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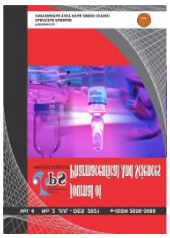

PHYTOCHEMICAL SCREENING AND EFFICACY AS AN ANTIOXIDANT FROM GUAVA LEAVES

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ABSTRACT
Free radicals are molecules that have free electrons, very harmful to health. One of the efforts to overcome it is with antioxidants. Naturally, in the body, there are antioxidants, namely superoxide dismutase, glutathione, and catalase, but depending on food intake, especially containing phenolics and flavonoids. Traditionally, guava leaves are used to treat diarrhea, dysentery, lower cholesterol, irregular menstruation, wounds, and canker sores. Judging from these various properties, it is possible that guava leaves contain chemical compounds that have the potential as antioxidants, especially phenolic compounds, the authors tested the ability of guava leaves as antioxidants. Guava leaves were prepared into ethanol extract, fractionated with n-hexane, ethyl acetate, and water, and phytochemical screening was carried out on the ethanol extract and each fraction. Antioxidant testing was carried out using the *Radical Scavenger* using *1,1-diphenyl-2-picrylhydrazyl*. The test results showed that the ethanol extract contained alkaloids, tannins, flavonoids, steroids, saponins, and glycosides. The n-hexane fraction contains alkaloids and glycosides. the ethyl acetate fraction contains tannins. the water fraction contains tannins and glycosides. As antioxidants, ethanol extract and water fraction were categorized as strong with IC50 ethanol = 42.06 g/ml, water fraction = 49.41 g/mL, n-hexane and ethyl acetate fractions were categorized as medium with IC50 fraction n-hexane = 58.15 g /mL, ethyl acetate fraction = 51.60 g/ml.

Keywords : Guava leaf, antioxidant, *Radical Scavenger, 1,1-diphenyl-2-picrylhydrazyl.*

INTRODUCTION

In various mass media, many dangers arising from food intake and an unhealthy environment have been disclosed due to the formation of free radicals. This is especially experienced by people in urban areas who have a lot of busyness and tend to choose instant foods that are easy to prepare and contain a lot of food additives that contain free radicals, and air pollution which also contains free radicals (Safitri, 2002).

Free radicals are molecules, atoms, or groups of atoms that have unpaired electrons that will attract electrons from other compounds in the vicinity, for example from proteins, lipids, carbohydrates, and DNA (*deoxyribonucleic acid*), which are compounds found in the cell nucleus. , so that these cells will be damaged which will eventually cause various diseases, including cancer, cataracts, diabetes mellitus, kidney, asthma, lung disorders, liver and intestinal inflammation (Kumalaningsih, 2006).

One of the efforts to overcome the dangers of free radicals is by giving antioxidants. Antioxidants are atoms, molecules, or chemical compounds that can donate electrons to free radical molecules so as to break the chain reaction of free radicals, thereby inhibiting the rate of oxidation reactions by reacting with reactive free radicals and then forming an unreactive and relatively stable compound. 2005).

Synthetic antioxidant compounds that are

well known are butyl hydroxytoluene (BHT) and butyl hydroxyanisole (BHA). These two antioxidant compounds are widely used in the food and beverage industry. However, several research results have proven that these two antioxidants have unwanted side effects, namely the potential to be carcinogenic to reproduction and metabolism. Based on acute and chronic toxicity tests on experimental animals, the maximum use of this antioxidant in a food mixture is 200 ppm (Hernani, 2004).

Naturally, in our bodies, there are antioxidant compounds that play an active role in tackling the problem of free radicals, namely the presence of the enzyme superoxide dismutase or SOD, glutathione, and catalase that can protect cells from free radicals attack. However, this depends on lifestyle and diet or intake of foods that contain lots of vitamin C, vitamin E, beta-carotene compounds, phenolics, and flavonoids. Plants are the main source of antioxidants because the leaves, flowers, fruit, and seeds contain many chemical compounds that have antioxidant activity, namely tocopherols, ascorbic acid, carotenoids, polyphenolic compounds, and flavonoids. (Anonymous, 2001), an example is guava leaves (Psidium guajava L.) because it has traditionally been proven to treat various diseases, namely acute and chronic diarrhea, dysentery, flatulence in infants and children, elevated blood cholesterol levels, irregular menstruation, frequent urination (anyang-anyangan). sores, and canker sores. Judging from these various properties, it is likely that guava leaves contain various chemicals, especially those with phenolic groups that have potential as antioxidants (Dalimartha, 2006).

