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Identification of Phytochemical Compounds, Extraction Yield, and Total Soluble of Red, Brown, and Green Seaweeds

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A B S T R A C T

This study examined the effects of seaweed species and solvent types on the yield and phytochemical profile of seaweed extracts obtained via Ultrasound–Microwave Assisted Extraction (UMAE). Five types of seaweed Eucheuma cottonii, Gelidium sp., Gracilaria sp., Sargassum sp., and Ulva lactuca were extracted using methanol and ethanol. Phytochemical screening was conducted for alkaloids, phenols, flavonoids, saponins, tannins, steroids, and terpenoids. The results showed that the extract yield ranged from 18.16% to 48.37%, with the highest yield of 48.37% observed in the methanol extract of E. cottonii. Total soluble solids ranged from 14.0% to 37.5%, with the highest value of 37.5% found in the methanol extract of E. cottonii. Phenolic compounds were detected in most extracts; flavonoids were present in all methanol extracts and some ethanol extracts; saponins were found in E. cottonii, Gracilaria sp., Sargassum sp., and Ulva lactuca; steroids were present in the ethanol extracts of Gracilaria sp. and Sargassum sp.; and terpenoids were detected in E. cottonii, Gelidium sp., and U. lactuca. The results indicate that the type of solvent affects the yield, total dissolved solids, and phytochemical profile of the extracts; thus, the Ultrasound–Microwave Assisted Extraction (UMAE) method has the potential to be an effective green extraction method for various types of seaweed.

Contribution to Sustainable Development Goals (SDGs):
SDG 8: Decent Work and Economic Growth
SDG 9: Industry, Innovation, and Infrastructure
SDG 12: Responsible Consumption And Production
SDG 14: Life Below Water

1. INTRODUCTION

1.1. Research Background

Seaweed is a marine biological resource with high economic and functional value and has the potential to be developed as a functional food ingredient. Indonesia is the world's second-largest producer of seaweed, with a production volume of 9.6 million metric tons, supported by significant cultivation potential. However, the majority of Indonesia's seaweed exports are still in the form of raw or dried materials, meaning the value added generated has not yet been optimized [1,2]. In addition to being rich in fiber and minerals, seaweed also contains various bioactive compounds that act as natural antioxidants and may help prevent degenerative diseases. The five species most commonly utilized in Indonesia are *Eucheuma cottonii, Gracilaria sp., Gelidium sp., Sargassum sp.,* and *Ulva lactuca*, which represent the phyla *Rhodophyceae, Phaeophyceae*, and *Chlorophyceae* [3,4].

Differences among seaweed species result in variations in the composition of bioactive compounds and their biological activities, such as antioxidant, antimicrobial, prebiotic, and immunomodulatory properties. However, these bioactive compounds are generally trapped within a complex cell wall matrix, requiring effective extraction methods to achieve optimal results Conventional methods such as maceration and Soxhlet extraction are still widely used, but they have various limitations, including long extraction times, high solvent requirements, and the risk of degradation of bioactive compounds. These factors

pose challenges to the utilization of seaweed as a source of high-value functional compounds.

Advances in energy-assisted extraction technologies offer more efficient and environmentally friendly alternatives. Ultrasound-Assisted Extraction (UAE) utilises cavitation to increase cell wall permeability, while Microwave-Assisted Extraction (MAE) uses rapid, selective microwave heating to accelerate the release of bioactive compounds [5]. The combination of these two methods, known as Ultrasound–Microwave Assisted Extraction (UMAE), has been reported to increase extract yield, shorten processing time, and better preserve the stability of bioactive compounds compared to conventional methods [6,7]. However, studies on the application of UMAE to various types of local Indonesian seaweed remain relatively limited.

In addition to the extraction method, the type of solvent also plays a crucial role in determining the success of the extraction process, as each compound has different polarity characteristics. The use of solvents with varying degrees of polarity can result in differences in extraction efficiency as well as in the profiles of the extracted secondary metabolites. The Ultrasound–Microwave Assisted Extraction (UMAE) method is a green extraction technology that combines ultrasonic waves and microwaves to enhance extraction efficiency by accelerating mass transfer, thereby potentially reducing processing time and solvent consumption compared to conventional extraction methods. Therefore, this study aims to examine the effects of various types of seaweed (E. cottonii, Gracilaria sp., Gelidium sp., Sargassum sp., and U. lactuca) and solvent types on yield, total dissolved solids, and secondary metabolite profiles based on the results of phytochemical screening using the Ultrasound –Microwave-Assisted Extraction (UMAE). The results of this study are expected to serve as a scientific foundation for the development of seaweed extraction technology and to support the use of UMAE as an efficient green extraction method for producing extracts with optimal characteristics from various types of seaweed.

1.2. Literature Review

Seaweed is a source of natural bioactive compounds that have the potential to be used as functional foods. Several types of seaweed that are widely cultivated and utilized in Indonesia include Eucheuma cottonii, Gracilaria sp., Gelidium sp., Sargassum sp., and Ulva lactuca. These five species are known to contain various secondary metabolites such as phenols, flavonoids, tannins, saponins, alkaloids, steroids, and terpenoids, which act as antioxidants, antimicrobials, anti-inflammatories, and immunomodulators [8,9,10]. The content and biological activity of bioactive compounds in seaweed are influenced by the species, growing conditions, and the extraction method used.

