

Determination of Tannin Content of Gringsingan Stem Extract (*Mesosphaerum suaveolens* (L) Kuntze) by the UV-Vis Spectrophotometry

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ABSTRACT: The Gringsingan plant (*Mesosphaerum suaveolens* (L.) Kuntze) is widely used in traditional medicine and is known for its diverse pharmacological properties. Traditionally, it has been utilized to treat various ailments such as tumors, malaria, headaches, cancer, fever, stomach pain, yellow fever, rheumatism, constipation, urethritis, diarrhea, and kidney disorders, as well as to act as an analgesic, antiseptic, antispasmodic, and depurative agent. The present study aimed to determine the total tannin content in the stem extract of Gringsingan obtained through Microwave-Assisted Extraction (MAE) and to evaluate its potential as a bioactive compound source. Microwave-assisted extraction was selected because of its advantages, including shorter extraction time, reduced solvent consumption, and efficient recovery of active compounds compared to conventional methods. This experimental research was conducted in the laboratory and consisted of several stages: sample preparation, qualitative testing for tannin presence, and quantitative determination of total tannin content. Qualitative identification using 1% FeCl₃ reagent produced a dark blue to greenish-black coloration, confirming the presence of tannins in the Gringsingan stem extract. The result was further supported by thin-layer chromatography (TLC) testing with the same reagent, which yielded consistent positive results. Quantitative analysis of the total tannin content was carried out using UV-Visible spectrophotometry, with absorbance measured at the maximum wavelength for gallic acid. The analysis revealed that the tannin concentration in the Gringsingan stem extract was 7.487 mg GAE/g extract, indicating that this plant possesses measurable levels of tannins that contribute to its traditional medicinal value.

KEYWORDS: Gringsingan; extraction; phytochemical; spectrophotometry; tannin.

1. INTRODUCTION

The Gringsingan plant (*Mesosphaerum suaveolens* (L.) Kuntze), commonly known as *Hyptis suaveolens*, has long been utilized in traditional medicine for its diverse therapeutic benefits. It has been reported to treat various ailments such as tumors, malaria, headaches, cancer, fever, stomach pain, yellow fever, rheumatism, constipation, urethritis, and kidney disorders. In addition, the plant has been traditionally employed as an analgesic, antispasmodic, depurative, antiseptic for burns, and as a remedy for coughs, epistaxis, nausea, and diarrhea (Danong et al., 2021). Phytochemical screening studies have demonstrated that the stems of *M. suaveolens* contain several bioactive secondary metabolites, including alkaloids, flavonoids, tannins, terpenoids, and steroids, which are known to exhibit significant pharmacological activities and may serve as valuable precursors in drug development.

Various extraction methods have been developed to isolate tannin compounds from plant materials, including maceration, percolation, and the more recent green extraction technology known as Microwave-Assisted Extraction (MAE). The MAE method offers several advantages over conventional extraction techniques, such as reduced extraction time, lower solvent consumption, improved extraction efficiency, and minimal thermal degradation of sensitive bioactive compounds (Pratini, 2017).

Previous studies have reported varying tannin contents in different plant extracts using the MAE method. For example, galangal oleoresin contained 5.26% tannins (Huda et al., 2018), pelawan leaf extract 41.30% (Enggiwanto et al., 2018), pine bark extract 27.215 mg/g (Pratini, 2017), and balakka fruit extract 36.86 mg/g (Iriany et al., 2021). Meanwhile, Aulia Primayani and Chatri (2018) reported that the tannin content in the leaves of *M. suaveolens* was 0.520%. However, to date, no studies have reported the tannin content of *M. suaveolens* stem extract obtained using the MAE method.

Therefore, this study aims to determine the tannin content of *Mesosphaerum suaveolens* (L.) Kuntze stem extract using the Microwave-Assisted Extraction method. The findings are expected to provide new insights into the optimization of extraction techniques and the potential utilization of *M. suaveolens* stems as a sustainable source of natural tannins for pharmacological and industrial applications.



2. EXPERIMENTAL SECTION

2.1 Population dan sample

The sample population used in this research was the Gringsingan plant (*Mesosphaerum suaveolen* (L) Kuntze), then the sample used was Gringsingan stems originating from Sinjai Regency.

