Phytochemical profiles and ethnomedicine preliminary studies on seagrass species in the Southern Coast of Lombok Island Indonesia

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1. Introduction

In marine ecosystem, seagrass is the primary producer in supporting fish food sources and various types of aquatic biota. Seagrass is one of the most widespread coastal vegetation types, which have developed unique physiological, morphological, and ecological adaptations to grow and thrive in submerged marine conditions, and provide essential ecological functions for the coastal and marine
environment [1][2]. In this ecosystem, there lives a variety of marine biota with economic value, such as fish, crustaceans, mollusks (Pinna sp, Lambis sp, Strombus sp), Echinoderms (Holoturia sp, Synapta sp, Diadema sp, Acrbaster sp, Linckia sp) and worms (Polichaeta) [3]. Seagrass bed in the southern coastal waters of the Lombok island has a wealth of echinoderms (21 species) [4], fish resources (104 species) [4] and bivalves (40 species) [5].

The species richness of seagrass on the southern coast of Lombok island ranges from 8-10 species. Seagrass species with an even distribution are *Enhalus acoroides*, *Syringodium isoetifolium*, *Cymodocea serrulata*, *Cymodocea rotundata*, and *Halophylla ovalis*. Seagrass biomass has often been used as food and medicine by coastal indigenous societies [6]. Seagrass plants have been widely used for traditional medicine, for example, treating infections caused by pathogenic microbes, fever, anti-inflammatory, muscle pain, skin diseases/itching, anti-virus, diarrhea, anti-diabetes, wound healing, sedative, and anti-cancer. [7].

The description above shows the many uses of seagrass plants and their ecosystems. Therefore, various parties must continue to carry out studies related to seagrass ecosystem use and management, and involved in conservation program of seagrass. According to WHO estimation, about 80% of the world population depends on traditional medicine systems as primary health care [8]. Nowadays, there are a lot of researches on bioprospecting new drug sources as an alternative in drug development in the future. Besides that, ethnomedicine studies are also one of the trends to explore information from indigenous people about the use of plants. Therefore, the aim of this research where to investigate the profile of seagrass bioactive compounds and to analyze the etnomedicinal aspect of the seagrass species.

2. Materials and Methods

This research consists of two main activities. first, a laboratory-based study to evaluate the phytochemical profile of seagrass species. Second, a preliminary study of the ethnomedicinal aspects of seagrass species on the population/community in the south coast of Lombok island. Seagrass sampling locations were at Kuta Beach and Gerupuk Beach. Phytochemical tests for secondary metabolite compounds were carried out at the Laboratory of Immunobiology, Faculty of Mathematics and Natural Sciences, University of Mataram. Six seagrass samples (*Syringodium isoetifolium*, *Enhalus acoroides*, *Halophila decipiens*, *Cymodocea rotundata*, *Thalassiahemprichii*, and *Cymodoceacerulata*) were taken from the two locations mentioned above, then qualitative and quantitative tests were performed. The main steps in the experiment are drying of seagrass plant samples, maceration, evaporation and phytochemical tests including Alkaloids, Flavonoids, Phenolics, Tannins, Saponins, and Terpenoid Tests. Furthermore, the total phenol and flavonoid content were tested. The seagrass ethnomedicine study was conducted by interviewing residents in the southern coastal area of the island of Lombok.

2.1 Preparation of Seagrass Extract

Prior to the extraction experiment, the seagrass sample was dried for approximately five days to reduce the water content of the seagrass. After drying, the seagrass is sliced into small pieces and then mashed until it becomes a fine powder. Each type of seagrass powder was extracted by the maceration method using 96% ethanol solvent (polar) with a material: solvent ratio of 1:10. Remaceration was carried out two times for optimal results. After that, the liquid extract was evaporated by evaporation using a *rotary evaporator* at a temperature of 40°C (± 1 hour). The section obtained was then subjected to a phytochemical screening test to determine the content of compounds in seagrass.
2.2 Phytochemical Screening Test

2.2.1 Preparation of Test Solution

Weighed 0.1 grams of each ethanol extract of *Syringodium isoetifolium* and *Enhalus acoroides*, then dissolved with 10 mL of carrier solvent (Ethanol 96%) until the final concentration of the solution was 1%.