A fast, simple, and easy method to measure antioxidant activity is the free radical scavenger method (*Radical Scavenger*) using *1,1-diphenyl-2-picrylhydrazyl* (DPPH) as a free radical. This method has been widely used to test the ability as an antioxidant of a compound or component of various solid or liquid samples (Darmawan, 2004).

Based on the above, the researchers were interested in conducting phytochemical screening and testing the antioxidant activity of the ethanol extract and the n-hexane, ethyl acetate, and water fractions from guava leaves. (*Psidium guajava* L.). Antioxidant testing was carried out using the free radical scavenger method (*Radical Scavenger*) using *1,1-diphenyl-2-picrylhydrazyl* (DPPH).

RESEARCH METHODS

The chemicals used are of pro analytical quality (pa) unless otherwise stated are E-Merck products, namely: concentrated sulfuric acid, concentrated hydrochloric acid, ethyl acetate, iron (II) chloride, methanol, sodium hydroxide, magnesium powder, zinc powder, methanol, n-hexane, ethyl acetate, and quality pro-analysis Sigma production: 1.1-diphenyl-2-picrylhydrazyl (DPPH), distilled water (Daera Health Laboratory-Medan).

The tools used are laboratory glassware, blender (National), freeze dryer- (Modulyo, Edward, serial No.398), rough balance (Ohaus), electric balance (Vibra), spectrophotometer visit* (Shimadzu).

The stages of work carried out were: collecting and processing samples, making extracts by percolation followed by fractionation using n-hexane + water and ethyl acetate, identification of chemical compounds belonging to the alkaloid group, flavonoids. glycosides, tannins, saponins, steroids/triterpenoids from ethanol extract, n-hexane fraction, ethyl acetate fraction, and water fraction, as well as antioxidant activity testing using the *Radical Scavenger*,

Antioxidant Activity Testing

Prepared a solution with a concentration of 40 g/ml, then measured the absorbance at wavelength of 400-800 nm, so that the maximum absorbance is obtained as a wavelength.

DPPH solution with a concentration of 40 g/ml, the absorbance was measured with a visible spectrophotometer at a wavelength of 516 nm with an interval of 5 minutes to 30 minutes in order to obtain various absorbance values.

Prepared test solution (ethanol extract of guava leaves and hash! fraction with various extractives) each with a concentration of 4 g/mi, 8 g/ml, 12 g/ml and 16 g/ml in a 25 ml volumetric flask. 4 ml of 40 g/ml DPPH (*1.1-diphenyl-2-picrylhydrazyl*) solution was added, then the volume was made up with methanol to the mark line. Then the absorbance was measured with a visible spectrophotometer at a wavelength of 516 nm starting from 5 minutes after the addition of DPPH with an interval of 5 minutes to 30 minutes. The ability of the test material as an antioxidant was calculated based on the decrease in the absorption of the DPPH solution due to the

addition of the test material. The absorption value of the DPPH solution before and after the addition of the test material was calculated as percent inhibition (% inhibition). Then, the regression line equation was calculated with the sample concentration (pg/ml) as the abscissa (X-axis) and the inhibition value as the ordinate (Y-axis). Furthermore, the ability of the test material as an antioxidant is calculated with the Inhibitory concentration 50% (IC50) using the formula:

50 = ax + b

Description: a = Absorptivity
b = Thickness of the cuvette
x = Concentration

RESULTS AND DISCUSSION

Phytochemical screening results show that the ethanol extract contains alkaloids , tannins, flavonoids, steroids, saponins, and glycosides, meaning that they are very potential as antioxidants. The n-hexane fraction is not positive for the presence of tannins and flavonoids.

No.	EXAMINATION	Results
1	Alkaloids	+
2	Flavonoids	+
3	Tannins	+
4	Saponins	+
5	Glycosides	+
6	Anthraquinone	-
7	**Steroids/Triterpenoids**	**+**

The results above indicate that Guava leaves have potential as antioxidants, namely the presence of chemical compounds belonging to the flavonoid group which are generally known can act as antioxidants, namely as free radical scavengers because of the hydroxyl groups they contain. Flavonoids in this case act as reducing agents so that they can donate hydrogen to free radicals (Silalahi, 2006).

