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ANALYSIS OF PHYTHOLOGY COMPONENTS AND POTENTIALS OF ANTIOXIDANT ACTIVITIES OF Thalassia hemprichii EXTRACT

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Abstract— Seagrass is rich of marine resources and capable of producing secondary metabolites so that it can be used as functional food. Secondary metabolites that are generally produced by organisms play a role in self-defense from the environment or from the attack of other organisms including attached organisms/ epiphytes and prevent infection from pathogens and can be used as antioxidants. This study aims to analyze the content of bioactive compounds and the potential of antioxidant activity of Thalassia hemprichii extract. The examination of bioactive compounds is using phytochemical tests and examination of antioxidant activity is using the diphenylpicrylhydrazil (DPPH) method. Phytochemical test results showed that Thalassia hemprichii extract contained bioactive compounds in the form of flavonoids, saponins, tannins and polyphenols. The results of the antioxidant activity test of Thalassia hemprichii extract obtained IC50 values of 80.0331 indicating that Thalassia hemprichii extract has strong antioxidant activity. This research proves that Thalassia hemprichii contains bioactive compounds that have the potential to be antibacterial and antioxidant.

Keywords— *Thalassia hemprichii*, bioactive compounds, antioxidants

1. Introduction

Many candidates of herbal medicine have been explored in Indonesia with effects through various pathomechanisms in both several human diseases and animals experimental [1-5].
One of the water plants that has important benefits that can replace the source of natural antibacterial and antioxidants is seagrass. Thalassia hemprichii seagrass has an abundant amount of antibacterial and antioxidant source and is often dominant in mixed seagrass beds. Thalassia hemprichii is known to contain potential bioactive compounds as antibacterial, antifungal, antiprotozoa, antiviral, antifertility, and medicinal

substances that affect the cardiovascular system. One of the marine resources that is still not widely used in Indonesia is seagrass resource. Thalassia hemprichii also has bioactive potential as an antioxidant and contains phenolic compounds and has the potential ability as an antibacterial [6]. Seagrass is able to produce secondary metabolites so that it can be used as functional food. Secondary metabolites that are generally produced by organisms play a role in self-defense from the environment as well as from other organisms including organisms that attach to others / epiphytes and prevent infection from pathogens and can be used as antioxidants [7].

The human body can be exposed to microorganisms, causing infectious diseases from the mild level to sepsis and death. Similarly, excessive free radical reactions in the body are the cause of degenerative diseases such as heart disease, stroke, and cancer. The body needs an important component to ward off disease and the influence of free radicals. Antioxidants are able to save human body cells from the danger of free radicals. The antioxidants will react with free radicals and change free radicals that are unstable to be more stable. Examples of free radical neutralizing reactions by antioxidants are Diphenylpicrylhydrazyl compounds (free radicals) in action with antioxidants that donate one electron to form a more stable Diphenylpicrylhydrazine (non-radical) compound. Antioxidants can be obtained from natural and synthetic sources. Sources of natural antioxidants obtained from the sea include seaweed, sponges, and microalgae [8-9].
Considering the potential and high content of bioactive compounds in Thalassia hemprichii extracts, it is very necessary to study to develop Thalassia hemprichii extracts into standardized herbs that have antibacterial and antioxidant effects.

2. Materials and Method

2.1 Materials and Tools

The material used was Thalassia hemprichii seagrass from Wakatobi waters. The solvent used in the maceration process was 95% ethanol (technical, Brataco). In phytochemical screening, the following ingredients are needed, namely hydrochloric acid p.a. (Merck), sulfuric acid p.a. (Merck), acetone P p.a. (Merck), boric acid P, oxalic acid P, ether P, anhydrous acetic acid p.a. (Merck), chloroform (Brataco), Dragendroff reagents, Mayer reagents, 10% iron (III) chloride solution. The tools used are drip pipettes, stirring rods, measuring pipettes, horn spoons, porcelain cups, measuring cups, erlenmeyers, beaker cups, test tubes, electric scales (ADAM AFP-360L), ovens (BINDER).

2.2 Research Procedures

Making *Thalassia hemprichii* Ethanol Extract: *Thalassia hemprichii* Seagrass was first washed and dried, then weighed as much as 100 grams, followed by the maceration process for 24 hours with 100 mL 95% ethanol. The extract obtained was then evaporated in an oven at a temperature of 45-50 ° C to have a volume of approximately 10 mL. The extract was then cooled at room temperature, extraction is a process of separating the desired substance from a plant material [10].

Phytochemical screening of *Thalassia hemprichii* extract: Phytochemical tests on *Thalassia hemprichii* extract included examination of alkaloids, flavonoids, triterpenoids, saponins, tannins and polyphenols.

