EFFECTS OF EXPOSURE METHODS TO DIFFERENCES IN MATERIAL ANALYSIS OF PLANT TANIN IN LEAVES OF *PSIDIUM GUAJAVA* L. ACCORDING TO UV-VIS SPECTROSCOPICPHOTOMETRY FROM TANA TORAJA SOUTH SULAWESI

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Introduction

Psidium guajava L. leaves have been widely used in traditional medicine. Seed pink leaves contain tannins, essential oils, fatty oils and malt oils. The results of research showed that seed leaves can inhibit the activity of reverse transcriptase enzymes and deoxyribonucleic acid (DNA) polymerases and inhibit growth of dengue viruses that contain either deoxysiribonucleotic acid or ribonuclear acid (RNA) (1).

One of the active compounds contained in seed roses is tannins. According to the Department of Health (1989), the most commonly used part of the plant as a medicine is its leaves, as the leaves are known to contain 9-12% tannins, essential oils, fatty oils and malic acid. Claus and Tyler's research in 1965 stated that tannins have antiseptic properties that prevent damage caused by bacteria or fungi (2).

Tanins are a large group of complex compounds that are evenly distributed across plants. Almost every plant family has a species that contains tannins. Tanins are usually found in specific parts of plants such as leaves, fruits, stems and stems. Tanins are plant polyphenols that bind and dampen proteins. In the world of tannin medicine, it works to treat diarrhea, stop bleeding, and treat stomach disease (3).

One of the factors that influences the levels of tannins is the method used in the extraction process. Maseration and sochletation are two commonly used methods. Based on Nurhasnawati H. research, showing differences in the activity of ethanol leaf extracts by maseration and sochletation methods. The reason for choosing maseration and sochletation extraction methods is because they have many advantages compared to other methods of extraction. The main advantage of the maseration extraction method is that the procedures and equipment used are simple, the method of extraction is not heated so that the natural material does not become degraded. Cold extraction allows a lot of compounds to be extracted, although some compound has limited solubility in the extraction solvent at room temperature. The method of heat extraction (sochletation) is the best extraction method to obtain more extract results and also the solvent is used less (material efficiency) time used faster, the sample is extracted perfectly because done repeatedly (4).

The determination of tanin levels can be done by the method of visible ultraviolet spectroscopic photometry. To be able to read its absorption at the visible ultraviolet wavelengths, the tannins must be reacted with the color-forming reagent, the denis folin. The color formation is based on oxidation reduction reactions, where tannins act as reducers. Denis folins as oxidators, the oxidized tannins transform the phosmolybdat in denis folians into blue-coloured phos molybdenums that can absorb radiation at visible ultraviolet wavelengths (3).

Based on the background description above it is necessary to carry out research on the influence of the detection method on the results of the analysis of the tanin levels in leaves of *Psidium guajava* L. by UV-VIS spectophotometry.

Materials and methods

The tools used in this study are: mixer bars, porcelain cups, chemical glasses, measuring glass, cork, filter paper, reaction tubes, drop pipettes, rotary evaporators, micropipets, measure cloves, pyrex, UV-VIS spectroscopic photometry. As for the materials used in the study: Aquadest, Etanol 70%, leaf extract of *Psidium guajava* L., p.a. denis folin, and saturated Na_2CO_3.

Sample processing

The samples used were samples of rose leaves of seeds taken around Lamunan, Makale district of Tana Toraja South Sulawesi. *Psidium Guajava* L. leaves have been collected by wet disorting to separate dirt or other foreign material on the sample. Then a wash is performed to remove the scraps attached to the sample. After that, there's a process to facilitate the drying process. Drying is done using a oven at a temperature of 45^0C for 2 days because the drying temperature is best not to exceed 60^0C so as not to damage the chemical content in simplisia. Drying is done so that the rose leaves of seeds become more durable and not easily mushroomed. After drying the material is checked again by selecting the really dry material and discarding the unwanted foreign objects (dry sorting), then stored in a dry container and closed closely (5).