Extraction is the process of separating bioactive compounds from a material matrix using an appropriate solvent based on the principle of solubility. The effectiveness of extraction is influenced by the extraction method, type of solvent, processing time, temperature, and the characteristics of the raw material [11]. Conventional extraction methods such as maceration and Soxhlet extraction are still widely used, but they have limitations, including long extraction times, high solvent consumption, and the risk of bioactive compound degradation due to prolonged heating [12].

One of the emerging modern extraction technologies is Ultrasound–Microwave Assisted Extraction (UMAE), which combines ultrasonic waves and microwaves in a single extraction process. Ultrasonic waves induce cavitation, which can disrupt cell walls and enhance mass transfer, while microwaves generate volumetric heating that accelerates the release of bioactive compounds from the material matrix [13]. The combination of these two mechanisms produces a synergistic effect that increases yield, shortens extraction time, reduces solvent use, and preserves the stability of heat-sensitive bioactive compounds compared to conventional methods.

In addition to the extraction method, the type of solvent also plays a crucial role in determining the success of the extraction process. Differences in solvent polarity affect the solvent's ability to dissolve and extract various chemical components from the seaweed matrix, thereby impacting the extraction yield, total dissolved solids, and the profile of compounds detected through phytochemical screening. The selection of an appropriate solvent also affects mass transfer efficiency during the extraction process, meaning that the amount of compounds successfully extracted may vary depending on the type of solvent used. Therefore, combining the Ultrasonic-Microwave Assisted Extraction (UMAE) method with the selection of the right solvent is a key factor in obtaining seaweed extracts with optimal physicochemical characteristics.

1.3. Research Objective

This study aims to analyze the photochemical compounds in the seaweeds *Eucheuma cottonii, Gelidium sp., Gracilaria sp., Sargassum sp.,* and *Ulva lactuca using* ultrasound-microwave assisted extraction with different solvents, methanol and ethanol.

2. MATERIALS AND METHODS

2.1. Materials

The materials used in this study included the seaweed species *Eucheuma cottonii., Gelidium sp., Gracilaria sp., Sargassum sp., and Ulva lactuca.* The solvents used for extraction were methanol (Merck), and ethanol (Merck). Chemicals employed for analysis cimporised HCl (Merck), magnesium powder, FeCl (Merck), chloroform (Merck), ammonia, sulfuric acid (Merck), Meyer's, Wagner's, Dragendorff's, anhydrous acetic acid (Merck), follin (Merck), sodium carbonate (Merck), vanillin (for analysis), DPPH, aluminum chloride (for analysis), and distilled water.

2.2. Research Procedures

This study used a completely randomized design (CRD) with a factorial pattern involving two factors: Factor I consisted of five levels and Factor II consisted of two levels, each repeated twice, resulting in 20 experimental units. The data obtained from the analysis were processed using Analysis of Variance (ANOVA) with a significance level of 5% to determine the presence of interactions and significant differences between each treatment. If significant differences were found, a 5% DMRT post-hoc test was conducted.

2.3. Production of Seaweed Flour

The seaweed collected from Sayang Heulang Beach in Garut, West Java, was first cleaned by soaking it in fresh water for 1 hour to reduce its salt content and remove any remaining dirt and foreign matter. Afterward, the seaweed is cut into smaller pieces to ensure more even drying. The samples are then dried in an oven

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at 60°C for 6 hours until the seaweed is completely dry. Next, the dried seaweed is ground into a powder using a blender. The resulting powder is then sifted through a 60-mesh sieve to achieve a uniform particle size.

2.4. Seaweed Flour Extraction

5 g of seaweed powder was weighed and then extracted using 70% methanol or 70% ethanol as the solvent, with a solid-to-solvent ratio (w/v) of 1:10. The mixture of material and solvent was placed in a sealed container and extracted using an ultrasonic sonulator at a frequency of 40 kHz and a temperature of 40°C for 15 minutes. Next, the ultrasonic extract was further extracted using a microwave at 300 watts for 5 minutes. The resulting mixture was then filtered using filter paper to separate the filtrate from the residue. The filtrate was separated from the remaining solvent using a rotary evaporator at 40°C until a concentrated extract was obtained, which was collected in an Erlenmeyer flask [14].The resulting extract was subsequently used for various tests according to the study parameters.

2.5. Total Yield

Yield is calculated by comparing the final weight of the product to the initial weight of the raw materials. The yield can be calculated using the following formula:

$$\%\text{Rendemen} = \frac{weigh\ \ of\ the\ concentrated\ extract\ (gr)}{Initial\ weigh\ \ of\ the\ crude\ drug\ (gr)} \times 100\%$$ [15]

2.6. Phytochemical Qualitative Tests

2.6.1. Qualitative Test for Alkaloids

A 50 mg sample was dissolved in 2 drops of 2 N sulfuric acid (H_2SO_4) until homogeneous. The sample solution was then placed on a test plate and the alkaloid reagents Dragendorff, Meyer, and Wagner were added. The test results were considered positive for alkaloids if a red to orange precipitate formed upon the addition of the Dragendorff reagent, a yellowish-white precipitate upon the addition of the Meyer reagent, and a brown precipitate upon the addition of the Wagner reagent [16].

