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# Identification of Phytochemical from Seaweed Extracts ( Eucheuma cottonii., Gracilaria sp., and Gelidium sp. ) Using Microwave-Assisted Extraction (MAE)

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**A B S T R A C T**

This study looks at the chemical profiles of three types of red seaweed—Eucheuma cottonii, Gracilaria sp., and Gelidium sp.—using a method called microwave-assisted extraction (MAE) with a mix of ethanol and water for 7, 8, and 9 minutes. Qualitative screening was performed for seven secondary metabolites: alkaloids, flavonoids, phenols, tannins, saponins, triterpenoids, and steroids. The results showed flavonoids and phenolic compounds were found in all the seaweed samples, but saponins were only found in the Gelidium sp. extracts. In contrast, alkaloids, tannins, triterpenoids, and steroids were not detected in any seaweed species. Phenolic compounds exhibited the highest positive intensity, while the flavonoid content appeared relatively low. Solvent composition and extraction duration influenced the extraction efficiency of the MAE method for bioactive compounds. These findings provide a valuable basis for future studies on the potential bioactivities of red seaweeds as natural sources in functional food and pharmaceutical applications.

**Contribution to Sustainable Development Goals (SDGs):**
**SDG 3:** Good Health and Well-being
**SDG 9:** Industry, Innovation, and Infrastructure
**SDG 12:** Responsible Consumption and Production
**SDG 14:** Life Below Water

## 1. INTRODUCTION

### 1.1. *Research Background*

Indonesia is renowned for its rich biodiversity, including abundant marine resources such as seaweed along its coastal regions. Seaweed has long been utilized in various sectors, including food, cosmetics, and traditional medicine. One of the key components present in seaweed is phytochemical compounds, which include alkaloids, flavonoids, tannins, saponins, and terpenoids [1]. These secondary metabolite compounds exhibit a range of biological activities, including antioxidant, antimicrobial, and anti-inflammatory properties, thereby warranting further investigation [2].

Preliminary analysis of secondary metabolite content in natural materials can be conducted through qualitative phytochemical screening. This testing provides fundamental information on the presence of potentially bioactive compounds, serving as an initial step before quantitative analyses or further biological activity assays [2]. Employing efficient extraction methods is also crucial for obtaining these bioactive compounds in maximal yields and within shorter extraction times.

Microwave-assisted extraction (MAE) is a modern technique increasingly employed for extracting bioactive compounds from natural materials. MAE utilizes microwave energy at frequencies around 2450 MHz, which can interact with polar molecules and ions in both the solvent and the sample [3]. The use of MAE has been reported to be effective in extracting various bioactive compounds, including flavonoids and polyphenols, which are generally sensitive to high temperatures and oxidative degradation [5].

In addition to containing potentially bioactive compounds, seaweed such as Gracilaria sp. also holds significant economic value when processed into food products. Rosida et al. (2021) conducted a study on using Gracilaria sp. as a raw material in the production of nori, employing different stabilizing agents, namely chitosan, gelatin, and pectin. The study revealed that the addition of 3% gelatin resulted in nori with good textural quality, high protein content, and sensory profiles favored by consumers [6]. These findings highlight that seaweed has potential as a bioactive source and can be developed into innovative value-added food products.

This study aims to conduct a qualitative phytochemical analysis of three types of seaweed, namely Eucheuma cottonii, Gracilaria sp., and Gelidium sp., extracted using the MAE method with a mixed solvent of ethanol and water (1:1). Extraction times of 7, 8, and 9 minutes were employed to optimize the yield of bioactive compounds, particularly total flavonoids. The findings of this research are expected to provide an initial profile of bioactive compounds in seaweed extracts and support their potential utilization as natural ingredients for functional food and pharmaceutical applications.

