

Analysis of Flavonoid Content of Red Seaweed (*Gelidium amansii* J.V.F. Lamouroux) growing in Krakal Beach, Gunung Kidul, Yogyakarta with a comparison of extraction methods

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Abstract. Red seaweed (*Gelidium amansii* J.V.F. Lamouroux) is a plant found on the Krakal coast of Gunung Kidul, Yogyakarta. This seaweed is known to contain flavonoids and has antioxidant activity. The extraction method used can affect the levels of bioactive compounds produced. Therefore, a comparison between maceration and soxhlet extraction methods is necessary to determine the effectiveness of each method in extracting flavonoids from *Gelidium amansii* J.V.F. Lamouroux. The purpose of this study was to determine which method is more optimal in extracting flavonoid compounds.

Methods: This study used two extraction methods: maceration (soaking at room temperature) and soxhlet (repeated heating with a soxhlet apparatus), using 96% ethanol as the solvent. Flavonoid content was quantitatively determined using UV-Vis spectrophotometry. Data were analyzed using the Shapiro-Wilk test for normality, homogeneity test, and the Mann-Whitney test to determine differences between methods.

Results: The flavonoid content of the maceration method was $4.77\% \pm 0.033\%$, while that of the soxhlet method was $3.75\% \pm 0.02\%$. The normality test showed that the data were not normally distributed ($p = 0.00$), but were homogeneous ($p = 0.06$). The Mann-Whitney test showed a significance value of 0.043 ($p > 0.05$), indicating a significant difference between the two methods.

Conclusion: The maceration method produced numerically higher flavonoid levels than soxhletation, resulting in a statistically significant difference. Therefore, both methods can be used depending on efficiency and the nature of the extracted compounds.

Keywords: *Gelidium amansii*, flavonoids, extraction, maceration, soxhletation.

INTRODUCTION

Krakal Beach is one of the beaches in Gunung Kidul, Yogyakarta. It has a vast marine area and abundant natural resources. One marine resource with great potential for development and high economic value is seaweed (Harikedua & Segar, 2018). Seaweed is a group of single- or multi-celled plants containing chlorophyll. These plants contain a wide variety of organic substances, including polysaccharides, hormones, vitamins, minerals, and bioactive compounds that are important for health.

One of the main compounds found in red seaweed is flavonoids, which play a vital role in various aspects. Flavonoids are natural polyphenol compounds found most abundantly in green plants. These compounds are known to have various benefits, including acting as free radical scavengers, inhibiting enzymes involved in hydrolysis and oxidation, and possessing anti-inflammatory properties (Wahid & Raudah, 2021).

Flavonoids are compounds that are susceptible to degradation at high temperatures. The heating process can trigger damage to the flavonoid structure and result in a decrease in the levels of active compounds and the loss of most of their biological activity (Priamsari et al., 2019).

Factors to consider in extraction include the solvent and the extraction method. When selecting a solvent, considerations include toxicity (poisoning), ease of evaporation, polarity, selectivity, and solvent cost (Agustina et al., 2018). In a study conducted by Trifani (2012), 96% ethanol was chosen to extract flavonoid compounds because it is selective, non-toxic, has good adsorption capacity and high extraction capacity, and can extract non-polar, semi-polar, and polar compounds. The solvent used in this study was 96% ethanol because it can produce a concentrated extract due to its easier penetration into the sample cell walls than lower concentrations of ethanol.

There were two extraction methods used in this study maceration and soxhletation. The maceration method is an extraction technique that involves soaking powdered medicinal plants in a solvent at room

temperature. This method was chosen because it can prevent damage to compounds that are heat-labile. Furthermore, another advantage of the maceration method is its simple procedure and equipment, making it easy to implement (Asworo & Widwastuti, 2023). The soxhlet method is an extraction technique that involves repeatedly soaking the sample in a heated solvent. This process allows the desired components from the sample to be completely isolated (Yulinar & Suharti, 2023).

