Antioxidant Activity Test of Chayote (Sechium edule (Jacq.) Swartz) Ethanol Extract using DPPH Method

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Abstract. The chayote (*Sechium edule* (Jacq.) Swartz) is thought to have excellent antioxidant activity. Fresh chayote has been used empirically by the public as an antihypertensive and to reduce cholesterol levels. This is because chayote contains several vitamins including vitamin A, B, C, and several secondary metabolites. The antioxidant activity found in chayote can prevent the oxidation of a molecule thus stopping the free radical chain reaction and maintaining the working system in the body. This study aims to determine what secondary metabolites are contained in the ethanol extract of chayote and to determine their antioxidant activity. The chayote used is young and old. The research stages were extraction by maceration method, phytochemical screening, determination of functional groups using the FT-IR method, and testing for antioxidant activity using the DPPH method (1,1-diphenyl-2-picrylhydrazyl). The results of the phytochemical screening of the chayote's ethanol extract contained chemical compounds of the alkaloids, flavonoids, saponins, tannins, and glycosides. The FT-IR results of the chayote extract showed the presence of functional groups O-H, C-H, C-N, C = C, C≡C, and C-O. The results of the examination of antioxidant activity using the DPPH method for the ethanol extract of young and old chayote fruit obtained IC_{50} values of 1281.1 and 847.5 μg/ml.
Keywords: ethanol extract of chayote, Phytochemical Screening, antioxidant activity, DPPH method

1. Introduction

Free radicals are molecules that have one or more unpaired electrons, are very unstable and very reactive so that they can cause damage to cell components such as DNA, lipids, proteins and carbohydrates, free radicals that can cause cell damage and also damage biomolecules in the body. ultimately can lead to degenerative disease [1] . Sources of free radicals can come from pollution, dust or are produced continuously as a consequence of normal metabolic sme [2] . The human body continuously forms free radicals through normal metabolic processes, inflammation, malnutrition and the resulting response to external influences in the form of environmental pollution, ultraviolet rays, cigarette smoke and vehicle smoke. These radical compounds will attack the components of the human body organs and cause various degenerative diseases such as cataracts, atherosclerosis, cancer and heart disease. In the human body, we need a substance that helps protect the body from free radical attack. Substances that can reduce the reactivity of free radicals are antioxidants.

Antioxidants can neutralize free radicals so that atoms with unpaired electrons get an electron pair so they don't become reactive anymore. The antioxidant system as a defense mechanism against free radical attack, naturally exists in the human body [3] . Sources of antioxidant antioxidants, namely those made by the body itself are enzyme systems in the human body such as the enzyme superoxide dismutase ; natural antioxidants in the form of flavonoids, phenolic acids, coumarin, tannins, enol tokof , vitamin C and beta-carotene; synthetic antioxidant chemicals that are usually added to food, for example Butylated Hydroxyanisole (BHA), Butylated Hydroxytoluene (BHT) and Propyl Galat [4] .

One of the plants thought to have antioxidant activity is chayote. Chayote (*Sechium edule* (Jacq.) Swartz) is a plant species in the Cucurbitaceae family which is commonly used to treat diseases. Chayote contains several vitamins, including vitamin A, vitamin B and vitamin C [5]. The use of chayote as a disease treatment is used empirically by boiling the fruit. It can lower blood pressure, relieve urinary retention, a burning sensation when urinating, dissolve kidney stones and arteriosclerosis [6]. And the use of crushed chayote is placed on the bleeding gums to stop the bleeding [7]. However, to extract secondary metabolites, the extraction is carried out with ethanol because ethanol is a universal solvent that can extract polar, nonpolar and semi-polar compounds [8]. The extract in chayote showed the presence of alkaloids, flavonoids, sa ponin and terpenoids [9]. Generally, these metabolite compounds are polar so that in this study a polar solvent was used, namely water and ethanol [10].

