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Profile of Secondary Metabolite Compounds of Seagrass *Enhalus acoroides* Extracted with Different Solvents from Suli Waters, Central Maluku Regency

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ABSTRACT

Seagrass, especially *Enhalus acoroides*, is one of the important components in coastal ecosystems that provide various ecological and economic benefits. Secondary metabolite compounds produced by *Enhalus acoroides* can vary based on the environmental conditions where they grow. This study aimed to determine the profile of secondary metabolite compounds of *Enhalus acoroides* extract in the Suli Waters, Central Maluku Regency. This research was conducted experimentally in the laboratory and analyzed descriptively. Water quality was assessed based on temperature, dissolved oxygen levels, salinity, and pH, while identification of secondary metabolite compound groups was carried out qualitatively using phytochemical tests. Water quality showed that the seawater temperature in the waters of Suli Village reached 30°C; dissolved oxygen levels were 6.1mg/ L; salinity was $30^{o}/_{oo;}$ and pH was 6.8. It was observed that the methanol extract of *Enhalus acoroides* contains flavonoids, saponins, phenolics, tannins, steroids, terpenoids, and alkaloids. Meanwhile, flavonoids, phenolics, tannins, steroids, and terpenoids were detected in the ethyl acetate extract of *Enhalus acoroides*. Environmental conditions and the use of different types of solvents can affect the composition of the secondary metabolite compounds produced.

INTRODUCTION

Coastal and marine resources in Indonesia are natural assets that have great potential for management and utilization in development **(Oktawati *et al*., 2018)**. In general, these resources consist of three main ecosystems: mangroves, seagrasses, and coral reefs **(Kartika *et al*., 2023)**. Each of these ecosystems has various benefits, namely coral reefs have the potential as ingredients for diarrhea medicine **(Asih *et al*., 2021)**; seagrass can be used in cosmetic products **(Badriyah *et al*., 2023a)**; and mangroves have the potential as herbal ingredients that are rich in antioxidants **(Denny, 2024)**. Seagrass is a flowering plant (angiospermae) with single-lobed leaves (monocotyledons) that can grow well and permanently under the surface of the sea. Seagrass forms a carpet-like expanse in the sea, which can consist of one species or various species **(Tangke, 2010)**. **Ramadhan *et al*. (2021)** explained that as an organism that lives permanently at the

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bottom of the waters, seagrass has a greater potential to produce active compounds in its body.

Enhalus acoroides has been widely researched and reported to contain active compounds, including tannin and alkaloid compounds **(Badriyah *et al*., 2023b)**. *Enhalus acoroides* from Sepanjang Beach-Yogyakarta contains tannins, saponins, triterpenoids, flavonoids, and steroids **(Permana *et al*., 2020)**. Bioactive compounds in *Enhalus acoroides* have significant potential to be developed as antibacterials, anticancer, and antioxidants. **Hitijahubessy *et al*. (2021)** confirmed that *Enhalus acoroides* extract is effective in inhibiting the growth of *Vibrio* sp. bacteria, thus showing its potential in controlling *Vibrio parahaemolyticus* bacteria. According to **Nurfitriani *et al*. (2017)**, the content of secondary metabolites in marine organisms is influenced by several factors, where environmental factors are one of the main causes. Secondary metabolites are chemical compounds produced by organisms in response to various environmental conditions and stress, and they play an important role in ecological interactions, such as protection against predators, competition with other organisms, and adaptation to the environment **(Petersen *et al*., 2020)**. According to **Yang *et al*. (2018)**, environmental factors such as salinity, temperature, lighting, and others have significant impacts on the production and composition of secondary metabolites in marine organisms. Changes in salinity or temperature can affect the synthesis of bioactive compounds produced by marine organisms, such as algae, sponges, and coral reefs. This is also explained by **Windyaswari *et al*. (2019)** that water quality containing various nutrients and pollutants can also play a role in changing the secondary metabolite profile.

Atun (2014) explains that the type of solvent used can also affect the secondary metabolite content of *Enhalus acoroides* because the solubility of these compounds varies depending on the solvent used. Polar solvents such as water or methanol are effective in extracting polar compounds, while non-polar solvents such as chloroform or ethyl acetate are better at extracting non-polar compounds. This is also emphasized by **Dewatasari (2020)** who elucidated that secondary metabolites that usually have different solubility properties will be easier to extract with solvents that match their polarity. The purpose of this study was to determine the profile of secondary metabolite compounds of *Enhalus acoroides* extracted with different solvents from the waters of Suli Village, Central Maluku.

