Bioactive compounds in some marine plants and invertebrates

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Abstract. Bioactive compounds are substances found in living organisms, such as marine plants or animals. The objective of this study was to identify bioactive compounds with antibacterial and antifungal properties from seagrass, mangroves, sea cucumber, and gastropods to investigate their potential to promote health. Sample collection was carried out in June 2024 in the waters of Jepara. The method involved extracting bioactive compounds using a methanol solvent and conducting antibacterial and antifungal tests using the disk diffusion method on various strains of *Escherichia coli* and *Staphylococcus aureus* bacteria, as well as the fungus *Candida albicans*, which commonly cause diseases in humans. Phytochemical tests were performed to identify the types of compounds present in seagrass, mangroves, sea cucumbers, and gastropods. A gas chromatography–mass spectrometry (GC–MS) analysis revealed that samples of marine plants and invertebrates contained compounds with antibacterial and antifungal properties. Flavonoid compounds were present in all samples examined, while alkaloids, steroids, and tannins were also detected in several samples. Furthermore, saponin compounds, which can act as antimicrobial agents, were found only in sea cucumber samples. In conclusion, marine plants and invertebrates demonstrate significant potential as sources of bioactive compounds that could be utilized in clinical and pharmaceutical settings to promote health. These compounds offer a new alternative for treating bacterial diseases, especially in light of the increasing challenge posed by bacterial resistance to antibiotics. Additionally, the use of these bioactive compounds can support natural health maintenance and help prevent bacterial and fungal infections that can lead to human diseases. This makes them effective in maintaining overall health and preventing diseases caused by harmful microorganisms.
Key Words: antibacterial, antifungal, health, marine plants, invertebrates.

Introduction. Bioactive compounds are substances found in living organisms, such as plants or animals, that exhibit significant biological activity in humans or other organisms. These compounds can have various beneficial effects on health, including anti-inflammatory, antioxidant, and anticancer properties (Pringgenies et al 2023). Antibacterial compounds refer to substances that can kill or inhibit the growth of bacteria and are commonly used in treating bacterial infections, whether natural or synthetic. Conversely, antifungal compounds refer to substances that can kill or inhibit the growth of fungi and are frequently used in treating fungal infections in humans or animals (Pringgenies et al 2023). While there are similarities between bioactive, antibacterial, and antifungal compounds, each category has a slightly different focus. Bioactive compounds represent a broad category that includes various compounds with positive health effects. In contrast, antibacterial and antifungal compounds are more specific in their ability to target and combat bacteria and fungi, respectively. In terrestrial environments, secondary metabolites are commonly found in higher plants, while in marine environments, they are found in immobile biota (Pringgenies et al 2023). Mangrove plants are marine plants that grow in coastal areas and contain various bioactive compounds with antibacterial and antifungal properties. Studies have demonstrated that extracts and active compounds from mangrove plants can inhibit the growth of pathogenic bacteria and fungi, making them potentially useful in the development of medicines and health products (Pringgenies et al 2023). Seagrass, often found in shallow waters and nutrient-rich areas, also contains compounds with antimicrobial effects that can combat pathogenic bacteria and fungi (Santosa et al 2024). Moreover, seagrass-

associated fungi have demonstrated antibacterial activity against *Vibrio alginolyticus* and *Vibrio parahaemolyticus* (Setyati et al 2023a).

Sea cucumbers are marine invertebrates that inhabit the sea. Research has found that these animals contain bioactive compounds with strong antibacterial and antifungal properties. The active compounds found in sea cucumbers, particularly in their bodies, can inhibit the growth of harmful bacteria and fungi. Various types of compounds present in sea cucumbers, including fatty acids, glycosides, and polysaccharides, have demonstrated effective antimicrobial activity (Pringgenies et al 2017). Additionally, a consortium of bacterial symbionts found in the stomachs of sea cucumbers (Djunaedi et al 2021) and the symbiotic bacteria *Bacillus aquimaris* and *Virgibacillus chiguensis* have been studied for their impact on meat quality (Pringgenies et al 2021a). Gastropoda is a class of invertebrate animals that includes mollusks like snails and slugs. Some species of gastropods can produce bioactive compounds with antibacterial and antifungal properties (Rashad et al 2023). These compounds are typically found in the mucus or special glands in the bodies of gastropods and can be utilized in developing drugs or health products to treat bacterial and fungal infections in humans (Fatina et al 2024).