Extraction Process

Maserati Method

Psidium guajava L. leaves, which were then dried, were each weighed 200 grams and extracted by maseration method, i.e. by soaking the sample with 70% ethanol until the whole sample was soaked, then sealed and stored for 3 days while occasionally mixed and then filtered. The amps are inserted back into the maseration device and done as they were until the fluid is uncoloured. The extraction result is attached to a rotavapor and then applied onto a water irrigator until a thick extract is obtained (6).

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Sochletation Method

paper, tied with thread, inserted into a round clove on the socket, plus 750 mL of 70% ethanol. Sochletations are carried out until the cycle droplets are no longer colored. The liquid extract obtained is then squeezed using a rotary evaporator at a temperature of 40ºC until a thick ethanol extract is produced (4).
After the extracts are obtained then the calculation of the irrigation with the formula as follows (7) :

$$\%\ Rendement\ = \frac{\text{Obtained extracted}}{\text{Extracted simplisia}}\ X\ 100\%$$

Qualitative Analysis of Tannins
The filter obtained from the graphite extraction is taken in a quantity of 5ml, inserted into the reaction tube and then saturated with a $FeCl_3$ solution. After that, the color change of the solution, the change of color to green indicates the presence of tannins in the solution of the extract (8).

Manufacture of Denis Folin
Sodium tungstate is taken as much as 5 g and mixed with 1 g of phosphomolibdat acid. Dissolved using aquadest in a chemical glass. After perfectly dissolved inserted into a 50 mL pumpkin then added 2,5 mL phosphatic acid and added quadest until the limit(9).

Production of saturated Na_2CO_3 solution
The preparation of a saturated Na_2CO_3 solution is done by weighing as much as 7.5 g of Na2Co3 then dissolved with aquadest in a chemical glass and heated to 60^0C. After the perfect solution is inserted into a 100 mL pumpkin (3).

Manufacture of 1000 ppm standard solution of phosphoric acid
Fatty acid 0.1 grams dissolved in 100 ml of aquades. This standard solution must always be made new every time it is tested. Manufactured a series of thinning 150 ppm, 200 pppm, 250 pptm, 300 ppm and 350 ppm. Each 1 ml of the dilution series is taken and placed in a 10 ml grated pumpkin container containing 7.5 ml of aquabidestilata. In the pumpkin, add 0.5 ml of denis folin, soak for 3 minutes and add 1 ml of saturated Na_2CO_3 solution, incubate for 15 minutes. Then the absorption is read at a wavelength of 740 nm (10).

Maximum absorption wavelength setting
Taking one of the concentrations of the raw solution, the absorption is measured at a wavelength range of 400-800 nm (11).

Standard Curve Making
The default curve is made by linking the standard solution concentration to the absorption result obtained from measurements using UV-VIS spectroscopes at a wavelength of 740 nm (11).

Determination of Tannin Content
A total of 0.01 grams of maserat is weighed and dissolved with aquabidestilata up to 10 ml. If not perfectly soluble The sochletation tool is installed, then a powder of *Psidium guajava* L. leaves 50 grams wrapped with cylindrical
can be assisted with a tool that works to homogenize the solution. Stepped 1.0 ml of the sample carefully, put into a 10 ml container that had contained 7.5 ml of aquabidestilata. Add 0.5 ml of denis folin peraction, insist for 3 minutes, infuse 1.0 ml of saturated Na_2CO_3 solution. Incubated for 15 minutes, then read its absorption at the maximum wavelength. Calculated using the standard curve obtained so that the concentration of the measured sample is known (10).