Chayote water has a diuretic effect that is useful for smooth urination. Chayote is also useful for preventing heart disease, stroke and can increase immunity [11]. The benefits of chayote are very widely used. Diuretic effects allegedly caused by saponins contained in chayote water the conjoined causes it to used by the community to decrease the levels of cholesterol or lowering blood pressure. The chayote is used by the community in a fresh condition. The chayote pulp and skin are mashed and the juice is taken. The juice produced is consumed directly. But the use of chayote as a treatment for disease also by boiling the fruit.

This study aims to determine the antioxidant activity contained in extracts that can be available and stored for a longer time and to determine the content of secondary metabolites by phytochemical screening and determination of functional groups using the FTIR method . The research procedure was carried out by extracting the substances contained with ethanol as a universal solvent because it can attract all components with different solubilities. The separation of the solvent from the extract was carried out in a water bath at a temperature of 70-90°C. Isolation method that can be done by the general public. Having obtained the extract conducted phytochemical screening to determine the content of secondary metabolites in the condensed extracts and determination of functional groups by FTIR . Then the antioxidant activity was determined by the DPPH (1,1-diphenyl-2-picryhydrazyl) method. The DPPH method is an easy, simple, fast method and the results are reliable.

2. Materials and Methods

The materials used in this research are chayote (young and old), 96% ethanol, aquadest, chloroform, hydrochloric acid, sulfuric acid, mayer reagent, bou chardate reagent , iron (III) 1% chloride, chlorhydrate 70 %, 2N sodium hydroxide, lead (II) acetate, reagent dragendorf, reagent molisch, reagent Liebermann-Burchard, DPPH, Vitamin C (ascorbic acid).

2.1 Sample Making

Part used is fruit squash *(Sechium edule* (Jacq.Swartz *)* young and old. The collection of samples se how purposive, ie without comparing to other regions. The raw material fruit squash the young and old each 1 kg weighed, washed with running water, then dried and mashed. The chayote extract was made by maceration using 96% alcohol sprays. 1000 grams of chayote fruit which have been mashed with 7500 ml of 96% ethanol and left for 5 days protected from light, while stirring repeatedly. After 5 days it is filtered and the pulp is squeezed. Then the dregs are added with 2500 liters of water, stirred. The vessel is closed and left in a cool place, protected from light for 2

days. Then the precipitate is separated [12] . The maserate obtained was concentrated with a water bath at a temperature of ± 70-90 °C until a thick extract was obtained.

2.2 Phytochemical Screening

Determination of secondary metabolites contained in the extract viscous include: Test Alkaoids, Test Flavonoids , Test saponins, Test Steroids / Triterpenoids, Test Tannins and Test glycosides. All phytochemical screening stages followed standard procedures [13-14].

2.3 Infrared Spectroscopy Analysis

A qualitative analysis test was conducted to determine the secondary metabolite compounds contained in the sample using Infrared Spectroscopy[15-16]. Tests are carried out to determine the functional groups possessed by the compound in the sample solution according to the procedure [17].