Results of Determination of Maximum Absorption Wavelength The

results of the measurement of the maximum absorption of 40 ppm DPPH solution in methanol using a UV-Vis spectrophotometer obtained the maximum absorption at a wavelength of 514 nm. The results of this maximum absorption measurement are not much different from the measurement results obtained by previous researchers.

Operating Time of DPPH Solution in Methanol

the7th minute 7) and from the measurement results obtained the best working time (stable) for 18 minutes, namely at 18 minutes to 36 minutes after the addition of methanol solvent. The absorption curve for the operating time of the DPPH solution in methanol can be seen in Figure 2 below (data attached):

Results of Antioxidant Activity Analysis of Test Samples

Based on the results of the measurement of DPPH absorbance at the 18th minute and 36th minute with the addition of an ethanol extract test solution guava leaves and vitamin C with concentrations of 40 ppm, 80 ppm, 120 ppm and 160 ppm which were compared to the DPPH control (without the addition of the sample/test solution) could be analyzed for the antioxidant activity of the test sample. To see the relationship between DPPH absorbance and the addition of the concentration of the test sample in analyzing its antioxidant activity, it can be seen from the curves in Figures 3 and 4 for guava leaf ethanol extract and Figures 5 and 6 for vitamin C as follows:

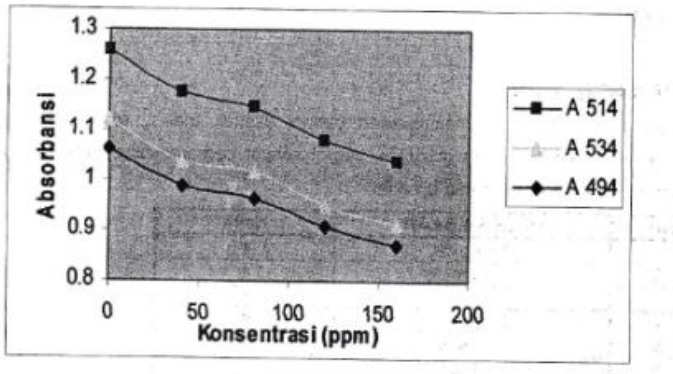

Figure 3. The results of the analysis of the antioxidant activity of guava leaf samples seeds at the 18th minute

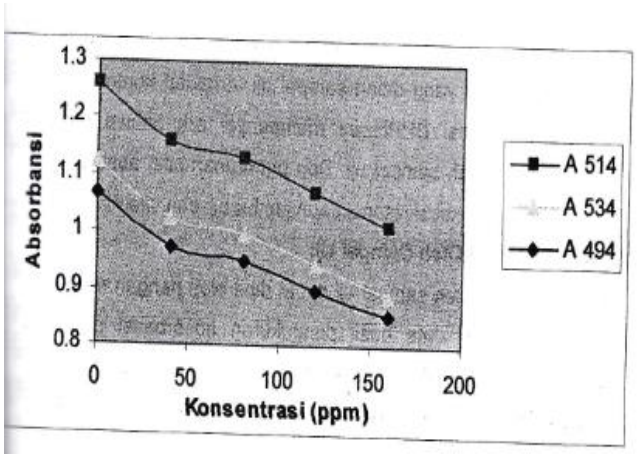

Figure 4. The results of the analysis of the antioxidant activity of the guava leaf extract sample at the 36th minute

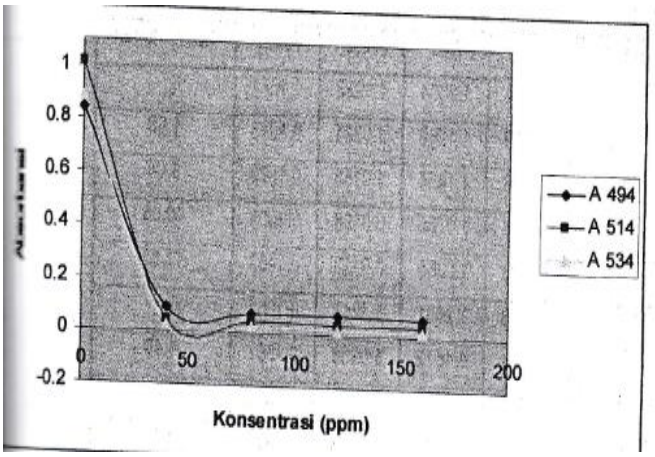

Figure 5. The results of the analysis of the antioxidant activity of vitamin C at the 18th minute

