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Study of Antioxidant activity and determination of Phenol and Flavonoid content of Pepino's Leaf extract (*Solanum muricatum* Aiton)

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Abstract: Pepino (*Solanum muricatum* Aiton) is the newly plant developed in Indonesia. Pepino's Fruit (*Solanum muricatum* Aiton) has been used traditionally as remedy of mellitus diabetic, hypertention and sprue. The aims of this study are to detemine antoxidant activity and total phenol and flavonoid contents of pepino's leaf (*Solanum muricatum* Aiton) extracts. Pepino's leaves (*Solanum muricatum* Aiton) was extracted by using stratified maceration method with n-hexane, ethyl acetat, and methanol as solvents. Determination of total phenol and flavonoid contents used spechtrophotometry method and antioxidant activity used DPPH radical scavenging method. The results show that rendamen yield of n-hexan extract is 2.7 %, ethyl asetat 5.39 % and methanol 9.91 %.
Keywords: antioxidant, phenol, flavonoid, pepino (*Solanum muricatum* Aiton).

INTRODUCTION AND EXPERIMENTAL

Indonesia has the complete source of biodiversity, including a large number of plant species. Indonesia has more than 30,000 plant species of marine life below. Approximately 9,600 species was known as medicine and there are about 20,000 species of plants that have been known yet the benefits so that we need scientific research to determine the efficacy of these plants for the advancement of remedy in Indonesia, with the fact that the effort to know information about the chemical compounds and bioactivity of plants through scientific research becomes very important [1].Various plants in Indonesia have been used to improve public health, one example is the pepino (Solanum muricatum Aiton), the fruit is still one family with eggplant that has many health benefits including curing diabetes, stroke, high blood pressure, heartburn (indigestion), cancer, kidney, constipation, and hemorrhoids [2]. This plant comes from the Andes (South America) in the region of Peru and Chile. In Indonesia, this plant is a newcomer developed in 2000, so just a few of the research about pepino (Solanum muricatum Aiton), especially in Indoneisa. Fruits and leaves of pepino (Solanum muricatum Aiton) contain alkaloids, flavonoids, and tannins [3.] Phenols and flavonoids are compounds that can counteract the free radicals. The human body has several defense mechanisms against free radicals. The important defense is the enzyme system, called superoxide dismutase (SOD), catalase, and glutathione peroxidase [4.]However, the human body requires antioxidant intake to help prevent free radical overload. Pepino's fruit has a total phenolic contents 24.68 mg GAE / g and the total flavonoid contents 53.60 mg RE / g, with IC_{50} of 0.44 mg / ml [5].The

highest antioxidant activity of various extracts of pepino's fruit (Solanum muricatum Aiton) in a row is 70% ethanol, 22.11 g / m, ethyl acetate, 23.81 mg / ml, water, 28.31 ug / ml, chloroform, 30 , 06 ug / ml, petroleum ether, 32.80 ug / ml and hexane, 38.92 ug / ml [6]. Based on these descriptions, the antioxidant activity and the determination of total phenol and flavonoid contents in pepino's leaves (Solanum muricatum Aiton) have been done. In addition, because pepino's fruit (Solanum muricatum Aiton) is a seasonal fruit, so that with the study on the pepino's leaves (Solanum muricatum Aiton) is expected that pepino plant (Solanum muricatum Aiton) can be developed as a source of medicinal materials optimally.

Figure 1. Pepino (*Solanum muricatum* Aiton)

Materials. Pepino's leaves (Solanum muricatum Aiton) was obtained from Malino Kab. Gowa determined in the laboratory of Phytochemistry, UMI.

Chemicals. 10% aluminum chloride, boric acid, gallic acid, 2 N hydrochloric acid, sulfuric acid, concentrated acetone, sterile distilled water, DPPH (2,2-Diphenyl-1-Picryl-Hydrazyl), ethanol 96% and 70%, ether, ethyl acetate, potassium acetate 1 M, quercetin, ferichlorida solution, methanol, absolute methanol, n-hexane, 7% sodium carbonate, Bouchardat reagents, reagent Folin-Chiocalteu, rutin, oxalate fine powder, magnesium powder.

Extraction. Powder of pepino's leaves (Solanum muricatum Aiton) as much 170 grams was extracted by using stratified maceration method. Samples were extracted by using n-hexane, ethyl acetate and methanol as the solvent. Pepino's leaves (Solanum muricatum Aiton) was extracted by using n-hexane, and then the residue was extracted again by using ethyl acetate, and the residue was extracted by using methanol. The filtrate obtained was evaporated by using a rotary evaporator to obtain a viscous extract.

