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ANTIOXIDANT POTENTIAL OF BIOACTIVE COMPOUNDS IN ETHANOL EXTRACTS OF SEAGRASS AND MACROALGAE FROM LAMPUNG WATERS

Potensi Aktioksidan dari Senyawa Bioaktif dalam Ekstrak Etanol Lamun dan Makroalga dari Perairan Lampung

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ABSTRACT

The sea in Indonesia covers 75% of its total land area. According to data from the Ministry of Marine Affairs and Fisheries (2019), Indonesia has approximately 5.8 million km² of water area out of a total territory of 7.81 million km². The biodiversity in Indonesia's coastal areas is utilized by coastal communities in their daily lives. Coastal resources also have potential in the health sector, such as seagrass, macroalgae, and taurine, which can be used as raw materials for natural medicine. The purpose of this study is to analyze the antioxidant potential based on the IC50 value of taurine, ethanol extracts of the seagrasses Enhalus acoroides and Cymodocea rotundata, as well as the macroalgae Padina australis and Sargassum duplicatum. The method used is the antioxidant activity assay using DPPH (2,2-Diphenyl-1-picrylhydrazyl). The results of this study indicate that, after testing with DPPH, only the ethanol extract of Cymodocea rotundata had an IC50 value of < 200 µg/mL, specifically -652.95 µg/mL, whereas the IC50 values of taurine, the ethanol extract of Enhalus acoroides, and the ethanol extracts of the macroalgae Padina australis and Sargassum duplicatum were > 200 µg/mL, indicating no antioxidant activity. In conclusion, only the ethanol extract of Cymodocea rotundata has the potential to be a candidate for antioxidant raw material.

Keywords: Bioactive, DPPH (2,2-Diphenyl-1-picrylhydrazyl), Macroalgae, Seagrass, Taurine

ABSTRAK

Laut di Indonesia memiliki luas 75% dari total keseluruhan daratannya. Berdasarkan data Kementerian Kelautan dan Perikanan (2019), Indonesia memiliki luas perairan sekitar 5,8 juta km² dari 7,81 juta km² total luas wilayahnya. Keanekaragaman hayati di kawasan pesisir Indonesia dimanfaatkan oleh masyarakat pesisir dalam kehidupan sehari-hari. Sumber daya pesisir juga memiliki potensi di bidang kesehatan, seperti lamun, makroalga, dan taurin yang dapat dimanfaatkan sebagai bahan baku obat alami. Tujuan dari penelitian ini yaitu untuk

menganalisis potensi antioksidan berdasarkan nilai IC_{50} dari taurin, ekstrak etanol lamun *Enhalus acoroides* dan *Cymodocea rotundata*, serta ekstrak makroalga *Padina australis* dan *Sargassum duplicatum*. Metode yang digunakan adalah metode aktivitas antioksidan dengan DPPH *(2,2-Diphenyl-1-picrylhydrazyl)*. Hasil dari penelitian ini menunjukkan bahwa setelah dilakukan pengujian dengan DPPH *(2,2-Diphenyl-1-picrylhydrazyl)*, hanya ekstrak etanol lamun *Cymodocea rotundata* yang memiliki nilai IC_{50} < 200 µg/mL yaitu -652,95 µg/mL, sedangkan nilai IC_{50} dari taurin, ekstrak etanol lamun *Enhalus acoroides*, serta ekstrak etanol makroalga *Padina australis* dan *Sargassum duplicatum* > 200 µg/mL yang berarti tidak memiliki aktivitas antioksidan. Kesimpulan dari penelitian ini adalah hanya ekstrak etanol lamun *Cymodocea rotundata* yang berpotensi menjadi kandidat bahan baku antioksidan.

Kata Kunci: Bioaktif, DPPH *(2,2-Diphenyl-1-picrylhydrazyl)*, Lamun, Makroalga, Taurin

INTRODUCTION

The Indonesian coast has various promising natural resource potentials, not only in the fields of fisheries and tourism, but also in the field of health. Seagrass and macroalgae are examples of coastal biodiversity in Indonesia which are known to have bioactive content that can be utilized in the field of health. Based on research by Safia & Musrif (2020), macroalgae contain various bioactive compounds such as alkaloids, flavonoids, phenol hydroquinone, and tannins. Meanwhile, seagrass contains bioactive compounds in the form of flavonoids, alkaloids, steroids, and saponins (Nurafni & Rinto, 2018). Coastal communities have utilized the bioactive content of seagrass and macroalgae for various needs, such as a source of food, traditional medicine, and raw materials in the pharmaceutical and cosmetic industries (Silaban, 2024; Katili, 2021). In addition, taurine is also a compound that is known to have potential as a raw material for natural medicines, one of which is as an anticancer (Bareta et al., 2023).

