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Mapping and Identification of Bioactive Seagrass Compounds *Enhalus acoroides* in the Waters of Morotai Island

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ABSTRACT

The types and volumes of bioactive compounds can vary in plants that inhabit different areas. This research aimed to map and analyze the content of bioactive compounds in the seagrass *Enhalus acoroides* in the waters around Morotai Island. The study was conducted from June to August 2024, with sampling points spread across six sub-districts: Pilowo and Waringin Villages (South Morotai), Sambiki Tua and Sangowo Villages (East Morotai), Tanjung Saleh and Gorua Villages (North Morotai), Pangeo and Sopi Villages (Morotai Jaya), Usbar Pantai and Wayabula Villages (West South Morotai), and Posi-Posi and Saminyamau Villages (Rao Island). The extraction and identification of bioactive compounds from the seagrass were performed at the Faculty of Fisheries and Marine Science Laboratory. Seagrass mapping was conducted using ArcGIS software. The identification of bioactive compounds was carried out qualitatively using chemical detectors, including alkaloids, flavonoids, saponins, steroids, triterpenoids, phenols, and quinones. The results indicate that *Enhalus acoroides* is found in the waters of Morotai Island, specifically in the districts of South Morotai (Pilowo and Waringin), South West Morotai (Wayabula), Rao Island (Posi-Posi and Saminyamau), East Morotai (Sangowo and Sambiki Tua), and North Morotai (Tanjung Saleh). The yield of *Enhalus acoroides* leaf extract was 61.17%, higher than the rhizome yield of 48.28%. Among the accessions, the highest yield of *Enhalus acoroides* extract was from the Wayabula accession (19.84%), while the lowest was from the Saminyamau accession (1.72%). The methanol extract of *Enhalus acoroides* contains bioactive compounds, including alkaloids, saponins, steroids, triterpenoids, phenols, and quinones.

INTRODUCTION

Seagrass is one of the plants in shallow marine ecosystems that plays a vital role in marine life. Seagrass is a higher plant (Anthophyta) that lives and grows immersed in the marine environment, has leaves, rhizomes, and roots and reproduces generatively (seeds) and vegetatively (shoots). One type of seagrass is *E. acoroides*. *Enhalus acoroides* is a critical species in the Indo-Pacific and the dominant species throughout the

Indian Ocean and tropical parts of the Pacific Region **(Sarinawaty *et al.*, 2020)**. Ecologically, the presence of seagrass contributes to and plays a vital role as a contributor of nutrients for fertility in coastal and marine environments. The function and benefit of seagrass in shallow water ecosystems is as a primary producer, as a habitat for biota, and providing shelter and a place to attach to various kinds of organisms and a nurturing area **(Nurul *et al*., 2018)**. **El-Regal *et al*. (2014)** reported that nine types of fish larvae were found in the seagrass habitat of Mabahiss Bay, Egypt.

Seagrass, beyond its ecological role, holds significant economic value. It is used as a material for matting, feed, cosmetics, and pharmaceutical ingredients. Its widespread growth in Indonesia, including the Morotai Island Regency, presents numerous economic opportunities. Ongoing research on mapping seagrass species, such as Halophila Espinosa, in South Morotai waters **(Rakim, 2023)** and the unexplored potential of *Enhalus acoroides* in the waters of Morotai Island further enhances its economic allure.

The research results of **Mandea *et al*. (2022)** reported that in Juanga waters, Morotai Island, six species of seagrass were found, namely *Cymodocea serrulata*, *Cymodocea rotundata*, *Holodule pinifolia*, *Thalassia hemprichii*, *Enhalus acoroides*, and *Halophila minor*. **Nur *et al*. (2023)** reported that in the waters of Zum-Zum Island, Morotai Island, seven types of seagrass were found, namely *Halodule pinifolia*, *Syrigodium isoetifolium*, *Halophila ovalis*, *Enhalus acoroides*, *Cymodocea rotundata*, *Cymodecea serulata,* and *Thalassia hemprichii*. **Nurafni and Nur (2018)** and **Firmansyah *et al*. (2022)** also reported that six types of seagrass were found in the waters of Dodola Island. **Sukandar *et al*. (2008)** stated that biodiversity can be interpreted as chemical diversity capable of producing chemicals, both for the needs of humans and other organisms, such as medicines, cosmetics, and essential ingredients for synthesizing more useful organic compounds.

Significant strides have been made in recent years in exploring the potential of seagrass, particularly in uncovering its bioactive substances. Notably, **Nurafni and Nur (2018)** discovered that seagrass accessions from Morotai Island exhibit antifouling activity. Similarly, **Nur *et al*. (2021)** found that *Cymodecea rotundata* and *Halodule pinifolia* also possess antifouling properties. Furthermore, **Nurafni *et al*. (2021)** reported that *E. acoroides* are antibacterial against *Staphylococcus aureus*. **Dewi (2013)** researched the potential of *Thalassia hemprichii* and *Enhalus acoroides* seagrass leaves to inhibit biofilm formation in the weak to moderate category. **Ali *et al*. (2012)** also identified the potential of *Cymodocea rotundata* seagrass in inhibiting the growth of Aegypti larvae.

The use of seagrass cannot be separated from the bioactive compounds it contains. The research results of **Ravikumar *et al*. (2008)** showed that the content of bioactive compounds in seagrass originating from Southern Indian waters has potential antibacterial properties. **Nurafni and Nur (2018)** showed that seagrass from the waters of Morotai Island contains alkaloids, flavonoids, saponins, and steroids. **Nurafni *et al*.**

(2021) reported that seagrass contains alkaloids, saponins, and steroids. **Firmansyah (2022)** elucidated that *Thalassia hemprichii* in the waters of Morotai Island contains bioactive compounds. **Nur and Rahmawati (2019)** postulated that the bioactive compounds in seagrass originating from southern Indian waters have potential antibacterial properties. Furthermore, **Nurafni and Nur (2018)** clarified that the seagrass type E. *acoroides* from Morotai waters has antimicrobial activity.

The waters of Morotai are a treasure trove waiting to be explored, especially in the seagrass ecosystem. *Enhalus acoroides*, found in the waters of Morotai Island, have the potential to develop bioactive compounds. However, research on mapping *E. acoroides* types and their use in Morotai Island waters is still in its infancy. Therefore, there is a pressing need for further research to map *E. acoroides* seagrass species from Morotai Island waters and identify their bioactive compounds, paving the way for their future utilization.