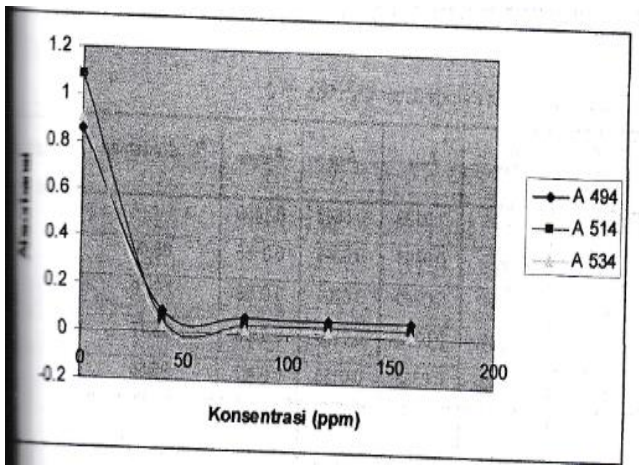

Figure 6. The results of the analysis of the antioxidant activity of vitamin C at 36 minutes

Figure concentration increase. This decrease in the absorbance value of DPPH means that the DPPH free radical capture/damage has occurred by the test sample. And the decrease in the absorbance value of DPPH indicates the presence of antioxidant activity from the ethanol extract of guava leaves and vitamin C.

Results of DPPH Free Radical Attenuation Analysis by Test Samples The

analysis of DPPH free radical attenuation by the test sample can be obtained by first calculating the value of $A_{Calculate}$ (calculated absorbance) from the absorbance measurement data at wavelengths of 494 nm, 514 nm and 534 nm at the 18th and 36th minutes. From the analysis that has been carried out, the percent reduction value is obtained for each increase in the concentration of the test sample as shown in the following table (calculations attached):

Table 3. Results of Free Radical Attenuation Analysis by Guava Leaf

Extract	Sample	A_{494}	A_{514}	A_{534}	$A_{Calculate}$	%
18	DPPH	1.0644	1.2604	1.1223	0.1671	-
	40	0.9859	1.1769	1.0394	0.1643	**1.68**
	80	0.9636	1.1464	1.0153	0.1570	**6.04**
	120	0.9066	1.0785	0.9498	0.1503	**10.05**
	160	0.8693	1.0365	0.9113	0.1462	**12.5168**
36	1.2601	1.12061297	1.2601	1.12061297	_	_
	_	_	_	_	_	_
	attenuati	0.9509	1.1306	0.9988	0.1558	**6.26**

	on					
	120	0.8986	1.0728	0.9473	0.1499	**9.75**
	160	**0.8536**	**1.0122**	**0.8920**	**0.1394**	**16.07**

Information: Data is the result of 3 measurements

Table 4. Results of Free Radical Induction Analysis by Vitamin C

Sample Minute	(ppm)	A_{494}	A_{514}	A_{534}	$A_{Calculate}$	% damping
18	**DPPH**	0.8436	1.0129	0.8843	0.1490	_
	40	0.0879	0.0408	0.0246	0.0155	**89.60**
	80	0.0694	0.0390	0.0201	0.0058	**96.10**
	120	0.0681	0.0 373	0.0190	0.0063	**95.77**
	160	0.0642	0.0333	0.0158	0.0067	**95.50**
36	DPPH	0.8582	1.0840	0.9002	0.2048	_
	40	0.0935	0.0511	0.0262	0.0088	**' 95.70**
	80	0.0696	0.0379	0.0198	0.0684	**0.0068**
	96.73	0.067.0.0 02	0.0198	0.0	67.0.0096 4	**0.067**
	68	_	_	_	_	_