Seagrass and macroalgae are known to have potential as anticancer and antioxidants. Several previous studies have shown that seagrass and macroalgae extracts can inhibit the growth of cancer cells. According to research by Widiastuti et al. (2021) compounds such as saponins, flavonoids, and tannins found in seagrass *Enhalus acoroides* are cytotoxic and play an important role in inhibiting the proliferation of HeLa cervical cancer cells. Testing using the BSLT method on seagrass extracts *Enhalus acoroides* and *Cymodocea rotundata* showed that both seagrass extracts were toxic with LC_{50} values of 133.73 µg/mL and 126.77 µg/mL respectively (Bareta et al., 2024). Ethanol extracts of macroalgae *Sargassum duplicatum* and *Padina australis* have been shown to have cytotoxic properties against HeLa cervical cancer cells, with IC_{50} values of 1,108.7 µg/mL and 681.1 µg/mL, respectively, making them potential sources of raw materials for anticancer drugs (Saputra et al., 2024). In addition to their potential as anticancer agents, seagrass and macroalgae also have the potential as antioxidants. In addition, macroalgae Sargassum sp. extracted with ethyl acetate has the potential as an antioxidant raw material with an IC_{50} value of 204.12 µg/mL (Sedjati et al., 2024). Macroalgae *Padina* sp. extracted with different solvents showed antioxidant activity. The *Padina* sp. extract using ethyl acetate showed moderate antioxidant activity with an IC_{50} value of 137.02 µg/mL. Meanwhile, the extracts obtained using n-hexane and methanol solvents are included in the very weak antioxidant category, with IC_{50} values of 1234.41 µg/mL and 1554.45 µg/mL, respectively (Hidayati et al., 2017).

However, the utilization of seagrass and macroalgae in the waters of South Lampung and Pesawaran, Lampung, is still relatively minimal. In fact, these two areas have coastal ecosystems rich in biodiversity, including various types of seagrass and macroalgae that contain bioactive compounds and have the potential as raw materials for natural medicines. This lack of utilization could be due to limited information regarding the bioactive content and its health potential. Based on this, this study aims to determine the antioxidant potential of

taurine, seagrass, and macroalgae originating from the waters of South Lampung and Pesawaran, Lampung.

METHODS

Time and Place

This research was conducted in May 2022. The process of making ethanol extract, phytochemical tests, and testing the antioxidant activity of DPPH (2,2-Diphenyl-1-picrylhydrazyl) on taurine and ethanol extracts of seagrass and macroalgae were carried out in the Biomolecular Laboratory and Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung.

Research Design

This research used a factorial completely randomized design. The factors in this study consisted of 5 (five) solutions, namely taurine, seagrass extracts *Enhalus acoroides* and *Cymodocea rotundata*, and macroalgae *Padina australis* and *Sargassum duplicatum*. The taurine used in this study was commercial taurine which is known to have potential as an anticancer candidate according to the research of Maysa et al. (2016). The seagrass *Enhalus acoroides* and the macroalgae *Sargassum duplicatum* used in this study were chosen because they are the most abundant seagrass and macroalgae species found in the waters of Dollar Beach Padada, Ketapang District, South Lampung. The other two species, namely the seagrass *Cymodocea rotundata* and the macroalgae *Padina australis*, were chosen because they are the most abundant seagrass and macroalgae species found in the waters of Tegal Mas Beach, Teluk Pandan District, Pesawaran. Seagrass and macroalgae were collected from both waters because this area is a tourist destination famous for its abundance of natural resources.