2.2.2 Alkaloid Test

The test was carried out by taking 1 mL of each sample of the test solution into two different test tubes. After that, each extract was added with ten drops of Dragendorff reagent. If each solution forms an orange precipitate, it is positive for alkaloids [9].

2.2.3 Flavonoid Test

The test is carried out by taking 1 mL of each test sample into two different test tubes. Then heated for about 5 minutes. After being heated, each was added with 0.1 grams of magnesium metal powder and five drops of concentrated HCl. If each solution forms a yellow-orange to red color, then it is positive for flavonoids [9].

2.2.4 Phenolic Test

The test is carried out by taking 1 mL of each test sample into two different test tubes. Then add 2-10 drops of a solution of FeCl₃. 5% A positive reaction is indicated by the formation of a green or bluish-green color [9].

2.2.5 Tannin Test

Tests are carried out by taking 1 mL of each test solution sample into two different test tubes. Then the model was added with ten drops of 10% FeCl₃. The example is declared positive for tannin if it produces a blackish-green or blue-black color [9].

2.2.6 Saponin Test

A total of 2 ml of the test solution is added to 10 mL of hot water. Then shaken vigorously for 10 seconds, a stable foam as high as 1-10 cm for 30 minutes will be formed. It does not disappear after adding one drop of 2 N hydrochloric acid, indicating the presence of saponins [9].

2.2.7 Terpenoid Test

A total of 1 ml of the test solution sample was put into a different test tube. After that, each extract was added with three drops of concentrated HCl and one drop of concentrated H₂SO₄. If each solution forms a red or purple color, it is positive that it contains terpenoids [9].

2.2.8 Total Phenolic Content Test Total Flavonoid Content Test

A total of 25 L of the sample was mixed with 125 L of aqua bikes and 7.5 L of sodium nitrite (5%). The mixture was incubated for 5 minutes while continuously shaking so that it was completely homogeneous. In the same well, 15 L of 10% aluminum chloride (AlCl₃) solution was added, and the mixture was again incubated for 50 minutes at room temperature in the dark. After 50 minutes, 50 L of 1 M sodium hydroxide (NaOH) was added to each sample well. The entire test solution was read for absorbance with a UV-Vis spectrophotometer at a wavelength of 510 nm [10].

2.2.9 Total Phenolic Content Test

A total of 20 L of seagrass extract (EL) sample with a concentration of 1mg/ml was put in a 96 well plate well, and 100 L of Folin-Ciocalteus solution was added. Homogenized and incubated at room temperature for 5 minutes. After 5 minutes, 80 L sodium carbonate (75g/L) was added to the wells to be re-incubated for 60 minutes at room temperature and in the dark. After 60 minutes, the absorbance was read under a UV-Vis spectrophotometer at a wavelength of 765 nm. Total phenol value was expressed in mg gallic acid equivalent per gram extract (mg GAE/100 g of EL) [10].
2.3 Data Collection on Ethnomedicine of Seagrass Plants on Lombok Island

Respondents as the primary source of information consisted of local communities on the southern coast of Lombok Island (Kuta, Grupuk & Awang). In collecting data related to seagrass ethnomedicine, it was carried out in 2 stages. The first stage is collecting seagrass specimens from coastal areas either directly or taking photos, and the second stage was interviews activity with ten respondents and community leaders in the location. The age range is 35 to 65 years. Information from community leaders and medical experts (village healers). In this study, interviews were conducted using a structured and free technique. Structured interviews using instruments in the form of questionnaires with semi-open questions are intended to obtain data and dig up information about the species and parts of seagrass plants used as traditional medicine. In connection with the use of certain species of seagrass for certain diseases, at the time of the interview, specimens and botanical photographs of seagrass plants were shown to the respondents. The results of the interviews were then recorded on a questionnaire instrument, which were then summarized, and the conclusions were drawn regarding the use of each seagrass species [11].