2.4 Testing Antioxidant Activity With Visible Spectrophotometry

The Principle of the DPPH Free Radical Trapping Method. The ability of the test sample to reduce the oxidation process of DPPH (1,1-diphenyl-2-picrylhidrazil) as free radicals in a solution of methanol or ethanol (resulting in reduction of DPPH purple) with IC_{50} (the concentration of the test sample that is able to reduce free radicals is 50%) is used as a parameter to determine the antioxidant activity of the test sample. Condensed extract of antioxidant activity carried out in stages as follows: **1) Preparation of DPPH solution** : Dit draw at 20 mg DPPH dissolved in 50 ml of ethanol , derived DPPH solution with a concentration of 400 ug / ml . **2) Preparation of Blank Solution** : 40 µg / ml of DPPH solution in 1 ml pipette, then dissolved with 10 ml in a tentukur flask, obtained a concentration of 4 µg / ml. **3) Determination of the maximum DPPH** absorption wavelength : a **DPPH** solution with a concentration of 40 µg / ml was measured at a wavelength of 400-800 nm. **4) Determination of *Operating Time*. 5) Preparation of Young and Old Chayote Ethanol Extract Sample Solution** : Weighed 1 g of the test sample each, then put it in a 100 ml measuring flask dissolved with ethanol to the mark line (concentration 10000 µg/ml). **6) DPPH Absorbance Measurement After Sample Addition :** 1 ml pipette sample solution; 1.5; 2; 2.5; and 3 ml, then each was put into a 10 ml fluidized flask (to obtain a concentration of the test solution 1000, 1500, 2000, 2500, and 3000 µg/ml). Then into each measuring flask 1 ml of DPPH solution (concentration 400 µg/ml) was added, then the volume was sufficient with ethanol until the mark line, left in a dark place. Measurements were made in 3 repetitions. The absorbance is measured at the maximum wavelength obtained and allowed to stand for the operating time obtained. **7) DPPH Absorbance Measurement After Vitamin C Addition :** A total of 50 mg of vitamin C crystals are weighed, put in a 50 ml flask, with ethanol up to the mark line (concentration 1000 µg/ml), 5 ml pipettes put into a 50 ml shaped flask, add ethanol to the line mark (concentration 100 µg/ml), pipette back from each solution of 1; 1.5; 2; 2.5; 3; and 3.5 ml were put in a 10 ml flask with ethanol added to the boundary line and then added 1 ml of DPPH solution (concentration 400 µg/ml) with a concentration of vitamin C 10, 15, 20, 25, 30 and 35 µg/ml. Then the absorbance is measured at the maximum wavelength. The measurement is done 3 times after settling in according to the operating time obtained. **8) Determination of Percent inhibition:** The ability of antioxidants and vitamin C samples was measured as a decrease in absorbance of DPPH solution (purple DPPH reduction) due to the addition of the sample solution. The absorbance value of the DPPH solution measurement results before and after the addition of the sample solution was calculated as percent inhibition.

From the results of the calculation of the percent inhibition obtained in the sample, it is continued with the calculation of the linear regression line equation with the sample concentration (µg/ml) as x axis and the inhibition value as y axis. The ability of the test material as antioxidant activity by calculating the *inhibitory concentration 50 %* (IC_{50}) uses the following formula: 50 = ax + b . **9) Determination of IC_{50}** Value is a number that indicates the concentration of the test sample (µg

/ ml) which provides 50% DPPH reduction (able to inhibit or reduce the oxidation process by 50%). The results of the calculation are entered into the obtained regulatory equation.

3. Results and Discussion

3.1 Phytochemical Screening of Chayote

Phytochemical Screening of Chayote Extract . Secondary metabolites contained in young and old chayote can be seen in Table 1. Phytochemical screening is a way to identify bioactive compounds that have not been seen through a test or examination.

Table 1 . Results of Phytochemical Screening of Young and Old Chayote

No	Chemical Compound Group	Ethanol extract of young chayote	Ethanol extracts of Old Chayote
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Tannins	+	+
4	Saponins	+	+
5	Steroid/Triterpenoids	-	-
6	Glycosides	+	+

Information:
(+) Contains the substance being examined
(-) Does not contain the substance being examined

3.2 Functional Groups Based on FT-IR

Determination of the cluster in the sample extracts of squash old using a FT-IR can be seen respectively in figure 1 in the form of the spectrum of FT-IR following. This spectrum represents the results of secondary metabolites in chayote