2.6.2. Qualitative Test for Phenols

A 50 mg sample was dissolved in 0.25 mL of 70% ethanol until homogeneous. Next, 2 drops of a 5% $FeCl_3$ solution were added to the solution, and the color change was observed. The test result was considered positive for tannins if a green or bluish-green color formed in the solution [16].

2.6.3. Qualitative Test for Flavonoids

A 50 mg sample was mixed with 0.05 mg of magnesium powder, then dissolved in 0.2 mL of amyl alcohol—a mixture of 37% HCl and 95% ethanol in equal volumes—and 4 mL of 70% alcohol was added. Once homogeneous, the sample was observed on the amyl alcohol layer. The test result was considered positive for flavonoids if a red, yellow, or orange color formed on the amyl alcohol layer [16].

2.6.4. Qualitative Test for Saponins

A 50 mg sample was placed in a test tube, then 20 mL of hot water was added and the mixture was shaken until foam formed. Next, the stability of the foam was observed for 30 minutes, and 1 drop of 2 N HCl was added. The test result is considered positive for saponins if the foam remains stable for 30 minutes and does not disappear after the addition of 2 N HCl [16].

2.6.5. Qualitative Test for Tannins

A 50 mg sample was mixed with 20 mL of hot water, and the mixture was filtered to obtain the filtrate. Two drops of a 1% $FeCl_3$ solution were then added to the filtrate, and the color change was observed. The test result was considered positive for terpenoids if the solution turned blue or blackish green [16].

2.6.6. Qualitative Test for Steroids and Triterpenoids

A 50 mg sample was mixed with 2 mL of chloroform and placed in a test tube. Next, 5 drops of acetic anhydride and 3 drops of 2 N concentrated sulfuric acid (H_2SO_4) were added; the mixture was then homogenized, and the color change was observed. The test result is considered positive for steroids or triterpenoids if a reddish-brown color forms in the early stage of the reaction and subsequently turns blue or green. A blue color indicates the presence of steroid compounds, while a reddish-brown color indicates the presence of triterpenoid compounds [16].

2.7. Analysis of Total Dissolved Solids (AOAC, 1984)

Measurement of total dissolved solids using a refractometer according to AOAC. The total dissolved solids content of star fruit with added extract was determined using a handheld digital refractometer (Model: PAL-1, Atago Co., Ltd., Tokyo, Japan). The procedure is as follows: one drop of distilled water is applied to the prism of the refractometer and immediately wiped off with a tissue; then, 1–3 drops of the liquid sample are applied to the prism, and the reading is taken in a well-lit area [17].

3. RESULT AND DISCUSSION

3.1. Yield of Seaweed Extract

Based on the results of the analysis of variance using a two-way ANOVA, it can be seen that there is a significant interaction ($p \leq 0.05$) between the seaweed type treatment and the solvent type treatment, and that each treatment has a significant effect on the yield of the resulting seaweed extract. The mean values of seaweed extract yield resulting from the seaweed type and solvent type treatments are presented in Table 1.

Table 1. Result of yield from seaweed powder extracts

Types of seaweed	Types of solvent	Yield (%) (Average ± $\overline{SD}$)
Eucheuma Cottonii	Metanol	48.37 ± 0.56^{i}
	Etanol	41.17 ± 0.30^{h}
Gelidium sp.	Metanol	32.16 ± 0.70^{e}
	Etanol	22.56 ± 0.82^{c}
Gracilaria sp.	Metanol	37.49 ± 0.54^{g}
	Etanol	33.45 ± 0.14^{f}
Sargassum sp.	Metanol	21.25 ± 0.22^{b}
	Etanol	18.16 ± 0.08^{a}
Ulva lactuca	Metanol	32.93 ± 0.07^{ef}
	Etanol	27.86 ± 0.55^{d}

Description: Averages followed by different letters indicate a significant difference (p≤0,05).

Based on Table 1, the total yield of seaweed extract ranged from 18.16–48.37%. The total yield for the *Sargassum sp.* treatment using ethanol as the solvent was lower, at 18.16 ± 0.08%, while the total yield for the *Eucheuma cottonii* treatment using methanol as the solvent was higher, at 48.37 ± 0.56%. The effect of seaweed type and solvent type on total yield is shown in Figure 1.

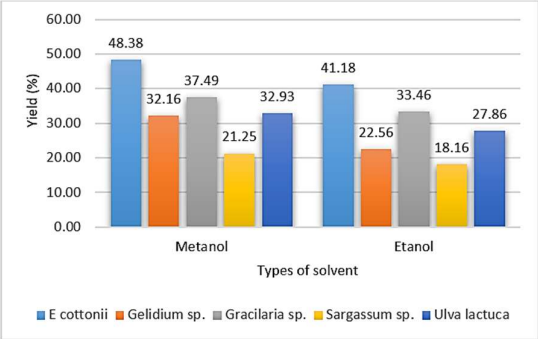

Figure 1. Relationship between seaweed type and solvent type on yield

The extract yields obtained in this study ranged from 18.16% to 48.37%, with the highest yield produced by the methanol extract of Eucheuma cottonii (48.37 ± 0.56%) and the lowest by the ethanol extract of Sargassum sp. (18.16 ± 0.08%). In general, all treatments yielded relatively high extraction yields, exceeding the minimum threshold for a "good" extraction yield (>10%) [18]. The high yields obtained indicate that the Ultrasound Microwave-Assisted Extraction (UMAE) method is capable of effectively extracting bioactive components from seaweed.