The results of a study conducted by Putri et al. (2024) on "Comparison of Maceration and Soxhlet Extraction Methods on Total Phenolic Content of Sargassum sp Ethanol Extract" showed that the maceration extraction method produced the highest phenolic content, at 3.7179 mg GAE/g, while the Soxhlet method produced 3.5156 mg GAE/g.

Based on the above description, a comparative study was conducted to determine the total flavonoid content of 96% ethanol extract of red seaweed.

METHODS

Equipment

The equipment used for phytochemical screening included an analytical balance (*Ohaus*), a dropping pipette, a Pyrex measuring cylinder, a Pyrex test tube, and an Erlenmeyer flask (Pyrex). The equipment used for Soxhlet extraction included a condenser, a casing, a siphon, a Soxhlet flask, an F-tube, a fat flask, and a heating mantle.

The maceration equipment consisted of a vessel, a rotary vacuum evaporator (*IKA Laborthechnik*), an analytical balance (*Ohaus*), a blender (*Philips*), a Shimadzu cuvette, a Pyrex beaker, a Pyrex Erlenmeyer flask, a Pyrex measuring flask, an oven, a porcelain dish, and filter paper.

The equipment used to determine total flavonoid levels included a UV-Vis spectrophotometer (UV Mini SHIMADZU), volumetric pipettes (Pyrex) (*Socorex*), analytical balance (*Ohaus*), cuvettes, droppers, glass beakers (Pyrex), measuring cylinders (Pyrex), Erlenmeyer flasks (Pyrex), volumetric flasks (Pyrex), spatulas, glass funnels (Pyrex), porcelain dishes (Pyrex), and label paper.

Materials

The materials used in this study were dried red seaweed (*Gelidium amansii* J.V.F. Lamouroux) obtained from Krakal Village, Gunung Kidul Regency. The solvent used in this study was 96% ethanol.

- 1) Color test materials: 10% NaOH, Mg powder, HCl powder.
- 2) Total flavonoid test materials: Extract sample, quercetin, 96% ethanol, AlCl₃, sodium acetate.

Preparation of Red Seaweed Powder (*Gelidium amansii* J.V.F. Lamouroux)

The raw material used in this study was dried red seaweed (*Gelidium amansii* J.V.F. Lamouroux). Dry sorting was performed on red seaweed that was reddish-brown in color, free from black spots or rot, and free from sand or other organisms.

The dried seaweed was ground using a blender (Wardhani, 2014). After blending, the seaweed was ground into powder and sieved using a 40-mesh sieve.

Red Seaweed (*Gelidium amansii* J.V.F. Lamouroux) Extraction

Using the Maceration Method

100 grams of red seaweed (*Gelidium amansii* J.V.F. Lamouroux) powder was poured into an Erlenmeyer flask and 750 ml of 96% ethanol was added. The flask was then slowly stirred until the solvent completely covered the red seaweed powder. The flask was then tightly closed and protected from direct sunlight. The soaking process lasted for 3 days, stirring occasionally every 8 hours. After 3 days, the mixture of the powder and ethanol was filtered, producing a macerate. The residue was soaked again in 250 ml of ethanol for 1 day, filtered again, and the macerate was obtained. It was then separated from the residue and concentrated using a rotary evaporator at 40°C until a thick extract was obtained. (Puspitasari & Proyogo, 2013)

Extraction of Red Seaweed (*Gelidium amansii* J.V.F. Lamouroux) Using the Soxhlet Method

100 grams of powdered medicinal plant was wrapped in filter paper and tied at both ends with string. The sample was then placed in a Soxhlet apparatus, and 1000 ml of 96% ethanol was added to the round-bottom flask and Soxhlet tube to moisten the sample. The Soxhlet process was carried out at 50°C until the droplets had completed 6 cycles or the droplets were colorless or clear. The filtrate was then evaporated using a rotary evaporator at a temperature not exceeding 40°C until a thick extract was obtained (Puspitasari & Proyogo, 2013).

