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Qualitative Phytochemical Screening of Cladophora glomerata Macroalgae Extract Obtained Using Microwave-Assisted Extraction (MAE)

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ABSTRACT

This study aimed to qualitatively identify classes of secondary metabolites in *Cladophora glomerata* extract obtained via Microwave-Assisted Extraction (MAE) using distilled water as the solvent for 5 minutes. We used a 3×3 factorial design with three replicates, resulting in nine treatment combinations. Treatments included solid-to-solvent ratios of 1:30, 1:40, and 1:50 g/mL and microwave power levels of 300, 450, and 600 watts. We conducted qualitative phytochemical screening for six classes of secondary metabolites: phenolics, tannins, flavonoids, alkaloids, saponins, and triterpenoids. The results showed that phenolics, tannins, and saponins were qualitatively detected in all extracts across all treatment combinations. Conversely, flavonoids, alkaloids, and triterpenoids were not detected in any tested extraction combination. These findings provide preliminary qualitative evidence of phenolics, tannins, and saponins in *Cladophora glomerata* extract obtained via MAE using distilled water.

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 has abundant and diverse macroalgal resources, as most of its territory consists of water bodies. Macroalgae are sources of bioactive molecules with biological properties and have potential applications in the food, cosmetic, and pharmaceutical industries [1]. One type of macroalga found in Indonesia is *Cladophora glomerata*. This species is a green macroalga frequently found in aquatic ecosystems; under favorable environmental conditions, it can generate significant biomass in a short period [2]. Its abundance and diverse bioactive components make *C. glomerata* a promising algal resource for further study.

Cladophora glomerata contains a variety of secondary metabolites. Studies on ethanolic extracts of *Cladophora glomerata* have reported several classes of phytochemical compounds, such as tannins, saponins, and flavonoids [3].

Research on freshwater *Cladophora glomerata* has shown sterols and other bioactive components [4], and phenolic compounds have also been isolated and identified from the species [5]. The presence of these various phytochemicals suggests that *Cladophora glomerata* could serve as a source of compounds with diverse biological activities. Various studies have reported that the *Cladophora* genus possesses pharmacological potential, including antioxidant, antimicrobial, and anti-inflammatory activities, among others [6]. However, the presence of a compound in the raw material does not guarantee its detection in the extract, as the phytochemical profile can be influenced by the extraction method, solvent type, and extraction conditions employed.

Phytochemical screening can identify secondary metabolites in natural materials. This method relies on reactions between compounds in the extract and specific reagents, resulting in color changes, precipitate formation, or other characteristic reactions that indicate the presence of specific classes of compounds [8]. Phytochemical screening provides an initial overview of an



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extract's secondary metabolite profile; however, the results are qualitative and do not directly indicate compound concentrations. Therefore, phytochemical screening provides preliminary information on the presence of specific compounds.

One method for extracting bioactive compounds from natural materials is Microwave-Assisted Extraction (MAE). MAE utilizes microwave energy to heat the extraction system, thereby accelerating the release of compounds from the material matrix into the solvent. MAE has been reported to accelerate the extraction of specific compounds, such as carrageenan from *Hypnea musciformis* [10].

Studies on algal extraction technologies also indicate that MAE is a viable approach for obtaining high-value metabolites with relatively short extraction times [17]. However, MAE results vary by material and depend on factors such as the compound being extracted, the solvent used, the solid-to-solvent ratio, and microwave power. While increasing microwave power boosts system energy and accelerates heating, excessive power levels can potentially compromise the stability of heat-sensitive compounds [28, 31]. Consequently, the relationship between microwave power and extraction yield is unlikely to be strictly linear, necessitating further evaluation based on the specific material matrix used.

Solvent choice is another critical factor in extraction, as it determines the solvent's ability to dissolve specific compounds. Distilled water is a polar solvent suitable for extracting polar compounds; polar solvents often yield higher amounts of active compounds [7]. Distilled water is also favored due to its abundance and non-toxicity, as well as its ability to minimize the use of organic solvents, thereby avoiding issues related to organic solvent residues in the final extract [9]. Nevertheless, extraction yields obtained with distilled water cannot be directly equated with those obtained with organic solvents, given differences in polarity and compound solubility.

Previous studies have reported the bioactive content and activity of *C. glomerata* [3-6, 26] and the application of microwave-assisted extraction (MAE) to algae and other natural materials [10, 17, 28-33]. However, information on the phytochemical profile of *C. glomerata* obtained via MAE using distilled water as the solvent, particularly how microwave power and solid-to-solvent ratios affect it, remains limited. While findings from earlier studies help explain the general principles of MAE, they may not apply directly to *C. glomerata* because of differences in matrix characteristics and metabolite composition.

