Screening of Active Compounds from Sargassum Seaweed Collected from Lampung Bay

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Abstract. Sargassum is a genus of brown algae with significant potential as a bioprospecting material. This seaweed is abundant in Lampung Bay, although it has not yet received the same attention as commercial seaweeds like Gracilaria sp. and Eucheuma sp. The purpose of this study is to inventory the types of active compounds in Sargassum extracts using solvents with different polarities. Extraction was carried out using the maceration method with methanol (polar) and n-hexane (non-polar) solvents in a 1:3 ratio. Methanol yielded a more optimal quantity and concentration of filtrate compared to n-hexane. Qualitative testing of active compounds was conducted through phytochemical assays, indicating the presence of flavonoids, alkaloids, polyphenols/tannins, and saponins in Sargassum. The target flavonoid compound was confirmed by quantitative analysis using UV-Vis spectrophotometry, which revealed a flavonoid content of 7.84 grams in the methanol-solvent sample and 3.61 grams in the n-hexane sample. To support its use in aquaculture activities, further testing for antibacterial activity is required..

Keywords: Antibiotic, Active compound, bioprospection, Sargassum

INTRODUCTION

Seaweed is a simple plant that lacks distinct differentiation between roots, stems, and leaves, all of which are collectively referred to as thallus Seaweed is categorized into three main groups: Chlorophyta (green algae), Phaeophyta (brown algae), and Rhodophyta (red algae) [1]. In Indonesian waters, common types of seaweed include Gracilaria, Gelidium, Eucheuma, Hypnea, Sargassum, and Turbinaria. Seaweed contains compounds with antiviral, antifungal, antibacterial, and antifouling properties [2]. One type of seaweed with notable antibacterial activity is Sargassum duplicatum [3]. Sargassum polycystum is rich in bioactive compounds such as alginate, fucoidan, fucoxanthin, and phlorotannin [4]Additionally, Sargassum has been reported to contain active compounds such as steroids, alkaloids, phenols, triterpenoids, flavonoids, saponins, and tannins, which exhibit antibacterial, antiviral, and antifungal properties ([3];[5]).

The abundant presence of Sargassum seaweed in the waters of Lampung Bay has not received as much attention compared to commercial seaweeds such as Gracilaria sp. and Eucheuma sp. [6]. Sargassum grows wild along the coast, and in several European countries, it has even been classified as an invasive species due to its rapid growth, allowing it to compete with native species and alter community composition and ecosystem dynamics [7].

Secondary metabolites are compounds produced by living organisms under specific conditions. One qualitative method to test for secondary metabolites in natural substances is phytochemical screening. Seaweeds in the Phaeophyta division produce algin or alginate, laminarin, cellulose, and mannitol. Commonly, Phaeophyta species utilized for alginate production include Macrocystis, Turbinaria, Padina, and Sargassum sp. [8]. The potential applications of seaweed continue to expand, reaching fields such as pharmaceuticals, cosmetics, and medicine.

Secondary metabolites are predominantly found in higher terrestrial plants. This differs in marine environments, where secondary metabolites are mainly found in immobile organisms [9]This phenomenon is due to the fact that immobile organisms remain in the same environment over generations, unless they are moved by natural forces such as currents, waves, or natural disasters. This condition drives these organisms to protect themselves by producing bioactive compounds within their secondary metabolites. Similarly, brown algae Sargassum employs this survival strategy. Sargassum collected from Lampung Bay is exposed to various hydro-oceanographic events specific to the characteristics of a semi-enclosed bay. This alga defends itself in part by producing bioactive compounds that serve as protection against environmental fluctuations.

Other bioactive compounds include flavonoids, tannins, and phenols, each with a distinct mechanism of action, yet all exhibiting biocidal properties. Bactericidal bioactive compounds are those capable of disrupting bacterial defenses and cellular structures, ultimately leading to cell damage and, subsequently, the death of the targeted bacteria [5].