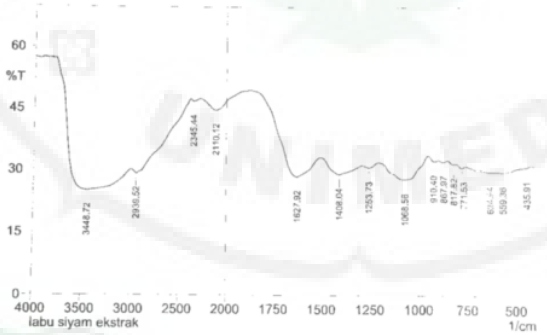

Figure 1 . Spectrum of Ethanol Extract of Old Chayote

The results of FT-IR spectroscopic analysis in Figure 1 and 2 of the ethanol extract of young and old chayote fruit have OH alcohol group in the 3448 cm^{-1} wave number region, there is an aliphatic CH group in the 2939 cm^{-1} wave number region. There is a C≡ C group alkenes in the area of wave number 2110 cm^{-1}. There is a C=C Alkenes group in the wave number region of 1627 cm^{-1}. There is a C-H Alkenes group in the wave number region of 1408 cm^{-1}. There is a C-N of alkaloids in the wave number region of 1253 cm^{-1}. There is a C-O Ether group in the wave number region of 1068 cm^{-1}. There is an Aromatic C-H group in the region of wave number 867, 817, and 771 cm^{-1}. There is a C-X chloride group at the wave number region of 624 and 559 cm^{-1}.

3.3 DPPH Method Antioxidant Activity Test Results With Visible Spectrophotometry

3.3.1 Results of Determining the Maximum Wavelength of the DPPH. The results of measuring the

maximum absorption wavelength of 40 µg / ml DPPH (1,1-diphenyl-2-picrylhydrazyl) solution in ethanol solute resulted in the maximum absorption at a wavelength of 516.50 nm. The maximum wavelength measurement results can be seen in Figure 2.

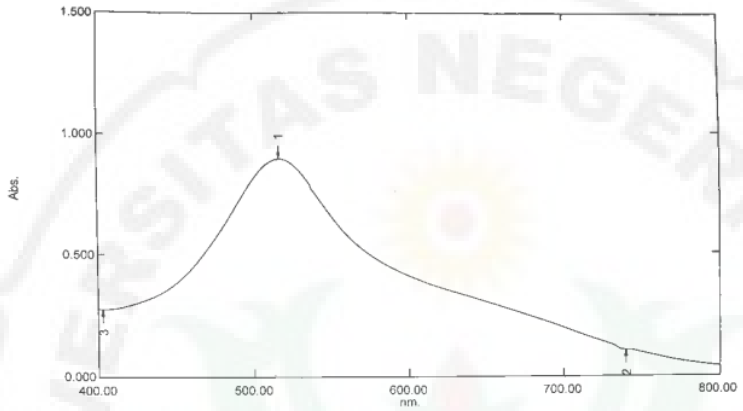

Figure 2 Maximum Wavelength Curve of DPPH

The obtained 516.50 nm wavelength, including one in the visible wavelength range of 400-800 nm, and included in the DPPH wavelength range (1,1-diphenyl-2-picrylhydrazyl) which ranges from 515-520 nm [18-19].

3.3.2 Results of Determination of Operating Time. Determination of the operating time aims to determine the time required for the sample to react with the DPPH radical with a maximum measured at a maximum wavelength of 516.50 nm. Ditujukkan stability with time constant absorbance value at a certain time span during 0- 6 0 minutes. The results of determining the operating time obtained an absorbance of 0.8442 in the 12th minute. Then at that minute the compounds that had antioxidant activity in determining the most stable operating time reacted with the DPPH radical.

3.3.3 DPPH Absorbance Measurement Results After Addition of Test Samples and Vitamin C. DPPH absorbance measurement after sample addition was carried out at a maximum wavelength of 516.50 nm with a concentration of ethanol extract and young chayote of 1000, 1500, 2000, 2500, and 3000 μg/ml and vitamin C with a concentration of 10, 15, 20, 25, 30, and 35 μg/ml. The results of DPPH absorbance after addition of the test sample and vitamin C, each of which can be seen on Table 2 and Table 3.

Table 2. Results of DPPH Absorbance Measurement After Addition of Ethanol Extract of Young Chayote

Concentration of the test solution	Absorbance Measurement			Absorbance Average
	1	2	3	
DPPH	0.897	0.897	0.897	0.897
1000	0.373	0.369	0.369	0.370
1500	0.293	0.301	0.304	0.299
2000	0.258	0.261	0.263	0.260
2500	0.172	0.172	0.171	0.171
3000	0.139	0.139	0.138	0.139