MATERIALS AND METHODS

Time and place

This research was carried out from June to August 2024, from sampling *Enhalus acoroides* seagrass to identifying its bioactive compounds. Survey and collection of seagrass were conducted in 6 sub-districts: South Morotai, East Morotai, North Morotai, Morotai Jaya, South West Morotai, and Rao Island Districts. Two points (villages) were selected from each sub-district, as shown in Fig. (1). Extraction and identification of the bioactive compounds of seagrass *E. acoroides* were carried out at the Faculty Fisheries and Marine Science (FPIK) Pasific Morotai University Laboratory.

Tools and materials

The tools and materials used in this research were a diving knife, snorkel, sample bag, water quality tester, test tube, flask bottle, bowl, jar, spatula, test tube rack, aluminum foil, filter paper, funnel, Erlenmeyer flask, beaker, fan, dropper pipette, spatula, laptop, and Arcgis map 10.8. While, the materials used in this research were seagrass *Enhalus acoroides*, magnesium powder, natrium hydroxide (NaOH), methanol, amyl alcohol, chloroform, l meyer, distilled water, sulfuric acid, ammonia, alcohol, FeCl, ethanol, HCl, anhydrous acetic acid, and ammonia.

Research procedures

Collection of seagrass samples

The seagrass type *E. acoroides* was taken from Morotai Island's waters, consisting of 12 points (villages) from 6 sub-districts. Observation points where *E. acoroides* seagrass species are found were marked. *E. acoroides* seagrass were taken using a diving knife to make the removing process of the rhizome and roots easier. Collection of *E.*

acoroides in the field was carried out freely, and enough samples were taken for extraction purposes.

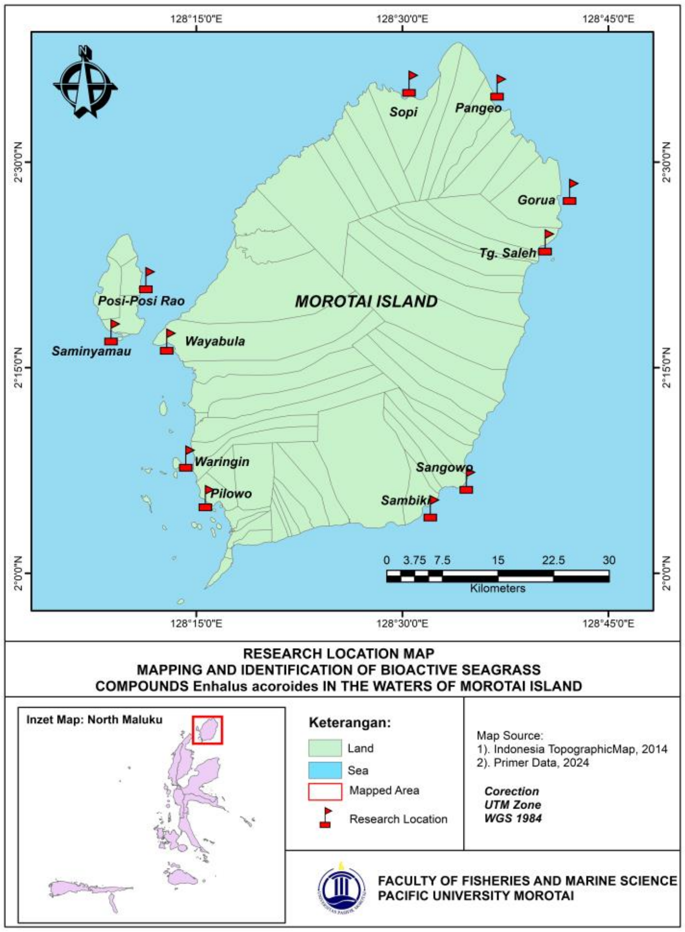

Fig. 1. Map of Morotai Island research location

Seagrass distribution mapping

Mapping the distribution of seagrass species, *Enhalus acoroides*, was conducted in the waters of Morotai Island using the ArcGis 10.8 application. From the survey/observation results, a distribution map was made at each point based on whether or not *E. acoroides* seagrass was found.

Sample preparation

Seagrass samples obtained from the observation location were washed thoroughly using fresh water. Apart from that, *E. acoroides* seagrass was also cleaned from epiphytes attached to the leaves and from a mixture of other seagrass or algae removed during sampling. Next, *E. acoroides* was dried in the sun and covered with black cloth. After drying, *E. acoroides* were separated between leaves and rhizomes and were labeled according to the collection location (accession).

Seagrass sample extraction

Leaf and rhizome samples of *E. acoroides* were cut into small pieces and blended separately to become powder (simplisia). Extraction was carried out using the maceration method using methanol solvent for 72 hours. The initial weight of the simplicia was weighed first and soaked in methanol (ratio of simplicia: solvent = 1:5). Next, it was filtered using filter paper, and the filtrate was evaporated in a water bath until the solvent was entirely evaporated. The extract obtained was weighed and stored in a flacon bottle.

Identify groups of bioactive compounds

The crude seagrass extract was subjected to phytochemical testing to unveil the diverse array of bioactive compounds it harbors. The qualitative identification of seagrass bioactive compounds using chemical detectors revealed a rich spectrum, including alkaloids, saponins, flavonoids, steroids, triterpenoids, phenols, and quinones. This diversity underscores the potential of seagrass as a source of bioactive compounds for further tests.

Exploration and exploitation

1) Alkaloid test

A total of 0.1 gram of extract was mixed with 5ml of chloroform and 5ml of ammonia, then heated, shaken, and filtered. Five drops of 2 N sulfuric acid was added to each filtrate, then shaken and let to stand. The top of each filtrate was taken and tested with Meyer's reagent. The formation of orange, brown, and white precipitates indicated the presence of alkaloids.

2) Saponin test

A 0.1 gram of extract was boiled in a water bath with 20ml. The filtrate was shaken and allowed to stand for 10 minutes. The formation of stable foam means there was positive saponin.

3) Flavonoid test

A total of 0.1 grams of extract was added to 20ml of water, boiled for 5 minutes, and then filtered. 5ml of filtrate was added to 0.05mg powder, and three drops of concentrated HCl were added to each filtrate, then shaken. The formation of red, yellow, or orange indicated flavonoids' presence.

