Tannin Extract Of Guava Leaves (Psidium Guajava L) Variation With Concentration Organic Solvents

Meigy Nelce Mailoa, Meta Mahendradatta, Amran Laga, Natsir Djide

Abstract: Research has been conducted to determine the levels of tannins in leaves of guava (*Psidium guajava* L) using a variation of the concentration of organic solvent. The method used for qualitative analysis with the tannins are formed by the intensity of the color is blackish green $FeCl_3$ compounds. While the principle of quantitative determination of tannins is tanat acid standard curve at a wavelength of 724,5 nm. In the quantitative analysis of tannins used variations of organic solvent (ethanol and acetone with a concentration of 30%, 50%, 70%). Levels of tannins in the sample solution was calculated with Tannates Acid Equivalent (EAT). The results showed levels of tannins in leaves of guava with 30% ethanol, which is 3.228 mg/g, 50% is 2.970 mg/g, 70% is 2,333 mg/g. While the levels of tannins in guava leaves with 30% acetone solvent which is 2,781 mg/g, 50%: 2,738 mg/g, 70%: 2,405 mg/g.

Index Terms: Tannin, Guava Leaves,Organic Solvents

1. INTRODUCTION

Plants produce a wide range of active compounds that provide pharmacological effect. Generally, the active compounds do not play an important role in the metabolism of plants, so it is often referred to as secondary metabolites (Stepp and Moerman, 2001[1]; Liu *et al.,* 1998[2]. Secondary metabolites have long been known as a source of effective medical therapies and important, such as anti-bacterial and anti-cancer (Cragg, 1997)[3]. This compound is continuously being the main source of many essential efficacious drugs (Harvey, 2000)[4]. In the practice of traditional medicine, people have made use of the active compounds from various plants in the form of medicine, to cure diseases. Active compounds in the plant has been a source of inspiration for disease therapy difficult or expensive treatment (Raskin *et al.,* 2002)[5]. Active compounds of plants can be grouped into four categories, namely: phenols, alkaloids, terpenoids, and non-protein amino acids. The classification is based on the precursor, the basic structure and biosynthetic pathway (Edwards and Gatehouse, 1999;[6], Smith, 1976).[7] These compounds have a wide variation in chemical diversity, distribution and function (Smith, 1976)[7]. Phenol group is characterized by the presence of an aromatic ring with one or two hydroxyl groups. Phenol group consists of thousands of compounds, including flavonoids, phenylpropanoid, phenolic acids, anthocyanins, quinones pigment, melanin, lignin, and tannins, which are widely distributed in various plant species (Harbone, 1996)[8].

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Tannins are generally defined as polyphenolic compounds have high molecular weight (over 1000) and can form a complex with the protein. Based on the structure, tannins can be divided into two classes, namely taninterkondensasi (condensed tannins) and tannin-terhidrolisiskan (hydrolysable tannins) (Hagerman *et al*., 1992[9]; Harbone, 1996)[8].Guava leaves (*Psidium guajava*) is part of the guava tree commonly used as a traditional medicine to cure diarrhea and thrush. Guava leaves (*Psidium guajava*) containing the active chemical saponins, flavonoids, tannins, eugenol, and triterpenoids. Polyphenolic compounds dominate guava leaves are flavonoids (> 1.4%) and tannins (BPOM 2004)[10]. Tannin compounds are polyphenolic compounds that are in plants, food and drink (Makkar and Becker, 1998)[11] is soluble in water and organic solvents (Haslam, 1996)[12]. Tannins can be obtained from almost all kinds of green plants, plants low level and high level with varying levels and quality. Tannins are polyphenolic compounds that are very complex. Because of the phenol group, the tannins can react with formaldehyde (condensation polymerization) to form thermosetting products that can be used as an adhesive. Antibacterial effectiveness of tannin contained in the leaves of plants such as guava is influenced by the concentration of tannins. The higher levels of tannin antibacterial activity will increase. Because of its importance in the treatment of guava leaf, the quality, safety and benefits should be improved through research and development. To improve the quality, safety and effectiveness of guava leaves as a natural medicine Indonesia, standardization needs to be done to the raw material, either in the form or in the form of crude drug extracts or galenic preparations. One of the factors that affect the quality of medicinal plant extracts is the concentration of the solvent used for extraction (Gaedcke *et al.,* 2003)[13]. It is therefore necessary to study to determine the concentration of organic solvent for efficient extraction of tannins from guava leaves extract in order to get good quality. This study aims to determine the type of solvent with a suitable concentration to obtain tannin extract of guava leaves are good quality. Quality parameters of guava leaf extract is measured is the acquisition of extractive (yield of extract) and levels of tannins in leaves of guava.