3. Results and Discussion

On the southern coast of Lombok Island, six species of seagrass were found. The six species are *S. isoetifolium*, *E. acoroides*, *H. decipiens*, *C. rotundata*, *T. hemprichii*, and *C. serulata*. Samples were successfully taken from the south coast of the beach in Central Lombok at low tide. The mapping of the six species is presented in Figure 1.

![Figure 1. The Location map of the research](image)

In this study, environmental parameters were measured, including salinity, temperature, depth, pH, and substrate at each sampling location which are presented in Table 1.

| Location | Environmental Parameter |
|----------|-------------------------|
|          | Temperature (°C) | pH | Salinity (‰) | Depth (m) | Substrate | DO (ppm) | Phosphate (ppm) | Nitrate (ppm) |
| Kuta     | 30          | 8  | 40           | 0.2-5     | Sandy     | 6.5      | 0.01           | 43.50         |
| Gerupuk  | 26          | 7  | 31.36        | 0.2-5     | Sandy     | 6.5      | 0.01           | 43.49         |

Based on the table 1, the temperature value on the Kuta beach is 30°C, and at Gerupuk Beach is 26°C which can be categorized as a normal temperature for the tropical region. Lee et al. [12] stated that seagrass has an average temperature range between 23 °C and 32 °C that supports growth, photosynthesis, and reproduction processes.
The pH value of the research sampling locations, namely on the Kuta beach was at the value of 8 and on Gerupuk beach was at the value of 7. Based on the Quality Standards of the Decree of the Minister of the Environment Number 51 in 2004, the pH range for marine biota, 7–8.5, is included in the normal category. The salinity at both locations was $40^0/000$ at Kuta beach, while at Gerupuk beach, the saltiness was $31.36^0/000$. According to Ramdhoni [13], the average salinity value in the waters of Lombok Island is $40^0/000$.

The depth obtained at the two research sites is the average depth value ranging from 0.2-5m with the depth at low tide. Then the substrate where the seagrass species were found at the two sampling locations was found to be a sandy substrate. The value of dissolved oxygen content in seagrass beds constantly fluctuates. Fluctuations in dissolved oxygen content in the waters are thought to be caused by the use of dissolved oxygen by seagrass for respiration of roots and rhizomes, respiration of aquatic biota, and nitrifying bacteria in the nitrogen cycle process in seagrass beds [14]. Dissolved oxygen (DO) values measured at Kuta Beach and Grupuk Beach are still sufficient for seagrass growth.

Phosphate and nitrate levels based on Kep.MNLH No. 51 of 2004, for marine life is 0.015 mg/l and 0.008 mg/l. According to Amelia et al. [15], the primary source of nitrate in waters comes from household waste and agricultural waste. Phosphate is the primary source of organic matter decomposition in sediments. The main source of phosphate and nitrate naturally comes from the waters themselves through the process of decomposition, weathering, plant decomposition, the remains of dead organisms, land waste disposal (domestic, industrial, agricultural, livestock, and feed residues), which bacteria will decompose into substances. Nutrients in the form of nutrients utilized by marine plants such as seagrass for growth and development [16]. Grupuk Beach and Kuta beach have a normal phosphate value, obtained a value of 0.01 ppm. High human activities influence the value of this phosphate content, so it has many main sources of nitrate and phosphate in it.

The results show that the nitrate content at the Kuta and Grupuk beach locations is 43.50 and 43.49 ppm, respectively. This value belongs to the category of high nitrate. High nitrate levels in seagrass ecosystems are caused by many litters produced from fallen and decaying seagrass leaves. Nitrate content is also used as a parameter of seagrass density. According to [17], if nitrate increases, the thickness of seagrass will increase in value. The results of the qualitative test are presented in Table 2.