Fish farming activities are constantly threatened by microbial diseases, which are illnesses caused by microorganisms, particularly bacteria. Vibrio spp. is a pathogenic bacterium responsible for vibriosis, a disease that affects fish and crustaceans [10]. To combat pathogenic Vibrio spp., antibiotics are commonly administered. However, excessive use of antibiotics in aquaculture can lead to bacterial resistance to commercial antibiotics and pose health risks to humans due to antibiotic accumulation in cultured organisms [4]. Therefore, the antibacterial compounds found in Sargassum can potentially serve as a natural antibiotic in fish farming practices. Consequently, fundamental research on the screening of active compounds in Sargassum from Lampung Bay requires further confirmation.

METHODS

This research was conducted from June to August 2024. Sample collection was carried out along the coast of Lampung Bay. The commonly used extraction method, maceration, was employed in this study. Drying, extraction, and antibacterial assays were conducted at the Fish Health Laboratory of the Department of Fisheries and Marine Science. Phytochemical tests, compound separation using a rotary evaporator, and quantitative analysis of compounds using spectrophotometry were performed at the Industrial Chemistry Laboratory, Lampung State Polytechnic.

The equipment used in this research includes a rotary evaporator (Janke & Kunkel RV 06ML), centrifuge (Yenaco YC-1180T), spectrophotometer (Genesys 10S UV-VIS), color reader (Accuprobe HH06), vortex mixer (Barnstead Thermolyne Maxi Mix II), blender, analytical balance (SHIMADZU), aluminum foil, tissue, filter paper, sample bottles, oven, desiccator, 40 mesh sieve, volumetric flask, measuring flask, test tubes (Iwaki), cuvettes, Erlenmeyer flasks, Whatman No. 1 paper, separatory funnel, volumetric pipette (Pyrex), micropipette (Transferpette), microtubes, spatula, glass and plastic funnels, stirring rods, knives, basins, glass plates, trays, and beakers (Pyrex).

The research process begins with the collection of Sargassum samples from the waters of Lampung Bay, specifically around the Polinela floating net cages. The extraction phase starts with weighing the seaweed in its wet state (before drying) and in its dried state. The seaweed is cleaned and dried for approximately 7 days using the air-drying method. Once dried, the seaweed is ground using a blender. Several factors in the extraction process affect the yield, including the type of solvent, the weight-to-solvent volume ratio, temperature, agitation, extraction time, and sample size [11].

The comparison between wet weight and dry weight is calculated as the percentage of moisture content reduction. The dried seaweed is blended into a powder and then soaked in n-hexane and 96% methanol for 7 days (7 x 24 hours) at a ratio of 1:3 (1 part Sargassum powder to 3 parts solvent). The mixture is then filtered to obtain a filtrate that is separated from the residue. The separation of active compounds is performed by evaporating the filtrate using a rotary evaporator. The moisture content is calculated as the percentage of the difference between wet weight and dry weight divided by the wet weight .

$$\text{Water content} = \frac{\text{dry weight}}{\text{wet weight}} \text{ x } 100\%$$

The yield calculation is determined by expressing the weight of the extract as a percentage of the dry weight of the seaweed:

$$\text{Yield} = \frac{\text{weight of extract}}{\text{dry weight}} \text{ x } 100\%$$

The variables observed in the Sargassum extract include moisture content, extract yield [12], color intensity in phytochemical tests, and total flavonoids from quantitative spectrophotometry tests. Quantitative tests are conducted on target compounds using spectrophotometry. The UV-Vis spectrophotometer operates on the principle of light absorption, where a portion of the light or radiation passed through a solution is absorbed, some is reflected, and some is transmitted [13].

RESULTS AND DISCUSSION

MOISTURE CONTENT AND YIELD OF CRUDE EXTRACT FROM SARGASSUM

The measurement of moisture content in dried Sargassum in this study was found to be 13.33%. This value was obtained from the percentage of the difference between the wet weight of 1200 g for each treatment and the final weight after drying. The reduction in moisture content during the 7-day drying process effectively decreased the moisture content by 90%. This moisture content falls within the typical range for dried seaweeds. The water content of fresh seaweed is similar to that of most plants, ranging from 80-90%, and after air drying, it reduces to 10-20%. The yield of the extraction depends on the solubility properties of its bioactive components. According to [14], the chemical composition of seaweed varies among individuals, species, habitats, harvest age, and environmental conditions.