2.2 Materials and tools

The materials used in this research were aluminum foil, aquadest, aallic acid, FeCl₃ 1%, filter paper, 15% Na₂CO₃ solution, TLC plate, methanol, capillary pipe, Folin coicalteu reagent, sample of gringsingan stem (*Mesosphaerum suaveolen* (L) Kuntze), Tissue. The tools used in this research were stirring rods, 1 liter plastic bottles, porcelain cups, chambers, funnels, drying freezers, chemical beakers 50 mL, chemical beakers 500 mL, measuring cups 10 mL, measuring flasks 100 mL, 254 nm and 366 nm UV lamps, coarse and fine cloths, microwaves, micropipettes 100-1000 µL, tweezers, dropper pipette, horn spoon, Iron horn spoon, UV-Vis spectrophotometer, test tube, analytical balance, blue tip, vial.

2.3 Sample preparation

Gringsingan (*M. suaveolens* (L) Kuntze) stem samples that have been collected are then wet sorted and washed to remove other impurities that are still attached to the sample. The stem sample is then cut into small pieces with a knife. After that, the sample was dried and then ground with a blender. After grinding 300 grams of simplicia powder, put it in a 500 mL beaker with 300 mL Aquades solvent, then put it in the oven at 800 watts for 6 minutes. The filtrate is filtered then the residue is extracted again with the same treatment

2.4 Qualitative analysis

2.4.1 Reagent FeCl₃

2 mg was put into a test tube then dissolved in distilled water, 3 drops of 1% FeCl₃ solution were added, the solution produced a blackish green color so it could be concluded that the sample contained tannin compounds (Kusumo *et al.*, 2017).

2.4.2 Thin Layer Chromatography (TLC)

A total of 2 mg of dry Gringsingan stem extract was dissolved in distilled water, then spotted on an F254 silica gel TLC plate and then left to dry. The TLC plate is then inserted into the chamber containing the eluent which has been completely eluted. The eluent used is Methanol pa: distilled water in a ratio of 8:2 + 2 drops of glacial gallic acid. After being eluted to the boundary line of the TLC plate, it was removed from the chamber and dried, the spots were observed with ultraviolet light at wavelengths of 254 nm and 366 nm and 1% FeCl₃ spots were visible (Lestari, T., & Sidik, Y. 2013).

2.5 Quantitative analysis

2.5.1 Preparation of a standard solution of gallic acid

Weighed 10 mg of gallic acid, dissolved it in distilled water to a volume of 100 mL so that a parent standard of 100 ppm was obtained. The stock standard solution of gallic acid was pipetted at 4 ppm, 5 ppm, 6 ppm, 7 ppm, and 8 ppm as a standard solution of gallic acid (Fatonah *et al.*, 2021).

2.5.2 Determination of wavelength and standard curve of gallic acid standards

A standard solution of 8 ppm gallic acid was added to 1 mL of Folin Ciocalteu reagent, then shaken and left for 5 minutes, then added 2 ml of 15% Na₂CO₃ solution, shaken homogeneously, then added aqua demineralisata until exactly 10 mL and incubated for 60 minutes then made a blank solution 1 mL of Folin Ciocalteu reagent, then add 2 ml of 15% Na₂CO₃ solution and shake until homogeneous. After that, the blank solution and standard gallic acid solution were put into different cuvettes and then read at a wavelength of 400-800 nm.

Standard solutions of gallic acid 4 ppm, 5 ppm, 6 ppm, 7 ppm, and 8 ppm each were put into a 10 mL volumetric flask, 1 mL of Folin Ciocalteu reagent was added, then shaken and left for 5 minutes, then 2 ml of the solution was added. 15% Na₂CO₃ was shaken homogeneously, then added aqua demineralisata to exactly 10 mL and incubated for 60 minutes. After that, the blank solution and standard gallic acid solution were put into different cuvettes and then read at a wavelength of 777 nm (Fatonah *et al.*, 2021).