RESULTS AND DISCUSSION

Red Seaweed (*Gelidium amansii* J.V.F. Lamouroux) Powder Preparation

A total of 500 grams of dried herbal medicine was taken as a sample for further processing. Before use, the material first underwent a dry sorting process, which involves selecting materials based on predetermined inclusion criteria to ensure that only materials with appropriate quality and characteristics were used in this study. This dry sorting was carried out to separate impurities, foreign matter, and incompletely dried herbal medicine, thus obtaining clean, usable material for further processing (Lindawati & Ni'ma, 2022).

After the sorting process is complete, the selected material is ground using a blender until a fine powder is obtained. This grinding process aims to reduce the particle size, facilitating subsequent extraction or analysis. The resulting powder is then sieved using a 40-mesh sieve to achieve a uniform and consistent particle size. After the sieving process is complete, the powder that passes through the sieve is weighed again to determine the final weight of the processed fine material. After weighing, the powder weighs 210 grams. The resulting powder is first calculated for drying loss before being used for extraction, and the water content in the powdered medicinal plants is calculated.

Seaweed Extraction Results (*Gelidium amansii* J.V.F. Lamouroux) Using the Maceration Method

The maceration method is an extraction technique that involves soaking the crude drug in a solvent without direct heating, making it suitable for materials containing active compounds that are sensitive to high temperatures, such as flavonoids. Maceration has several relevant advantages, including a simple process, no need for large amounts of heat, and a better ability to maintain the stability of the flavonoid structure, which is easily degraded by heating. Because flavonoids in red seaweed are potentially damaged when extracted at high temperatures, the maceration method can produce more optimal and stable flavonoid levels than the soxhletation method, which involves repeated heating (Chandra et al., 2019). Thus, maceration is a more environmentally friendly alternative extraction method for the bioactive compounds in (*Gelidium amansii* J.V.F. Lamouroux).

The choice of solvent in the extraction process is an important factor that significantly influences the efficiency of extracting bioactive compounds. In this study, 96% ethanol was used as the solvent because ethanol is a polar solvent suitable for dissolving flavonoid compounds, which also polar. This selection is based on the principle of "like dissolves like," meaning that solvents will be more effective in dissolving compounds with similar polarity (Permana & Jambe, 2016). Ethanol is widely used in natural product extraction because it not only has broad solubility (capable of dissolving polar to semi-polar compounds), but is also relatively safe and non-toxic.

According to (Agustien, 2021), the disadvantage of using 96% ethanol as a solvent is its relatively high price, and if used in large quantities, it becomes uneconomical. Selecting an appropriate solvent is a crucial factor in the extraction process. The solvent used must be able to extract most of the desired secondary metabolites in the herbal extract. The solvent used must be able to dissolve the desired substance, have a low boiling point, be inexpensive, non-toxic, and non-flammable. Therefore, 96% ethanol was chosen as the ideal solvent for extracting flavonoids from red seaweed (*Gelidium amansii* J.V.F. Lamouroux) in this study.

The extraction process is carried out using the maceration method, which involves soaking the powdered medicinal plants in a solvent at room temperature for a specified period of time. 100 grams of dry, sifted powdered medicinal plants are carefully weighed and then placed into a glass jar that has

been wrapped with aluminum foil to protect it from light exposure, which can cause degradation of the active compounds.

Next, 750 mL of 96% ethanol was added as a solvent, then the mixture was stirred thoroughly. After the initial mixing process, the container was tightly covered with aluminum foil and left protected from light. The mixture was stirred periodically every 8 hours to ensure even solvent distribution and accelerate the diffusion of the active compounds from the herbal extract into the solvent. The maceration process was carried out for 3 days (72 hours) at room temperature.