From the extraction results using 96% methanol, it can be observed that the bioactive components of Sargassum tend to be polar. The yield of the dried material from Sargassum was 4.23% for the methanol solvent and 1.13% for the hexane solvent. These results were derived from the weight comparison of the extracted material, which was 6.77 g for the methanol solvent and 1.8 g for the hexane solvent, against a dry weight of 160 g.

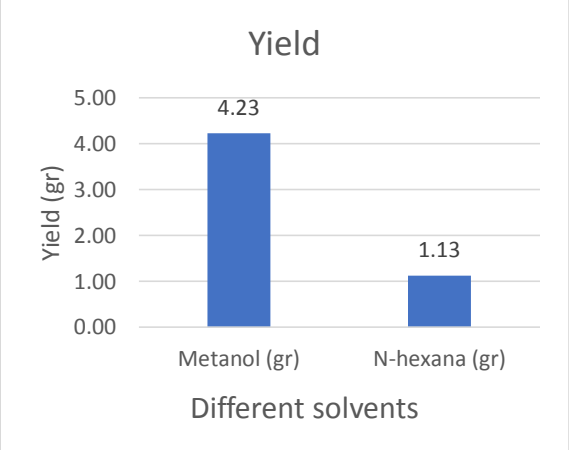

FIGURE 1. Yield

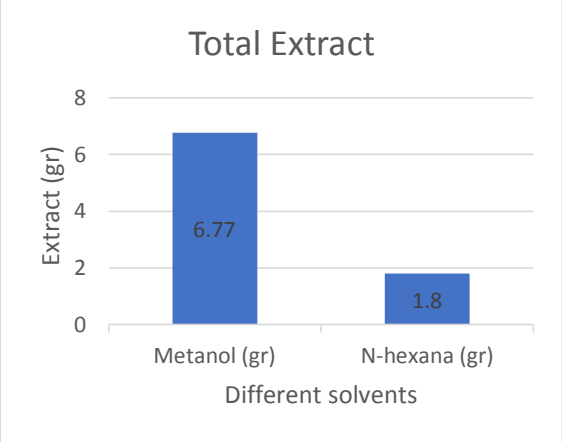

FIGURE 2. Total Extract

EXTRACT YIELD

The analysis of variance indicates that the type of solvent treatment has a highly significant effect ($p < 0.01$) on the average yield of Sargassum extract. The average yield of Sargassum extract from three repetitions is presented in the table below:

TABLE 1. Average of yield

Solvent	Duration og maceration	Average of yield
Methanol	7 x 24 jam	4,23 ± 0,70
Hexana	7 x 24 jam	1,13 ± 0,41

Based on **TABLE 1** above, it is evident that the methanol solvent yields the highest average yield of 4.23%,

which is significantly different from the hexane solvent treatment yield of 1.13%. The yield of the extract with methanol is in the form of a very thick paste, whereas the extract from hexane is in a liquid form. This difference is attributed to the polar nature of the compounds in the extract; polar compounds will dissolve in polar solvents.

The difference in solvent type affects the amount of extract produced. Methanol solvent yields a higher extraction rate compared to hexane solvent, which has lower polarity. This indicates that the compounds in the Sargassum extract have polarities closer to that of methanol, as the extraction of compounds is based on the similarity of polarity with the solvent. Polar compounds will dissolve in polar solvents [15]. According to [16], the solvent liquid during maceration penetrates the cell walls and enters the cell cavities containing active substances. The active compounds will dissolve due to the concentration gradient between the active substance solution inside and outside the cell, causing the concentrated solution inside the cell to be displaced outward. This process continues until a concentration equilibrium is achieved between the solutions inside and outside the cell.

Seaweed has a high-water content. Methanol is capable of dissolving polar compounds and can extract water molecules that are bound by hydrogen, resulting in the formation of hydrogen bonds. During extraction, the hydrogen bonds that hold the active components within the Sargassum tissue, which also has a high-water content, are extracted and dissolved in the methanol solvent [17]. In contrast, hexane is a relatively unreactive solvent that primarily reacts with acids, bases, and reducing agents.