This study focused on natural compounds or those produced by organisms that have significant biological effects. The term 'antibacterial' refers to compounds that can inhibit or kill bacteria, while the term 'antifungal' refers to compounds that can inhibit or kill fungi. In this research, marine plants like mangroves and seagrasses, as well as invertebrate animals such as sea cucumbers and gastropods, were believed to have the potential to produce compounds with antibacterial and antifungal properties for medicinal purposes. Samples of marine plants such as mangroves and seagrasses, as well as invertebrate animals such as sea cucumbers and gastropods, were chosen because these organisms are known to produce bioactive, antibacterial, and antifungal compounds. Mangroves and seagrasses often contain various bioactive compounds that can be utilized in medicine and healthcare (Ariyanto et al 2018; Ariyanto et al 2019; Ningsih et al 2020; Gono et al 2022; Pringgenies et al 2024). Similarly, sea cucumbers and gastropods are recognized for their potential antibacterial and antifungal properties. The selection of these samples was expected to yield valuable information for the research on the potential health benefits of marine plants and invertebrates. The research aimed to identify bioactive compounds with antibacterial and antifungal properties in seagrasses, mangroves, sea cucumbers, and gastropods, alongside exploring their potential applications in healthcare.

Material and Method

Collection of samples. Samples of seagrass, mangroves, sea cucumber, and mollusks were collected from the waters of Jepara Beach, Central Java, in June 2024. The samples were randomly taken from their habitats, placed in plastic ziplock bags for identification, and stored in an ice box. The identification process, which was conducted immediately after sampling, is presented in Figure 1. Two types of seagrasses, *Cymodocea rotundata* (1a) and *Enhalus acoroides* (1b), one type of mangrove, *Acanthus ilicifolius* (1c), one type of sea cucumber, *Holothuria atra* (1d), and two types of mollusks, *Turbo setosus* (1e) and *Tectus fenestratus* (1f), were found. Subsequently, the extraction process, phytochemical tests, antibacterial and antifungal tests, and gas chromatography–mass spectrometry (GC–MS) methods were performed at the Tropical Marine Biotechnology Laboratory in Building J, Faculty of Fisheries and Marine Sciences, and the Integrated Laboratory at Diponegoro University, Semarang.

Sample extraction. The extraction of samples from mangroves, seagrass, sea cucumbers, and mollusks began with a multi-stage maceration process. A dry sample weighing 100 g was soaked in 350 mL of n-hexane solvent for 24 hours. After 24 hours, the mixture was filtered, and the residue was separated. The residue from the n-hexane soaking was then soaked again with ethyl acetate and methanol in the same manner as the initial treatment with n-hexane. The extraction method utilized in this study was a solid-liquid extraction method employing a rotary evaporator for extraction. Following

filtration with filter paper, the filtrate was evaporated using a rotary evaporator at a temperature of 25ºC. The resulting extract was transferred from the Erlenmeyer flask to a vial and treated with methanol. Afterwards, the vial containing the extract was stored in a refrigerator at 4ºC (Pringgenies et al 2021b).

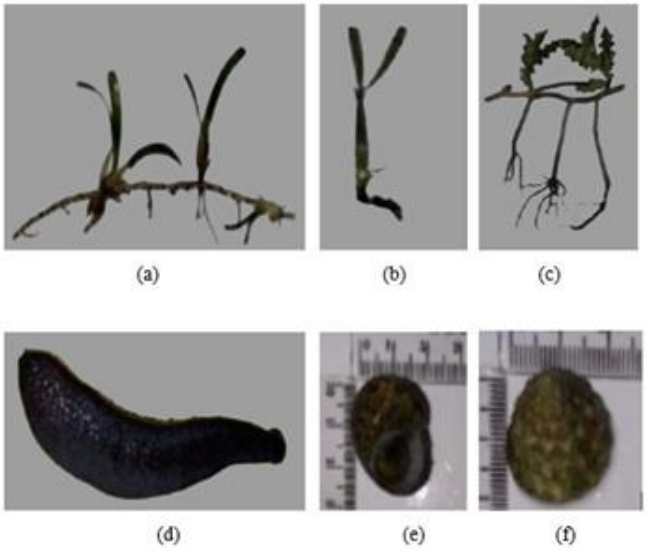

Figure 1: a. *Cymodocea rotundata* (Ascherson & Schweinfurth, 1870); b. *Enhalus acoroides* (Royle, 1839); c. *Acanthus ilicifolius* (Linnaeus, 1753); d. *Holothuria atra* (Jaeger, 1833); e. *Turbo setosus* (Gmelin, 1791); f. *Tectus fenestratus* (Gmelin, 1791).