Compared to both conventional extraction methods and single-solvent-assisted extraction, the UMAE method used in this study demonstrated superior performance. In Eucheuma cottonii, the methanol extract yield of 48.37% and the ethanol yield of 41.17% were significantly higher than the extraction yields from maceration, which were 6.6% using methanol and 4% using ethanol [19]. These values are also higher than the ethanol extraction yields of 9.23% [20] and 3% [21]. In fact, when compared to the Ultrasound-Assisted Extraction (UAE) method, the resulting yield reached only 11.6% [22]. These results indicate that the combination of ultrasonic waves and microwaves provides higher extraction efficiency compared to the use of ultrasonic waves or maceration alone.

A similar trend was also observed in Gelidium sp. The methanol and ethanol extract yields obtained in this study were 32.16% and 22.56%, respectively, which were higher than the ethanol extract yield from maceration, which was 11.44% [23]. These values were also higher than the yield of the extract obtained through ultrasonic-assisted extraction combined with maceration, which was 4.03% [24]. For Gracilaria sp., the methanol extract yield of 37.49% and the ethanol extract yield of 33.45% were also higher than the ethanol extract yield of 0.9% [25].

Among the brown seaweeds, Sargassum sp. yielded 21.25% (methanol) and 18.16% (ethanol). These values are significantly higher than the ethanol extraction yields of 0.565% [26], 1.39% [27], and 2.5% [28]. Meanwhile, among the green seaweed group, Ulva lactuca yielded 32.93% using methanol and 27.86% using ethanol. These values are higher than the extraction yields from ethanol maceration, which were 25% [29], 13.55% [30], and 6.75–7.30% [31].

A significant difference between this study and previous research indicates that the extraction method plays a crucial role in determining the amount of compounds successfully extracted. Conventional maceration methods generally rely on the passive diffusion of the solvent into the material matrix, requiring a long extraction time—ranging from 24 hours to several days. During this process, mass transfer occurs slowly, so some bioactive compounds remain trapped within the seaweed's cellular tissue. This results in lower extraction efficiency and affects the resulting yield.

In contrast, the UMAE method utilizes the synergy of two extraction energy sources: ultrasonic waves and microwaves. Ultrasonic waves produce the phenomenon of acoustic cavitation, which involves the formation, growth, and collapse of microbubbles within the extraction medium. The collapse of these bubbles generates mechanical forces capable of disrupting cell wall structures and increasing the porosity of the seaweed tissue. This damage to the cell structure accelerates the penetration of the solvent into the material matrix while facilitating the release of intracellular compounds into the solvent.

At the same time, microwaves generate dielectric heating through interaction with polar molecules and ions present within the cell tissue. Heating occurs from the inside of the cell outward (internal heating), thereby rapidly increasing the cell's internal pressure. This increase in pressure causes the cell walls to rupture and accelerates the release of bioactive components into the solvent. When these two mechanisms occur simultaneously, mass transfer resistance is reduced, and the extraction rate increases significantly compared to both conventional extraction methods and single-assisted extraction methods.

The increase in yield achieved through the UMAE method indicates that the amount of seaweed constituents successfully extracted is also greater than that obtained using conventional extraction methods or single-aid extraction. Seaweed is known to contain various polar bioactive compounds such as phenols, flavonoids, tannins, saponins, alkaloids, and steroids, as well as the major polysaccharides that characterize each group of seaweed, namely carrageenan in Eucheuma cottonii, agar in Gelidium sp. and Gracilaria sp., alginate and fucoidan in Sargassum sp., and ulvan in Ulva lactuca. The high yield obtained indicates that the combination of ultrasonic and microwave waves is capable of enhancing the release of these compounds from the cellular matrix into the extraction solvent.

This phenomenon occurs because the damage to the cell wall structure caused by ultrasonic cavitation and internal heating by microwaves makes secondary metabolites and structural polysaccharides which were previously trapped within the cell matrix more easily soluble and able to diffuse into the solvent. Consequently, the total amount of dissolved solids transferred into the solvent increases, resulting in a higher yield compared to maceration or single-solvent extraction methods. This finding is further supported by the phytochemical analysis results from this study, which revealed the presence of various classes of secondary metabolites in the resulting extract. Therefore, the high yield obtained not only reflects an increase in the amount of extract produced but also indicates improved recovery of bioactive compounds and polysaccharides that have the potential to confer functional activity to the seaweed extract.

The advantages of UMAE are demonstrated not only by the high yield it produces but also by the efficiency of the extraction process. Most comparative studies use extraction times ranging from 24 hours to 5 days, whereas UMAE is capable of producing higher yields in a much shorter extraction time. This efficiency is a crucial aspect in the development of industrial-scale extraction technology because it can reduce energy consumption, minimize solvent use, and increase process productivity. Thus, the application of UMAE has the potential to provide greater economic benefits compared to conventional extraction methods.

Therefore, the main novelty of this study lies in the successful application of the UMAE method to extract various groups of tropical seaweed using different polar solvents, yielding consistently higher yields compared to most previous studies that used maceration, Soxhlet extraction, or ultrasound-assisted extraction. These results reinforce the potential of UMAE as a new-generation extraction technology that is more efficient, faster, and sustainable for obtaining bioactive compounds from seaweed that can be utilized in the development of functional foods, pharmaceutical raw materials, cosmetics, and nutraceutical products.