Identification of Chemical compounds, conducted by the deposition and the color reaction method[7] modified.

a. Alkaloid test

The extract was added 1 mL of 2 N hydrochloric acid and 9 mL of distilled water. Heated for 2 minutes, cooled and filtered. Immediately take 3 drops of the filtrate on a watch glass. Added 2 drops of Bouchardat (If there are deposits of brown to black, it contains alkaloids).

b. Phenol test

The extract was dissolved by using 70% ethanol. Resulting solutions were taken as much 1 mL then added 2 drops of FeCl3. The formation of green or blue-green color indicates the presence of phenolic compounds.

c. Flavonoid test

Dissolve the extract with the solvent, respectively, evaporated to dry 1 mL of the experiment solution, wet the residue with acetone P, added a little finely powdered boric acid, fine powder oxalate P, heated carefully, avoid excessive heat. Mix the remaining was obtained with 10 mL of ether P. Observe with 366 nm UV light, if the solution flourecency intensiveyellow, indicating the presence of flavonoids.

d. Saponin test

The extract was added to a test tube. Then add 10 mL of hot water. Refrigerate and shake vigorously for 10 seconds. If the solution formed foam for 10 minutes, as high 1-10 cm after the addition of 1 drop of 2 N hydrochloric acid, the foam does not disappear then the extract contains saponins.

e. Glycoside test

The extract was dissolved by the solvent, respectively, then evaporated 0.1 mL, dissolve the remain in 5 mL of anhydrous acetic acid P. Add 10 drops of sulfuric acid P, there is a blue or green color indicates the presence of glycosides (Liebermann Burchard reagent).

f. Tannin test

The extract was added 100 mL of hot water boiled for 5 minutes and filtrated. Filtrate was added a solution of FeCl3, if there is a greenish black color indicates the presence of tannins.

Determination of Total Phenols and Flavonoids

Determination of total phenol and flavonoid content in the leaf extract of pepino (Solanum muricatum Aiton) refers to the Chang et al[8] and Nugroho et al[9] procedure with some modifications.

a. Determination of Total Phenol Content

1. Preparation of standard curve of gallic acid

Standard solution was prepared by weighing 10 mg of gallic acid dissolved in 96% ethanol to a volume of 10 mL. The stock solution was pipetted 0.25 mL of 96% ethanol, added with up to 25 mL volume. Preparated concentrations of 1, 2, 3, and 4 ppm then added with 0.4 mL of Folin-Ciocalteau reagent allowed 4-8 minutes, add 4.0 mL of 7% Na_2CO_3 solution, add sterile distilled water to a volume of 10 mL and allowed for 2 hours at room temperature. Measured wavelength of maximum absorbance at 730 nm against a blank consisted of sterile distilled water and reagent Folin-Chiocalteau, then made the calibration curve.

2. Determination of total phenol content

Solution of methanol and ethyl acetate extracts of leaves of pepino (Solanum muricatum Aiton) made by weighing each 10 mg of extracts were dissolved in 10 mL of 96% ethanol. Pipetted respectively - each 1 mL added by 96% ethanol to a volume of 10 mL. Pipetted respectively - each 1.5 mL of methanol and ethyl acetate extracts, respectively added with 0.2 mL of Folin-Ciocalteau reagent allowed for 4-8 minutes, add 2.0 mL of 7% Na_2CO_3 shake until homogeneous, add sterile distilled water up to 5 mL and allowed for 2 hours at room temperature. Measured wavelength of maximum absorbance at 730 nm, and then made the calibrated curve.

b. Determination of total flavonoids

1. Preparation of standard curve rutin

Rutin standard solution prepared by weighing 10 mg rutinly diluted with 96% ethanol to a volume of 10 mL. Stock solution pipetted of 1 mL added by 10 mL ethanol 96%. Concentration of the resulting solution was made above concentrations of 3.75, 5, 6.25, 7.5, 8.75 and 10 ppm to 100 ppm then added with 3 mL of 96% ethanol, added 0.2 mL of 10% $AlCl_3$ solution, add 0, 2 mL of 1 M CH3COOK, added up to 10 mL sterile distilled water allowed for 30 minutes at room temperature. Measured wavelength of maximum absorbance at 415 nm, against a blank consisted of sterile distilled water and 10% $AlCl_3$ and created a calibration curve.

