

Determination of Flavonoids Content of Gringsingan Stems (*Mesosphaerum suaveolens* (L) Kuntze) Extracted by Microwave Assisted Extraction

Selpida Handayani*, Aminah, Nurul Fadillah Badu

Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar 90231, Indonesia

* Corresponding Author. E-mail: selpida.handayani@umi.ac.id

Received: 13 November 2024 / Revised: 10 December 2024 / Accepted: 15 January 2025

ABSTRACT: Gringsingan plant (*Mesosphaerum suaveolens* (L) Kuntze) is a plant belonging to the *lamiaceae* family originating from Sinjai district, South Sulawesi. This plant is empirically used by the people of Sinjai district as a medicine to relieve stomach aches. The modern extraction method used is microwave-assisted extraction, which is an extraction method that uses microwave radiation to speed up extraction by heating the solvent quickly and efficiently. The aim of this research was to determine the value of total flavonoid content in Gringsingan (*Mesosphaerum suaveolens* (L) Kuntze) stems extract based on microwave-assisted extraction. This research was carried out experimentally in the laboratory. The stages of this research began with preparing test samples, testing the quality of flavonoid compounds, and ended with determining the total flavonoid content of gringsingan stems, which then measured their absorption using a UV – Vis spectrophotometer. In this study, the results were obtained that the Gringsingan stem extract was positive for containing flavonoids based on identification results using the Wilstater reagent. This sample was also declared positive for containing flavonoids based on the thin layer chromatography (TLC) test using the $AlCl_3$ specific reagent. Determination of the total flavonoid content of Gringsingan stem extract based on microwave assisted extraction. Obtained was 17.949 mgQE/g.

KEYWORDS: Gringsingan stem; total flavonoids; microwave-assisted extraction; spectrophotometer.

1. INTRODUCTION

Gringsingan plant (*Mesosphaerum suaveolens* (L) Kuntze) is one of the plants that has medicinal properties that has been used by the community. This plant is a plant belonging to the *lamiaceae* family which was obtained from Sinjai district, South Sulawesi. This plant is empirically used by the Sinjai people as medicine to relieve stomach aches. This plant has been used in traditional medicine to treat various types of diseases and conditions because it is an important source of alkaloids, flavonoids, phenols, saponins, terpenes and sterols (Bazerra., 2022).

One of the chemical compounds contained in the gringsingan plant (*Mesosphaerum suaveolens* (L) Kuntze) is flavonoids. Flavonoid compounds are phenolic compounds which have various pharmacological activities such as inhibiting heart disease, cancer, reducing plasma oxidation and antioxidants (Rhaihana., 2023).

Extraction method that can be used to extract chemical compounds such as flavonoids is the microwave assisted extraction method, which is a modern extraction method that has been developed. Microwave assisted extraction (MAE) is an extraction method that utilizes microwave radiation to speed up the extraction process by heating the solvent quickly and efficiently. This extraction method is suitable for extracting bioactive compounds because it has better temperature control (Putri et al., 2021).

Based on research that has been conducted (Suhaendar et al., 2022), the flavonoid content in the leaves of the slobber plant using the microwave-assisted extraction method is 0.75%; this value is greater than the maceration and ultrasound-assisted extraction (UAE) extraction methods.

Based on this statement, there has been no research that has determined the flavonoid content of gringsingan stems extract (*Mesosphaerum suaveolens* (L) Kuntze) using the microwave-assisted extraction (MAE) method, so this research was carried out to determine the flavonoid-content of gringsingan stem (*Mesosphaerum suaveolens* (L) Kuntze) with the microwave-assisted extraction (MAE) method.

2. EXPERIMENTAL SECTION

2.1. Population and Sample

The population is the gringsingan plant (*Mesosphaerum suaveolens* (L) Kuntze), and the samples used in this research were stems from the gringsingan plant (*Mesosphaerum suaveolens* (L) Kuntze) obtained from Sinjai district, South Sulawesi.

