetate fraction of ketepeng cina leaves (*Cassia alata*) leaves contains flavonoid and phenolic compounds. The flavonoid content of the ethyl acetate fraction of the ketepeng cina leaves was 2.665 mgRE/g and the phenolic content was 3.729 mgGAE/g (Rahmawati et al, 2015). The results of the antioxidant activity of the ketepeng cina flower using the DPPH method obtained IC_{50} values from the ethanol extract, n-hexane fraction, ethyl acetate fraction, and water fraction of 60.08 g/ml, 127.27 g/ml, 33.43 g/ml and 66.78 g/ml. The antioxidant test was carried out using the DPPH method with UV-Vis spectrophotometry at a wavelength of 400-700 nm, from the results of these measurements the maximum wavelength used will be determined (Ria Safitri, 2020).

Based on this research, encourages researchers to study further the antioxidant potential of the extract and the ethanol extract fraction of ketepeng cina leaves using the ABTS method.

RESEARCH METHOD

This in vitro laboratory experimental study tested the antioxidant activity of the ethanol extract and the ketepeng cina leaves fraction using the ABTS assay.

A. Tools and materials

Rotary evaporator, UV/VIS Spectrophotometer, Analytical Balance, Volumetric flask, Cuvette, Micropipette, Ketepeng cina leaf powder, Quercetin, Ethanol 96%, Ethyl Acetate, N-Hexane, $AlCl_3$, Sodium acetate, ABTS, Potassium persulfate, Methanol, Phosphate buffer pH 7.4.

B. Extraction

A total of 300 grams of ketepeng cina leaf powder was extracted by maceration using 96% ethanol solvent for 3 x @ 24 hours at room temperature with occasional stirring. The macerate was concentrated with a rotary evaporator at a temperature of 40 – 60^{O}C and thickened over a water bath.

C. Fractionation

10 g of thick extract was dissolved with 150 mL of distilled water and then put in a separating funnel. The first fractionation was with the addition of 150 mL of n-hexane, then shaken and allowed to stand until 2 layers were formed (aquadest layer below and an n-hexane layer above) take the n-hexane layer. The water layer is then fractionated with the addition of 150 mL of ethyl acetate into the layer, then shaken and allowed to stand until 2 layers are formed (aquadest layer below and an ethyl acetate layer above) take the ethyl acetate layer. The water fraction, ethyl acetate fraction, and n-hexane fraction were then concentrated using a rotary evaporator and concentrated in a water bath.

D. Determination of Flavonoid Content

1. Quercetin Standard Curve

From a standard solution of 50 ppm quercetin, then several concentrations were made, namely 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm. From each concentration of the standard solution of quercetin, 1 mL was pipetted. Then 1 mL of 10% $AlCl_3$ and 1 mL of 1M sodium acetate were added. Samples were incubated for 25 minutes. The absorbance was determined using the UV-Vis spectrophotometric method at a wavelength of 435 nm (Aminah et al, 2017).

2. Determination Of Sample Flavonoid Content

10 mg of the sample was dissolved in 10 mL of ethanol so that a concentration of 1000 ppm was obtained. 1 mL of this solution was pipetted and then 1 mL of 10% $AlCl_3$ solution and 1 mL of 1 M sodium acetate was added. The samples were incubated for 25 minutes. Measure the absorbance at a wavelength of 435 nm. The flavonoid content was calculated using the linear regression equation of the quercetin standard curve where the concentration is the x-axis and absorbance is the y-axis. Absorbance results from sample measurements were entered into linear regression. The absorbance of the sample is y, so that the total flavonoid content obtained is expressed as the number of mg equivalent of quercetin (QE) in each gram of sample (Noer et al., 2018).