2. Determination of total flavonoid content

Solution of the pepino leaf extracts (Solanum muricatum Aiton) made by weighed each of 100 mg of ethyl acetate and methanol extracts diluted to a volume of 10 mL with 96% ethanol. Pipetted 1 mL of each solution then added to the volume of 10 mL. Pipetted respectively 1 mL of methanol and ethyl acetate extracts, respectively added by 1.5 mL of 96% ethanol, add 0.1 mL of 10% $AlCl_3$ solution, add 0.1 mL of 1 M CH_3COOK, add sterile distilled water to 5 mL and allowed for 30 minutes at room temperature. Measured wavelength of maximum absorbance at 415 nm, and create a calibrated curve.

Antioxidant activity test: Test of antioxidant activity of n-hexane, ethyl acetate and methanol extracts of pepino's leaves (Solanum muricatum Aiton) was tested by using DPPH radical scavenging method, based on Brand William[10] procedure with some modifications. Sample solution was pipetted 0.5 ml of various concentrations, then added 3.5 ml of DPPH, mixed by using vortex and incubated for 1 hour at 37^oC. Absorbance was measured at a wavelength of 517 nm.

RESULT AND DISCUSSION

Pepino's leaves (Solanum muricatum Aiton) were extracted by using stratified maceration method. Maceration method is chosen because it is a cold extraction method which does not damage the chemical compounds. Moreover, this method is more simple and easy to do. Stratified extraction is expected to separate bioactive compounds in the same sample based on the level of polarity, without the bioactive compounds dissolved in the other solvent which is not the solvent[11]. Wherein, the solvent used was n-hexane, ethyl acetate and methanol.

Identification of the chemical compounds was tested by staining reactions and deposition method[12] with some modifications aiming to determine the chemical compounds contained in the pepino's leaves (Solanum muricatum Aiton). The chemical identification assay were tested are alkaloids, phenols, flavonoids, glycosides, saponins, and tannins.

Table 1. Phytochemical Screening of Pepino's Leaf Extracts (Solanum muricatum Aiton)

Test	Extracts		
	n-hexsan	Ethyl acetat	Methanol
Alkaloid	-		
Phenol	-		
Flavonoid	-		
Glicoside	+		
Saponin	-		
Tanin	-		

Determination of phenol compounds was tested to determine the levels of total phenolic compounds in the samples with gallic acid as a comparator which is one of the natural phenol, stable and relatively inexpensive compared than the other[13] . The extracts were added to the Folin - Ciocalteu reagent producing a yellow color indicating the extracts contain phenol, after that, the extracts were added to a solution of 7 % Na_2CO_3 produced a blue color. Chiocalteau-Folin reagent can not react with the phenolic compounds present in the extracts in acid , therefore 7 % Na_2CO_3 was added to provide alkaline conditions that phenolic compounds present in the extract may react with Folin- chiocalteau reagent marked by a color change from yellow to blue.

Table 2. The results of the determination of Total Phenol Content% (w / w) of Pepino Leaf's Extracts (Solanum muricatum Aiton)

No.	Extracts	Absorbance (Y)	% phenol content
1.	Methanol	0,153 0,152 0,158	4,64
2.	Ethyl acetat	0,076 0,0072 0,077	3,36

Analysis of total flavonoids was conducted by Chang et al[8] colorimetric method with rutin as a comparison which is one of the most frequent type of flavonoid found in plants . Generally in the form of rutin glycosides or quercetin 3 - rutinside . In the measurement of total flavonoids in the extract solutions were added $AlCl_3$ 10 % then the formation of a complex between $AlCl_3$ and flavonoids containe in the sample are characterized by color changes to yellow . While the addition of potassium acetate as a stabilizer meant that the solution of sample extracts colore yellow constant[14]

The antioxidant activity assay on the Pepino's Leaf Extracts (Solanum muricatum Aiton) were conducted with several variations of concentration and quercetin as the standard . The solvent used was methanol , because methanol is a polar solvent that can dissolve polar and non -polar compounds such as those in n - hexane , ethyl acetate and methanol extracts, and methanol does not interfere the reaction[15].