2.2. Materials and Tools

The materials used in this research were aluminum foil, distilled water, concentrated HCl, magnesium powder, methanol p.a., aquabidest, $AlCl_3$ 10%, potassium acetate and quarcetin powder. The tools used in this research are glassware (pyrex), spot-visible spray bottle, porcelain cup, chamber (Camag), freezer dry, camera, 10 mL measuring flask

(iwaki), 254 nm and 366 nm UV lamps, Micropipette (dragon lab), tweezers, capillary tube, drop pipette, UV – Vis spectrophotometer (Jasco V – 350), test tube, analytical balance (carat series), vial.

2.3 Qualitative analysis

2.3.1 Sample preparation

The research material was Gringsingan stems which had been collected and then carried out wet sorting and washing to remove other impurities that were still attached to the sample. After that, the shape is changed by cutting it into small pieces and then mashing it using a blender. After grinding 30 grams of simplicia powder, put it in a beaker with 300 mL of distilled water, then put it in the oven at 800 watts for 6 minutes. The filtrate is filtered and the residue is extracted again with the same treatment.

2.3.2 Wilstater test

One mL of extract is added with a few drops of concentrated HCl plus a little Mg powder. A positive reaction if there is a orange color change

2.3.3 Thin layer chromatography (TLC)

Water extract of gringsingan stem (*Mesosphaerum suaveolens* (L) Kuntze) was dissolved in distilled water and then spotted on a G60 F₂₅₄ silica gel plate and eluted using methanol: aqua dest (8:2) and two drops of acetic acid. Then the plate was dried and sprayed with AlCl₃ specific reagent. The photostopic results of the presence of flavonoid compounds are indicated by yellow fluorescent spots at UV 366.

2.4 Quantitative Analysis

2.4.1 Preparation of 10% AlCl₃ reagent.

Weigh 10 grams of aluminum chloride and dissolve 10 mL of distilled water.

2.4.2 Preparation of 1 M potassium acetate reagent.

Weigh 0.98 grams of potassium acetate then dissolve it in sterile distilled water to make 10 mL.

2.4.3 Determination of wavelength and standard curve of quercetin

Determination of the maximum wavelength of quercetin was carried out by running a quercetin solution in the wavelength range of 400-800 nm. This maximum wavelength will be used to measure the absorption of the sample. The standard solution of quercetin is 1000 ppm, then several concentrations are made, namely 30 ppm, 35 ppm, 40 ppm, 45 ppm and 50 ppm. From each concentration of quercetin standard solution, 3 mL of methanol was added, 0.2 mL of 10% AlCl₃ reagent was added, 0.2 mL of 1 M potassium acetate reagent was added, and the amount was filled with distilled water to 10 mL. After that, it was incubated for 30 minutes at room temperature. , then measure the absorbance on a UV – Vis spectrophotometer with a wavelength of 431 nm, then create a calibration curve and relate the quercetin concentration to the absorbance (Fawwaz *et al.*, 2023).

2.4.4 Determination of flavonoid content of water extract of gringsingan stem (*Mesosphaerum suaveolens* (L) Kuntze) using UV-Vis spectrophotometry

A blank solution was made first, then 20 mg of gringsingan (*Mesosphaerum suaveolens* (L) Kuntze) stem extract was weighed, then dissolved in 10 mL of methanol (2000 ppm), from this solution pipetted 1 mL, 3 mL of methanol was added, 0.2 was added. mL of 10% AlCl₃ reagent, 0.2 mL of 1 M potassium acetate reagent and fill with distilled water to 10 mL then incubate for 30 minutes at room temperature. Next, the absorbance of the blank solution and sample solution was measured using UV – Vis spectrophotometry with a wavelength of 431 nm. The solution was made in 3 replications so that the flavonoid content obtained was obtained as the equivalent of quercetin/g extract.