This study qualitatively identified the phytochemical profile of *Cladophora glomerata* extracts obtained by MAE using distilled water as the solvent over 5 minutes. The study employed solid-to-solvent ratios of 1:30, 1:40, and 1:50, and microwave power levels of 300, 450, and 600 watts. This research evaluates the phytochemical profile of *C. glomerata* extracts obtained by MAE with distilled water across various combinations of microwave power and solid-to-solvent ratios. This research is expected to provide preliminary information on the presence of secondary metabolites in *C. glomerata* extracts and serve as a foundation for further studies on their characterization and potential bioactivity, particularly regarding their utilization in the food and pharmaceutical sectors.

1.2. Literature Review

Cladophora belongs to a group of green macroalgae containing carbohydrates, proteins, lipids, and fibers, and holds potential as a source of bioactive substances [12, 13]. Several studies have reported secondary metabolites, such as tannins, saponins, flavonoids, terpenoids, and glycosides, in the ethanolic extract of *C. glomerata* [3]. Additionally, sterols and phenolic compounds have been reported in *C. glomerata* [5], while the *Cladophora* genus is known to possess various other bioactive components [2, 6]. Variations in the types of compounds detected may arise from differences in species, growth conditions, and the extraction methods or solvents employed. Consequently, the reported presence of a specific metabolite class in *C. glomerata* using a particular solvent does not necessarily imply that the same class of compounds will be detected in an extract obtained using distilled water.

Microwave-Assisted Extraction (MAE) is an extraction method that utilizes microwave energy to facilitate the release of compounds from the material matrix into the solvent. This method is widely used to extract bioactive compounds because it accelerates heating, allows shorter extraction times, and requires less solvent than several conventional extraction methods [16, 17]. MAE has also been applied to extract specific components from algae, such as carrageenan from *Hypnea musciformis* [10] and various phytochemicals from *Cladophora* sp. using distilled water [9], demonstrating its applicability to macroalgal matrices.

Several factors influence extraction success, including time, temperature, solvent type, particle size, the solid-to-solvent ratio, and microwave power [18, 19]. Microwave power determines the energy delivered to the system and can influence the heating rate during extraction. Increasing power under certain conditions can accelerate heating and facilitate the release of compounds from the material matrix; however, excessively high power levels can also compromise the stability of heat-sensitive compounds [19, 20]. Consequently, the relationship between microwave power and extraction efficiency is not always linear, meaning that higher power settings cannot be directly assumed to yield extracts with higher compound content.

The solid-to-solvent ratio is a crucial parameter in the MAE method, primarily because the solvent absorbs microwave radiation. Under certain conditions, increasing the amount of solvent can improve contact between the material and the solvent, facilitating mass transfer of compounds into the liquid phase. However, excessive solvent does not necessarily increase extraction yields; excessive microwave absorption by the solvent can hinder microwave penetration into the material matrix during the MAE process [18, 20]. Consequently, the solid-to-solvent ratio must be optimized based on the material's characteristics, the type of solvent, and the applied power level.

Solvent characteristics also play a vital role in the MAE process. The solvent must absorb microwave energy to ensure efficient extraction. Distilled water, for instance, can cause rapid solvent heating, which promotes the transfer of compounds from the material into the solvent [11]. However, distilled water does not guarantee optimal extraction for all metabolite classes, as extraction efficiency ultimately depends on the polarity and solubility of individual compounds. Therefore, the phytochemical profile of extracts obtained using distilled water may differ from that of extracts obtained using organic solvents.

Given these factors, it is important to investigate how varying microwave power and solid-to-solvent ratios affect *C. glomerata* extracts. This study employed power levels of 300, 450, and 600 watts and solid-to-solvent ratios of 1:30, 1:40, and 1:50, with a fixed extraction time of 5 minutes using distilled water. These variations were used to qualitatively assess the secondary metabolite profile in the resulting extracts. This approach is necessary because different MAE conditions yield distinct phytochemical profiles for *C. glomerata*.

1.3. Research Objective

This study aims to qualitatively identify the phytochemical profile of *Cladophora glomerata* extract obtained via the Microwave-Assisted Extraction (MAE) method using distilled water as the solvent, with solid-to-solvent ratios of 1:30, 1:40, and 1:50, and microwave power levels of 300, 450, and 600 watts.