Information: Data is the result of 3 measurements

From table 1 (guava leaf) and table 2 (vitamin C) above, it can be seen that each increase in concentration and with increasing time indicates an increase in the reduction of DPPH free radicals by the test sample. To see the correlation curve of the test sample concentration (ppm) with the percentage of free radical scavenging DPPH can be obtained by plotting the value of the percent reduction and the concentration of the test sample which can be seen in Figure 7 for guava leaf extract and Figure 8 for vitamin C below:

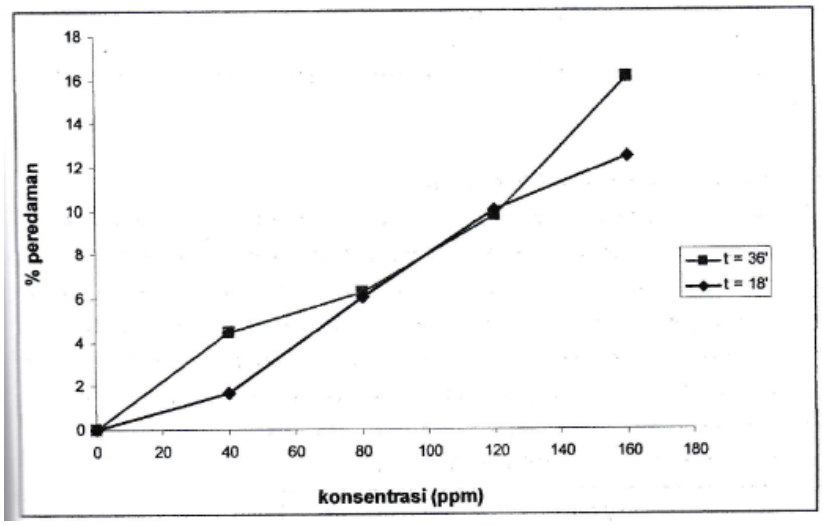

Figure 7 The relationship between the concentration of guava leaf ethanol extract and the percent reduction at the 18th and 36th minutes

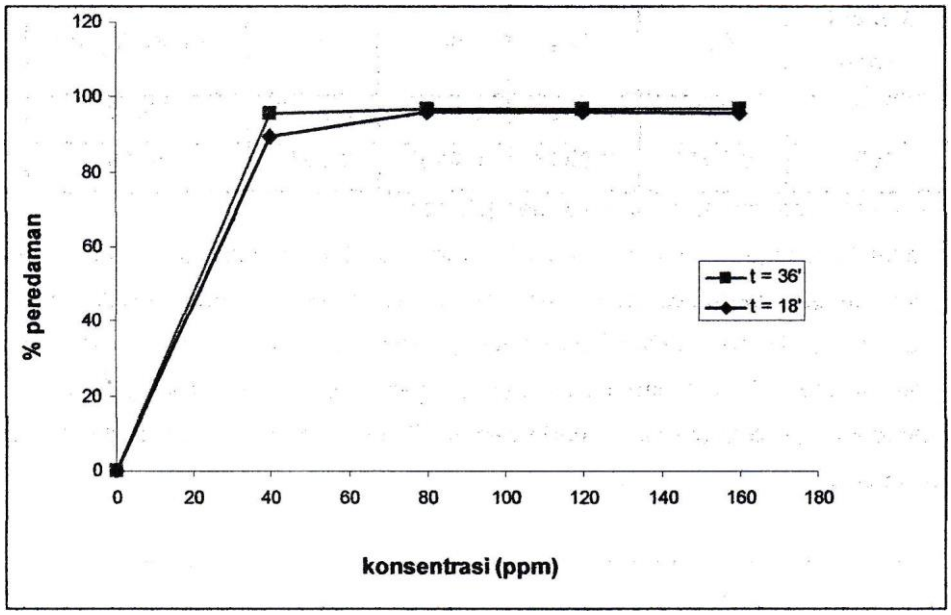

Figure 8. The relationship between the concentration of vitamin C (ppm) and the percent reduction at the 18th and 36th minutes