Data Analysis
The data type of this study uses the primary data obtained from the absorption value by making a standard curve of the relationship between rate and absorbance and obtaining the linear equation y = bx + a made based on the data of the absorbtion and concentration of the standard solution (6).
Data analysis is aimed at determining the differences in tannin levels in seed rose leaves using the calculation = $\frac{(a.v)\ /\ 1000}{G} \times FP$

Result and Discussion
The content of chemical compounds that are toxic to the biological response must be capable of having chemical specifications, i.e. information on the composition (type and level) and as a drug must meet the safety criteria. Similarly with the plant extracts themselves in the form of pharmaceutical products in addition to having to meet the monographical requirements of raw materials (simplisia), also have the requirements of general and specific standard parameters (12).

Tannins are active compounds of secondary metabolites that are known to have several properties namely as astrigenes, antidiarrhea, antibacterials, and antioxidants. Soluble in water and organic solvents. In this study the simplicity of rose leaves of seeds is dried in a windy way. Drying means cutting the occurrence of enzymatic reactions (microbial activity) and cutting mushroom growth so that it can be stored longer and not easily damaged so that its chemical composition does not change (13).

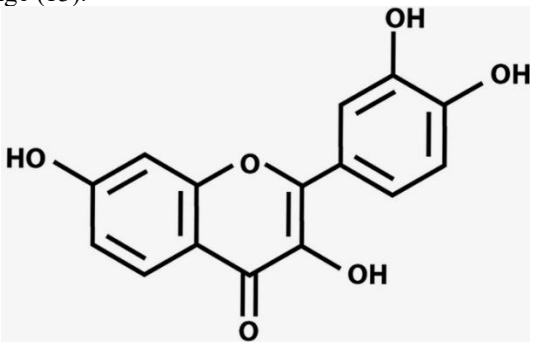

Figure 1. Structure of tanin compounds (10)

Phytochemical testing is a qualitative test to suspect the presence of tannin compounds in leaf extracts. The phytochemical tests carried out in this study are the

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addition of extracts with $FeCl_3$ reagents shown with a change of color i.e. green or blue ink. Phytochemicals using $FeCl_3$ are used to determine whether the sample contains phenol groups shown with green or old blue after the addition with $FeCl_3$, so that when the phytochemistry test with $FeCl_3$ gives a positive result it is possible in the specimen there is a phenolic compound and possible one of them is a tannin. Because tannins are polyphenol compounds. This is reinforced by the (10) opinion that states that the classic way to detect phenolic compounds is simple by adding extract with 1% $FeCl_3$ solution in water, which produces strong green, red, purple, blue and black colours. The formation of green or blue ink on the extract after adding $FeCl_3$ is due to the formation of a complex compound with a Fe3+ ion (14). The results of the qualitative testing of tannins are in line with previous research. The results of previous studies showed that in a qualitative analysis trial the extract of *Psidium Guajava* L. leaves that was saturated with $FeCl_3$ underwent a change in the color of the solution to a blue-blue black indicating that the extracts contain tannins.

The formation of blue-green or black ink on the extraction after being added with $FeCl_3$, as a tannin, will form a complex compound with a Fe^{3+} ion (15).

Table 1. The results of the qualitative testing of tannins

Extraction Method	Tannin Testing Results	The resulting color
Maceration	+	Dark blue
Sochletation	+	Dark blue

It forms a complex compound between tanin and $FeCl_3$ due to the presence of the Fe^{3+} ion as the central atom and the tanin has an O atom that has a free electron pair that can coordinate to the center atom as its ligature. The Fe^{3+} ion in the above reaction binds the three tanins that have 2 donor atoms namely the O atom at the position 4' and 5' hydroxized, so there are six free electron pairs that can be coordinated to the centre atom (16).