E. Antioxidant Activity Testing with ABTS Method

The test procedure was carried out based on the method of Emad A and Shalaby 2013. ABTS solution was prepared by mixing 5 ml of 7 mM ABTS stock solution and 5 ml of 2.45 mM potassium persulfate solution, the mixture was incubated for 12 - 16 hours. A total of 0.1 ml of sample solution (15, 10, 15, 20, and 25 ppm) was mixed with 0.9 ml of ABTS solution. The mixture was incubated in the dark for 20 minutes, then the absorbance was measured at a wavelength of 740 nm

with a UV-visible spectrophotometer. Measurements were carried out three times with vitamin C as a comparison. The damping percentage value represented by the IC50 value is calculated by the following formula:

$$\% \text{ Inhibiton} = \frac{\text{abs control} - \text{abs sample}}{\text{abs control}} \text{ x } 100\%$$

The IC_{50} value was calculated using the linear regression equation between concentration versus % inhibition. IC_{50} value is obtained from the value of x after replacing y = 50 (Shalaby and Shanab, 2013)

RESULT AND DISCUSSION

Maceration was carried out by soaking the powder leaves of ketepeng cina as much as 300 grams using 96% ethanol for 3 × @ 24 hours with stirring 3 times a day. Stirring aims so that the sample can be mixed with the solvent so that it can prevent not mixing properly between the ketepeng cina leaves and 96% ethanol. The maceration method was chosen because it is the simplest and is one of the cold methods so that it can minimize damage to active compounds that are not resistant to heating. Meanwhile, 96% ethanol was chosen because it is a universal solvent and is non-toxic. The extraction result is a thick liquid of as much as 42.23 grams with a yield of 14.07%.

The phytochemical screening process was carried out using a test tube to determine the class of secondary metabolite compounds contained in the ethanol extract of ketepeng cina leaves. From the results of phytochemical screening that has been carried out, it is found that the ethanol extract of the ketepeng cina leaf contains compounds of the alkaloid, terpenoid, steroid, flavonoid, phenolic, saponin, and tannin class (Table 1). The fractionation process was carried out by liquid-liquid partition method using ethanol, ethyl acetate and hexane as solvents.

Table 1 Results of Identification of Phytochem,ical Compounds

Compound	Reagent	Description
Flavonoid	Powder Mg + HCl	(+) Formed red color
Fenolik	FeCl3 1 %	(+) Formation of blue-black color
Saponin	Aquadest	(+) Formation of 1 cm foam that does not disappear, added an acid solution
Alkaloid	Dragendorf	(+) Formed a red precipitate
Steroid	Lieberman–Burchard	(+) Formed purple color

Determination of the total flavonoid content in the ethanol extract of ketepeng cina leaves was carried out by the colorimetric method. The principle of this method is the formation of a stable acid complex between AlC_{l3} with a C-4 ketone group and a

hydroxyl group at C-3 or C-5 in flavones and flavonols. Meanwhile, $AlCl_3$ forms an unstable bond with the ortho dihydroxy group in ring A or B on the flavonoid so that a color is formed which can be measured by UV/VIS spectrophotometer. As a standard, quercetin is used because quercetin is a flavonoid belonging to the flavonol group (Sapiun et al., 2020). The addition of sodium acetate stabilizes the complex formation between $AlCl_3$ and flavonoids (Lindawati and Ma'ruf, 2020). In determining the levels of flavonoids contained in the ketepeng cina leaf extract, it is necessary to determine the maximum wavelength, operating time, and the manufacture of quercetin standard curves that will be used in the test.

From the results of the determination of the maximum wavelength and operating time obtained a maximum wavelength of 435 nm following research conducted by Das et al., 2013 and an operating time of 25 minutes for incubation [17]. In making the standard querstin curve, the maximum wavelength is 435 with an incubation time of 25 minutes. From the determination of the standard quercetin curve, the linear regression equation y = 0.0154x + 0.2071 is obtained with a correlation value of $R^2 = 0.9823$ Figure 1.