2. MATERIALS AND METHODS

2.1. Materials

The primary material used in this study was *Cladophora glomerata* obtained from an algae cultivation shop in Mojokerto City, East Java, Indonesia. Preliminary sample identification was based on morphological characteristics, comparing the sample's features and visual documentation with descriptions and images of *Cladophora glomerata* in the literature. The observed characteristics included color, filament shape, branching pattern, and texture. These characteristics were compared with the morphological traits of *C. glomerata* reported in the literature—specifically, a filamentous thallus with dense or profuse branching and a slightly rough texture [14,15,39].

Distilled water was used as the solvent for the extraction process. Chemicals used for phytochemical screening included iron(III) chloride (FeCl₃, Merck), n-hexane (Merck), ethanol (Merck), hydrochloric acid (HCl, Merck), magnesium powder (Merck), Meyer's, Wagner's, and Dragendorff's reagents (Nitra Kimia), glacial acetic acid (Merck), sulfuric acid (H₂SO₄, Merck), and sodium hydroxide (NaOH, Merck).

2.2. Research Procedure

The study used a 3 × 3 factorial design with two factors: solid-to-solvent ratio (1:30, 1:40, and 1:50) and extraction power setting on a Samsung MS23K3515AS microwave (300, 450, and 600 watts). These factors produced nine treatment combinations, each performed in triplicate. The observed variables included the qualitative presence of six classes of phytochemical compounds: phenolics, tannins, flavonoids, alkaloids, saponins, and triterpenoids. Phytochemical screening results were reported qualitatively based on positive (+) or negative (-) outcomes.

2.3. Sample Preparation

Cladophora glomerata were cleaned of adhering impurities and foreign matter and washed under running water. They were then cut into segments approximately 1 cm in length and dried in a cabinet dryer at 60°C for 4 hours. The dried samples were ground and sieved to achieve a particle size of 80 mesh. The resulting *C. glomerata* powder was stored at 4°C in a tightly sealed container until extraction.

2.4. Moisture Content

Moisture content was determined using an oven method. An empty dish was dried in an oven at a stable temperature of 105°C for 2 hours, transferred to a desiccator for 30 minutes, and then weighed. Approximately 2 grams of *Cladophora glomerata* powder sample were placed into the dish. The dish containing the sample was placed in an oven at 105°C for 16–24 hours. Subsequently, the dish was transferred to a desiccator for 30 minutes and then weighed [21].

2.5. Extraction

Extraction of *Cladophora glomerata* powder was performed using the Microwave-Assisted Extraction (MAE) method. One gram of *C. glomerata* powder was placed in an Erlenmeyer flask, and distilled water was added as the solvent at solid-to-solvent ratios of 1:30, 1:40, and 1:50 g/mL. The mixture was then homogenized using a stirrer. Next, the mixture was irradiated with microwaves for 5 minutes at 300, 450, and 600 watts. The resulting extract was separated to obtain the filtrate used for the qualitative screening of secondary metabolite groups [22].

2.6. Qualitative Screening of Phytochemical

Phytochemical screening provided preliminary qualitative evidence of secondary metabolite groups in the *C. glomerata* extract. The secondary metabolite groups tested included alkaloids, flavonoids, phenolics, tannins, saponins, and triterpenoids. Test results were determined based on characteristic positive reactions, such as color changes, precipitate formation, or foam formation. This testing provided an initial indication of secondary metabolite groups. The phytochemical screening conducted in this study served as a preliminary qualitative method to indicate the presence of several classes of secondary metabolites in the *C. glomerata* extract.

2.6.1. Qualitative Phenolic Test

To 5 mL of the *Cladophora glomerata* extract filtrate, add 5 drops of 3% iron(III) chloride (FeCl₃) solution. A positive result indicating the presence of phenolic compounds was indicated by the solution changing color to red, green, purple, blue, or black [23].

2.6.2. Qualitative Tannin Test

To 5 mL of the *Cladophora glomerata* extract filtrate, add 5 drops of 1% iron(III) chloride (FeCl₃) solution. A positive result indicating the presence of tannins was indicated by the solution changing color to dark blue or greenish-black [23].

2.6.3. Qualitative Flavonoid Test

To the *Cladophora glomerata* extract filtrate, add 5 mL of ethanol, followed by 0.2 g of magnesium (Mg) powder and 3 drops of hydrochloric acid (HCl). A positive result indicating the presence of flavonoids was indicated by the solution changing color to red, yellow, or orange [24].