Table 2. Calculation of yield

Extraction Method	Sampling Weight (g)	Extract Weights (g)	Extract yield (%)
Maceration	200	12.5813	6.29
Sochletation	200	14.8314	7.42

The above table shows that the method of sochletation produces greater irrigation according to the maseration method, the higher the extraction temperature will cause the movement of the molecules faster, so also with the presence of the circulation (movement) of the solvent can increase the rate of mass transfer of the antioxidant compounds from the cells of the seed rose leaf, thus the contact of the dissolved substance (solute) in the sample with the solute becomes more frequent and more extract is obtained. In this study, the total tannin content was determined based on the addition of color-forming reagents, namely folin denis. The principle of the folin denis method is the formation of a complex blue-coloured compound that measures its absorption in the area of visible light. The determination of tanin levels can be done by the method of visible ultraviolet spectroscopic photometry. To be able to read its absorption at the visible ultraviolet wavelengths, the tannins must be reacted with the color-forming reagent, the denis folin. Color formation is based on oxidation reduction reactions, where tannins act as reducers. Folin denis as an oxidizer, the oxidized tannins will transform the phosmolybdat in the folin denise into a blue-coloured phosmolenic that can absorb radiation at visible ultraviolet wavelengths (3).

The fatty acid reacts with the Denis Folin reagent (Folin Ciocalteau) producing a yellow color indicating that it contains a phenolic, after which it is added with a solution of Na_2CO_3 as a base atmospheric agent.

Table 3. Tannic acid measurement results

Standard Solution	Concentration (ppm)	Absorbance (nm)	Linear Regression Equations
Tanat Acid	150	0,2141	$y = 0,0004x + 0,1516$ $R^2 = 0,9926$
	200	0,2413	
	250	0,2648	
	300	0,2805	
	350	0,3038	

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During the reaction, the hydroxyl group in the phenolic compound reacts with the Denis Folin reaction (Folin Ciocalteau), forming a blue-coloured molybdenum-tungsten complex with an unknown structure and can be detected by a spectroscopic photometer.

The greater concentration of the phenolic compound, the greater the concentration, the more phenolic ions will reduce the heteropoly acid (phosphomolibdat-phosphatungstat) to the molybdenum-tungsten complex, so that the color produced is more concentrated (17).