### *1.2. Literature Review*

Red seaweeds such as *Eucheuma cottonii*, *Gracilaria sp.*, and *Gelidium sp.* are known to contain bioactive compounds, including polyphenols, flavonoids, alkaloids, tannins, saponins, and triterpenoids. These compounds act as antioxidants due to their hydroxyl groups, which can neutralize free radicals through electron or hydrogen atom donation [5,7]. Qualitative studies have shown that ethanol extracts of *Gracilaria sp.* contain alkaloids, phenols, saponins, flavonoids, and triterpenoids [8], while *Gelidium sp.* has been reported to exhibit high total phenolic and flavonoid contents [9]. Conducting qualitative phytochemical screening is essential for the preliminary identification of secondary metabolites, thus guiding the selection of extraction methods and further bioactivity assays. Such screening typically involves specific color reactions, for example, the Dragendorff reagent for alkaloid testing, the Shinoda reaction for flavonoids, and foam observation for saponins.

The extraction method is crucial in obtaining active compounds from natural materials. Conventional methods typically require long extraction times and high temperatures, which may degrade thermolabile compounds [10]. As an alternative, the Microwave-Assisted Extraction (MAE) method has gained increasing attention. The working principle of MAE is based on rapid heating generated by microwave energy, which leads to increased temperature and pressure within the cells. Electromagnetic waves at a frequency of 2450 MHz can penetrate the cell walls of the plant material and directly heat the solvent and water content within the sample. This process enhances the diffusion and solubilization of target compounds more efficiently [11]. Moreover, the uniform heating produced by microwaves can accelerate the disintegration of cell walls and release active compounds. Water has a high dielectric constant as a solvent, making it highly effective in absorbing microwave energy and improving extraction efficiency [12, 13].

Microwave-assisted extraction (MAE) advantages include shorter extraction times and the ability to preserve the stability of bioactive compounds compared to conventional methods. However, critical factors such as solvent type and extraction duration must be optimized, considering that some active compounds, such as phenols and flavonoids, are susceptible to degradation at high temperatures [14]. Studies have demonstrated that variation in extraction time also affects the yield; excessively long durations may lead to increased degradation of heat-sensitive compounds [15].

### *1.3. Research Objective*

This study aims to analyze the phytochemical compounds in the seaweeds *Eucheuma cottonii, Gracilaria sp.*, and *Gelidium sp.* using microwave-assisted extraction with 7, 8, and 9 minutes.

## 2. MATERIALS AND METHODS

### *2.1. Materials*

The materials used in this study included the seaweed species *Eucheuma cottonii*, obtained from Sepanjang Market, Sidoarjo Regency, East Java, and *Gracilaria sp.* and *Gelidium sp.*, sourced from Sukabumi city. The solvents used for extraction were ethanol (Merck) and distilled water. Chemicals employed for analysis comprised sulfuric acid ($H_2SO_4$, Merck), sodium hydroxide (NaOH, Merck), ethanol (Merck), Meyer's, Wagner's, and Dragendorff's reagents (Nitra Kimia), ferric chloride ($FeCl_3$, Merck), n-hexane (Merck), methanol (Merck), hydrochloric acid (HCl, Merck), magnesium powder (Merck), and glacial acetic acid (Merck).

### *2.2. Prosedur Penelitian*

This study was conducted using a Completely Randomized Design (CRD) with two treatment factors: seaweed species (**Eucheuma cottonii**, **Gracilaria sp.**, and **Gelidium sp.**) and extraction time (7, 8, and 9 minutes). Each treatment was performed in triplicate. The observed variables included qualitatively identifying alkaloids, flavonoids, phenols, tannins, saponins, triterpenoids, and steroids in the resulting extracts.

### *2.3. Ekstraksi Tepung Rumput Laut*

The extraction of seaweed powder was carried out using the Microwave-Assisted Extraction (MAE) method. A total of 0.5 grams of seaweed powder was placed into an Erlenmeyer flask, followed by 12.5 mL ethanol and 12.5 mL distilled water (1:1 solvent ratio). The mixture was stirred using a magnetic stirrer at 10 rpm for 20 minutes. Subsequently, the mixture was irradiated with microwaves at 300 watts for varying durations of 7, 8, and 9 minutes [1]. The resulting extract filtrate was analyzed for its phytochemical content [16].