3.2. Phytochemical Compounds In Seaweed Extract

The effects of seaweed type and solvent type on the presence of alkaloids, flavonoids, phenolics, tannins, saponins, triterpenoids, and steroids are presented in Table 2.

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Table 2. Result of qualitative phytochemical tests from seaweed powder extracts

Types seaweed	Types solvent	Alkaloid	Fenol	Flavonoid	Saponin	Tanin	Steroid	Terpenoid
Eucheuma Cottonii	Metanol	+	+	+	+	-	-	+
	Etanol	-	+	-	+	-	-	+
Gelidium sp.	Metanol	+	+	+	-	+	-	+
	Etanol	+	-	+	-	-	-	+
Gracilaria sp.	Metanol	-	+	+	+	-	-	-
	Etanol	-	-	-	+	-	+	-
Sargassum sp.	Metanol	+	+	+	+	+	-	-
	Etanol	-	+	+	+	+	+	-
Ulva lactuca	Metanol	-	+	+	+	+	-	+
	Etanol	+	-	-	+	+	-	+

Description: (-) : does not contain the tested compound
(+) : contains the tested compound

3.2.1. Alkaloids

Phytochemical test results showed that alkaloids were detected in the methanol extracts of *Eucheuma cottonii, Gelidium sp*., and *Sargassum sp*., as well as in the ethanol extracts of *Gelidium sp.* and *Ulva lactuca*. Meanwhile, no alkaloids were detected in the ethanol extracts of *E. cottonii* and *Sargassum sp*., nor in either of the *Gracilaria sp.* extracts. Positive results were indicated by the formation of a precipitate following the addition of Mayer's, Dragendorff's, and Wagner's reagents, which signaled the presence of alkaloids. Differences in results among species and solvents are thought to be influenced by variations in secondary metabolite content as well as the solvents' ability to extract alkaloids. Methanol, which has higher polarity, is generally more effective at dissolving polar alkaloids, although in *Ulva lactuca*, alkaloids were detected only in the ethanol extract. Furthermore, negative results may be due to low alkaloid levels or levels below the detection limit of the testing method [32].

The alkaloids found in seaweed have protective effects against oxidative stress, inflammation, and abnormal cell proliferation. This helps prevent a number of degenerative diseases, including cancer, metabolic syndrome, and cardiovascular disorders. Seaweed alkaloids are promising candidates for the development of functional foods and marine-based nutraceutical products due to their dual action as antioxidants, anti-inflammatory agents, and anticancer agents. To maximize the application of seaweed alkaloids in the food and health industries, further research is still needed to fully understand their chemical structure, mechanism of action, and bioavailability [33]

3.2.2. Phenols

The results of the qualitative tests showed that phenolic compounds were detected in almost all seaweed extracts, except for the ethanol extracts of *Gelidium sp., Gracilaria sp*., and *Ulva lactuca.* A positive result was indicated by the formation of a blue to blackish-green color after the addition of the $FeCl_3$ reagent, indicating the formation of a complex between iron (III) ions and phenoxide ions [34]. Phenolic compounds act as antioxidants because they can donate hydrogen atoms to stabilize free radicals and inhibit chain oxidation reactions [35]. Differences in phenol content among species are thought to be influenced by variations in natural pigments, such as phycobiliproteins, chlorophyll, and fucoxanthin, as well as secondary metabolic activity involved in the formation of bioactive compounds. In addition, the type of solvent also affects phenol extraction. Methanol, which has higher polarity, tends to be more effective at extracting phenolic compounds than ethanol because it is better able to dissolve the hydroxyl (-OH) groups in phenolic compounds [36].

Phenols are a group of secondary metabolites commonly found in plants and have the potential to be used as functional food components due to their antioxidant, anti-inflammatory, antimutagenic, and antitumor activities. The activity of polyphenols in food is influenced by their stability, bioactivity, food structure, and their interactions with proteins, lipids, and carbohydrates. However, natural phenolic compounds are easily degraded during processing and storage due to the effects of heat, light, oxygen, and pH. In addition, the use of excessive amounts of phenols can cause a bitter taste in food products [37].

3.2.3. Flavonids

Qualitative flavonoid tests yielded positive results for the methanol extracts of *Eucheuma cottonii, Gelidium sp., Gracilaria sp., Sargassum sp.*, and *Ulva lactuca*, as well as the ethanol extracts of Gelidium sp. and Sargassum sp. Positive results were indicated by the appearance of a red or orange color following the addition of Mg powder and HCl due to the reduction of the benzopyrone ring in the flavonoid compounds [38]. Differences in results among treatments indicate that the flavonoid content and the solvents' ability to extract them vary among the different types of seaweed. Methanol tends to be more effective at extracting flavonoids because it has higher polarity than ethanol; consequently, flavonoids in *Eucheuma cottonii, Gracilaria sp*., and *Ulva lactuca* were detected only in the methanol extracts.
Flavonoids are polyphenolic compounds found in seaweed; these compounds possess anti-inflammatory, antidiabetic, and antioxidant properties, and support cardiovascular and digestive health. Seaweed extracts such as those derived from the genus Sargassum sp. have the potential to serve as a source of bioactive compounds for functional foods. As part of their defense mechanisms and response to microbial infections, plants produce flavonoids that act as antimicrobial agents against various microorganisms. This class of polyphenols, known as flavonoids, has a wide range of biological effects, including antiviral, antibacterial, antitumor, anti-inflammatory, and antioxidant properties [39].