Table 3. Results of Determination of Total Flavonoid Content % (w / w) of Pepino's Leaf Extracts *(Solanum muricatum Aiton)*

No.	Extract	Absorbance (Y)	% flavonoid contennt
1.	Methanol	0,150 0,158 0,150	2,005
2.	Ethyl acetate	0,085 0,093 0,106	0,93

Activity of the leaf extracts of pepino (Solanum muricatum Aiton) as antioxidants can be found in the ability to reduce DPPH radical, where DPPH solution acts as a free radicals which will react with antioxidant compounds, so it will turn into a DPPH *2,2 - Diphenyl - 1 - Picryl hydrazyl* nature non - radical is not danger. The increasing amount of *2,2 - Diphenyl - 1 - PicrylHydrazyn* will be marked with a purple color change in the solution became pale yellow [15] .

The parameters used to determine the antioxidant activity of the extracts is IC_{50} , defined as the concentration of substrate or sample solution that will lead to reduced activity of DPPH by 50 % . The greater the antioxidant activity of the IC_{50} value will be smaller [15].

Table 4. Results of DPPH Radical Scavenging of Pepino's Leaf Extracts (Solanum muricatum Aiton)

Sample	Concentration (µg/mL)	Absorbance		% DPPH Scavenging	IC_{50} (µg/mL)
		Blank	Sample		
n-hexane extract	20	1,058	0,964	8,885	139,587
	50		0,944	10,775	
	100		0,933	11,815	
	150		0,909	14,083	
	Regression equation y = 0,298 X+ 8,403				
Ethyl acetate extract	20	1,058	0,957	9,546	266,25
	50		0,943	10,869	
	100		0,935	11,626	
	150		0,929	12,193	
	Regression equation y = 0,152 X + 9,530				
Methanol extract	20	1,058	0,979	7,467	60,389
	50		0,925	12,571	
	100		0,893	15,595	
	150		0,863	18,431	
	Regression equation y = 0,634 X + 7,173				
Quercetine	2	1,081	0,938	13,228	1,852
	4		0,874	19,149	
	6		0,797	26,272	
	10		0,694	35,800	
	Regression equation y = 2,8347x + 8,0217				

The results showed that the three extracts tested, methanol extract has the highest antioxidant activity, then the n-hexane and ethyl acetate extracts. Where, methanol extract has moderate antioxidant activity with IC_{50} value of 67.550 mg / mL, n-hexane extract has weak antioxidant activity with IC_{50} value of 139.58 mg / mL, and the ethyl acetat extract has no potention as an antioxidant because it has the IC_{50} value of 266.25 mg / mL. This is consistent with the levels of total phenols and flavonoids found in the leaves of pepino, in which the methanol and ethyl acetate extracts of leaves of pepino were 4.64% and 3.36%. While the flavonoid compounds in the methanol and ethyl acetate extracts are 2.005% and 0.93%. Quercetin was used as the standard has a very strong antioxidant activity with IC_{50} value of 1.852 mg / mL.

The high antioxidant activity of the methanol extract presumably relates with chemical compounds of phenols, flavonoids and tannins. Where, greater in number than those found in ethyl acetate and n-hexane extracts. Phenolic compounds have antioxidant activity because it is able to donate hydrogen radicals to neutralize free radicals and the phenolic radicals form will be stabilized by resonance[16]. Tannins can act as an antioxidant because of its ability to stabilize the lipid fraction and its activity in the inhibition of lipoxygenase[17]. Alkaloid compounds, particularly indole, has the ability to halt the chain reaction of free radicals efficiently[18].

Antioxidant activity of n-hexane extract smaller than the methanol extract. Based on phytochemical test, n-hexane extract contains glycoside compounds. Glycoside is a compound consist of a combination of two parts of the compound, they are sugar and non-sugar (aglycone). The compounds provide antioxidant activity of the extracts of n-hexane is non-polar compounds. The Less polar aglycone such as isoflavones, flavanones, and flavones tend to be more soluble in non-polar solvents such as ether and chloroform[19]. All types of phenolic compounds have antioxidant activity but with different degrees [20]. The conclution from this reaearch :

1. Total phenolic content of the methanol and ethyl acetate extracts of leaves of pepino (Solanum muricatum Aiton) of 4.64% and 3.36%, while the content of total flavonoids was 2.005% and 0.93%.

2. Antioxidant activity in methanol extract obtained with IC_{50} value of 67.550 mg / mL, n-hexane extract 139.58 mg / mL, and the ethyl extract 266.25 mg / mL.

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