Table 2. The qualitative phytochemical test of 6 seagrass species from southern of Lombok Island

| No | Type or Test | S. isoetifolium | E. acoroides | H. decipiens | C. rotundata | T. hemprichii | C. serulata |
|----|-------------|----------------|--------------|--------------|--------------|---------------|-------------|
| 1  | Alkaloids   | -              | -            | -            | -            | -             | -           |
| 2  | Flavonoids  | +              | +            | +            | +            | +             | +           |
| 3  | Phenols     | +              | +            | +            | +            | +             | +           |
| 4  | Tannins     | +              | +            | +            | +            | +             | +           |
| 5  | Saponins    | -              | -            | -            | -            | -             | -           |
| 6  | Terpenoids  | -              | -            | -            | -            | -             | -           |

3.1. Phytochemical Compounds Test

Flavonoid, Phenolic and Tannin compounds were found in six types of seagrass. In seagrass, *S. isoetifolium*, flavonoid compounds are found in seagrass roots. The mechanism of action of flavonoid compounds as antimicrobials is to inhibit the nucleic acid synthesis and damage the membrane, causing the release of intracellular compounds [18]. In phenolic, mechanism of action of phenolic compounds as an antimicrobial is to denature the protein so that the protein components of microbial cells are damaged and cause cell death [19]. While in tannins, mechanism of action of tannin compounds as antimicrobials is destroying the main components of the cell wall consisting of chitin, glucans, lipids, thereby inhibiting microbial growth [20].
Alkaloid, saponin and terpenoid compounds were not found in the six types of seagrass collected. According to Rompas et al [21], the compounds contained in seagrass are chalcone group compounds that are toxic and have a very bitter taste, where their use is to avoid the threat of herbivores and protect plants from insect attacks. The mechanism of action of alkaloids is by denaturing proteins, causing damage to the proteins that make up cells [18]. In saponin, this compound indicated by the absence of foam formation when shaken. The mechanism of action of saponin compounds as antimicrobials is to reduce cell surface tension, causing leakage and release of cell intracellular compounds [18]. While in terpenoid, this compound indicate by the absence of red or purple indicates this. The mechanism of action of terpenoids as antimicrobials is by reacting with porins (transmembrane proteins) on the outer membrane of the bacterial cell wall, forming a solid polymeric bond, thereby causing the destruction of the porin [18].

Alkaloids, saponins, and terpenoids were not found in the six species of seagrass tested in this study (Table 2). Environmental factors are very influential on the biosynthesis of secondary metabolites. Extreme temperatures, salinity, and drought stress on the substrate for growth hurt the biosynthesis of certain secondary metabolites. Plants secondary metabolite is a compound that plays an essential role in the interaction of plants with abiotic stress; in addition, plant growth and development are also primarily mediated by the endogenous level of this secondary metabolite [22]. So it can be concluded that environmental conditions can also affect the levels of compounds in seagrass.

3.2. Test of Total Phenolic Concentration and Total Flavonoid Level

One of the important things in the bioprospecting of secondary metabolites is quantitative analysis to determine the total content of certain compounds. In this study, the quantitative analysis focused only on the total flavonoid and phenolic content. The standard used for the quantification of phenolic and flavonoids compounds were gallic acid and quercetin flavonoids respectively.

![Figure 2](image_url)

**Figure 2.** The analysis of Total flavonoid content (TFC) of 6 seagrass species. a) Calibration curve by using Quercetin Standard. b) The value of flavonoid content (based on 3 replication) through linear regression ($y = 0.005x + 0.033; \text{R}^2 = 0.991$). The data showed that *E.acoroides* had the highest TFC and was not significantly different from *S.isoetifolium*. 
The analysis of the total flavonoid content of 6 seagrass species tested, there are 2 species that have the highest total flavonoid content, namely S. isoetifolium and E. acoroides. While the other 4 species have a total flavonoid content below the 2 species (Figure 2). This difference in the total amount of flavonoid content can be caused by differences in environmental conditions where it grows, such as temperature, ultraviolet light, nutrients, water availability and CO$_2$ levels in the atmosphere [23]. So with these factors, there can be a precise diversity in the flavonoid content.

**Figure 3.** The analysis of Total phenolic content (TPC) of 6 seagrass species was measured using Folin-Ciocalteu reagent. a) Calibration curve ($y = 0.004x + 0.063; R^2 = 0.998$) of Gallic acid Standard. b) The value of phenolic content (based on 3 replication) through linear regression.

Analysis of the total phenolic content of 6 seagrass species observed, there is 1 species that has the highest total phenolic content, namely T. hemprichii (Figure 3). While other species have a total phenolic content below the T. hemprichii species. Substrate can affect the amount of secondary metabolites of a plant. Dense soil causes roots to easily absorb mineral salts and results in increased secondary metabolite content [24].