Table 1. Results of Red Seaweed Extraction

No	Metode	Powder	Extract	Yield
1	Maceration	100 g	28.07 g	28.07 %
2	Sokletation	100 g	17.91 g	17.91 %

Based on the extraction results obtained, it can be concluded that both methods, maceration and soxhletation, are capable of extracting active compounds from medicinal plants, but with varying effectiveness. The maceration method produced a higher yield of 28.07%, compared to the soxhletation method, which only produced a yield of 17.91%, despite the shorter soxhletation process (approximately 6 hours). This result may be due to the fact that heating during soxhletation causes degradation or oxidation of flavonoid compounds. The active flavonoid compounds in medicinal plants are sensitive to heat, so they are destroyed during the soxhletation process. Even with repeated soxhletation,

Soxhletation: Although repeated, soxhletation lasts a shorter time (generally 3–8 hours), so for compounds that dissolve slowly, the yield may be lower. Maceration, on the other hand, is more suitable for compounds that are not heat-stable because it is carried out at low temperatures (room temperature without heating). Maceration is carried out for a longer period (1–4 days), allowing more time for the compound to dissolve completely. This indicates that maceration is more efficient in terms of the quantity of extract produced, especially for heat-sensitive compounds because it is carried out without direct heating.

Conversely, soxhletation is more suitable for compounds that are temperature-stable and when extraction time is a primary consideration.

Determination of Total Flavonoid Content

Before determining the total flavonoid content, the initial step is to determine the maximum wavelength (λ max) of the quercetin standard solution. The maximum wavelength is the wavelength at which the absorbance reaches its highest value, which is obtained by reading the absorption spectrum of the quercetin standard solution using a UV-Vis spectrophotometer. This determination aims to ensure that the measurement is carried out at the correct wavelength.

The quercetin- AlCl_3 complex absorbs light maximally, resulting in more accurate measurement results (Asmorowati & Yety Lindawati, 2019). The maximum wavelength was determined by running a standard quercetin solution. One concentration, 30 ppm, was used as a representative concentration because it is the middle concentration in the range and therefore considered to represent the average value of the calibration curve. A 1 ml pipet of the 30 ppm quercetin standard solution was then added with 0.1 ml of 10% AlCl_3 , 0.1 ml of 1 M sodium acetate, and then ethanol p.a. was added to the 10 ml volumetric flask mark.

Operating time determination is performed to determine the point at which the sample reaches a stable state after reacting and forming a compound complex (Asmorowati & Lindawati, 2019). In this study, operating time determination was performed using a quercetin solution with a concentration range of 30 ppm.

The total flavonoid content of red seaweed extract using the maceration and soxhletation methods was calculated using linear regression from the previously measured quercetin standard curve. The results of the quercetin and flavonoid content equivalence can be seen in the table below:

Table 2. Results of Determination of Total Flavonoid Content Using the Soxhletation and maceration Method

Method	Concentration	total flavonoid content (%)	Average
Sokletation	1000 ppm/2.5 ml	37.37 37.37	3.75 % $\pm$ 0.02

		37.39	
Maceration	1000 ppm/2.5 ml	47.53 47.53 48.25	4.77 % ± 0.033

Determination of total flavonoid content from *Gelidium amansii* ethanol extract using the maceration method was performed using the AlCl₃ colorimetric method, which relies on the principle of complex formation between aluminum ions (Al³⁺) and the carbonyl group (C=O) at the C-4 position and the hydroxyl group (-OH) at the C-3 or C-5 position of the flavonoid ring, specifically the flavone and flavonol groups (Chang et al., 2002). This reaction produces a yellow complex compound whose color intensity can be measured using a spectrophotometer at specific wavelengths, indicating the amount of flavonoid compounds in the sample.

The absorbance results of the extract obtained through the maceration method showed values of 0.523, 0.523, and 0.528. These values are within the reasonable range for flavonoid absorbance testing, which is between 0.2 and 0.8, as recommended by the Directorate General of Food and Drug Monitoring (2014). The average of these three absorbance values was calculated to be 0.5247, which was then entered into the linear regression equation of the quercetin standard curve ($y = 0.0069x + 0.195$). This calculation yielded a flavonoid concentration of 47.77 mg/L.

Based on these results, the total flavonoid content in the extract produced through the maceration method was 4.77% ± 0.033%. This value reflects the average flavonoid content from three replicate tests, with uncertainty calculated based on the standard deviation relative to the obtained concentration. These values indicate that the maceration method is quite capable of extracting flavonoid compounds from *Gelidium amansii*, even without using a heating process. The advantage of this method is its ability to maintain the stability of flavonoid compounds, which are sensitive to high temperatures. Therefore, the maceration method can be used as an alternative when extraction is required that maintains the intact chemical structure of flavonoids.