2.5.3 Determination of tannin content of water extract of gringsingan

Weighed 10 mg of gringsingan stem extract (*Mesosphaerum suaveolen* (L) Kuntze) then dissolved in distilled water to a volume of 10 mL so that the stock solution was 1000 ppm. Then pipet 1 mL and add 1 mL of Folin Ciocalteu reagent, then shake and leave for 5 minutes, then add 2 mL of 15% Na₂CO₃ solution, shake until homogeneous and add distilled water to a volume of 10 mL, leave for 60 minutes. Next, the blank solution and the absorbance of the extract solution were observed at a wavelength of 777 nm. Then the solution was made in 3 replications so that the total tannin

content was calculated to be equivalent to gallic acid (GAE) (Fatonah *et al.*, 2021).

3. RESULTS AND DISCUSSION

In this research, the plant used was the Gringsingan plant (*Mesosphaerum suaveolens* (L) Kuntze) which is a type of the Lamiaceae tribe. The empirical use of this plant is as a medicine for tumors, malaria, headaches, cancer, fever, stomach ache, yellow fever, rheumatism, analgesic, antispasmodic, constipation, urethritis, antisudorific, depurative, relieves coughs, epistaxis, nausea, can also be used to exterminate insects (Danong *et al.*, 2021).

Then the samples used were stems from the Gringsingan plant originating from Sinjai Regency, South Sulawesi. The presence of tannin chemical compounds in gringsingan stems means that researchers want to know the total tannin content in gringsingan stems using the modern extraction method using a microwave or commonly known as *microwave assisted extraction* (MAE). MAE has the advantage of being able to produce extracts with high yields, using a relatively short time, and using minimal solvents compared to conventional extraction methods. MAE uses microwave radiation and temperature to break down simplicia cells during the extraction process so that functional compounds can more easily come out of the simplicia and diffuse into the liquid phase (solvent) (Rabeca Elfirta *et al.*, 2023).

The first step is to prepare the sample, then wash it to remove impurities that are still attached to the sample, then cut it into small pieces, then dry it and then blend it before extraction. The extract obtained from the extraction process is weighed to determine the yield. Calculation of the yield percentage aims to find out how much simplicia is needed for extraction to obtain the desired amount of extract (Egra *et al.*, 2019). The extraction results of gringsingan showed in the **Table 1**.

Table 1. Results of the water extract of Gringsingan stem (*Mesosphaerum suaveolens* (L) Kuntze)

Sample	Sample weight (g)	Solvent volume (mL)	Extract weight (g)	Percent yield (%)
Gringsingan stem	30 gram	300 mL	8.317 gram	2.772%

The next stage is qualitative testing of tannins with color suji and TLC. Identification of tannin compounds was carried out by adding 1% FeCl₃ reagent, the color change of the solution was due to a reduction reaction (**Figure 1**), tannins are a class of polyphenolic compounds which are able to reduce iron (III) to iron (II), with the presence of a phenol group from the tannin compound which binds with FeCl₃ to form a complex blackish green (Maulida *et al.*, 2020).

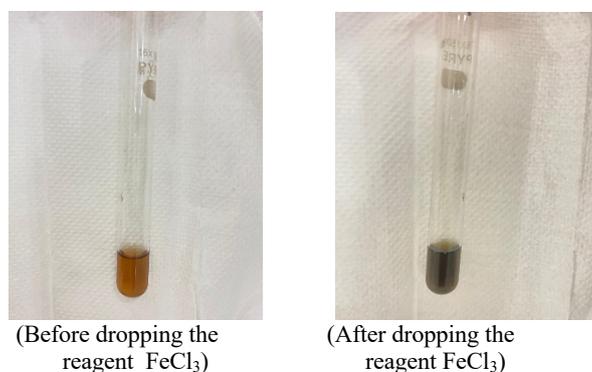


Figure 1. Results of tannin testing with phytochemical screening

Next, test using TLC or Thin Layer Chromatography using the eluent methanol: distilled water in a ratio of 8: 2 + 2 drops of gallic acid. Then it was observed under a UV lamp of 254 and 366 nm to produce spots. The eluent used was a polar solvent so that it was able to elute several compounds that were also polar such as tannin (Lestari, T., & Sidik, Y. 2013). Then dripped with FeCl₃ solution which is used to determine the presence of dots. Observation using a UV lamp is based on the principle that, at a wavelength of 254 nm, the plate gives fluorescence while the sample is dark in color, visible stains arise due to the interaction between the UV light and the fluorescence indicator on the TLC plate, while at a wavelength of 366 nm it gives On the contrary, where the stain gives fluorescence and the plate is dark in color, the visible stain arises due to the interaction between UV light and the chromophore group bound by the ausochrome present in the stain (Forestryana & Arnida, 2020).