4) Steroid/triterpenoid test

0.1 gram of extract was then filtered in 20ml of ether for 2 hours. Next, 5ml of the filtrate obtained was evaporated in an evaporator cup until being dry. Then, two drops of concentrated sulfuric acid and two drops of anhydrous acetic acid were added. The color change from purple to blue or green indicates the presence of steroids, while the formation of red indicates the presence of triterpenoid compounds.

5) Phenol test

A 0.1 gram of extract was added with ten drops of 1% FeCl. A positive extract contains phenol if it produces a green, red, purple, blue, or dark black color.

6) Quinone test

0.1 gram of extract was added to 10ml of ethanol, boiled for 5 minutes, and filtered. Next, 5ml of filtrate was added with five drops of NaOH solution. A red color formulation indicated the presence of quinone.

Data analysis

The extraction data and compound groups obtained were presented as figures and tables using Microsoft Excel 2007. These data were then meticulously analyzed descriptively, ensuring that every detail was in line with the research objectives. The percentage yield of the extract, calculated using the percentage yield formula, is a testament to the thoroughness of our analysis.

RESULTS AND DISCUSSION

Mapping the distribution of the seagrass *Enhalus acoroides* on Morotai Island

Distribution mapping is an activity process that provides information on the surface of the earth, where the activity aims to describe a picture of the Earth's surface and the natural resources found in a place, including the distribution of seagrass species found in the waters of Morotai Island Regency. The distribution of seagrass species *Enhalus acoroides* in the waters of Morotai Island Regency can be seen in Fig. (2). The distribution of seagrass species *Enhalus acoroides* in the waters of Morotai Island is found in the districts of South Morotai (Pilowo and Waringin), West South Morotai (Wayabula), Rao Island (Posi-Posi and Samiyamau), East Morotai (Sangowo and Sambiki), North Morotai (Tanjung Saleh and Gorua), meanwhile in Morotai Jaya the seagrass species *Enhalus acoroides* was not found. Data were collected in 6 sub-districts from 12 sampling points; the *Enhalus acoroides* type was most commonly found in 9 sampling points. *Enhalus acoroides* has a broader distribution and is most often found in Indonesian waters. This is in line with what was reported in Coremap-CTI (2017 to 2018), showing that the types *Enhalus acoroides* and *Thalassia hemprichii* are cosmopolitan types that are spread throughout Southeast Asia and the Indo-West Pacific region **(Coremap-CTI, 2021)**. **Julianinda *et al*. (2022)** reported the distribution of seagrass species found in South Morotai waters, including *Cymodocea rotundata*, *Enhalus acoroides*, *Halodule uninervis*, *Halodule pinifolia*, *Halophila ovalis*, *Sryngodium isoetifolium*, and *Thalassia hemprichii.*

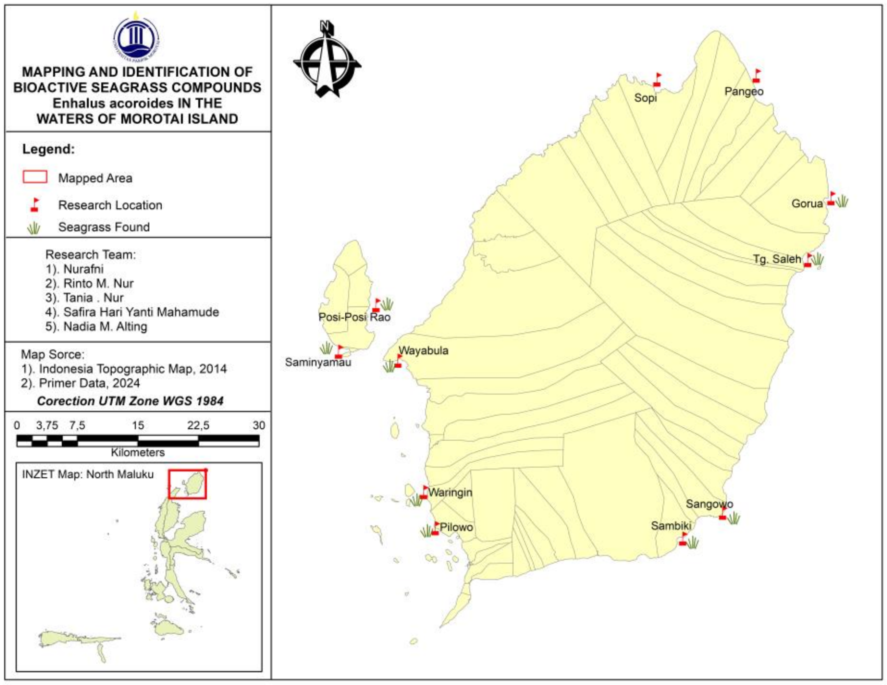

Fig. 2. Map of the distribution of seagrass *Enhalus acoroides* in the waters of Morotai Island

In Morotai Jaya waters (Pangeo and Sopi Villages) and South West Morotai waters (Usbar Pante Village), the *E. acoroides* type was not found due to the different forms of adaptation of seagrass such as substrate type and the influence of high waves. This coincides with the research results on the status of seagrasses in 2021. *E. acoroides* was not found at several points due to the form of adaptation and specific habitat ranges, such as substrate type and the influence of high waves **(Coremap-CTI, 2021)**. From the observations, it can also be seen that Morotai Jaya has waters with high waves because it faces the open sea. This is also caused by the morphological condition of *E. acoroides*, which has the most significant and extended leaves compared to all types of seagrass, thus affecting its adaptation to waters with high waves. **Dahlan and Nofrizal (2007)** reported that the organic material content in the substrate and relatively calm water conditions influence the distribution of *E. acoroides*.

Suniati (2018) reported that of the eight types of seagrass in the intertidal area of Pulau Dua, Enggano District, the *E. acoroides* type was found unevenly and was not found at all observation stations; only the *Thalassia hemprichii*, *Cymodocea serrulata*, and *Halodule uninervis* types were found evenly throughout—intertidal area. Moreover, **Rosalina *et al*. (2022)** noticed that in mapping, the distribution of seagrass species found in Kapoposang Island, there were six types of seagrass, namely *Thalassia hemprichii*, *Enhalus acoroides*, *Cymodocea rotundata*, *Halodule pinifolia*, *Halophila ovalis*, *Syringodium isoetifolium*, while the type of seagrass with the lowest distribution only found in certain spots, is *Enhalus acoroides*.