PHYTOCHEMICAL TESTING

Phytochemical testing was conducted to identify the active compounds present in the plant. The results of the phytochemical analysis of the Sargassum extract indicate the presence of bioactive compounds, namely alkaloids, flavonoids, tannins/polyphenols, and saponins, as presented in the table below:

TABLE 2. Phytochemical testing

Chemical compound	Reactiom	Metanol solvent	N-hexana solvent
Alkaloid	Meyer	+	-
	Dragendorf	+	+
Flavonoid	Metode Bate Smith & mertcalf	+	+
Tanin	(+) FeCl	+	+
Saponin	Forth test	+	+

Quantitative phytochemical testing was conducted as a screening for the general active compound content in seaweed. The phytochemical analysis included four active compounds: alkaloids, flavonoids, tannins, and saponins. The extract with methanol showed positive results for all four compounds. The extract with n-hexane also demonstrated positive results, except for the alkaloid test using Mayer's method. In the alkaloid test, a positive result is indicated by the formation of a red precipitate when using Mayer's reagent (HgCl2 + KI). Alkaloids contain nitrogen atoms and are basic, requiring the addition of sulfuric acid for extraction. The nitrogen atom, which has a lone pair of electrons in the alkaloid, replaces the iodide ion in Mayer's reagent, resulting in the formation of a red precipitate upon the addition of Mayer's reagent. This occurs because the nitrogen in the alkaloid reacts with the K+ metal ions from Mayer's reagent [18] [19].

In the flavonoid test results, the sample showed a positive outcome, indicated by a color change to reddish-black. Flavonoids are the target of this study based on the assumption of the antibacterial activity potential of the Sargassum sample. Flavonoids are polar compounds due to the presence of several hydroxyl groups. Therefore, flavonoids generally dissolve in polar solvents such as ethanol and methanol. These types of solvents function to release flavonoids from their salt forms. The addition of concentrated hydrochloric acid serves to protonate the flavonoids, resulting in the formation of flavonoid salts. After the addition of magnesium powder, a positive result is indicated by a color change of the solution to reddish-black. The reddish-black color produced signifies the presence of flavonoids as a result of reduction by concentrated hydrochloric acid and magnesium ([20]

Flavonoids are synthesized by plants as a defense system and in response to infections by microorganisms, making them effective as antimicrobial compounds against various microorganisms. Flavonoids are one of the polyphenolic compounds that exhibit a range of effects, including antioxidant, antitumor, anti-inflammatory, antibacterial, and antiviral properties.

QUANTITATIVE ANALYSIS OF FLAVONOID COMPOUNDS

In this study, the total flavonoid content as the target compound was determined using the colorimetric/UV-Vis spectrophotometry method. To determine the total flavonoid content in the sample, $AlCl_3$ was added to the sample solution. The addition of $AlCl_3$ serves to form a stable acid complex with the C-4 carbonyl group and the C-3 or C-5 hydroxyl groups of flavones and flavonols. Additionally, $AlCl_3$ forms a labile acid complex with the ortho-dihydroxyl groups on the A or B ring of flavonoids, resulting in maximum absorption at a wavelength of 430 nm. Potassium acetate is also added to stabilize the wavelength in the visible range. Prior to measurement, an incubation period of 30 minutes is performed to allow the reaction to reach completion, thereby maximizing color intensity [21].

In determining the total flavonoid content of *Sargassum* samples with two types of solvents, quercetin was used as a standard solution for comparison, as it is a type of flavonoid in the flavanol group found in many plant species [22]. Quercetin is also among the most effective compounds in capturing free radicals and inhibiting various oxidation reactions, as it can produce phenolic radicals stabilized by the resonance effect of its aromatic ring [23]; [24].