3.2.4. Saponins

Qualitative saponin tests yielded positive results for the methanol and ethanol extracts of *Eucheuma cottonii, Gracilaria sp., Sargassum sp.*, and *Ulva lactuca*, while the methanol and ethanol extracts of Gelidium sp. yielded negative results. Positive results were indicated by the formation of stable foam after shaking and the addition of HCl. Foam forms because saponins possess natural surfactant properties, namely hydrophilic groups that bind to water and hydrophobic groups that interact with air, thereby forming micelles and producing stable foam [9]. HCl was added to test foam stability, as saponins are capable of maintaining bubbles under acidic conditions [40]. Differences in results among treatments indicate variations in saponin content among the different seaweed species. Saponin content can be influenced by the type of pigment, the components of the cell wall, and the seaweed's ability to produce secondary metabolites as a defense mechanism against environmental disturbances. The negative results for *Gelidium sp*. indicate that saponin content was either undetectable or present at such low concentrations that it failed to produce stable foam during the test.

Seaweed is rich in saponins, amphiphilic bioactive compounds that are essential in the production of functional foods and nutraceuticals. By reducing fat absorption, increasing cholesterol excretion, and balancing the composition of the gut microbiota, saponins play a role in regulating lipid metabolism through mechanisms involving the gut-liver axis, thereby supporting metabolic health [41]. Saponins have physiological effects, such as hepatoprotective, antioxidant, antimicrobial, anticancer, antidiabetic, anti-obesity, and immunomodulatory effects, which help prevent degenerative diseases. Saponins are valuable in the food industry due to their emulsifying, foaming, and stabilizing properties, which can improve the texture and stability of food products.

3.2.5. Tanins

Qualitative tannin tests yielded positive results for the methanol extracts of *Gelidium sp., Sargassum sp*., and *Ulva lactuca*, as well as the ethanol extracts of Sargassum sp. and Ulva lactuca. Positive results were indicated by the formation of a blue-black

or green-black color following the addition of the $FeCl_3$ reagent. This color change occurs because the phenolic groups in tannins form complexes with Fe^{3+} ions [42]. Differences in results among treatments indicate variations in tannin content among the different types of seaweed, as well as differences in the solvents' ability to extract them. Tannins are polar compounds that are generally more soluble in highly polar solvents; thus, methanol tends to be more effective at extracting tannins than ethanol. Additionally, differences in seaweed taxonomic groups, pigment content, and response to environmental conditions can influence the formation of tannins as secondary metabolites that play a role in antioxidant activity.

In seaweed, particularly the Phaeophyceae class, florotanins have the potential to be utilized as functional foods. Padina sp. seaweed is known to have a high tannin content, making it a potential source of natural antioxidants capable of scavenging free radicals, preventing lipid peroxidation, and protecting cells from oxidative stress. These activities play a role in the prevention of degenerative diseases such as diabetes, cancer, and atherosclerosis. The tannin content in seaweed can also enhance nutritional value and health through cellular defense mechanisms [43].

3.2.6. Steroids and Triterpenoids

Qualitative steroid testing yielded positive results for the ethanol extracts of *Gracilaria sp*. and *Sargassum sp*., while the other treatments yielded negative results. A positive result is indicated by the formation of a bluish-green color after the addition of the Liebermann-Burchard reagent due to the formation of conjugated double bonds during the oxidation of sterols or steroids [44]. A positive reaction is also indicated by the formation of a green ring resulting from the interaction of unsaturated sterols with anhydrous acetic acid and sulfuric acid [45]. Differences in results among treatments indicate variations in steroid content among the different seaweed species as well as differences in the solvents' ability to extract them. Steroids act as components of cell membranes that help maintain cellular stability and function in response to changes in environmental conditions; thus, their content can be influenced by the physiological activity and adaptive capacity of each seaweed species. Qualitative tests for terpenoids yielded positive results in the methanol and ethanol extracts of *Eucheuma cottonii* and *Gelidium sp*., as well as in the ethanol extract of *Ulva lactuca*, while the other treatments yielded negative results. Positive results were indicated by the formation of a red to brownish color following the addition of the reagent. Differences in results among treatments indicate variations in terpenoid content among the different seaweed species. Negative results may occur because terpenoids are easily oxidized and volatile, so some compounds may degrade during sample preparation or storage. Terpenoids are secondary metabolites that play a role in the biosynthesis of natural pigments and in protection against environmental stress and oxidation. Therefore, differences in pigment composition and biosynthetic capacity among seaweed species may influence the presence of terpenoid compounds detected during testing.

Steroid compounds have anti-inflammatory, antibacterial, antifungal, and anticancer properties. The largest class of secondary metabolites is called terpenoids, which consist of isoprene units (C_5H_8). These substances are found in various parts of plants, particularly in pigments, seaweed, and essential oils. Terpenoids are classified as monoterpenes, sesquiterpenes, diterpenes, triterpenes, and so on, based on the number of isoprene units they contain. Terpenoids provide natural aromas, possess pharmacological properties (antimicrobial, anti-inflammatory, and antioxidant), and protect plants from pests and diseases [46].