Extraction of *Enhalus acoroides*

Extraction of *E. acoroides* was carried out using the maceration method with methanol solvent. This method was chosen because it has many advantages compared to other extraction methods. The main advantage of the extraction method is that the procedures and equipment used are simple; the extraction method is not heated, so the possibility of damage to thermolabile compounds can be avoided. In addition, methanol is used as a solvent because methanol is polar, and methanol can filter more bioactive compounds **(Kawaroe, 2015)**. The extraction results of *E. acoroides* bioactive compounds are presented in Table (1).

Based on the yield results of the extract obtained, differences were seen between the rhizome yield results and the yield results on leaves from specific locations. This is because seagrass leaves contain chlorophyll and carotenoid pigments. Chlorophyll itself is the green pigment of leaves in plants. Apart from that, epiphytic organisms were also found on seagrass leaves. The presence of these organisms causes plants to experience stress producing more bioactive compounds in response to this situation. **Mabrouk *et al*. (2014)** explained that excessive epiphytic organisms on the seagrass leaves' surface can inhibit the seagrass's photosynthesis process. **Dewick (2010)** argued that plants produce bioactive compounds as attractants, dyes, and defense agents against environmental stress. Fig. (3) shows that *E. acoroides* leaf extract yield was 61.17%, and rhizome was 48.28%. This result is different from what **Nur and Nugroho (2018)** reported; the yield of leaf extract obtained was less when compared to bulbil and tubers (as food reserves).

Table 1. Extraction results of *Enhalus acoroides* seagrass

Location		Sample Type	Amount		
			Simplicity (g)	Extract (g)	Rendement (%)
Rao Island	Posi-Posi	Leaf	58	1,44	2,48
		Rhizome	24	0,98	4,08
	Saminyamau	Leaf	74	0,56	0,76
		Rhizome	75	0,72	0,96
South Morotai	Pilowo	Leaf	33	3,76	11,39
		Rhizome	27	1,38	5,11
	Waringin	Leaf	55	1,64	2,98
		Rhizome	76	1,9	2,50
East Morotai	Sangowo	Leaf	33	4,02	12,18
		Rhizome	88	3,14	3,57
	Sambiki Tua	Leaf	100	4,1	4,10
		Rhizome	100	6,22	6,22
North Morotai	Tanjung Saleh	Leaf	52	5,1	9,81
		Rhizome	56	3,52	6,28
	Gorua	Leaf	53	3,64	6,87
		Rhizome	50	5,16	10,32
West South Morotai	Wayabula	Leaf	50	5,3	10,6
		Rhizome	50	4,62	9,24

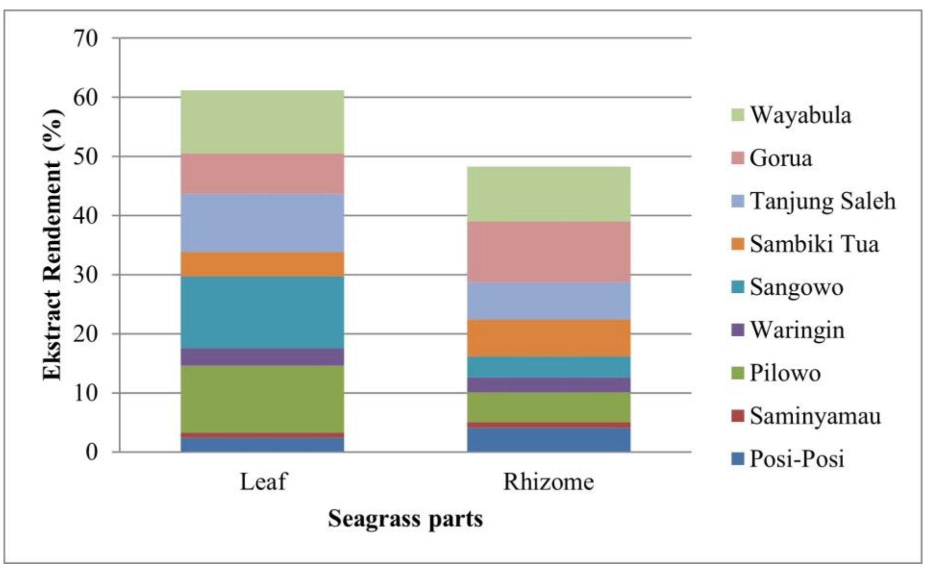

Fig. 3. Comparison of *Enhalus acoroides* leaf yields and rhizome extracts from Morotai Island waters

From Table (1), it can also be seen that there are differences in the yield of *E. acoroides* methanol extract from different accessions. Overall, the yield of methanol extract from *E. acoroides* from Posi-Posi accession was 6.56%,; it was evaluated inSaminyamau accession at 1.72%, Pilowo accession at 16.50%, Waringin accession at 5.48%, Sangowo accession at 15.75%, Sambiki Tua at 10, 32%, Tanjung Saleh accession at 16.09%, Gorua accession at 17.19%, and Wayabula accession at 19.84%. Overall, the yield of methanol extract from seagrass *E. acoroides* was obtained more from Wayabula Village. A comparison of the yield of *E. acoroides* seagrass methanol extract based on accessions can be seen in Fig. (4).

Differences in environmental factors can cause differences in the yield of these bioactive compounds. **Chang *et al.* (2016)** explained that environmental conditions influence the physiology and production of plant bioactive compounds. **Koroy *et al.* (2021)** reported that laor accessed from different locations showed differences in the percentage yield of bioactive compounds. Apart from the abiotic environment, such as temperature, pH, salinity, and substrate, the biotic environment also influences the synthesis of bioactive compounds. There is an increase in the number and type of bioactive compounds. Furthermore, **Zhang and Demain (2005)** elucidated that plants form secondary metabolites in response to the needs and challenges of their natural environment.