2. MATERIAL AND METHODS

Equipment and Materials

The tools used in this study include analytical scales, blenders, sieves, desiccator, petridish, stir bar, beaker, beakers, pipettes, Erlenmeyer flask, evaporator, filter paper, aluminum foil, vortex and UV-Vis spectrophotometer. Materials used in this study is the guava leaves, distilled water, technical ethanol 96%, technical acetone. Folin Ciocalteu reagent, Na_2CO_3 7%, 1% $FeCl_3$

Research procedures

1. Sample Preparation of Guava Leaf Wind dried guava leaves for 1 week. Once dried avocado seed blend to a powder and sieved using a sieve.
2. Guava Leaf Extraction. The sample extraction by maceration. Weighed as much as 50 g of guava leaves, soaked in 150 mL of ethanol and acetone with a concentration of 30%, 50% and 70% for 24 hours and then filtered to obtain a filtrate. Treatment was for 3 days. Filtrate obtained together then evaporated to obtain ethanol and acetone extracts. The evaporated extract was cooled in a desiccator before further analysis.
3. Determination of Moisture Content. Determination of water content was conducted using Sudarmadji (1989)[14]. Water content is determined by weighing 2 grams of avocado seeds. Sample is introduced into the oven at 105^oC for 3 hours, then removed from the oven and cooled in a desiccator for 30 minutes, then the weight of the sample was weighed. This treatment is done several times until a constant sample weight.
4. Phytochemical test Tannins Using $FeCl_3$ Phytochemical test is a qualitative test for the suspected presence of tannin in the extract of guava leaves. Phytochemical test conducted in this study that adding extracts with $FeCl_3$ reagent indicated by the color change of green or blue-black ink. Phytochemical test using $FeCl_3$ is used to determine whether a sample contains a phenol group is indicated by a green color blackish or dark blue after being added with $FeCl_3$, so if phytochemical with $FeCl_3$ test gives a positive result it made possible the samples contained phenolic compounds and possible one of them is tannin. Because tannins are polyphenolic compounds. This was confirmed by Harbourne, (1987)[8] classic way to detect simple phenol extract is added to a solution of 1% $FeCl_3$ in water, which cause the color green, red, purple, blue and black strong. Formation of green or blue-black ink on the extract after added with $FeCl_3$ as tannins will form complexes with Fe3+ ions.
5. Determination of Levels of Total Tannins Manufacture of Standard Solution. Carefully weighed 10 mg gallic acid, then dissolved in distilled water and paid back the volume up to 10 ml to obtain a level of 1 mg / ml as a stock solution. And the stock solution pipetted their respective ¬ 10, 15, 20, 25 and 30 ml, was added to 0.2 ml of Folin Ciocalteu (after diluted with distilled water 1:1), homogeneously mixed for 10 seconds and then allowed to stand for 5 minutes. Then add 2 ml of Na_2CO_3 7% w/ v (in distilled water), homogeneously mixed for 30 seconds, then paid back the volume to 5 ml with distilled water in a pint flask in order to obtain a final concentration of 2, 3, 4, 5, and 6 mg / ml. Allowed to stand for 95 minutes. Measured on a UV-Vis spektrofometer the maximum wavelength

Preparation of samples

Carefully weighed 0.1 mg of guava leaf extract was then added to 0.2 ml of Folin Ciocalteu (after diluted with distilled water 1:1), and then allowed to stand for 5 minutes. Then add 2 ml of Na_2CO_3 7% w/v (in distilled water), homogeneously mixed for 30 seconds, then paid back the volume with distilled water to 10 ml in a pint flask. From this stock solution pipetted 1 ml and diluted with water to 10 ml of distilled water. Allowed to stand for 95 minutes. Measured on a UV-Vis spektrofometer at maximum wavelength. Replication is done 3 times.