To evaluate differences in phytochemical profiles across extraction methods, the UMAE results were compared with those of several methods reported in previous studies, as shown in Table 3.

Table 3. Comparison of phytochemical profiles of seaweed extracts obtained using UMAE and other extraction methods.

Types seaweed	Method	Flavonoid	Alkaloid	Tanin	Saponin	Fenol	Steroid	Triterpenoid
E. cottonii	UMAE	+	+	-	+	+	-	+
	Maserasi (Panjaitan & Meze, 2023)	-	-	-	-	-	+	-
	UAE (Panjaitan & Meze, 2023)	+	-	-	+	-	+	+
Gelidium sp.	UMAE	+	+	+	-	+	-	+
	Maserasi (Lutfiyanti et al., 2012)	-	+	-	-	-	+	+
Gracilaria sp.	UMAE	+	-	-	+	+	-	+
	Soxhlet (Insani et al.2022)	-	-	-	+	+	+	-
	MAE (Sheravina et al., 2025)	+	-	-	-	+	-	-
Sargassum sp.	UMAE	+	+	+	+	+	-	-
	Maserasi (Ramlan et al., 2024)	+	-	-	+	+	+	-
	Soxhlet (Samudra et al., 2022)	+	-	-	+	+	+	-
Ulva lactuca	UMAE	+	-	+	+	+	-	+
	Maserasi (Rompas & Gasah, 2022)	+	-	-	-	-	+	+

Description: (-) : does not contain the tested compound
(+) : contains the tested compound

Based on Table 3, the UMAE method was generally able to detect a greater number of phytochemical classes compared to conventional extraction methods or single-aided extraction methods in most types of seaweed. In Eucheuma cottonii, Gelidium sp., Sargassum sp., and Ulva lactuca, extraction using UMAE yielded a more complete phytochemical profile compared to the maceration method. Similarly, in Gracilaria sp., the UMAE method was able to detect more compound classes than either the Soxhlet or MAE methods.

The more diverse phytochemical profile of the UMAE extract is believed to be related to the synergy between ultrasonic and microwave waves during the extraction process. Cavitation generated by ultrasound enhances cell wall disruption, while dielectric heating by microwaves accelerates the release of intracellular compounds into the solvent. The combination of these two mechanisms enhances mass transfer, allowing for the extraction of a greater number of bioactive compounds. These comparative results are consistent with the high yield and total dissolved solids obtained in this study, further reinforcing the potential of UMAE as a more efficient extraction method for obtaining bioactive compounds from various types of seaweed.

3.3. Total Dissolved Solids

Based on the results of a two-way analysis of variance (ANOVA), it was found that there was a significant interaction ($p \leq 0.05$) between the seaweed type and solvent type treatments, and that each treatment had a significant effect on the total dissolved solids in the obtained seaweed extracts. The mean values of the total dissolved solids in the seaweed extracts obtained from the different seaweed and solvent treatments are presented in Table 4.

Table 4. Total Dissolved Solids in Seaweed Powder Extract

Types of seaweed	Types of solvent	Total dissolved solids (%) (Average)
Eucheuma Cottonii	Metanol	31.00^{e}
	Etanol	13.50^{a}
Gelidium sp.	Metanol	25.00^{d}
	Etanol	14.00^{ab}
Gracilaria sp.	Metanol	27.50^{d}
	Etanol	17.00^{c}
Sargassum sp.	Metanol	37.50^{f}
	Etanol	14.00^{ab}
Ulva lactuca	Metanol	27.50^{d}
	Etanol	16.50^{bc}

Description: Averages followed by different letters indicate a significant difference ($p \leq 0,05$).

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Based on Table 4, the total dissolved solids in the seaweed extracts ranged from 14–37.5%. The total dissolved solids for the *Sargassum sp*. and *Gelidium sp.* treatments using ethanol as the solvent were lower, at 14%, while the total dissolved solids for the *Eucheuma cottonii* treatment using methanol as the solvent were higher, at 37.5%. The effects of seaweed species and solvent type on total dissolved solids are shown in Figure 2.

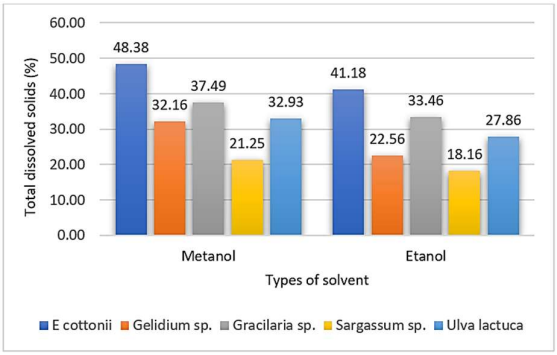

Figure 2. Relationship between seaweed type and solvent type and total dissolved solids

The results of the analysis showed that the total dissolved solids (TDS) values differed significantly ($p<0.05$) among the treatments. The highest TDS value was obtained from the Sargassum sp. extract using methanol as the solvent, at 37.50 ± 0.71, while the lowest value was obtained from the Eucheuma cottonii extract using ethanol as the solvent, at 13.50 ± 0.71. In general, all extracts using methanol as a solvent yielded higher TDS values compared to ethanol extracts from the same seaweed species. These results indicate that seaweed species, solvent type, and their interaction influence the amount of components successfully dissolved during the extraction process.