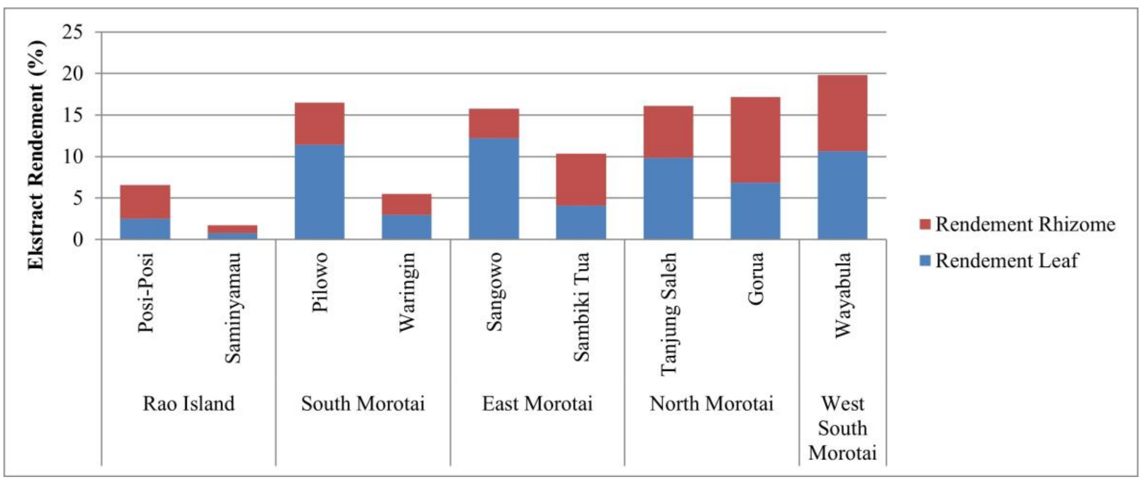

Fig. 4. *Enhalus acoroides* methanol extract yield is based on accessions from Morotai Island waters

The extracted sample will produce a different texture. What influences the texture of the extract is the content contained in the seagrass (Fig. 5). From these results, it can be seen that the texture of the *Enhalus acroides* methanol extract is paste and sticky (in the leaf extract). In contrast, the rhizome methanol extract has a paste-like texture, slightly watery and not sticky.

Fig. 5. Methanol extract of *Enhalus acroides*; a. Green (Leaves); b. Brown (Rhizome)

Identification of bioactive compound classes

They identified bioactive compound groups of seagrass *Enhalus acoroides* by conducting two tests on leaves and rhizomes. The results of identifying bioactive compounds from methanol extracts of leaves and rhizomes of *E. acoroides* from the waters of Morotai Island are shown in Table (2). However, in this study, there were extracts from several accessions for which the class of bioactive compounds was not identified, such as extracts of rhizomes and leaves of *E. acoroides* from the Saminyamau accession, rhizome and leaf extract of *E. acoroides* accession Sambiki Tua, leaf extract of *E. acoroides* accession Posi-Posi, and rhizome extract of *E. acoroides* accession Gorua. This was because the extract obtained was too little (Saminyamau and Posi-Posi accessions) and damaged (Sambiki Tua and Gorua accessions). Damaged extracts can be seen as turning watery and foamy, so they cannot be used.

Based on the results of identifying bioactive compound groups, it was found that alkaloid compounds were found in methanol extracts of *E. acroides* rhizomes (accessions Posi-Posi, Pilowo, Waringin, Sangowo, and Wayabula) and leaves (accessions Pilowo, Waringin, Sangowo, Tanjung Saleh, and Gorua). This group of compounds was not found in the methanol extract of the rhizome of *E. acroides* from the Tanjung Saleh accession and the leaves from the Wayabula accession. **Nur *et al*. (2021)** reported the presence of alkaloid compounds in the methanol extract of *E. acroides*. The results of previous research conducted by **Arifuddin (2013)** in the waters of Spermonde Island, Makassar, and **Dewi *et al*. (2013)** in Pramuka Island, Jakarta, also found the content of alkaloid compounds in the seagrass *E. acoroides*. This shows that alkaloid compounds are essential in seagrass growth and provide protection, infection prevention, and competition for space **(Ramadan *et al*., 2021)**. Alkaloids also function as growth regulators for plants **(Harborne, 2006)**.

The results of the identification of bioactive compounds showed that methanol extracts of *E. acoroides* rhizomes and leaves from all research locations did not contain flavonoid compounds—the absence of red, yellow, or orange color changes on the amyl alcohol layer. **Sari (2013)** reported that samples of *E. acroides* seagrass methanol extract reacted with reagents, tending to occur in alkaline conditions and showing a negative response to the reagents used. However, the results of this study are different from those of previous studies, which identified the presence of flavonoid compounds in *E. acoroides* extracts **(Qi *et al*., 2008; Dewi *et al*., 2012; Gustavina *et al*., 2018; Nur *et al*., 2021)**.

The results showed that all leaf extracts contained saponins, while the rhizome extracts of Pilowo and Wayabula accessions did not contain saponin compounds. **Nur *et al*. (2021)** detected the presence of saponin compounds in the methanol extract of *E. acroides* taken from the waters of Pandanga Village, Morotai Island. **Harborne (2006)** further explained that saponin compounds are surface-active compounds with soapy properties and can be detected based on their ability to form foam. Furthermore, **Daughari (2012)** postulated that saponins can dissolve in alcohol and water solvents.

Steroid and triterpenoid compounds were identified using concentrated sulfuric acid and anhydrous acetic acid reagents. A positive reaction from this test is that a color from purple to blue or green is formed, indicating the presence of steroids. In contrast, the formation of a red color indicates the presence of triterpenoid compounds. The identification results showed that the steroid compound group was only found in leaf extracts (Sangowo, Pilowo, Gorua, and Wayabula accessions) and was not found in the rhizome extracts of all accessions. Meanwhile, the triterpenoid compound group is only contained in the methanol extract of the rhizome of the Waringin accession. **Tiwari *et al*. (2011)** stated that terpenoid compounds could be found in water, ethanol, methanol, chloroform, and ether extracts. **Malikun (2011)** reported differences in the types and levels of terpenoids from *Brucea javanica* (L.) Merr. of each vegetative organ and accession. **Pradana *et al*. (2018)** noticed that *E. acoroides* extract contains flavonoids, triterpenoids, and steroids. In addition, **Nur *et al*. (2021)** reported the presence of steroid compounds in the methanol extract of *E. acoroides*.

Terpenoid compounds are generally found freely in plant tissues. **Harborne (2006)** explained that chemically, terpenoids are generally fat-soluble and found in the cytoplasm of plant cells. Terpenoids play a role in plants as growth regulators (abscission sesquiterpenoids and gibberellins) and photosynthetic aiding pigments (carotenoids). Due to its essential function for plant survival, it is possible that this group of compounds can be found in many leaf organs. However, in this study, no triterpenoid compounds were found in the methanol extract of *E. acoroides* leaves.