MATERIALS AND METHODS

Research location description

Suli Village is one of the villages in Salahutu District, Central Maluku Regency, located on the coast. The coastal waters of Suli Village face the strait that separates Haruku Island and Seram Island. This causes the coastal waters of Suli Village to be directly influenced by the Seram Sea which enters through the strait, allowing it to be influenced by quite intensive tidal currents. However, due to the adaptation of seagrass morphology and the shape of its roots, the seagrass community can grow well in these waters. This is due to the type of basic water substrate which is dominated by muddy sand and carbonate substrates.

Tools and materials

The tools used during the experiment were a shaker (*Brushless DC motor*), autoclave (*SMIC model YX-28*), test tube (*Pyrex®*), analytical balance (*A&D company limited*), rotary evaporator (*BUCHI R-215*), aluminum foil, DO meter (*Hanna*), refractometer (*AMTAST DBS1*), pH meter (*Hanna*) and thermometer. While the materials used were *Enhalus acoroides*, methanol, ethyl acetate, distilled water, H_2SO_4 2N, $FeCl_3$ 1%, HCl 2N and Mg.

Work procedures

Water quality measurement

Measurement of water quality parameters includes water temperature using a thermometer, dissolved oxygen (DO) using a DO meter (*Hanna*), pH using a pH meter (*Hanna*) and salinity using a refractometer (*AMTAST DBS1*).

Preparation of test materials and extraction of Enhalus acoroides

The test materials used in this study were *Enhalus acoroides* obtained from the water of Suli State, Central Maluku. The samples were taken to the laboratory to be washed and cut into small pieces, after which they were air-dried. After drying, the samples were ground with a blender to obtain powder.

50g of powder *Enhalus acoroides* were put into a 100mL Erlenmeyer flask. Then 50mL of methanol solvent was added. The mixture was shaken with a shaker for 3 hours at a speed of 120rpm (rotation per minute) and macerated for 24 hours. Then the maceration residue was filtered with a Buchner funnel and re-dissolved using the same solvent until it was clear. The filtrate obtained was combined in an extract storage container **(Ukratalo *et al*., 2023)**. The above treatment was also repeated for the use of ethyl acetate solvent. The obtained methanol and ethyl acetate extracts were concentrated using a rotary evaporator at 30-40ºC, and N_2 gas was passed through until the solvent evaporated completely. Then the yield was calculated using the equation:

$$Rendement = \frac{Weight\ of\ crude\ extract\ obtained}{Sample\ weight\ used} \times 100\%$$

***Phytochemical test* (Kaihena *et al*., 2022)**

Identification of secondary metabolites using qualitative phytochemical tests with the principles of color reactions, precipitation, and foam formation includes testing of alkaloids, steroids/triterpenoids, polyphenols/tannins, flavonoids, and saponins. With the following work steps:

a. Alkaloid

2mL of extract solution was added with 3 drops of 2N H_2SO_4 then heated, followed by testing with Mayer and Wagner reagents. Positive test results were obtained whenever a precipitate was formed on the addition of Mayer's reagent and a brownish precipitate was observed upon the addition of Wagner's reagent.

b. Triterpenoid/ Steroid
Lieberman-Burchard reagent was added to 2mL of the extract solution. Positive test results are obtained if a green precipitate is formed (containing steroids) or a red precipitate is formed (containing triterpenoids).

c. Polyphenols/ Tannins
1mL of extract solution was added with two drops of 1% $FeCl_3$ reagent. The formation of green or blue color indicates the presence of phenol compounds.

d. Flavonoid
Extract solution of 1mL was added with a little Mg powder and 2mL of 2N HCl. Flavonoid compounds will produce orange to red color.

e. Saponins
An extract solution of 1mL was added with distilled water and shaken vigorously. The presence of saponin compounds is indicated by the formation of 1-10cm foam that is stable and not less than 10 minutes.

RESULTS AND DISCUSSION

1. Water quality of Suli State, Central Maluku

Measurement of the water quality of Suli Village, Central Maluku, referring to Government Regulation of the Republic of Indonesia Number 22 of 2021 concerning the Implementation of Environmental Protection and Management, can be seen in Table (1).