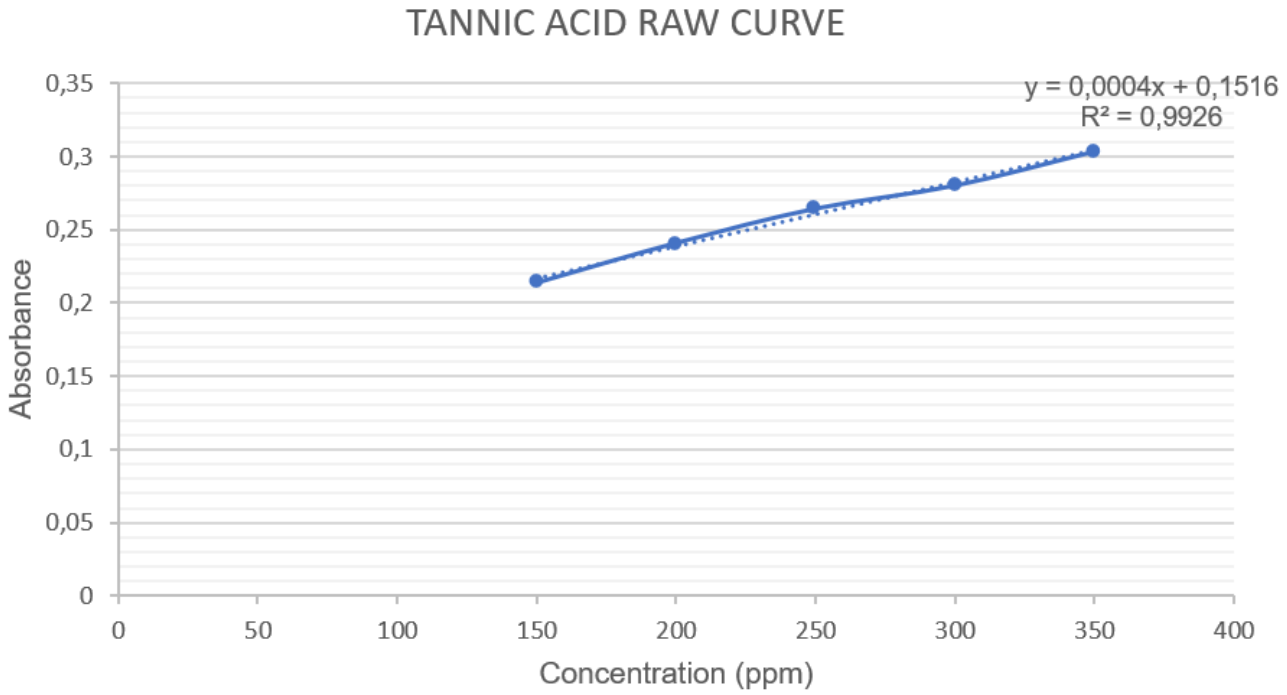

Figure 4. Tannic acid standard curve at a wavelength of 740 nm

At the measurement of total tanin absorption for the determination of the calibration curve of the fatty acid with a wavelength of 740 nm and from the calibrating curve obtained the curve equation is y = 0,0004x + 0,1516 with the value of the relation coefficient R^2 = 0,9926. A linearity value close to 1 indicates the relationship between the concentration of the solution of the fatty acid and the absorption value.

Table 4. Results of measuring total tannin content

Extract	Replication	Abcorbance	Average	Total Tannin Content (mg EAT/g)
Maceration	I	0,2724	0,2751	3,0875
	II	0,2759		
	III	0,2769		
Sochletation	I	0,2996	0,3005	3,722
	II	0,3012		
	III	0,3008		

Based on the results of the calculation of the tanin levels of some extracts showed that the total tanin content produced from the extraction is higher sochletationally this is due to the presence of an influence of the temperature of extraction that can be adjusted so as not to damage the required components with the addition of the extracting temperature of the necessary components can be extracted perfectly and at the extraction sochletation occurs refrigeration which causes the components not easily decomposed. To see the comparison obtained with the sochletation extraction to the tanin levels of the leaves of *Psidium guajava* L., then the test of the tanins levels using two methods of extraction. From the results of the analysis obtain the levels of tanins using sochletation method. This proves that the method of sochletation extraction with ethanol solvents produces extracts of seed rose leaves with a higher tannin content than the maseration method. This is in line with the chemical properties of tannins that are very soluble, and will increase when dissolved in hot water (18), and states that phenol compounds tend to be soluble in polar solvents (10).

Conclusion

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Based on the results of the research, it can be concluded that: The method of extraction affects the total tannin level of *Psidium guajava* L. leaf extract where the most optimal in extraction is the method of sochletation with the highest yield ratio compared to the maseration method. The total tannins level obtained from seed pink leaf by performing the extraction method maserated with the yielded level of 3,0875 mg EAT/g and the yielted rate of 3,722mg EAT /g.

Effects of exposure methods to differences in material analysis of plant tanin in leaves of P*sidium guajava* l. According to UV-VIS spectroscopic photometry from Tana toraja south Sulawesi

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Psidium guajava L. leaf is one of the kinds of plants that are traditionally nutritious for treating diseases, previous research stated that *Psidium guajava* L. has tannin compounds that are known to act as astrigenic, anti diarrhea, antibacterial, and antioxidant. The aim of this study is to determine the effect of the detection method on the difference in the results of the analysis of the levels of tannins in seed leaves (Psidium guajava L.) by UV-Vis spectroscopic photometry and to know the amount of the tannins produced. The extraction methods used were maseration and sochletation. The results showed total tannin levels in the extract using the Maseration method of 3,0875 mg EAT/g sochlettation method at 3,722 mg EAT/g. It can be concluded that the extraction uses a sochletation method that has the highest tanin content compared to the sochleteation method.

Keywords: *Psidium guajava* L., extraction method, tannins, UV-Vis spectrophotometry

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