The phenolic compound group test results were also positive from the rhizome extracts of Posi-Posi, Pilowo, Waringin, Sangowo, and Wayabula accessions. Meanwhile, Tanjung Saleh's accession was not found. Meanwhile, the results of tests for phenolic compounds from the rhizome extracts of Pilowo, Tanjung Saleh, Gorua, and Wayabula accessions showed positive results. While, negative results were shown from the rhizome extract of the Tanjung Saleh accession and the leaf extract of the Waringin and Sangowo accessions. One derivative of phenolic compounds is quinone. The results of identifying this group of compounds from the methanol extract of *E. acoroides* showed that the rhizome extract from all accessions contained quinones. In contrast, the leaf extract was only found in the extract from the Sangowo accession. This result is also in line with the research results of **Firmansyah (2022)** who found that, not all of the phenolic and quinone compounds were found in the methanol extract of Thalassia hemprichii from different accessions.

The content of secondary metabolites in plants or animals is greatly influenced by environmental parameters, salinity, extreme weather, and growing location **(Mahmiah *et al*., 2023)**. Environmental factors influencing bioactive compounds' stability are temperature, light radiation, air (especially oxygen, carbon dioxide, and water vapor), and humidity. Other factors that can influence stability, namely pH, water properties, biotic conditions, and the presence of other chemicals that are contaminants or from mixing different products, can actively influence the stability of active ingredient preparations **(Nurfadilah, 2013)**. Poor environmental conditions affect the production of bioactive compounds for organisms. Apart from that, the success of extraction is also influenced by the type of solvent used in the extraction. **Mahmiah *et al*. (2023)** reported that the ethanol extract of *E. acoroides* leaves contains alkaloids, flavonoids, saponins, terpenoids, saponins, and polyphenols.

Water parameters

Water parameters play a crucial role in this research. Environmental conditions are essential in reducing the production and yield of secondary metabolites. During growth and development, plants interact with the environment's surroundings, whereas seagrasses interact directly with abiotic environmental components such as water, light, temperature, and substrate. Key elements include marine water parameter measurement data, including temperature, salinity, pH, and substrate. The results of these measurements, as shown in Table (3), are as follows: water temperature ranged from 30-34ºC, pH from 8.64-9.38, salinity from 25-40‰, and the substrate was sandy to muddy sand. In general, the parameter conditions in the Morotai Island research location are conducive to the life of seagrass resources.

Handayani *et al.* (2016) stated that the results of water temperature measurements exceeding 30ºC were due to the shallow water topography, which allowed sunlight to reach the bottom. **Mossi *et al.* (2009)** reported that higher temperatures affect the content of secondary metabolites, particularly tannins. Environmental parameters such as temperature, nutrient levels, and CO2 concentration influence the presence of compounds in plants **(Rahakbauw & Wataguly, 2016)**.

Coastal water salinity values are strongly influenced by the influx of fresh water from rivers, as found in the research location. The salinity value in the location ranges from 25-40 ‰ and is still suitable for the growth of seagrass, especially the *E. acoroides* type. According to **Hutomo (1999)**, seagrass can grow in different water salinities, most of which have a tolerance range of 10-40‰. However, decreasing or high salinity can affect the production of secondary metabolite compounds. According to **Santos (2004)**, a decrease in salinity can affect the process of photosynthesis so that glucose stimulates the activity of the PAL enzyme. This enzyme is a precursor for the biosynthesis of flavonoid compounds. The photosynthesis process produces compounds that play a role in growth and other compounds that do not play a role in growth or are known as secondary metabolites **(Aho & Beck, 2011)**.The substrates found in the location under study, sand and mud, significantly influence the growth of seagrass leaves due to their high levels of N and P nutrients. The presence of sediment is crucial for seagrass, serving as a habitat and a nutrient supplier. A lack of nutrients can affect the glucose production process, which stimulates the activity of the PAL enzyme (Phenylalanine ammonia-lyase) responsible for the biosynthesis of secondary metabolite compounds including flavonoids **(Santos, 2004)**.

Table 2. Identification of bioactive compound groups

Group Compound	Seagrass Parts	Accession									Information
		Posi Posi	Saminya mau	Pilowo	Waringin	Sangowo	Sambiki Tua	Tanjung Saleh	Gorua	Wayabula	
Alkaloids	Rhizome	+	*	+	+	+	*	-	**	+	The presence of an orange precipitate indicates the presence of alkaloids.
	Leaf	*	*	+	+	+	*	+	+	-	
Flavonoids	Rhizome	-	*	-	-	-	*	-	**	-	The formation of red, yellow, or yellow indicates the presence of flavonoids.
	Leaf	*	*	-	-	-	*	-	-	-	
Saponin	Rhizome	+	*	-	+	+	*	+	**	-	The formation of stable foam indicates the presence of saponin.
	Leaf	*	*	+	+	+	*	+	+	+	
Steroids	Rhizome	-	*	-	-	-	*	-	**	-	A purple-to-blue or green color change indicates the presence of steroids
	Leaf	*	*	+	-	+	*	-	+	+	
Triterpenoids	Rhizome	-	*	-	+	-	*	-	**	-	The formation of a red color indicates the presence of triterpenoids.
	Leaf	*	*	-	-	-	*	-	-	-	
Phenol	Rhizome	+	*	+	+	+	*	-	**	+	The formation of a dark green, red, or black color indicates the presence of phenol
	Leaf	*	*	+	-	-	*	+	+	+	
Quinones	Rhizome	+	*	+	+	+	*	+	**	+	The red color indicates the presence of quinone.
	Leaf	*	*	-	-	+	*	-	-	-	

Note: (-) does not contain bioactive compounds, and (+) contains bioactive compounds.
* Identification of bioactive compound groups was not carried out because the extract samples obtained were tiny.
** Identification of bioactive compound groups was not carried out because the samples obtained were damaged.

Table 3. Parameter measurement results

Location		Parameter			
		Temperature (°C)	Salinity (‰)	Water pH	Substrate
Rao Island	Posi Posi	30	30	8,93	Sandy
	Saminyamau	30	40	9,38	Sandy
South Morotai	Pilowo	31	28	8,34	Muddy
	Waringin	32,6	28,7	6,52	Muddy
East Morotai	Sangowo	32	25	8,64	Muddy sand
	Sambiki Tua	34	35	9,17	Muddy sand
North Morotai	Tanjung Saleh	31,3	34,7	6,92	Muddy sand
	Gorua	31,5	29,5	6,24	Muddy sand
Morotai Jaya	Sopi	31	33,6	6,64	Muddy sand
	Pangeo	28,3	23,5	6,83	Sandy
West South Morotai	Wayabula	32	31	6,54	Sandy
	Usbar Pante	30	28,7	8,14	Muddy sand

The pH measurements in each location showed different values; in some locations, it showed a value of 9.38, while in other locations, it showed a value of 6.5. It becomes more acidic due to the increased carbon dioxide (CO2) that seawater absorbs. According to **Andika *et al*. (2020)**, the pH value of seawater is closely related to dissolved carbon; the more carbon dioxide, the lower the pH value. The presence of secondary metabolite compounds such as flavonoids in plants varies, which can be caused by differences in water conditions such as ultraviolet light, water availability, and carbon dioxide (CO_2) content **(Bhat *et al*., 2009)**.