2.6.4. Qualitative Alkaloid Test

The extraction filtrate was divided into three test tubes for testing using Mayer's, Wagner's, and Dragendorff's reagents. In the Mayer's test, 2 drops of Mayer's reagent were added to the

Cladophora glomerata extract filtrate. A positive result indicating the presence of alkaloids was indicated by the formation of a white precipitate. In the Wagner's test, 2 drops of Wagner's reagent were added to the extract filtrate. A positive result was indicated by the formation of a brown precipitate. In the Dragendorff's test, 2 drops of Dragendorff's reagent were added to the extract filtrate. A positive result was indicated by the formation of an orange precipitate [23].

2.6.5. Qualitative Saponin Test

Shake a 10 mL sample of the *Cladophora glomerata* extract filtrate vertically for 10 seconds and allow it to stand for 10 minutes. A positive result indicating the presence of saponins was indicated by the formation of stable foam. Next, add one drop of 1% hydrochloric acid (HCl). If the foam remained stable after adding HCl, the test result was considered positive for the saponin group [23].

2.6.6. Qualitative Triterpenoid Test

Ten millilitres of the *C. glomerata* extract filtrate were treated with 10 drops of glacial acetic acid, followed by the addition of 10 drops of sulfuric acid (H₂SO₄). A positive result indicating the presence of triterpenoids was evidenced by the formation of a green color in the sample [23].

3. RESULT AND DISCUSSION

Bioactive compounds from *Cladophora glomerata* macroalgae were extracted from the powdered material using the MAE method with distilled water as the solvent for 5 minutes. This study used solid-to-solvent ratios of 1:30, 1:40, and 1:50, with extraction power levels of 300, 450, and 600 watts. The qualitative phytochemical screening results are presented in Table 1.

Table 1. The qualitative phytochemical screening results

Power (watts)	Solid to solvent ratio (g/mL)	Phenolics	Tannins	Flavonoids	Alkaloids	Saponins	Triterpenoids
300	1:30	+++	+++	-	-	+++	-
	1:40	+++	++	-	-	+++	-
	1:50	+++	++	-	-	+++	-
450	1:30	+++	+++	-	-	++	-
	1:40	+++	+++	-	-	++	-
	1:50	+++	+++	-	-	+++	-
600	1:30	++	+++	-	-	++	-
	1:40	+++	+++	-	-	++	-
	1:50	+++	+++	-	-	+++	-

Note: (-) : tested compound not identified

(+) : small amount of tested compound identified

(++) : slightly larger amount of tested compound identified

(+++): large amount of tested compound identified

3.1. Moisture Content

Based on the analysis, the moisture content of *Cladophora glomerata* powder was 9.73%. This value is higher than that reported by Sirbu, who obtained a moisture content of 5.71% for (*Cladophora vagabunda*) [25]. However, the moisture content obtained in this study falls within the maximum limit for *Cladophora glomerata* powder established by Yarnpakdee, who stated that the moisture content of dried *Cladophora sp.* raw material should be below 10% [26]. Moisture content varies with drying method, temperature, and duration. Longer drying times and higher drying temperatures increase water evaporation from the macroalgae, significantly reducing moisture content [27].

3.2. Phenolic

Qualitative screening results indicated a positive reaction for phenolic compounds in all *Cladophora glomerata* extracts across all combinations of microwave power and solid-to-solvent ratios. A positive result was evidenced by a color change to blue following the addition of 3% FeCl₃. Thus, the extracts obtained at 300, 450, and 600 watts with solid-to-solvent ratios of 1:30, 1:40, and 1:50 indicated the presence of phenolic compounds.

At the 600-watt power setting with a 1:30 solid-to-solvent ratio, the observed positive reaction showed the least distinct color change compared to the other treatments. This suggests that extraction conditions influence the intensity of the observed phenolic reaction. Increasing microwave power enhances heating, thereby facilitating the release of components from the material matrix. However, higher microwave energy levels may also affect the stability of certain heat-sensitive components [28, 29].

The solid-to-solvent ratio can also influence the transfer of components from the material matrix into the solvent. At a 1:30 ratio, the solvent volume is lower than at 1:40 and 1:50 ratios. Theoretically, this condition can limit contact between the material matrix and the solvent, thereby affecting the transfer of extracted components [20]. Therefore, the less pronounced color change observed with the 600-watt and 1:30 ratio combination is likely attributable to the heating conditions and the volume of solvent employed.