2.3 Phytochemical Test Procedures [11-12]

Preparation of phytochemical test solution: Preparation of test solution for phytochemical screening was done by dissolving 500 mg of Thalassia hemprichii ethanol extract in 50 mL of 95% ethanol.
Alkaloid examination: A total of 2 mL of the test extract solution was evaporated on a porcelain cup

until a residue was obtained. The residue was then dissolved with 5 mL of 2N HCL. The solution obtained was then divided into 3 test tubes. The first tube was added with dilute acid which functions as a blank. The second tube was added Dragendroff reagent as much as 3 drops and the third tube was added Mayer reagent as much as 3 drops. The formation of orange deposits in the second tube and yellow deposits in the third tube indicated the presence of alkaloids.

Flavonoid examination: As much as 1 mL of the test extract solution was taken and made the rest wet with acetone P. Add a little bit of boric P acid powder and a fine powder of oxalic P acid, heat cautiously over the water bath and avoid overheating. Mix the residue obtained with 10 mL ether P. Observe with 366 nm UV light. Intensive yellow fluorescent solution indicated the presence of flavonoids.

Triterpenoid examination: Triterpenoid examination was carried out with the Lieberman-Burchard reaction. A total of 2 mL of the test solution was evaporated in a vaporizer cup. The residue was dissolved with 0.5 mL chloroform, add 0.5 mL anhydrous acetic acid. Next, 2 mL of concentrated sulfuric acid was added through the tube wall. The formation of a brownish or violet ring at the boundary of the solution indicated the presence of a triterpenoid.

Saponin examination: A total of 10 mL of the test extract solution in a test tube was shaken vertically for 10 seconds then let it for 10 seconds. Formation of 1-10 cm height foam that was stable for not less than 10 minutes, indicated the presence of saponin. On the addition of 1 drop of HCL 2N, the foam does not disappear.

Examination of tannins and polyphenols: A total of 3 mL of the test extract solution was divided into 3 parts namely tube A, tube B, tube C. Tube A was used as a blank, tube B was reacted with 10% iron (III) chloride solution. Dark blue or greenish black showed the presence of tannins and polyphenols, whereas in tube C only gelatin salt was added. If precipitate forms in tube C, the extract solution contains tannins.

2.4 Antioxidant Test Procedure (IC50) DPPH Method [13]

Preparation of 500 ppm of Master Solution: Samples weighed as much as 0.005 g, then dissolved in 10 mL of methanol to obtain a sample of 500 ppm.

Antioxidant Testing (IC50) : 500 ppm samples were piped 0.1, 0.2; 0.4; 0.8; and 1.6 mL into different test tubes for varying concentrations of 10, 20, 40, 80, and 160 ppm, then 1 mL DPPH 0.4 mM was added, then add methanol to reach the volume of the 5 mL solution. Then it was homogenized, allowed to stand in a dark place for 30 minutes. Then, the absorbance was measured with a spectrophotometer at the maximum wavelength of (515 nm).

3. RESULT AND DISCUSSION

3.1 Phytochemical Test

The results of phytochemical analysis on Thalassia hemprichii extract showed the presence of bioactive compounds containing flavonoids, saponins and tannins and polyphenols (Table 1).

No	Phytochemical Test	Result	Conclusion
1.	Alkaloid	with Dragendroff reagents, there was no orange deposits formed	(-)
		With Mayer reagents, there was no yellow deposits formed	(-)
2.	Flavonoid	Intensive yellow fluorescence	(+)
3.	Triterpenoid	Brownish ring was not formed	(-)
4.	Saponin	Foam formed as high as 1.5 cm for 30 seconds	(+)
5.	Tannin and Polyphenol	Greenish black	(+)

Information: (+) = contains the referred compound; (-) = does not contain the referred compound

Table 1. Phytochemical screening results of *Thalassia hemprichii* extract

Phytochemical test results in Table 1 show that Thalassia hemprichii extract contains bioactive compounds including flavonoids, saponins, tannins and polyphenols. Flavonoids are proactive polyphenol compounds in most plants and cannot be synthesized or produced by humans [14]. Flavonoids can inhibit DNA gyrase, causing nucleic acid synthesis to be disrupted, play an active role as an antifouling and as an isolate against attachment of organisms [15]. Saponins can inhibit DNA polymerase so that nucleic acid synthesis is disrupted [16]. Tannins can inhibit the formation of bacterial cell wall polypeptides that cause cell wall lysis and bacterial cells die. The mechanism of antibacterial action of tannins is by repressing proteins through reaction with cell membranes, activating microbial cell adhesin, disrupting protein transport in the inner layer of cells, targeting the wall polypeptides cells so that the formation of cell walls becomes less perfect which causes bacterial cells to become lysis due to osmotic and physical pressure and the bacterial cells will die [17]. Secondary metabolites have various biological activities that can be utilized by humans. Various biological activities of secondary metabolites include anticancer, antibacterial, antioxidant and antifungal [18].