3. RESULTS AND DISCUSSION

Guava Leaf Extraction According Harbone (1987)[8], to extract the tannins in a total network of plants required a solvent capable of dissolving polar compounds senywa especially tannins. Water is a good solvent for most of the tannins, but the best solvent is a mixture of organic solvents and water. Extraction is the process of separating a substance based on differences in solubility of the two immiscible liquids are different. The principle of extraction is the polar compounds dissolve in polar solvents and non-polar compounds in non-polar compounds. Extraction method used in this study is extracted by maceration method. Maceration is a simple extraction method. Maceration is done by immersing the sample in an organic solvent. Organic solvents will penetrate the cell wall and into the cavity of the cell that contains the active substance so that the active substance will dissolve. Due to the difference between the solution concentration of active substance in the cell, then the solution is terpekat pushed out. The advantage this extraction method, is the method and the equipment used is simple and easily cultivated (Cheong et.al, 2005)[15]. Material to be macerated 50 grams soaked in a mixture of organic solvents (acetone, ethanol): water (1:3) with a concentration of 30%, 50% and 70% for 24 hours and treatment was repeated up to 3 times (Harborne 1987)[8]. Maceration is used because this process has a fairly high absorption effectiveness of the active substances contained in the leaves of guava include tannins. Fluid results maceration then evaporated with rotary evaporator (Rotavapor) to obtain a crude extract of guava leaves thick and brown, then to dry it using Frezdryer. Concentration aims to determine the% yield as well as ease in terms of storage when compared in an extract that is still strong (there are still solvent). The yield difference on one of the six samples because the content of bioactive extracted by solvents, so that the results obtained yield was varied. Yield obtained can be seen in Table 1.Presentase yield calculated from the weight of dry extract extracted by looking at the weight of the initial sample.

Table 1. Sample Results Ekstrasktif Guava Leaf With Variations Solvent Concentration

Solvent	Dry Sample Weight (g)	Extract Weight (g)	Yield (%)
Ethanol 30%	50	4.593	9.186
Ethanol 50%	50	4.944	9.888
Ethanol 70%	50	5.686	11.371
Acetone 30%	50	3.351	6.703
Acetone 50%	50	4.08	8.160
Acetone 70%	50	5.048	10.095

Yield results obtained as shown in Table 1 indicate that the solvent composition of 70% ethanol has a higher yield is 11.37% rather than solvent composition lainnya.Pada this study all results rendemennya extractive or brownish yellow as shown on the Figure 1.

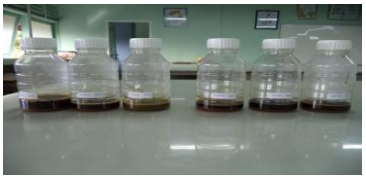

Figure 1. Results of Guava Leaf Extract Rough Guava Leaf Water Content

Determination of water content of guava leaves is performed to determine the number of water content in dried guava leaves. Percentage of moisture content of dry beans can be seen in Table 2. Determination of water content is useful to know the resistance of a material in storage and is the best way for a material handling to avoid the influence of microbial activity. Table 2 shows that the water content in guava leaves ranged from 4.08% -5.43% this shows the actual water content in the leaves of guava has been qualified as <10%. Number of low water levels will make the material more resistant stored in a relatively long period of time so that might be damaged by fungi during storage is very small.

Table 2. Dried Guava Leaf Water Content

No	Sample	Moisture (%)
1	Phase 1 Dried Guava Leaf Drying	5,43%
2	Phase 2 Dry Guava Leaf Drying	4,08%

Qualitative test Tannins Using $FeCl_3$

Phytochemical test conducted in this study that adding crude extract of guava leaves with 1% FeCl3 reagent. Results indicate a change in the color of blackish green, as shown in Figure 2.