3. RESULTS AND DISCUSSION

In this research, the samples used were stems from the Gringsingan plant (*Mesosphaerum suaveolens* (L) Kuntze), which belongs to the *Lamiaceae* family, obtained from Sinjai district, South Sulawesi. This plant is empirically used by the people of Sinjai district as a medicine to relieve stomach aches. One of the chemical compounds contained in the gringsingan plant (*Mesosphaerum suaveolens* (L) Kuntze) is flavonoids, so the aim of this research is to determine the levels of flavonoids in the stems of the gringsingan plant (*Mesosphaerum suaveolens* (L) Kuntze). The initial treatment in this research is to determine the extraction method. The extraction method chosen was microwave assisted extraction. Microwave assisted extraction is a modern extraction method that uses microwave radiation to speed up the extraction process. The energy produced in MAE is radiation energy and rotational energy. The presence of microwave radiation and rotating vibrations will cause the pressure on the cell walls to increase, then the cells will swell and more active compounds will come out (Putri *et al.*, 2021).

Gringsingan stems are extracted by grinding the stems first to facilitate the process of removing the active compound by the solvent from the plant tissue. Stems that are ground first are more easily filtered because the contact area between the surface of the cell tissue and the solvent is greater. The solvent used is distilled water. The extraction

method used in this research is Microwave Assisted Extraction. The weight of simplicia in the form of powder used was 30 grams, added with 300 mL of distilled water then microwaved. After being microwaved for 6 minutes, the sample was filtered using filter paper to separate the filtrate and residue. The filtrate obtained was then freeze-dried for approximately 24 hours.

The extract obtained from the Gringsingan stems sample was 8.317g with an extract yield of 2.772% (w/w) which can be seen in **Table 1**. The aim of determining the percent yield was to determine the levels of secondary metabolites that were successfully carried away by the solvent, but the type of secondary metabolites that were interested. This cannot yet be determined. Next, qualitative testing was carried out to determine the presence of flavonoids content in the extract of gringsingan stems (*Mesosphaerum suaveolens* (L) Kuntze). This qualitative testing is used Wilstater's reagent and thin layer chromatography method.

Table 1. Calculation results of percent yield of water extract of Gringsingan stem (*Mesosphaerum suaveolens* (L) Kuntze)

Sample	Weight (g)	Extract weight (g)	Yield percent (%)
Gringsingan stem	300	8.317	2.772

In the color test (test tube) using Wilstater's reagent, positive results were obtained (**Figure 1**). This test was carried out by adding concentrated Mg and HCl. The addition of concentrated HCl is used to hydrolyze flavonoids into their aglycones, namely by hydrolyzing O-glycosyl. Glycosyl will be replaced by H⁺ from the acid because of its electrophilic nature. Reduction with concentrated Mg and HCl can produce complex compounds that are red or orange (Lindawati & Ni'ma, 2022).

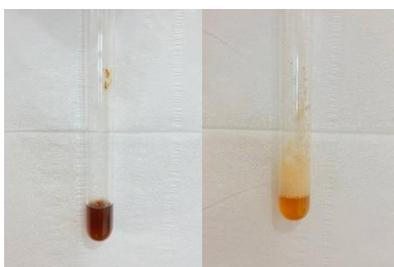


Figure 1. Qualitative result testing of flavonoid compounds in gringsingan stems by Wilstater reagent

Then, TLC testing which is one of the analytical methods used to separate components quickly based on the principles of adsorption and partition. The sample was spotted on an F₂₅₄ silica plate. The TLC plate is saturated first so that the solvent can be eluted at the same time so that the results obtained will be more accurate then the TLC plate is eluted using methanol eluent: distilled water (8:2) and two drops of acetic acid. The composition of the eluent used is polar so that it can separate polar flavonoids compounds. Next, the plates are dried and sprayed with the specific reagent AlCl₃ (Rhaihana., 2023).

Table 2. Qualitative result testing of flavonoid compounds in Gringsingan stems by TLC method.

Sample	Number of spots formed	AlCl ₃ preaction spraying (UV 366)	Rf value	Result
Gringsingan stems	1	yellow	0.636	(+)
	2	yellow	0.727	(+)

Next, a quantitative test was carried out on the water extract of Gringsingan stems (*Mesosphaerum suaveolens* (L) Kuntze) to determine flavonoids levels using the UV – Vis spectrophotometric method. The results of research on determining the flavonoids content of Gringsingan stems extract obtained a flavonoids content of 17.949 mgQE/g extract.