Table 3. Results of DPPH Absorbance Measurement After Addition of Vitamin C

Concentration of the test solution	Absorbance Measurement			Absorbance Average
	1	2	3	
DPPH	0.946	0.951	0.951	0.949
10	0.623	0.622	0.622	0.622
15	0.476	0.487	0.488	0.483
20	0.425	0.422	0.415	0.420
25	0.345	0.341	0.340	0.342
30	0.250	0.243	0.239	0.244
35	0.158	0.162	0.168	0.162

Based on Table 2 and Table 3 show the results of the measurement of the decrease in DPPH absorbance after the addition of the test sample, the decrease in the absorbance value of DPPH means that there has been a reduction of DPPH radicals by the test solution. The higher the concentration of the sample material added, the lower the absorbance value obtained. It means that antioxidant activity is getting higher.

3.3.4 Results of Determination of DPPH Free Radical Absorption Percentage by Test Samples and Vitamin C. The absorption value of the DPPH solution before and after the addition of the test solution and vitamin C was calculated as percent of absorption. The percent attenuation value obtained at each increase in the concentration of the test sample and vitamin C can be seen in Table 4.

Table 4. Analysis of % Inhibition Free Radical by Ethanol Extract of Chayote Young and Vitamin C

Sample	Concentration of Solution Test (ppm)	% Inhibition
Ethanol extract of young chayote	0 (DPPH)	0.00
	1000	57.97
	1500	66.66
	2000	71.01
	2500	80.93
	3000	84.50
Vitamin C	0	0.00
	10	34.45
	15	49.10
	20	55.74
	25	63.96
	30	74.28
	35	82.92

3.3.5 Result of IC_{50} value (Inhibitory Concentration). The IC_{50} value obtained based on the calculation of the linear regression equation by making the concentration of the test solution with the percentage of DPPH as a parameter of antioxidant activity, the sample concentration (μg/ml) as X axis and the absorbance value as Y axis. The inhibition percentage was obtained from the difference in absorption between the DPPH absorbance and the sample absorbance as measured by a Visible spectrophotometer [20]. The IC_{50} value is defined as the concentration of the test compound which can reduce free radicals by as much as 50%. The smaller the IC_{50} value, the higher the free radical

scavenging activity. The results of the IC_{50} value analysis on the antioxidant activity test of the ethanol extract of young chayote and vitamin C can be seen in Table 5.

Table 5. Results of Linear Regression Equation, Value IC_{50} of Ethanol Extract Young chayote and Vitamin C.

Sample	Regression Equations	IC_{50} (μg/ml)
Ethanol extract of young chayote	Y = 0.0264x + 16.179	1,281.098
Vitamin C	Y = 2.2652x + 7.813	18.623

In the same way calculated value of the IC_{50} ethanol extract of old chayote. Research results obtained by the value of the IC_{50} ethanol extract of old fruit squash is 847.5 μg/ml. Based on the results obtained value of the IC_{50} ethanol extract of the young and old chayote is 1281.098 μg/ml and 847.5 μg/ml and the IC_{50} value of vitamin C is 18.623 μg/ml. Table 5 shows that the ethanol extract of chayote have antioxidant activity in the category of very weak because of the value of the IC_{50} is greater than 150 (μg/ml). Meanwhile, vitamin C as a comparison has antioxidant activity in the very strong category because the IC_{50} value of is less than 50 μg/ml. This is because vitamin C is a pure compound and is very active in reducing free radicals, while the test sample extract of young chayote suffered secondary metabolite damage due to the thickening process of the extract at a temperature of 70-90 ºC so that it is no longer active in reducing DPPH free radicals.

4. Conclusion

The ethanol extract of young and old chayote contains secondary metabolites, namely alkaloids, flavonoids, tannins, saponins and glycosides. The results of FTIR from ethanol extract of chayote have functional groups are O-H, C-H, C=C , C≡C, C-N and C-O. The results of the examination of antioxidant activity with DPPH for the ethanol extract of the young and old chayote have IC_{50} value: 1281.098 and 847.5 μg/ml and vitamin C is 18.623 μg/ml. Antioxidant activity of them relatively weak because of the concentration inhibition (50%) is more substantial than 150 μg/ml.

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