Table 1. Measurement of water quality in Suli Village, Central Maluku

Parameter	Unit	Volume	Quality standards
Temperature	^oC	30	28-30
Dissolved Oxygen/DO	mg/L	6.1	>5
Salinity	$^o/oo$	30	33-34
pH	-	6.8	7-8.5

Table (1) shows that the sea water temperature in the waters of Suli State is 30^{o}C, the dissolved oxygen content is 6.1mg/ L, the salinity is 30o/oo and the pH is 6.8. The environment is one of the factors that influences the growth and development of *Enhalus acoroides*. Based on the measurement results, it can be said that the physical and chemical factors of the waters in Suli State do not inhibit the growth of *Enhalus acoroides*. This was emphasized in the study of **Sahertian and Wakano (2017)** who postulated that physical and chemical factors such as temperature, salinity, dissolved oxygen, current speed, and pH do not interfere with or inhibit the growth of *Enhalus acoroides* because all of its physical components have not exceeded the established quality standards.

Sea water temperature is the temperature measured at the surface or at a certain depth of the ocean **(Akbari *et al*., 2017)**. According to **Abraham *et al*. (2013)**, this temperature varies depending on geographic location, depth, time of year, and other factors such as ocean currents and weather. Based on the quality standards (Government Regulation of the Republic of Indonesia Number 22 of 2021), it was deduced that the temperature of sea water in Suli Village which reaches 30°C is still optimal for seagrass growth.

According to **Frederick *et al*. (2014)**, dissolved oxygen is a crucial element in waters (aquatic life) including seagrass which is a vital component of coastal ecosystems. Government Regulation of the Republic of Indonesia Number 22 of 2021 concerning Seawater Quality Standards for Biota shows that good DO levels for waters are >5 mg/L, with dissolved oxygen levels in the waters of Suli State at 6.1mg/ L, it can support optimal growth and health of *Enhalus acoroides*. **Artika *et al*. (2020)** explain that adequate dissolved oxygen allows *Enhalus acoroides* to get enough oxygen needed for metabolism and respiration processes.

Salinity, which measures the concentration of salt in seawater, plays a key role in determining the health and survival of seagrasses **(Cambridge *et al*., 2017)**. The salinity in the waters of Suli State is $30^{o}/_{oo}$ below the quality standard (Government Regulation of the Republic of Indonesia Number 22 of 2021) of $33\text{-}34^{o}/_{oo}$. This is because the *Enhalus acoroides* sampling was carried out in the afternoon during the rainy season in May 2024, and there was a flow of fresh water from the Negeri Suli River which affected the salinity value. This is in line with the research results of **Sari *et al*. (2021)**, who assessed that the distribution of salinity is influenced by several factors, namely water circulation, evaporation, rainfall, and river flow. *Enhalus acoroides* is a type of seagrass that can survive in a range of moderate to high salinity, so that a salinity of $30^{o}/_{oo}$ can support relatively stable conditions for its growth. According to **Darui *et al*. (2024)**, the salinity range that can be tolerated by seagrass is 10-40‰ and the optimum value is 35‰.

pH or acidity level has a big influence on aquatic plants and animals. The results of measuring the pH of seawater in the waters of Suli State were 6.8 below the quality standard set by the Government Regulation of the Republic of Indonesia Number 22 of 2021, with a range between 7-8.5. The pH value greatly affects the biochemical processes of water; at a pH range of <4.00, most aquatic plants will die because they cannot tolerate low pH **(Bongga *et al*., 2021)**. **Salahuddin *et al*. (2022)** stated that low pH can occur due to the presence of dissolved organic matter and CO_2.

2. Extract yield of *Enhalus acoroides*

The liquid obtained was then evaporated to obtain a thick extract. The yield obtained in the study is depicted in Table (2).

Table 2. Extract/ yield of *Enhalus acoroides*

Solvent	Extract (gr)	Yield (%)
Methanol	7.52	15.04
Ethyl Acetate	6.13	12.26

Table (2) shows that the yield value in methanol solvent was higher (15.04%) than in ethyl acetate solvent (12.26%). **Ruhardi and Sahumena (2021)**, in their study, explained that the yield of ethyl acetate solvent is smaller than that of methanol solvent due to the presence of methoxy groups in the chemical structure of ethyl acetate which causes hydrogen bonds to form with compounds in the sample. The high and low yields can be influenced by several factors such as differences in methods, temperatures, types of solvents, and extraction times **(Novitasari & Jubaidah, 2018)**. This was also stated by **Salamah and Widyasari (2015)** showing that other factors that may affect the yield value produced are the extraction method used, sample particle size, storage conditions and time, extraction time, comparison of the number of samples to the number of solvents used and the type of solvent used. The solvent polarity is an important factor as it equaled 5.2 for methanol, while that of ethyl acetate was 4.4 in polarity index.