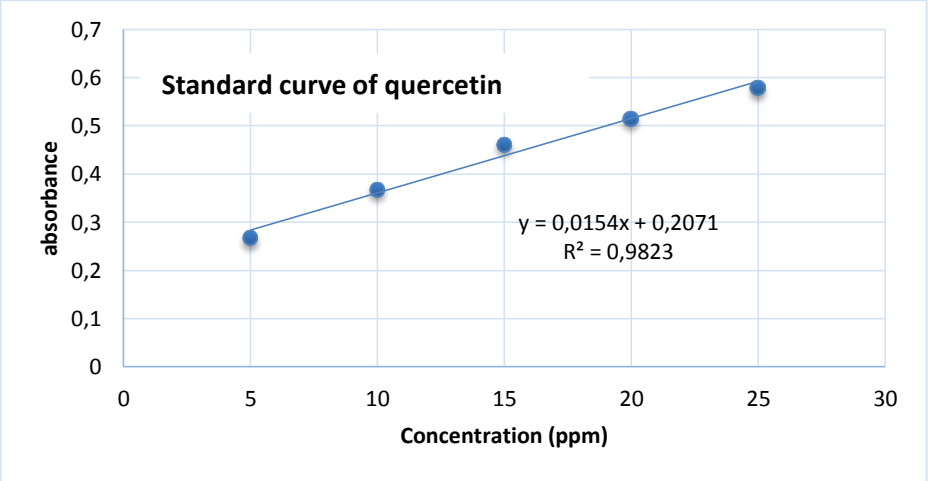

Figure 1. Graph of the determination of the standard curve of quercetin

Determination of the levels of flavonoids contained in the ethanol extract of ketepeng cina) by entering the resulting absorbance into the quercetin curve equation as the y-axis. The measurement results showed that the ethanol extract of ketepeng cina contained flavonoids of 11,054 ± 0.228 QE. The results of measuring the content of flavonoids can be seen in table 2.

Table 2. Content of Flavonoid Ethanol Extract of Ketepeng Cina Leaves (*Cassia alata L.*)

Sample	Abs	Flavonoid Content (g/mg quercetin) QE	Average ± SD
Replication 1	0,374	10,838	11.054 ± 0.228
Replication 2	0,381	11,292	

Replication 3	0,377	11,032

Determination of antioxidant activity in the study using the ABTS method. The principle of measuring the antioxidant activity of the ABTS method is the reduction of ABTS free radicals so that the blue color of ABTS free radicals disappears. ABTS free radicals occur from the reaction of ABTS diammonium salt with potassium persulfate which produces a blue color (Sridhar and Charles, 2019). Before measuring antioxidant activity with the ABTS method, it is necessary to determine the maximum wavelength and operating time that will be used in the test. From the results of determining the maximum wavelength and operating time, the maximum wavelength is 740 nm with an operating time of 20 minutes. Vitamin C was used as a comparison because vitamin C is one of the compounds with high antioxidant capacity. In addition, Vitamin C has free hydroxyl groups that can bind free radicals and the more hydroxy groups will increase antioxidant activity (Lung and Destiani, 2017).

The results of measurements of antioxidant activity on the sample show the following results. The ethanol extract obtained an IC_{50} value of 23,709 ppm, the water fraction obtained an IC_{50} value of 49,844 ppm, the ethyl acetate fraction obtained an IC_{50} value of 14,534 ppm and the n-hexane fraction obtained an IC_{50} value of 106,598 ppm. The test results show that the ethyl acetate fraction has the lowest IC_{50} value of 14,534 ppm. While Vitamin C as a comparison obtained an IC_{50} value of 4,323 ppm. Based on the table of antioxidant capacity, the ethyl acetate fraction is included in the category of very strong antioxidant, namely < 50 ppm (Molyneux, 2004). The results of antioxidant measurements can be seen in table 3.

Table 3. Results of Measurement of Antioxidant Activity Using the ABTS Assay

Sample	IC_{50} (ppm)
Vitamin C	4,323
Ethanol Extract	23,709
Water Faction	49,844
Ethyl acetate fraction	14,534
n-Hexane Faction	106,598

CONCLUSION

The ethanol extract of the ketepeng cina leaves had a flavonoid content of 11,054 ± 0.228 QE. From the antioxidant test using the ABTS method of ethanol extract and the ketepeng cina leaf fraction, it was found that the ethyl acetate fraction had the highest antioxidant activity with an IC50 value of 14.534 ppm

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