CONCLUSION

Based on the research results, several things can be concluded.

1. The distribution of seagrass species *Enhalus acoroides* in the waters of Morotai Island is found in the Districts of South Morotai (Pilowo and Waringin), South West Morotai (Wayabula), Rao Island (Posi-Posi and Samiyamau), East Morotai (Sangowo and Sambiki Tua), and Morotai North (Tanjung Saleh and Gorua). Meanwhile, in Morotai Jaya District, the seagrass species *Enhalus acoroides* was not found.
2. The total yield of *Enhalus acoroides* leaf extract (61.17%) was more significant than rhizome (48.28%). The yield of *Enhalus acoroides* methanol extract was also different for each accession. Posi-Posi accession 6.56%, Saminyamau accession 1.72%, Pilowo accession 16.50%, Waringin accession 5.48%, Sangowo Sambiki Tua accession 10.32%, Tanjung Saleh accession 16.09%, Gorua accession 17 .19%, and the Wayabula accession 19.84%.

3. The bioactive compounds in the methanol extract of *Enhalus acoroides* include alkaloids, saponins, steroids, triterpenoids, phenols, and quinones.

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REFERENCE

Aho, K. and Beck, E. (2011). Effects of ephiphyte cover on seagrass growth rate in two tidal zones. *Darthmouth Undergraduate Journal of Sciences.* 8(3):43-44.

Ali, M.S.; Ravikumar, S. and Beula, J.M. (2012). Bioactivity of seagrass against the dengue fever mosquito Aedes aegypti larvae. Asian Pacific Journal of Tropical Biomedicine:1-5.

Andika, Y.; M. Kawaroe, H.; Effendi, N.P. and Zamani (2020). Pengaruh kondisi ph terhadap respons fisiologis daun lamun jenis *Cymodocea rotundata*. Jurnal Ilmu dan Teknologi Kelautan Tropis, 12(2): 487-495.

Bhat, S.V.; Nagasampagi, B.A. and Meenakshi, S. (2009). Natural Products: Chemistry and Application. Narosa Publishing House, New Delhi. India.

Chang, J.; Mantri, N.; Sun, B., Jiang, L.; Chen, P.; Jiang, B.; Jiang, Z.; Zhang, J.; Shen, J., Lu, H. and Liang, Z. (2016). Effect of eleveted CO_2 and temperature on *Gynostemma pentaphyllum* physiology and bioactive compounds. *Jurnal Plant Physiology*, Volumes 196-197.

Coremap-CTI, LIPI. (2021). Status Padang Lamun Indonesia. Jakarta. Puslit Oseanografi.

Dahlan, Z. and Nofrizal (2007). Pemetaan sebaran lamun di perairan Teluk Tomini Provinsi Gorontalo. Jurnal Penelitian Sains. 10(1):166-173.

Dewi, C.S.U.; Soedharma, D. and Kawaroe, M. (2013). Komponen Fitokimia dan Toksisitas Senyawa Bioaktif dari Lamun *Enhalus acoroides* dan *Thalassia hemprichii* dari Pulau Pramuka, DKI Jakarta. Jurnal Teknologi Perikanan dan Kelautan. 3(2): 23-27.

Dewick, P.M. (2010). Medicine Natural Product: A Biosynthetic Approach. Second Edition. Wiley. England.

Doughari, J.H. (2012). Phytochemicals: Extraction Method, Basic Structures and Mode of Action as Potensial Chemotherapeutic Agents, Phytochemicals – A Global Perspective of Their Role in Nutrition and Health. Intech.

El-Regal, M.A.A.; Hellal, A.M. and Maaty, M.M. (2014). Abundance and diversity of reef fish larvae in Mabahiss Bay on the Egyptian Red Sea coast. Egypt. J. Aquat. Biol. & Fish. 18(4): 63 – 79

Firmansyah (2022). Identifikasi golongan senyawa bioaktif lamun *Thalassia hemprichii* di Perairan Pulau Morotai. Skripsi. Program Studi Ilmu Kelautan. Fakultas Perikanan dan Ilmu Kelautan, Universitas pasifik Morotai. Morotai.

Gustavina, N.L.G.W.B.; Dharma, G.B.S. and Faiqoh, E. (2018). Identifikasi Kandungan Senyawa Fitokimia Pada Daun dan Akar Lamun di Pantai Samuh Bali. Journal of Marine and Aquatic Sciences. 4(2), 271-277.

Handayani, D.R.; Armid and Emiyarti (2016). Hubungan Kandungan Nutrien dalam Substrat terhadap Kepadatan Lamun di Perairan Desa Lalowaru Kecamatan Moramo Utara. Jurnal Sapa Laut. 1(2): 42-53.

Harbone (2006). Metode Fitokimia: penuntun Cara Modern Menganalisis Tumbuhan. Terbitan Kedua Cetakan Keenam Diterjemahkan oleh: Kosasih. Penerbit ITB. Bandung.

Julianinda, Y.A.; Dewi, C.S.U.; Kasitowati, R.D. and Kurniawan, F. (2022). Studi Pustaka: Distribusi dan Sebaran Lamun Di Jawa Timur. Journal of Fisheries and Marine Research. 6(1):120-129.

Koroy K.; Nur R.M.; Nurafni, and Alican, I. (2021) .Characterization of morfology and identification of bioactive compounds of laor (*Eunice viridis*) from the waters of Morotai Island. International Conference on Fisheries and Marine. *IOP Conf. Ser.: Earth Environ. Sci*. 584

Mabrouk, L.; Brahim, M.B.; Hamza, A.; Mahfoudhi, M. and Bradai, M.N. (2014). A Comparison of Abundance and Diversity of Epiphytic Microalga Assemblages on The Leaves of the Seagrasses *Posidonia oceanica* and *Cymodocea nodosa* Asch in Eastern Tunisia. Hindawi Journal of Marine Biology. (1):1-10.