These results indicate that the soxhlet method has a higher extraction efficiency compared to the maceration method. This can be explained by the repeated heating and solvent circulation during soxhletation, which allows the solvent to penetrate plant tissue more effectively and optimally dissolve bioactive compounds such as flavonoids. Therefore, soxhletation is a highly suitable extraction method for polar to semi-polar compounds, such as flavonoids, from natural products like *Gelidium amansii* J.V.F. Lamouroux.

CONCLUSION

1. Both maceration and soxhlet extraction methods tested positive for flavonoid compounds.
2. The highest average total flavonoid content of red seaweed (*Gelidium amansii* J.V.F. Lamouroux) extract was obtained from the maceration method, at 4.77%, while the soxhlet method produced a flavonoid content of 3.75% in 1000 ppm/2.5 ml.

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No Conflict Interest

REFERENCES

- Agustian, I., Saputra, H.E. & Imanda, A. 2019. Pengaruh Sistem Informasi Manajemen terhadap Peningkatan Kualitas Pelayanan di PT. Jasaraharja Putra Cabang Bengkulu. Profesional: Jurnal Komunikasi dan Administrasi Publik, 6(1): 42-60.
- Asmorowati, H., & Lindawati, Y. (2019). Determination of total flavonoid content in avocado (*Persea americana* Mill.) using spectrophotometry method Penetapan kadar flavonoid total alpukat (*Persea americana* Mill.) dengan metode spektrofotometri. Jurnal Ilmiah Farmasi, 15(2), 51–63.
- Anisa, N. & Najib, S.Z. 2022. Skrining fitokimia dan penetapan kadar total fenol flavonoid dan tanin pada ekstrak etanol daun kersen (*Muntingia calabura* L.). Indonesian Journal Pharmaceutical and Herbal Medicine, 1(2): 96-104.

- Arrofiqi, M.R., Sakti, A.S. & Mayangsari, F.D. 2024. Kajian literatur aplikasi sejumlah metode ekstraksi konvensional untuk mengekstraksi senyawa fenolik dari bahan alam. *Jurnal Penelitian Farmasi & Herbal*, 7(1): 8-24.
- Chandra, A., Fitri, K., Widyastuti, F., Kulsum, U., & Saraswati, R. (2019). Optimasi Waktu Maserasi dan Jenis Pelarut Terhadap Kadar Flavonoid pada Ekstrak Daun Sirsak (*Annona Muricata* L). *Prosiding Seminar Nasional Teknologi Industri, Lingkungan dan Infrastruktur* (2): C.17.1-C 17.6.
- Chang, C.C., Yang, M.H., & Wen, H.M. (2002). Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. *Journal of Food and Drug Analysis*, 178–182.
- Citra, S. N., Permana, D. G. M., & Jambe, A. G. A. (2016). Pengaruh Jenis Pelarut Terhadap Kandungan Total Flavonoid dan Aktivitas Antioksidan Ekstrak Daun Matoa (*Pometia pinnata*). *Itepa: Jurnal Ilmu Dan Teknologi Pangan*, 5(1), 1–10.
- Dachriyanus, 2004. Analisis struktur senyawa organik secara spektroskopi, cetakan I. Padang: Cv. Trianda Anugrah Pratama.
- Fatmawati, A. & Aji, N. P. 2019. Penetapan kadar flavonoid total ekstrak etanol daun kelor (*Moringa oleifera* Lam) dengan metode kromatografi lapis tipis densitometri. In *Proc Conf Published online*, 1(1): 16-23
- The bibliography and citation follows the author-year APA style and we recommend using references, such as Mendeley, Zotero, EndNote etc. Make sure the number of references at least 20 and all are cited in the text
- Almukhambetov, B. M. T., & Nebessayeva, Z. (2015). The Application Of Figuratif Arts Capabilities In The Art-Pedagogical Activity Of A Teacher”. *Journal Procedia Social and Behavioral Science*, 197, 1525–1529.