3. Identification of secondary metabolite compounds of Enhalus acoroides extract

Identification of secondary metabolite compound groups was carried out qualitatively using phytochemical tests to determine the active compound groups contained in the *Enhalus acoroides* extract. The results of the qualitative secondary metabolite compound test are illustrated in Table (3).

Table 3. Results of secondary metabolite compound content tests of methanol and ethyl acetate extracts of *Enhalus acoroides*

No.	Compound groups	Methanol extract		Ethyl acetate extract	
		Color / sediment formed	Results	Color / sediment formed	Results
1	Flavonoids	Yellow	+	Yellow	+
2	Saponins	Foamy green	+	Green no foam	-
3	Phenolic	Blue	+	Blue	+
4	Tannin	Green	+	Green	+
5	Steroid	Deep blue	+	Deep blue	+
6	Terpenoid	Chocolate	+	Chocolate	+
7	Alkaloid	Foamy green	+	Green no foam	-

The results of the phytochemical test in Table (3) show that the methanol extract of *Enhalus acoroides* contains flavonoids, saponins, phenolics, tannins, steroids, terpenoids and alkaloids, while the ethyl acetate extract of *Enhalus acoroides* contains flavonoids, phenolics, tannins, steroids and terpenoids. The results of this study differ from the findings of **Nurafni and Nur (2018)**, who also studied secondary metabolite compounds in methanol extracts of *Enhalus acoroides* in the waters of Morotai Island. In their study, the compounds found included alkaloids, flavonoids, saponins, and steroids. This difference indicates a variation in the results of the study related to the types of compounds identified in the *Enhalus acoroides* extract. Table (3) also shows that the ethyl acetate extract of *Enhalus acoroides* contains flavonoids, phenolics, tannins, steroids, and terpenoids. However, these results differ from research by **Antarsih (2017)**, which also tested ethyl acetate extract from *Enhalus acoroides* leaves and found compounds including saponins, tannins, alkaloids, steroids, and glycosides.

The difference in results between this study and the research of **Antarsih (2017)** and **Nurafni and Nur (2018)** may be due to several factors; one possibility is the difference in the extraction method used. According to **Tjandrawinata and Nurkolis (2024)**, the extraction method can affect the type and concentration of compounds successfully extracted from *Enhalus acoroides* samples. This is in line with what was stated by **Amin *et al*. (2023)** that the extraction method used to obtain bioactive compounds from *Enhalus acoroides* involves techniques such as maceration with different solvents, viz. methanol, ethyl acetate, and n-hexane. **Chemat *et al*. (2017)** added that condition testing, such as temperature, pH, or extraction duration, may also contribute to differences in results. Another factor to consider is the difference in physical and chemical conditions of the waters where *Enhalus acoroides* grows. This was also confirmed by **Fredley *et al*. (2019)** elucidating that conditions such as salinity, temperature, and water quality can affect the chemical composition of seagrass. Research by **Suriani and Lukman (2020)** verified that differences in chemical compound composition can occur because the characteristics of seagrass habitats can impact the content of secondary metabolites found in seagrass. Differences in compound content between methanol and ethyl acetate extracts may be due to differences in extraction methods and how these compounds interact with environmental conditions. Research results by **Florensia and Wijaya (2023)** explain that the use of different solvents can affect the optimal extraction of secondary metabolite compounds. This finding is in line with that of research conducted by **Hartati *et al*. (2019)**, which states that differences in phytochemical screening results can also be caused by differences in the sensitivity of the extraction method used to the amount of chemical content of the natural materials tested.

CONCLUSION

It can be concluded that the sea water temperature in the waters of Suli State was recorded to be 30^{o}C in the afternoon, with the dissolved oxygen content at 6.1mg/ L, the salinity at 30$^o/_{oo}$ and the pH at 6.8. The methanol extract of *Enhalus acoroides* contains flavonoids, saponins, phenolics, tannins, steroids, terpenoids and alkaloids, while the ethyl acetate extract of *Enhalus acoroides* contains flavonoids, phenolics, tannins, steroids and terpenoids. Remarkably, environmental conditions and the use of different types of solvents can affect the composition of the secondary metabolite compounds produced.

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