The five factors in this study were tested for antioxidant activity using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method with 5 different concentrations, namely 250 µg/mL, 500 µg/mL, 750 µg/mL, 1000 µg/mL, and 1250 µg/mL with 3 repetitions. Determination of the concentration of the test solution was based on research by Mulyani et al. (2018) and Febrianti et al. (2020). Negative and positive controls were used as a comparison and to complement the antioxidant activity test using the DPPH method. The positive control used was ascorbic acid solution with 4 different concentrations, namely 2 µg/mL, 4 µg/mL, 6 µg/mL, and 8 µg/mL (Herawati & Saptarini, 2020).

Research Procedure

1. Preparation and Making of Extracts

Preparation and making of extracts were carried out according to the research of Bareta et al. (2023). Seagrass *Enhalus acoroides* and *Cymodocea rotundata*, as well as macroalgae *Padina australis* and *Sargassum duplicatum* were cleaned with running water, then dried in an oven until dry at a temperature of 50°C. After that, the seagrass and macroalgae were ground into powder.

The seagrass and macroalgae powders were then extracted using the maceration method using 80% ethanol solvent with a ratio of 1:10 for 3 days. The filtrate was concentrated using a rotary evaporator at a temperature of 40ºC. After that, the seagrass and macroalgae extracts were stored in an oven until they became increasingly concentrated into a paste at a temperature of 40ºC.

2. Antioxidant Activity Test with DPPH (2,2-Diphenyl-1-picrylhydrazyl) Method

The antioxidant activity test with DPPH (2,2-Diphenyl-1-picrylhydrazyl) was used because the DPPH method is a commonly used antioxidant activity test. Before the test was carried out, a stock solution was made with a concentration of 2000 µg/mL by dissolving taurine, seagrass extracts *Enhalus acoroides* and *Cymodocea rotundata*, and macroalgae

extracts *Padina australis* and *Sargassum duplicatum* in 70% ethanol. The stock solution was then made into 5 different test concentrations, namely 250 µg/mL, 500 µg/mL, 750 µg/mL, 1000 µg/mL, 1250 µg/mL and made 3 times.

Measurement of antioxidant activity was carried out by homogenizing 2 mL of 40 µg/mL DPPH solution and 2 mL of taurine solution, seagrass extracts *Enhalus acoroides* and *Cymodocea rotundata*, and macroalgae extracts *Padina australis* and *Sargassum duplicatum* each concentration in a test tube wrapped in aluminum foil. Positive control solutions of ascorbic acid were made with concentrations of 2 µg/mL, 4 µg/mL, 6 µg/mL, and 8 µg/mL. The test solution test tube was coated with aluminum foil to prevent light from entering and inhibiting the reaction that occurred, and incubated for 30 minutes at room temperature. The test solution, positive control, and negative control were tested by spectrophotometry with a UV-Vis spectrophotometer with a wavelength of 517 nm according to the research of Souhoka et al. (2019).

3. Data Analysis

After the absorbance value from the spectrophotometry results was obtained, the percentage of antioxidant activity was calculated using the following formula (Susanto et al., 2018):

$$\%\ \text{antioxidant activity} = \frac{\text{Control absorbance} - \text{Treatment absorbance}}{\text{Control absorbance}} \times 100\%$$

The regression equation is used to determine the IC_{50} value of the test solution and positive control of ascorbic acid with the formula y = ax + b. Because IC_{50} is a concentration that can inhibit 50% of DPPH free radicals, the y value is 50, while the x value represents the IC_{50} value (Hasanah et al., 2021). According to Molyneux (2004), a compound is said to have very strong antioxidant properties if it has an IC_{50} value <50 µg / mL, is strong if it has an IC_{50} value of 50-100 µg / mL, is moderate if it has an IC_{50} value of 100-150 µg / mL, and is weak if it has an IC_{50} value of 150-200 µg / mL.

RESULTS

Phytochemical Screening

Phytochemical screening was conducted qualitatively to determine the content of flavonoids, alkaloids, steroids, terpenoids, saponins, and tannins from seagrass extracts *Enhalus acoroides* and *Cymodocea rotundata*, as well as macroalgae *Padina australis* and *Sargassum duplicatum*. Data from phytochemical screening of ethanol extracts of seagrass and macroalgae were obtained from secondary data and can be seen in Table 1.