3.3. Tannin

Qualitative screening results indicated a positive reaction for the tannin group across all *C. glomerata* extract samples. A positive

result was evidenced by a color change to dark blue following the addition of 1% FeCl₃. We observed positive reactions for all combinations of microwave power (300, 450, and 600 watts) and solid-to-solvent ratios (1:30, 1:40, and 1:50).

At a power level of 300 watts with solid-to-solvent ratios of 1:40 and 1:50, the observed color reaction was relatively weaker compared to other treatment combinations. This variation in color change may be attributed to potential differences in the quantity or type of components extracted under varying treatment conditions. Solid-to-solvent ratio and microwave power are among the factors that can influence the extraction process using MAE [30].

Higher microwave power can generate more intense heating, thereby facilitating the release of components from the material matrix. However, the relationship between microwave power and tannin content is not necessarily linear. Some studies indicate that excessively high heating conditions can potentially affect the stability of certain components [31].

3.4. Flavonoid

Qualitative screening results indicated that flavonoids were not detected in any of the *C. glomerata* extracts. All treatment combinations, involving power levels of 300, 450, and 600 watts and solid-to-solvent ratios of 1:30, 1:40, and 1:50, yielded negative results based on the sample color change criteria for the flavonoid test.

The failure to detect flavonoids under the tested conditions may be related to the solvent type, extraction conditions, or the low abundance of flavonoids in the sample. Distilled water is a highly polar solvent, whereas solubility varies among different compound groups. Some studies report that organic solvents such as ethanol and methanol can be used effectively for flavonoid extraction [33]. Differences in solvent type may explain the failure to detect flavonoids in the extract obtained in this study.

MAE conditions can also influence the extracted components. Microwave energy enhances heating and facilitates the release of components from the matrix; however, varying extraction conditions can yield different extract results [32]. Discrepancies between these findings and previous studies may be attributed to differences in species, material matrix, solvent type, power, duration, and extraction conditions.

3.5. Alkaloid

Qualitative screening results indicated that alkaloids were not detected in any of the *C. glomerata* extracts. We obtained negative results across all combinations of microwave power (300, 450, and 600 watts) and solid-to-solvent ratios (1:30, 1:40, and 1:50) using the three tests employed: Mayer, Wagner, and Dragendorff.

Failure to detect alkaloids during qualitative screening may be attributed to the solvent used in this study. We used distilled water, a polar solvent. In contrast, other alkaloid extraction methods often utilize different solvent types and conditions, such as organic solvents or acidified solvent environments [34]. The absence of detectable alkaloids could also stem from low concentrations of these compounds in the sample, making them undetectable by qualitative testing.

3.6. Saponin

Qualitative screening results showed a positive reaction for saponins in all *C. glomerata* extracts. A positive result was indicated by the formation of stable foam after agitation, which remained stable even after adding 1% HCl, suggesting the presence of saponins across all combinations of microwave power and solid-to-solvent ratios tested.

In certain treatment combinations, specifically power levels of 450 and 600 watts with solid-to-solvent ratios of 1:30 and 1:40, the observed foam formation was relatively weaker compared to other treatments. This variation may be linked to differences in the quantity of components extracted under varying treatment conditions. The solid-to-solvent ratio influences the saponin extraction process [36].

A lower solvent volume can affect contact between the raw material and the solvent, as well as mass transfer of components during extraction [37]. Furthermore, higher microwave power levels can affect the stability of certain components [35].

3.7. Triterpenoid

Qualitative screening results indicated that triterpenoids were not detected in any of the *C. glomerata* extracts. Negative results were obtained across all combinations of microwave power (300, 450, and 600 watts) and solid-to-solvent ratios (1:30, 1:40, and 1:50), based on the color change criteria used in the triterpenoid test.

Failure to detect triterpenoids in the extracts may be attributed to the solvent used. Distilled water is a polar solvent, whereas certain triterpenoid groups are relatively nonpolar or semipolar, which may reduce their solubility in distilled water. Using solvents with different polarity characteristics can yield distinct metabolite profiles during extraction [38].

4. CONCLUSION

Based on the research findings, qualitative screening revealed phenolic compounds, tannins, and saponins in all *Cladophora glomerata* extracts obtained via MAE using distilled water for 5 minutes, across solid-to-solvent ratios of 1:30, 1:40, and 1:50 and power levels of 300, 450, and 600 watts. Conversely, we did not detect flavonoids, alkaloids, or triterpenoids under any tested extraction condition. These screening results provide preliminary qualitative evidence of these metabolite groups in *C. glomerata* extracts.

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