### *2.4. Phytochemical Qualitative Tests*

#### *2.4.1. Qualitative Test for Alkaloids*

The filtrate was divided into three test tubes for the following tests: a. Meyer's test: One to two drops of Meyer's reagent were added to the seaweed extract filtrate. The formation of a white precipitate indicates the presence of alkaloids.

Wagner's test: One to two drops of Wagner's reagent were added to the seaweed extract filtrate. The formation of a brown precipitate indicates the presence of alkaloids.

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Dragendorff's test: One to two drops of Dragendorff's reagent were added to the seaweed extract filtrate. The formation of an orange precipitate indicates the presence of alkaloids [17].

### *2.4.2. Qualitative Test for Flavonoids*

A volume of 5 mL of seaweed extract filtrate was mixed with 1 mL of concentrated hydrochloric acid (HCl) and added 5 mL of ethanol. The mixture was shaken vigorously and then allowed to separate. The formation of a pink-colored solution in the ethanol layer indicates the presence of flavonoid compounds [17].

### *2.4.3. Qualitative Test for Phenols*

The addition of 3% ferric chloride ($FeCl_3$) solution to 5 mL of seaweed extract filtrate. The appearance of green, red, blue, purple, or black coloration indicates the presence of phenolic compounds. The intensity of the color correlates with the concentration of phenols [17].

### *2.4.4. Qualitative Test for Tannins*

Add 1% ferric chloride ($FeCl_3$) solution to 5 mL of seaweed extract filtrate. The appearance of a dark blue or greenish-black coloration indicates the presence of tannins. The intensity of the color reflects the tannin concentration [17].

### *2.4.5. Qualitative Test for Saponins*

Ten milliliters of seaweed filtrate were placed in a test tube, shaken vertically for 10 seconds, then left to stand for 10 minutes. The formation of stable foam indicates the presence of saponins. Furthermore, the foam remains stable upon adding one drop of 1% hydrochloric acid (HCl) [17].

### *2.4.6. Qualitative Test for Triterpenoids and Steroids*

Ten milliliters of seaweed filtrate in a test tube was mixed with 10 drops of glacial acetic acid, followed by 10 drops of sulfuric acid ($H_2SO_4$). The formation of a green color indicates the presence of triterpenoids, whereas a red color indicates the presence of steroids [17].

## 3. RESULT AND DISCUSSION

Bioactive compounds were extracted from seaweed powder using the Microwave-Assisted Extraction (MAE) method with a solvent mixture of ethanol and distilled water (1:1). The extraction durations applied in this study were 7, 8, and 9 minutes. The effects of seaweed species and extraction time on the presence of alkaloids, flavonoids, phenols, tannins, saponins, triterpenoids, and steroids are presented in Table 1.

### ***3.1. Alkaloids***

The qualitative test for alkaloid compounds in the extracts of three seaweed species using different extraction durations showed negative results in all samples. This finding contrasts with El Shafay et al. (2016) study, which successfully extracted alkaloids from *Ulva lactuca* using the Soxhlet method with a weak acid solvent. The difference is attributed to variations in solvent type and extraction method, as alkaloids are more readily extracted using acidic or polar solvents [18].