Table 1. Phytochemical Screening of Ethanol Extracts of Seagrass and Macroalgae

Ethanol Extract	Flavonoids	Alkaloids	Steroids	Terpenoids	Saponin	Tannin
*Enhalus acoroides**	+	+	+	-	+	-
*Cymodocea rotundata**	+	+	+	-	+	+
*Padina australis***	+	+	+	-	+	-
*Sargassum duplicatum***	+	+	+	-	+	-

Description: (+) contains compound (-) does not contain compound
(*) Bareta, 2024 (**) Bareta, 2023

Phytochemical Activity Test with DPPH Method (2,2-Diphenyl-1-picrylhydrazyl)

Absorbance was measured using UV-Vis Spectrophotometer at a wavelength of 517 nm to determine the percentage of inhibition of each concentration in each taurine, seagrass ethanol extract, and macroalgae. A positive control using ascorbic acid was used with an IC_{50} value of -3.98 µg/mL. The lowest IC_{50} value was the ethanol extract of *Cymodocea rotundata* of -652.95

µg/mL, while the highest IC_{50} value was the ethanol extract of *Sargassum duplicatum* of 11,188.38 µg/mL. The IC_{50} values of taurine, seagrass ethanol extract, macroalgae ethanol extract and positive control ascorbic acid can be seen in Figure 1.

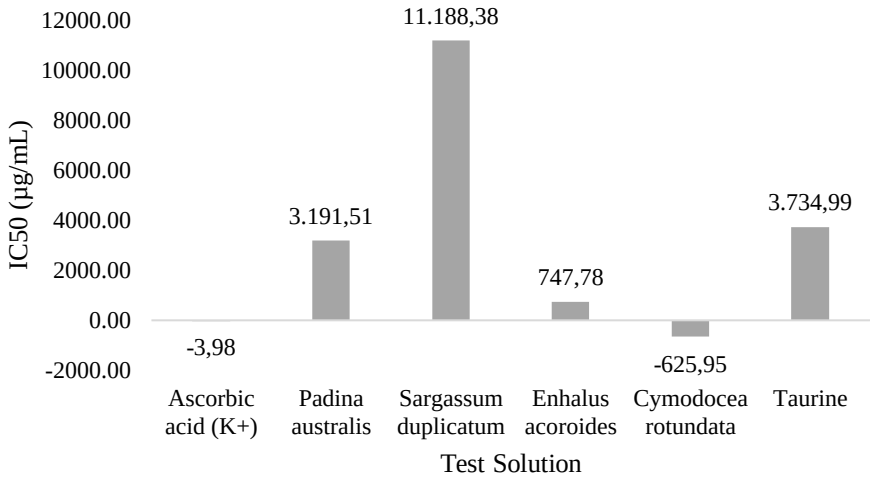

Figure 1. IC_{50} Values of Taurine, Macroalgae Ethanol Extract, and Seagrass Ethanol Extract

DISCUSSION

The extraction process of seagrass *Enhalus acoroides* and *Cymodocea rotundata*, as well as macroalgae *Padina australis* and *Sargassum duplicatum* was carried out using ethanol solvent. Ethanol solvent has polar properties and a relatively lower level of toxicity compared to other solvents. Solvents that have polar properties will only dissolve compounds that have polar properties (Gritter et al., 1991). Then bioactive compounds such as saponins, steroids, tannins, alkaloids, and flavonoids can be extracted. The physical and chemical conditions of the waters can affect the content of bioactive compounds in an organism, so the difference in results from each ethanol extract of seagrass and macroalgae is thought to be due to differences in the physical and chemical conditions of the waters where the seagrass and macroalgae were found.

The DPPH (2,2-Diphenyl-1-picrylhydrazyl) method was used because it is the most efficient and effective method for testing antioxidant activity compared to the FRAP (Ferric Reducing Antioxidant Power) and FIC (Ferrous Ion Chelating) methods (Maesaroh et al., 2018). The IC_{50} value is the concentration value of the test solution that can inhibit 50% of free radical reactions (Ramdan, 2024). DPPH (2,2-Diphenyl-1-picrylhydrazyl) is used as a free radical solution. The working principle is the color change of the DPPH (2,2-Diphenyl-1-picrylhydrazyl) solution whose radical properties are inhibited by the test solution which has antioxidant properties. The absorbance value of the color change indicates the strength or weakness of the antioxidant properties of the test solution. The lower the IC_{50} value, the higher the antioxidant activity, while the higher the IC_{50} value, the lower the antioxidant activity. A compound is categorized as having antioxidant activity if its IC_{50} value is less than 200 µg/mL (Molyneux, 2004). In this study, ascorbic acid was used as a control or comparison because it is known as a stable antioxidant agent (Maesaroh et al., 2018). The results showed that only the ethanol extract of seagrass *Cymodocea rotundata* had antioxidant activity with a very strong level with an IC_{50} value of -652.95 µg/mL. The IC_{50} value is different from the IC_{50} value of the methanol extract of *Cymodocea* sp. leaves according to Permana et al. (2016) which is 518.57 µg/mL. The minus value in the ethanol extract of *Cymodocea rotundata* is thought to be because the test concentration used was too high, so that the resulting IC_{50} value was too small and was classified as having very strong antioxidant activity compared to the positive