Mahmiah; Sa'adah, N.; Sunur, H.N. and Wijayanti, N. (2023). Profil Metabolit Ekstrak Etanol *Enhalus acoroides* (L.F.) Royle,1839 dari Nusa Tenggara Timur. Journal of Marine Research. 12(1):151-160.

Malikun (2011). Profil Terpenoid Organ Vegetatif *Brucea javanica* (L.) Merr. yang Tumbuh di Kalimantan Barat dan Yogyakarta. *Tesis*. Fakultas Biologi. Universitas Gadjah Mada. Yogyakarta.

Mandea, A.; Nurafni; Muhammd, S.H.; Koroy, K.; Sofianti, T. and Nur, R.M. (2022). Kepadatan jenis lamun di Perairan Desa Juanga Kabupaten Pulau Morotai. *Jurnal* Lemuru, 4(3):173-182.

Nur, R.M. and Nugroho, L.H. (2018). Cytotoxic activities of fractions from *Dioscorea bulbifera* L. chloroform and methanol extracts on T47D breast cancer cells. Pharmacognosy Journal. 10(1):33-38.

Nur, R.M.; Nurafni; Koroy, K.; Alwi, D.; Wahab, I.; Sulistiawati, S.; Dewi, R. and Rorano, M. (2021). The antibacterial activity of seagrass *Enhalus acoroides* against *Staphylococcus aureus*. 2nd International Conference on Fisheries and Marine. IOP Conf. Series: Earth and Environmental Science. 890

Nur, R.M.; Nurafni; Wahab, I.; Alwi, D.; Koroy, K.; Rahman, I.H.A.; Saibi, N. and Dewi, R. (2023) Antifouling activity of *Rhizipora micronata* in Morotai Island North Maluku. Aquaculture, Aquarium Conservation and Legislation. 16(1): 225-258.

Nurafni and Nur, R.M. (2018). Aktivitas antifouling senyawa bioaktif dari lamun di perairan Pulau Morotai. Jurnal Ilmu Kepulauan, 1(2).

Nurafni and Nur, R.M. (2018). Struktur komonitas lamun di Perairan Pulau Morotai. Prosiding seminar nasional kemaritiman dan sumber daya pulau-pulau kecil, 2(1).

Nurafni; Nur, R.M.; Koroy, K.; Alwi, D.; Muhammad, S. and Eso, N. (2021). Antifouling activity *Cymodecea rotundata* and *Halodule pinifolia* at Pulau Morotai. Journal of physics conference series vol 1.

Nurfadilah (2013). Uji bioaktiftas antibakteri ekstrak dan fraksi lamun dari Kepulauan Spermonde Kota. Skripsi. Universitas Hasanudin. Makassar.

Nurul, E.H.S.; Hermawan, E.K.; Bayu, P.; Happy, S.I.; Yulia, I.M.; Anggraini, K.; Rahmawati, S. and Suyarso (2018). Status padang lamun Indonesia. COREMAP CTI Lembaga Ilmu Pengetahuan Indonesia. ver02.

Pradana, N.E.; Wardiwira, F.F.; Hakim, L.; Imamah, A.N. and Istianisa, W. (2018). Efektivitas ekstrak lamun *Cymodocea rotundata*, *Thalassia hemprichii* dan *Enhalus acoroides* dari perairan Jepara sebagai antibakteri pada dillet ikan nila (*Oreochromis niloticus*) selama penyimpanan dingin. *Saintek Perikanan*. 13(2):143–147.

Rakim, F. (2023). Sebaran dan Kerapatan Spesies Lamun Halophila spinolosa di Perairan Morotai Selatan. Skripsi. Program Studi Ilmu Kelautan Fakultas Perikanan dan Ilmu Kelautan. Universitas Pasifik Morotai. Morotai

Ramadhan, R.; Dirgayusa, I.G.N.P. and Faiqoh, E. (2021). Skrining Fitokimia dan Uji Toksisitas Ekstrak Daun Lamun *Halophila ovalis* di Perairan Pantai Karma dan Pantai Serangan, Bali. Journal of Marine and Aquatic Sciences. 7(2):140-147.

Ravikumar, S.; Thajuddin, N.P.; Suganthi, S.; Jacob, I. and Vinodkumar (2008). Bioactive potential of seagrass bacteria against human bacterial pathogens. Journal of Environmental Biology. 31: 387-389.

Rosalina, S.D.; Rombe, K.H. and Hasnatang (2022). Pemetaan Sebaran Lamun Menggunakan Metode Lyzenga Studi Kasus Pulau Kapoposang, Provinsi Sulawesi Selatan. Jurnal Kelautan Tropis. 25(2):169-178.

Santos, C.V. (2004). Regulation off Chrlophyll Biosyntesis and Degradation By Salt Stress in Sunflower leaves. *Sci. Hortic*. 103:93-99.

Sari, D.W.S. (2013). Potensi Lamun *Enhalus acoroides* dan *Thalassia hemprichii* dari Perairan Pulau Pramuka Kepulauan Seribu sebagai Anti Oksidan dan Aktivitasnya dalam Menghambat Pembentukan Peroksida. Tesis. Universitas Padjadjaran. Bandung.

Sarinawaty, P.; Idris, F. and Nugraha, A. H. (2020). Krakteristik morfometrik lamun *Enhalus acroides* dan *Thalasia hemprichi* di Pesisir Pulau Bintan. Journal of Marine Research. 9(4): 474-48.

Sukandar, D.; Hermanto, S. and Lestari, E. (2008). Uji Toksisitas Ekstrak Daun Pandan Wangi (*Pandanus amaryllifolius* Roxb.) dengan Metode Brine Shrimp Lethality Test (BSLT). Valensi, 1(2).

Suniati (2018). Struktur Komunitas Lamun (Seagrass) di Daerah Intertidal Pulau Dua Kecamatan Enggano Kabupaten Bengkulu Utara. Skripsi. Fakultas Matematika dan Ilmu Pengetahuan Alam. Universitas Bengkulu. Bengkulu.

Tiwari, P.; Kumar, B.; Kaur, M. and Kaur, H. (2011). Phytochemistry Screening and Extraction: A Review. Internasional Pharmaceutical Sciencia. 1(1):1-15.

Zhang, L. and Demain, A.L. (2005). Natural Products: Drug Discovery and Therapeutic Medicine. Humana Press. https://link.springer.com/book/10.1007/978-1-59259-976-9.