**Table 1.** Results of qualitative phytochemical tests on filtrates from seaweed powder extracts.

| Rumput Laut | Waktu Ekstraksi (menit) | Alkaloid | Flavonoid | Fenol | Tanin | Saponin | Triterpenoid dan Steroid |
|---|---|---|---|---|---|---|---|
| *Eucheuma cottonii* | 7 | - | + | + | - | - | - |
| | 8 | - | + | + | - | - | - |
| | 9 | - | + | + | - | - | - |
| *Gracilaria sp.* | 7 | - | + | + | - | - | - |
| | 8 | - | + | + | - | - | - |
| | 9 | - | + | + | - | - | - |
| *Gelidium sp.* | 7 | - | + | + | - | + | - |
| | 8 | - | + | + | - | ++ | - |
| | 9 | - | + | + | - | +++ | - |

Keterangan:
(-) : tidak mengandung senyawa yang diuji
(+) : mengandung sedikit senyawa yang diuji
(++) : mengandung sedikit lebih banyak senyawa yang diuji
(+++) : mengandung banyak senyawa yang diuji

### ***3.2. Alkaloids***

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### ***3.3. Flavonoids***

The qualitative test for flavonoid compounds in the extracts of three seaweed species at different extraction durations yielded positive results in all samples. However, the intensity of the color reaction was relatively weak, indicating a low flavonoid content in the extracts. These results align with previous studies that reported the presence of flavonoids in red seaweed species such as *Gracilaria verrucosa* [27] and *Eucheuma cottonii* [1]. This is attributed to flavonoids being effectively extracted using polar or semi-polar solvents [16], consistent with the solvent system used in this study (ethanol and distilled water), which is semi-polar and polar. The low flavonoid content observed may also be related to the limited extraction duration employed. Although an increase in soluble compounds was observed at the 9-minute extraction time, significant accumulation of flavonoids was not yet achieved [28].

### ***3.4. Phenols***

The qualitative test for phenolic compounds in the extracts of three seaweed species at different extraction durations showed positive results in all samples. These findings are consistent with previous studies indicating that red seaweed contains phenolic compounds, although their levels vary depending on species and environmental conditions. According to Wijesekara et al. (2011), phenols can be effectively extracted using polar solvents [19]. This test result demonstrates that phenolic compounds can be efficiently extracted using the Microwave-Assisted Extraction (MAE) method.

### ***3.5. Tannins***

The qualitative test for tannin compounds in the extracts of three seaweed species at different extraction durations yielded negative results in all samples. This finding contrasts with several previous studies that successfully detected tannins in red seaweed using the maceration method [20]. This discrepancy indicates that the extraction technique, solvent type, and processing duration highly influence the effectiveness of tannin extraction. Additionally, variations in tannin content are affected by biological factors such as species, growth stage, and environmental conditions, including salinity and light exposure [21].

### *3.6. Saponins*

The qualitative test for saponin compounds in the extracts of two seaweed species (*Eucheuma cottonii* and *Gracilaria* sp.) showed negative results, whereas the extract of *Gelidium* sp. tested positive. These findings are consistent with previous studies reporting relatively high saponin content in several *Gelidium* species [22]. The test results for the Gelidium sp. samples indicated that longer extraction durations corresponded to higher saponin content, as evidenced by the increased volume of stable foam formed.

### *3.7. Triterpenoids dan Steroids*

The qualitative test for triterpenoid compounds in the extracts of three seaweed species at different extraction durations showed negative results in all samples. This finding contrasts with previous studies that successfully detected triterpenoids in seaweed using non-polar solvents such as chloroform, hexane, or ethyl acetate, which are more suitable for dissolving these lipophilic compounds [24]. Additionally, triterpenoid content has been reported in *Eucheuma cottonii* extracted by Soxhlet method using chloroform as the solvent [27]. The qualitative test for steroid compounds in the extracts of three seaweed species at different extraction durations also yielded negative results across all samples. This result differs from earlier research that identified steroid compounds in *Gracilaria corticata* using maceration with chloroform as the solvent [25] and in *Gelidiella acerosa* extracted with ethyl acetate [26].

## 4. CONCLUSION

Qualitative phytochemical testing is essential for the preliminary identification of secondary metabolite classes. The use of Microwave-Assisted Extraction (MAE) has proven effective in extracting flavonoids, phenols, and saponins. The solvent and extraction method may influence the absence of alkaloids, tannins, triterpenoids, and steroids. Additionally, variations in extraction duration can also affect the extraction outcomes.

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