control of ascorbic acid which had an IC_{50} value of -3.98 µg/mL. Meanwhile, taurine, ethanol extract of macroalgae *Padina australis*, macroalgae *Sargassum duplicatum*, and seagrass *Enhalus acoroides* used in this study were respectively 3,191.51 µg/mL, 11,188.38 µg/mL, and 747.78 µg/mL. This shows that the three extracts do not have antioxidant activity because the IC_{50} value obtained was > 200 µg/mL. The absence of antioxidant activity of the *Padina australis* extract in this study is in accordance with the ethanol extract of *Padina australis* taken from the waters of Tegal Mas, Lampung according to research by Alhafizoh et al. (2024) because it has an IC_{50} value > 200 µg/mL, namely 407.96 µg/mL. *Enhalus acoroides* extracted with methanol according to research by Permana et al. (2020) has moderate antioxidant activity with an IC_{50} value of 148.67 µg/mL. There is a difference in the IC_{50} value which is thought to be due to differences in the physical and chemical conditions of the waters where *Padina australis* and *Enhalus acoroides* were taken, which causes differences in the content of bioactive compounds in them. The drying process of seagrass and macroalgae in this study was carried out without heating and only using the help of solar heat. According to research by Firmansyah et al. (2016), the methanol extract of *Sargassum duplicatum* which was also dried without heating had an IC_{50} value of 143.03 µg/mL and was classified as having moderate antioxidant activity. The difference in IC_{50} values is thought to be due to differences in the solvents used in the maceration process. According to Pratama et al. (2021), taurine can function as an indirect antioxidant. This is because taurine can reduce membrane changes due to oxidative stress. The mechanism of taurine as an antioxidant includes stabilizing membrane permeability, its role as an osmoregulator, regulating calcium ions, and increasing the activity of antioxidant enzymes in the cellular system. The results of the antioxidant activity test of taurine in this study showed that taurine did not have antioxidant properties, this was thought to be due to storage conditions such as temperature and light intensity which were not optimal, thus reducing the effectiveness of taurine as a candidate or antioxidant agent.

Several factors can affect the results of antioxidant activity tests carried out using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method, including physical and chemical conditions in seagrass and macroalgae habitats, less than optimal treatment of test solutions, the wavelength used during the spectrophotometry process, the test concentration used, and the polarity of the solvent used during maceration.

CONCLUSION

Based on the results of the study, only the ethanol extract of *Cymodocea rotundata* seagrass showed very strong antioxidant activity with an IC_{50} value of -652.95 µg/mL. Meanwhile, taurine, ethanol extract of macroalgae *Padina australis*, *Sargassum duplicatum*, and seagrass *Enhalus acoroides* did not have antioxidant activity because the IC_{50} value obtained was more than 200 µg/mL. Several factors can affect the results of antioxidant activity tests carried out using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method, including physical and chemical conditions in seagrass and macroalgae habitats, less than optimal treatment of test solutions, the wavelength used during the spectrophotometry process, the test concentration used, and the polarity of the solvent used during maceration. Further studies are needed to isolate potentially toxic bioactive compounds from seagrass extracts *Enhalus acoroides* and *Cymodocea rotundata*, and macroalgae *Padina australis* and *Sargassum duplicatum*, and to identify their toxicity mechanisms to target cells or organisms. In addition, more in-depth clinical and toxicological trials are needed to determine the safety of using these bioactives in pharmaceutical or health applications, as well as exploring other potential benefits such as antimicrobial or anti-inflammatory activities that they may have.

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