Phenolics eluted in the TLC method will produce dark green, red-brown, purple, blue and black spots when sprayed with FeCl₃ (Pratiwi *et al.*, 2023). Gringsingan stem extract showed positive results for tannin after being dropped with FeCl₃ showing a blue color (**Figures 2, 3, and Table 2**).

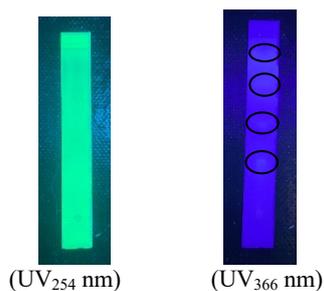


Figure 2. Results of tannin testing with TLC before dropping FeCl_3

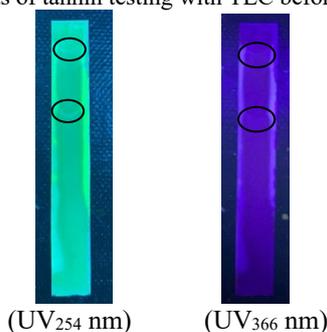


Figure 3. Results of tannin testing using TLC after dropping FeCl_3

Table 2. Test results for Gringsingan stem extract (*Mesosphaerum suaveolens* (L) Kuntze) using TLC

Sample	Spot		+ FeCl_3		Rf	Results
	UV 254	UV 366	UV 254	UV 366		
Gringsingan stem	-	4	2	2	0.672	+
					0.854	+

Next, determining the tannin content using UV-Vis spectrophotometry in the procedure, Folin ciocalteu reagent is used, the hydroxyl groups in phenolic compounds react with Folin ciocalteu reagent to form a blue molybdenum-tungsten complex which is detected with a spectrophotometer at a wavelength of 777 nm. Sodium carbonate is used to create alkaline conditions, because phenolic compounds react with Folin ciocalteu only in alkaline conditions. Gallic acid is used as a comparison because gallic acid has a phenol group, a compound that is stable, pure and cheaper than other comparisons (Amelia, 2015).

Determination of the maximum wavelength of gallic acid to which distilled water has been added and then added with Folin Ciocalteu reagent and sodium carbonate. This determination is made because the maximum wavelength in a compound varies. Determination of the maximum wavelength aims to determine the maximum absorbance which will later be used to determine tannin content (Listiana *et al.*, 2022; Fatonah *et al.*, 2021). Based on measurements of the maximum wavelength of gallic acid with a range of λ 400-800 nm, a maximum wavelength of 777 nm is produced (**Figure 3**)