Table 3. Calculation results of percent flavonoid content of Gringsingan stems (*Mesosphaerum suaveolens* (L) kuntze).

Sample	Replication	Absorbance	Total flavonoid content (mg QE/g extract)	Average total flavonoid content (mg QE/g extract)
Gringsingan Stems	1	0.336	17.291	17.949
	2	0.383	19.044	
	3	0.342	17.514	

4. CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that Gringsingan stem extract (*Mesosphaerum suaveolens* (L) Kuntze) contains flavonoid compounds based on the results of color tests using the Wilstater reagent and TLC using the specific reagent AlCl_3 . The flavonoid content of water extract of Gringsingan stems is 17.949 mgQE/g extract.

REFERENCES

- Almeida-Bezerra, J. W., Rodrigues, F. C., Lima Bezerra, J. J., Vieira Pinheiro, A. A., Almeida De Menezes, S., Tavares, A. B., Costa, A. R., Augusta De Sousa Fernandes, P., Bezerra Da Silva, V., Martins Da Costa, J. G., Pereira Da Cruz, R., Bezerra Morais-Braga, M. F., Melo Coutinho, H. D., Teixeira De Albergaria, E., Meiado, M. V., Siyadatpanah, A., Kim, B., & Morais De Oliveira, A. F. (2022). Traditional Uses, Phytochemistry, and Bioactivities of *Mesosphaerum suaveolens* (L.) Kuntze. In *Evidence-based Complementary and Alternative Medicine* (Vol. 2022). Hindawi Limited. <https://doi.org/10.1155/2022/3829180>
- Fawwaz, M., Pratama, M., Musafira, M., Wahab, I., Iriani, R., Aminah, A., Kusuma, A. T., & Arsul, M. I. (2023). Evaluation of Antioxidant Activity of *Vernonia amygdalina* Leaves and Its Flavonoid-Phenolic Content. *Indonesian Journal of Pharmaceutical Science and Technology*, *10*(2), 104.
- Lindawati, N. Y., & Ni'ma, A. (2022). Analysis of Total Flavanoid Levels of Fennel Leaves (*Foeniculum Vulgare*) Ethanol Extract by Spectrophotometry Visibel. *Jurnal Farmasi Sains Dan Praktis*, 1–12. <https://doi.org/10.31603/pharmacy.v8i1.4972>
- Putri, N. M., Wiraningtyas, A., & Mutmainah, P. A. (2021). Perbandingan Metode Ekstraksi Senyawa Aktif Daun Kelor (*Moringa Oleifera*): Metode Maserasi dan Microwave-Asissted Extraction (MAE) Comparison of Extraction Methods of Moringa Leaf (*Moringa oleifera*) Active Compounds: Maceration and Microwave-Assisted Extraction Methods. In *Jurnal Pendidikan Kimia dan Ilmu Kimia* (Vol. 4, Issue 2).
- Rhaihana, Bachtiar, A., Handayani, S., & Roskiana Ahmad, A. (2023). Penetapan Kadar Flavonoid Total Buah Dengan (*Dillenia serrata*) Menggunakan Metode Spektrofotometri UV-VIS. *Makassar Natural Product Journal*, *1*(2), 2023–2086. <https://journal.farmasi.umi.ac.id/index.php/mnpj>
- Suhendar, U., Utami, N. F., Sutanto, Dr., & Nurdayanty, S. M. (2020). Pengaruh Berbagai Metode Ekstraksi pada Penentuan Kadar Flavonoid Ekstrak Etanol Daun Iler (*Plectranthus scutellarioides*). *FITOFARMAKA: Jurnal Ilmiah Farmasi*, *10*(1), 76–83. <https://doi.org/10.33751/jf.v10>

This is an open access article which is publicly available on our journal's website under the Institutional Repository at <https://www.umi.ac.id/>