A concentration series of quercetin was prepared at 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm. The purpose of using a concentration series is to determine flavonoid content using the standard curve equation method to obtain a linear equation for calculating flavonoid concentration. Next, the maximum wavelength of quercetin was determined. The absorbance measurements of the quercetin standard solution are presented in the following table

TABLE 3. Standar Quecetin Flavanoid

Consentration (mg/L)	Absorbansi
10	0,0922
20	0,1215
30	0,1759
40	0,2125
50	0,2453

The determination of the maximum wavelength of quercetin was conducted by running it across the 400–800 nm wavelength range. The purpose of determining the maximum wavelength is to identify the absorption region, represented by the absorbance value of the standard solution measured using a UV-Vis spectrophotometer in the 400–800 nm wavelength range [22]. The results of the scan showed that the maximum wavelength of the quercetin solution was at 430 nm. The color of the quercetin standard solution was yellow, and the intensity of the yellow color increased with higher concentrations. The range of total flavonoid content based on absorbance values is approximately 0.2–0.8 [25]. The absorbance measurements of the *Sargassum* extract are shown in Table 4.

TABLE 4. Absorbance of Sargassum extract with different solvents

Sample	Replication	Absorbance	Flavonoid (mg/ml)	Total ofFlavanoid (mgQE/g eks)	Average flavonoid total (Mg QE/g eks)
Extract sargassum-Metanol	1	0.3630	0.078	0.803	7,84
	2	0.3640	0.078	7.828	
	3	0.3660	0.079	7.878	
Extract sargassum-Hexana	1	0.7710	0.180	3.601	3.61
	2	0.7730	0.181	3.611	
	3	0.7740	0.181	3.616	

The absorbance measurements were then plotted to obtain the linear regression equation. The calculation is

based on the Lambert-Beer law, which demonstrates a direct relationship between absorbance and analyte concentration. For the total flavonoid absorbance measurement in determining the quercetin calibration curve at a wavelength of 431 nm, the regression equation obtained was y = 0.004x + 0.0509. The standard solution of the flavonoid compound showed a linear relationship between absorbance and concentration, with a correlation coefficient of r = 0.9919. The (r) value close to one indicates that the regression equation is linear [21]. The standard quercetin absorbance graph is shown in Figure 4.

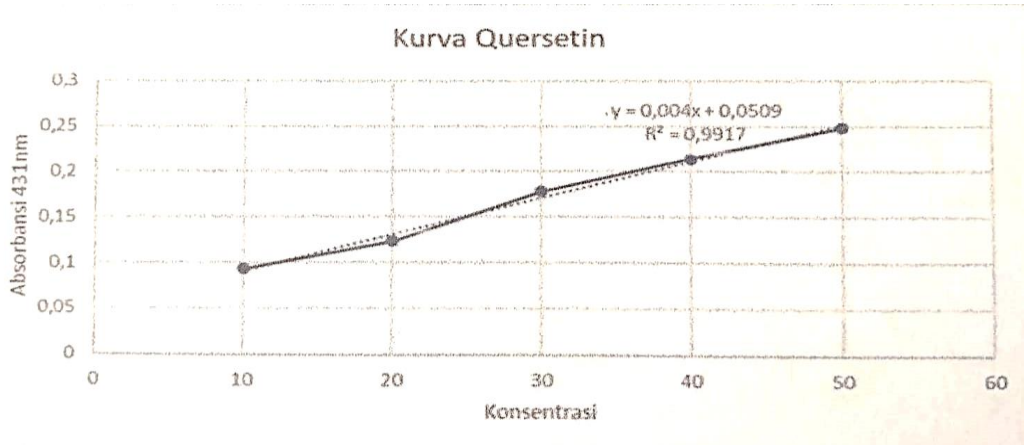

FIGURE 3. Quersetin curva

CONCLUSIONS

The conclusion of this study is that the active compounds screened from *Sargassum* collected from Teluk Lampung are alkaloids, flavonoids, saponins, and tannins. Methanol was found to be more effective than n-hexane as an extraction solvent for active compounds in terms of water content, yield, and qualitative test results. Quantitative analysis of the target flavonoid compound showed a total flavonoid content of 7.84 g in the methanol extract, compared to 3.61 g in the n-hexane extract.

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