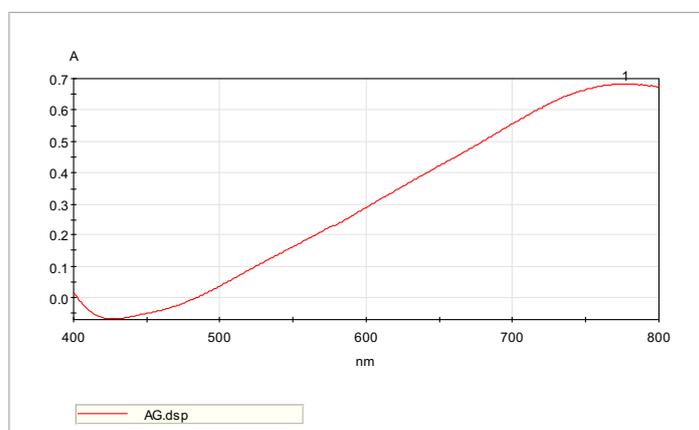


Figure 3. Results Maximum wavelength of gallic acid standard

The next stage is to create a standard curve for gallic acid. This curve is created to determine the correlation between the concentration of gallic acid and its absorbance. The standard curve equation obtained from five series of gallic acid concentrations is $y = 0.1042x - 0.0362$ with a correlation value (r) = 0.9982 from a value of $r^2 = 0.9965$ (Figure 4). The resulting absorbance data is classified as good, because all concentration series The smallest to largest values have an absorbance value of 0.2 – 0.8 while the correlation value obtained is 0.9982, this is said to be very good because a good correlation value is one that is almost close to 1. From the Lambert-Beer law if the absorbance The resulting range is between 0.2 – 0.8, so the graph will be in the form of a straight line (Listiana et al., 2022; Ardin et al., 2022; Fawwaz et al., 2022)

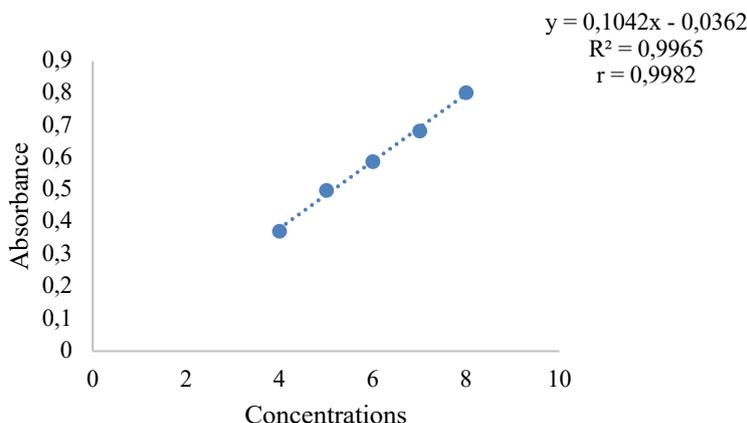


Figure 4. Gallic acid standard curve results

Followed by measuring the sample absorbance at a wavelength of 777 nm carried out with three replications, each of which obtained the absorbance value of replication 1, namely 0.754; replication 2 is 0.706 and replication 3 is 0.771 with a concentration value of 1000 ppm. The tannin content of replication 1 was 7.583 mgGAE/g, the tannin content of replication 2 was 7.122 mgGAE/g and the tannin content of replication 3 was 7.756 mgGAE/g for total tannin content an average of 7.487 mgGAE/g extract (Table 3).

Table 3. Calculation results of total tannin content of gringsingan stems (*Mesosphaerum suaveolens* (L) Kuntze)

Replication	Absorbance	Initial tannin content (mg/mL)	Tannin content mgGAE/g extract	Total tannin content mgGAE/g extract
1	0.754	0.007583	7.583	7.487
2	0.706	0.007122	7.122	
3	0.771	0.007746	7.746	

According to research by Aulia Primayani & Chatri, 2018, the total tannin content obtained in the leaves of gringsingan (*Mesosphaerum suaveolens* (L) Kuntze) was 0.520 mg/g using the maceration extraction method, whereas in this study the total tannin content in the stems of gringsingan (*Mesosphaerum suaveolens* (L) Kuntze) with the microwave assisted extraction method was higher, namely 7,487 mgGAE/g extract. *Mesosphaerum suaveolens* (L) Kuntze contains tannins which are used as anti-inflammatory agents and are also used topically for the treatment of burns (Shenoy et al., 2009)

4. CONCLUSION

Based on the results of this study, it can be concluded that the stem extract of Gringsingan (*Mesosphaerum suaveolens* (L.) Kuntze) obtained through Microwave-Assisted Extraction (MAE) was confirmed to contain tannin compounds, as indicated by the positive color reactions observed in qualitative tests using 1% FeCl₃ reagent and thin-layer chromatography (TLC), both of which produced characteristic dark blue to greenish-black spots. Furthermore, the quantitative analysis conducted using UV-Visible spectrophotometry revealed that the total tannin content in the Gringsingan stem extract was 7.487 mg GAE/g extract (milligrams of gallic acid equivalent per gram of extract). These findings demonstrate that the Gringsingan stem extract contains measurable amounts of tannins, supporting its potential as a natural source of bioactive compounds with possible pharmacological applications.

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Ethical Approval: Not applicable

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