Analysis of IC_{50} (inhibitory)concentration) Test sample

Analysis of the IC_{50} calculated based on the linear regression equation obtained by plotting the concentration of the test solution and the percent reduction of DPPH as a parameter of antioxidant activity, where the concentration of the test solution (ppm) as the abscissa and the percent reduction value as the ordinate. The results of the linear regression equation obtained for guava leaf extract are y = 0.0835X - 0.62 at the 18th minute and y = 0.0936 X - 0.182 at the 36th minute and for vitamin C is y = 0.4929X + 35.958 at the 18th minute and y = 0.4855X + 38.298 at the 36th minute. The results of the analysis of the IC_{50} obtained based on the calculation of the regression equation that has been carried out can be seen in the following table:

Table 5. IC50 value of guava leaf ethanol extract and vitamin C

Minutes	SAMPLE	IC_{50} (ppm)
18	Guava leaf ethanol extract Vitamins C	606.228 28.489
36	Guava leaf ethanol extract Vitamin C	536.132 24.103

From the table above shows that guava leaf ethanol extract has very weak antioxidant activity compared to vitamin C as a positive control which has very strong antioxidant activity. This is because the antioxidant activity in the ethanolic extract of guava leaves is only determined by antioxidant compounds that can be soluble (extracted) in ethanol (polar solvents) such as polyphenols (flavonoid compounds, anthocyanins), vitamin A and some vitamin C. that vitamin C is easily soluble in water but slightly soluble in ethanol. This vitamin is very sensitive, easily damaged when exposed to light, heat, air and oxygen (Ministry of Health, 1979). Because they have a number of unsubstituted hydroxyl groups or a sugar (flavonoids usually occur as flavonoid O-glycosides), flavonoids are polar compounds. And according to the law of "*dissolved like dissolved*", generally flavonoids are quite soluble in polar solvents such as ethanol, methanol, butanol,

acetone, dimethyl sulfoxide (DMSO), dimethylformamide (DMF), water and others. The presence of sugar bound to flavonoids tends to cause flavonoids to be more soluble in water (Markham, 1988). Meanwhile, other compounds that have antioxidant activity contained in guava leaves such as beta carotene and vitamin E may not be extracted in the ethanol extract. These compounds are soluble in fat or organic solvents (nonpolar) and insoluble in polar solvents such as ethanol (Kumalaningsih, 2006).

CONCLUSION

The results of phytochemical screening using ethanolic extracts contain alkaloids, tannins, flavonoids, steroids, saponins, and glycosides. The n-hexane fraction contains alkaloids and glycosides. The ethyl acetate fraction contains tannin compounds. The water fraction contains tannins and glycosides. The ethanol extract and the aqueous fraction of guava leaves have antioxidant activity in the strong category, the n-hexane and ethyl acetate fractions have moderate antioxidant activity.

REFERENCES

Anonymous. *Antioxidant Activity of Five Vegetable Traditionally Consumed by South-Asian Migrants in 2 in Bradford, Yorkshire*. UK. Online 2001.

Ministry of Health RI. (1989). *Materia Medika Indonesia*, Volume V. Jakarta: The Indonesian Ministry of Health. Page 513, 516, 536, 540, 549.

Ministry of Health RI. (1995). *Materia Medika Indonesia*, Volume IV. Jakarta: The Indonesian Ministry of Health. Pages 308, 310, 313.

Ministry of Health RI. (2000). *General Standard Parameters of Medicinal Plant Extracts*, Printing I. Jakarta: Ministry of Health RI. Pages 1, 10-13.

Geissman, TA (1962). *The Chemistry of Flavonoids Compounds*, New York: The Macmillan Company. P. 366.

Harborne, JB (1987). *Phytochemical Methods*, Translation of Padmawinata and Soediro. Bandung. Page 13, 147.

Hernani, Monoharjo. (2002). *Antioxidant Efficacious Plants*, Print I. Self-help Publisher. Case. 9-11.

http://www. people's minds. com/print/0604/17/cakrawala/penelitian.htm

Safitri, R. (2002). *Heart Disease Prevention Vegetables and Fruits*. People's Mind Cyber Media. Sauriasari. *Recognizing and Countering Free Radicals*, Online 2006. http://www.beritaiptek.com/zberitaiptek-2006-01-22-Mengenal-dan-Menangkal-Bebas-radicals.shtml

Silalahi, J. (2006). Functional Foods, Canisius. pp. 41-49, 54-55.

Sofia, D. *Antioxidants and Free Radicals*, Online 2002. http://www.chem-istry.org/?sect=article&ext=81