3.2 Potential Antioxidant Activity Test

Antioxidants are molecules that are able to slow down or prevent the oxidation process of other molecules. Oxidation is a chemical reaction that can produce free radicals that trigger chain reactions that can damage cells [19]. Testing the antioxidant activity of Thalassia hemprichii extract was carried out using the diphenylpicrylhydrazil (DPPH) free radical scavenging assay method. The method using DPPH is the most commonly used method for testing antioxidants [20]. Testing the antioxidant activity using the DPPH method was interpreted into the IC50 parameter or 50 inhibitory concentration. The results of the antioxidant testing of Thalassia hemprichii extract are shown in Table 2.

No	Concentration (µg/mL)	Absorbance (A) λ = 515 nm	Antioxidant Activity (%)	IC-50 Value (µg/mL)
1	10	0.355	16.08	80.0331
2	20	0.335	20.80	
3	40	0.299	29.31	
4	80	0.201	52.48	
5	160	0.085	87.75	

Table 2. Test results of the antioxidant activity potential of *Thalassia hemprichii* extract

Antioxidant activity was tested using the DPPH method which was interpreted into the IC50 parameter or 50 inhibitory concentration. The test results as in Table 2 above show that the absorbance percentage value was lower along with the increasing amount of extract concentration; ie. at a concentration of 10 µg / mL, it has an absorbance value of 0.355; a concentration of 20 µg / mL with an absorbance value of 0.335; a concentration of 40 µg / mL with an absorbance value of 0.299; a concentration of 80 µg / mL with an absorbance value of 0.201; a concentration of 160 µg / mL with an absorbance value 0.085. Unlike the case with antioxidant activity that the higher the concentration of the extract, the greater the presentation of antioxidant activity, ie. the concentration of 10 µg / mL has an antioxidant activity value of 16.08%, a concentration of 20 µg / mL with an antioxidant activity value of 20.80%, a concentration of 40 µg / mL with an activity value antioxidant 29.31%, concentration 80 µg / mL with antioxidant activity value 52.48%, concentration 160 µg / mL with antioxidant activity value 87.75%. The test results, as Amrun and Umiyah (2005) proposed that a decrease in absorbance indicates an increase in the ability to reduce DPPH free radicals [21]. Furthermore, Hanani (2005) revealed that the antioxidant activity of an extract was expressed in terms of the reduction of DPPH free radicals [22]. So, it can be interpreted that the greater the concentration of the extract resulted in greater antioxidant activity or a smaller percentage of absorbance. The extract of Thalassia hemprichii, Cymodocea rotundata, Enhalus acoroides Seagrass from Indonesia contains more phenolic compounds, thus showing a high DPPH free radical reduction activity [23].

The results of the antioxidant activity test of Thalassia hemprichii extract using DPPH method obtained IC50 values of 80.0331 which are classified as strong antioxidants. Molyneux (2004) classifies antioxidant activity based on IC50 values, which are very strong (IC50 <50 ppm), strong (50 ppm - 100 ppm), moderate (100 ppm -150 ppm), weak (150 ppm - 200 ppm), and very weak (IC50> 200 ppm). Furthermore, Molyneux argued that the smaller the IC50 value, the higher the antioxidant activity [26]. Figure 1 shows the decrease in free radicals following the increase in concentration of the sample extract. Thalassia hemprichii also has a unique secondary metabolite that can be used as an antioxidant and has an anticancer role. Invitro analysis using HeLa cells shows that semipolar and polar extracts show the potential as anti-cancer. The level of cell lethality with semipolar extract is higher than extracts polar, but not different from doxorubicin cancer drugs [24]. Previous study revealed that if there is more tannin content, the antioxidant activity is also greater because tannins are composed of polyphenol compounds which have free radical scavenger activity [25].
Other studies illustrate the potential of Thalassia hemprichii extract in reducing serum glucose levels. Experiments on animals given ethanol extract Thalassia hemprichii showed a decrease in glucose serum ($p < 0.01$). Post-treatment using Thalassia hemprichii also lowered cholesterol, triglycerides and LDL in animals. There was an increase in HDL in the post-treatment along with a decrease in creatinine, urea levels and body weight [26].

4. Conclusion

1. Based on the phytochemical tests, Thalassia hemprichii extract contains bioactive compounds in the form of flavonoids, saponins, tannins and polyphenols which have the potential to be antibacterial.
2. Based on antioxidant activity test on Thalassia hemprichii extract, the IC50 value obtained was of 80.0331 which indicates that Thalassia hemprichii extract has strong antioxidant activity.

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6. Authors' contributions

JJ, MH, BB and RN initiated and designed the study. JJ, MH, RN, BB, RS, RA, BB, YS, ARJ, RD, MRP and MS drafted the manuscript. JJ, MH, RN, BB, RD, DRH, MRP. BB, YS and ARJ supervised the field activities and the microbiology work. JJ, MH, DRH, OJ, EWF, RD, ARJ, MRP and MS helped to collect isolates. All authors have read and approved the final manuscript.

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