Based on Figures 2 and 3, it can be explained that there are differences in the total flavonoid and phenolic content as shown by the results of the phytochemical analysis of the seagrass species tested. This difference can be caused by environmental conditions that can affect the ability of a plant to produce certain types of secondary metabolites. The use of different solvents for extraction is influenced by the compositions and groups of chemical compounds [25]. In this study, the use of 96% ethanol as a solvent showed that to obtain high flavonoid content in seagrass, S. isoetifolium and E. acoroides species could be the right materials, while T. hemprichii species could be used to obtain high phenolic content.

### 3.3. Seagrass Ethnomedicine

Information on the use of seagrass as an ethnomedicine is still very minimal. In Castro and Ronnback's research[26], seagrass can serve as a remedy for various ailments, including stings, muscle aches, wounds, stomach problems, fever, malaria, and coughs. Another study described the use of seagrass by the Seri Indian tribe in Mexico as a treatment for diarrhea [27]. The use of seagrass as ethnomedical can be seen in Table 3.
Other diseases that can be overcome using traditional medicine from seagrass include low blood pressure, digestive disorders, skin diseases, iron deficiency, burns, ulcers, stomach acid. For severe medical conditions such as heart disease and low blood pressure, people usually peel the skin and eat the rhizome of *E. acoroides* plus a cup of water. In addition, residents also use seagrass to treat dandruff [28]. Seagrass also has good nutritional value for humans and livestock. Several ethnic groups in the Philippines [29] and Mexico [27] consume seagrass seeds because of their sweet and crunchy taste, like peanuts, and fishers think that eating them can increase their energy. The origins of *E. acoroides* are high in starch, with an energy content similar to flour, and when cooked, the seeds taste like sweet potatoes [29]. So that the role of seagrass as an ethnomedicine is very beneficial for the community, especially people on the south coast of Lombok island.

**Table 3.** Summary of Interview Result Regarding the Use of Seagrass as Traditional Medicine in Southern Coast of Lombok Island

| No | Seagrass Species | Name Local Name | Benefit from | Interview Location |
|----|-----------------|-----------------|--------------|--------------------|
| 1  | *S. isoetifolium* | Seagrass stick   | itchy medicine for | Kuta, Gerupuk, and Awang |
| 2  | *E. acoroides*   | Seagrass tetu    | Medicine for stomach pain, external wound medicine, itching and internal diseases (heart, cancer, and kidney), Fever, Flu, Tuberculosis. | Kuta, Gerupuk, and Awang. |
| 3  | *T. hemprichii*  | Banana seagrass  | Medicine for stomach pain, external wound medicine, itching medicine, and internal disease (heart, cancer, and kidney) | Kuta and Gerupuk. |
| 4  | *C. serrulata*   | Segras Mur.      | Cure for itching | Kuta, Gerupuk, and Awang |

The presence of an active compound can also be influenced by environmental factors, namely temperature, solar radiation, air (oxygen, carbon dioxide, and water vapor), and humidity [30]. Poor environmental conditions affect the production of secondary metabolites in organisms. In stormy conditions, seagrass will release its secondary metabolites to survive. The content of secondary metabolites is influenced by the environment and can be affected by the high level of association of seagrass beds. Threats from marine biota that live on seagrasses, also tend to produce bioactive compounds as a form of self-protection. Apart from environmental aspects, the type of solvent also significantly affects the levels of seagrass compounds [31]. The existence of groups of chemical compounds flavonoids, alkaloids, and tannins in species *S. isoetifolium* and *E. acoroides* indicate that these seagrass species have potential as natural chemicals antifouling, antibacterial, and antifungal [32].

4. Conclusion

The highest phenolic content was found in *T. hemprichii*. Meanwhile, the seagrass with the highest flavonoid content was *E. acoroides*. Among the six species in this study, there are four species of seagrass that residents commonly use as complementary materials for disease treatment. Based on the phytochemical profile and ethnomedicine studies, the four seagrass species can be an alternative source in obtaining bioactive compounds for the development of specific drugs in the future.
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