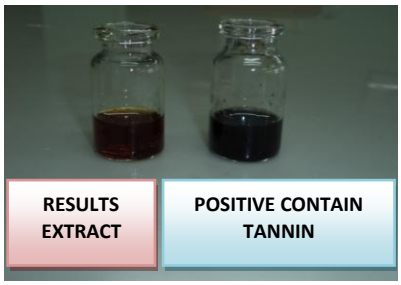

Figure 2. Qualitative test Tannins Using $FeCl_3$ Determination of Content of Total Tannins

Tannins are generally defined as polyphenolic compounds have high molecular weight (over 1000) and can form a complex with the protein. Determination of total content of tannins in leaves of guava using total phenol method using Folin Ciocalteu reagent and standard tanat acid. Determination of total phenol is used to determine the content of tannin contained in each sample. This method has advantages including better color rendition, can minimize the differences at the time of testing and more specific (Rita, 2006)[16]. Folin method does not distinguish between types of phenolic components. The more the number of phenolic hydroxyl group, the greater the concentration of phenolic components were detected (Khadambi, 2007)[17]. The determination of tannin content was measured using a standard curve tanat acid (mg / g).

Table 3. Acid Standard Tannates

Concentration	Absorbance 724,5 nm
2	0.224
3	0.323
4	0.416
5	0.488
6	0.61

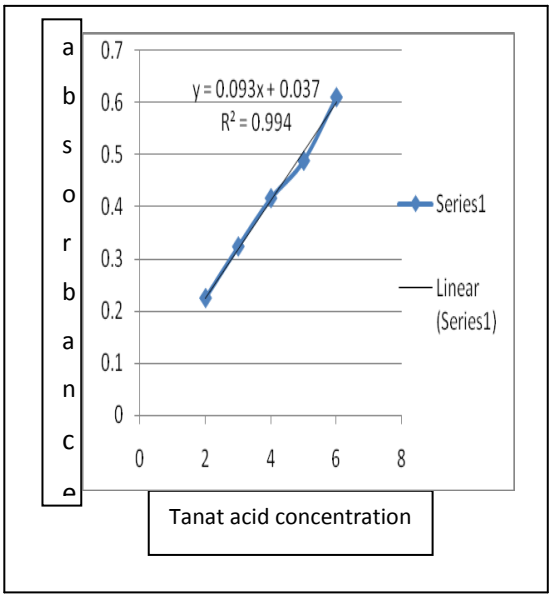

Figure 3. Relationship Between Concentration curve Tannates Acid (ug / ml) with absorbance

Total tannin content of guava leaf extract can be seen in Table 3. The content of total tannins expressed in mg / g of acid tanat.Tabel 3 shows that the highest content of total tannins contained in the 30% ethanol extract tanin higher levels compared with the other extracts.

Table 4. Total Tannin Content Testing Data In Guava Leaf Mean Number of Deuteronomy solvent (mg /g)

Solvent	Replay			Number	Average (mg/g)
	1	2	3		
Ethanol 30%	2.118	2.484	2.452	7.054	*2.351*
Ethanol 50%	1.688	1.710	1.785	5.183	1.728
Ethanol 70%	1.871	1.796	1.839	5.506	1.835
Aceton 30%	1.882	1.935	2.108	5.925	1.975
Aceton 50%	1.882	1.753	1.688	5.323	1.774
Aceton 70%	1.645	1.699	1.710	5.054	1.685

Based on table 4 it can be seen that by using different solvents and concentrations, the amount of extractable tannins are also different, although the solvent used is the same. By Markon *et al* (2007)[18] is affected by tannin extraction solvent polarity. Fisikomia properties marked with the solvent polarity index and momendipole as shown in Table 5.

Table 5. Physicochemical properties of Some Organic Solvents (Markon *et al* (2007)

Solvent	Index Snyder (solvent polarity)	Momen Dipole (Debye)
n-hexane	0,1	0,00
aceton	5,4	2,88
ethanol	5,2	1,69
methanol	5,6	1,70
water (H_2O)	9,0	1,87
ethanol 70%	7,3	
ethanol 50%	7,1	
ethanol 30%	7,9	

Acetone and ethanol is a polar solvent. Acetone is a polar-aprotic solvent that can not provide OH-ions, whereas ethanol is a polar protic solvent that can provide OH-ions, making it easier to interact with polar functional groups on the tannins. Therefore acetone to extract tannins lower than polar-protic solvent (ethanol).

4. CONCLUSION

Ethanol is the best solvent compared to acetone for tannin extraction from guava leaves. Ethanol with a concentration of 30% resulted in the amount of tannin 2.351 mg /g.

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