Total dissolved solids represent the total amount of compounds that have dissolved into the solvent during the extraction process. This parameter reflects the abundance of dissolved components present in the extract, including polysaccharides, phenolic compounds, flavonoids, pigments, soluble proteins, minerals, and other secondary metabolites. The higher the TDS value obtained, the greater the amount of components successfully extracted from the seaweed matrix and transferred into the solvent [47].

In general, the TDS values obtained are consistent with the extract yield results. Treatments that produced high yields tended to have high TDS values as well. This indicates that an increase in yield is not only due to an increase in the mass of the extract obtained but also to an increase in the amount of dissolved components within the extract. Yield describes the amount of extract successfully recovered from the raw material, while TDS describes the concentration of dissolved components contained in the extract. Therefore, these two parameters complement each other in evaluating the success of the extraction process.

Interestingly, although Eucheuma cottonii treated with methanol as a solvent yielded the highest extraction yield of 48.37 ± 0.56%, the highest TDS value was actually obtained from Sargassum sp. treated with methanol, at 37.50 ± 0.71. This finding indicates that a high yield is not always accompanied by the highest concentration of dissolved components. Yield refers to the total mass of extract obtained after evaporation, whereas TDS better reflects the amount of components present in dissolved form within the extract. Thus, the Sargassum sp. extract is presumed to contain a higher concentration of dissolved components even though its total yield is lower than that of Eucheuma cottonii.

These differences are likely related to the chemical characteristics of each type of seaweed. Red seaweeds such as Eucheuma cottonii are known to be rich in carrageenan, while Gelidium sp. and Gracilaria sp. contain agar as their primary polysaccharide. Meanwhile, the brown seaweed Sargassum sp. contains alginate, fucoidan, phlorotannins, and various phenolic compounds that are relatively easily soluble in polar solvents. The presence of these hydrophilic components is believed to contribute to the high measured dissolved solids content in Sargassum sp. extracts. The green seaweed Ulva lactuca also contains ulvan and various other polar components that contribute to its relatively high TDS value.

The high TDS values obtained in this study are believed to be related to the effectiveness of the Ultrasound Microwave-Assisted Extraction (UMAE) method. The combination of ultrasonic waves and microwaves enhances the release of intracellular components through the mechanisms of cavitation and dielectric heating. Ultrasonic cavitation causes mechanical damage to cell walls, thereby increasing tissue porosity, while microwaves increase intracellular pressure through heating from within the tissue. The synergy of these two mechanisms accelerates mass transfer and increases the amount of components successfully dissolved into the extraction solvent.

These results are consistent with various studies reporting that the combination of ultrasonic and microwave technologies can improve the recovery of bioactive compounds, polysaccharides, and soluble components compared to conventional extraction methods or single-assisted extraction. The combination of ultrasound and microwaves in the extraction of macroalgal biomass can improve extraction efficiency and the yield of value-added compounds compared to the use of a single technology [48]. Furthermore, intensive extraction methods can increase the release of phenolic compounds, polysaccharides, and other bioactive components due to enhanced mass transfer during the extraction process [49].

The high TDS values in this study were also supported by the results of qualitative phytochemical tests, which indicated the presence of various classes of secondary metabolites—such as flavonoids, alkaloids, saponins, tannins, steroids, and triterpenoids in the seaweed extract. Although the qualitative phytochemical testing did not reveal the concentration of each compound, the successful detection of these various classes of secondary metabolites indicates that the extraction process was able to release various bioactive components from the seaweed tissue. Therefore, the high TDS value obtained not only reflects an increase in the overall amount of dissolved components but also demonstrates the effectiveness of the UMAE method in extracting secondary metabolites and polysaccharides that have the potential to confer functional activity to the extract.

Overall, the combination of yield, total dissolved solids, and qualitative phytochemical parameters indicates that the UMAE method is capable of improving extraction efficiency for various types of seaweed. A high yield indicates the amount of extract successfully obtained; a high TDS indicates the abundance of dissolved components in the extract; and the phytochemical results indicate the successful extraction of various classes of secondary metabolites. Together, these three parameters reinforce the evidence that the UMAE method is effective for obtaining bioactive components from seaweed more optimally than conventional extraction methods.

4. CONCLUSION

The use of methanol as a solvent in the Ultrasound Microwave-Assisted Extraction (UMAE) method generally yielded higher extraction yields and total dissolved solids compared to ethanol for all types of seaweed tested. The highest yield was obtained from *Eucheuma cottonii* using methanol at 48.37 ± 0.56%, while the highest total soluble solids content was obtained from *Sargassum sp.* using methanol at 37.50 ± 0.71. Phytochemical screening results showed that the seaweed extracts obtained contained various classes of secondary

metabolites, namely flavonoids, alkaloids, saponins, tannins, steroids, and triterpenoids, with the number of detected compound classes varying among treatments. Overall, the combination of yield, total dissolved solids, and phytochemical profiles indicates that the UMAE method is effective in extracting bioactive components from seaweed. These findings are supported by a comparison with various previous studies, which show that the UMAE method tends to yield higher extraction yields than both conventional extraction methods and single-solvent-assisted extraction, thus potentially serving as a more efficient alternative extraction technology for the industrial-scale utilization of seaweed as a raw material source.

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