Antibacterial activity test on sample extracts. The tools and materials were sterilized to obtain pure and uncontaminated bacterial isolates. This was achieved by subjecting all equipment and materials to a 15 minute sterilization process at a temperature of 121ºC and a pressure of 1 atmosphere. Inoculation tools such as tube needles and spreaders were sterilized through incineration (i.e., exposure to fire). The work area was sterilized by spraying a disinfectant liquid, such as 70% alcohol, around it. The media used in this research were Nutrient Agar and Nutrient Broth. Nutrient Agar was used to create agar slants for fresh culture and storage of isolates. To prepare Nutrient Agar, 2.2 g of NA were mixed with 100 mL of distilled water, while Nutrient Broth was used as a bacterial culture medium and was made by mixing 0.25 g of peptone, 0.05 g of yeast, and distilled water to a total volume of 100 mL. Each media solution was homogenized using a hot plate stirrer until it boiled. The solution was then sterilized in an autoclave at 121ºC and 1 atm pressure for 15 minutes. After sterilization, the Nutrient Agar was poured into a Petri dish to solidify, while the Nutrient Broth solution was poured into a test tube (Pringgenies et al 2021b).

Planting test bacteria on nutrient agar (na) media. Bacterial inoculation testing was carried out using the streaking technique, which aimed to obtain pure cultures of *Escherichia coli* and *Staphylococcus aureus* for antibacterial activity testing. One colony of the test bacteria from the available stock in the Integrated Undip Laboratory was taken and streaked on a Petri dish previously added with NA media in a zig-zag pattern. During streaking, the petri dish was opened as little as possible to prevent contamination of the agar surface. The media inoculated with the test bacteria was then incubated at 30ºC for 24 hours. The Petri dishes were placed upside down to prevent condensation water from dripping onto the agar surface, which could potentially interfere with the growth zone of the test bacteria (Setyati et al 2019).

Sample extract antibacterial activity test. The positive control test was conducted using the antibiotic amoxicillin at a concentration of 80 µg by disk to determine the diameter of the inhibition zone against the test bacteria. The antibiotic was applied to a paper disk on agar media inoculated with the test bacteria. After 24 hours of incubation,

the diameter of the inhibition zone was measured. The antibacterial activity test of seagrass, mangrove, gastropod, and sea cucumber sample extracts was performed using the disk diffusion method (Kirby–Bauer test) with various concentrations. Sterilised NA media was poured into a Petri dish and allowed to solidify. The pure extract from the samples at a concentration of 100% was tested against pathogenic bacteria, including *E. coli* and *S. aureus* from the gram-negative and gram-positive bacteria groups, respectively, as well as the fungus *Candida albicans.* The methanol solution sample in paste form was diluted with the DMSO solution to create a stock solution of 25 µg μL^{-1} for each concentration. Antibacterial activity tests were conducted on samples obtained from seagrass, mangrove, sea cucumber, and gastropod extracts. The tests were performed on pathogenic bacteria using four different concentrations: 250 ppm (I), 500 ppm (II), 750 ppm (III), and 1,000 ppm (IV). Subsequently, the test medium was incubated at 37°C for 24 hours. After 24 hours, the medium was observed, and the diameter of the inhibition zone was measured using a caliper. A clear zone of inhibition was formed around the paper disk. Each treatment was repeated three times during the experiment.

Phytochemical test. The phytochemical test method was employed to analyze the compound content of the test sample. This involved performing color reaction tests by adding chemical reagents to plant extracts and observing any color changes. The tests included those for flavonoids, alkaloids, steroids, tannins, and saponins (Pringgenies et al 2023).

Gas chromatography–mass spectrometry (GC–MS) test. In the GC–MS test, the sample extract was placed into the instrument and then separated into components and solutions. All compounds contained in the components were identified. Researchers could then calculate the quantitative analysis of each component. The number of compounds contained in the extract is indicated by the peak in the chromatogram, while the names/types of compounds present are interpreted by the spectrum data from each peak using the library approach method in the GC–MS database (Pringgenies et al 2023). The GC–MS instrument generates a chromatogram, which shows the separation of different compounds present in the sample based on their molecular weight and polarity. Each peak on the chromatogram represents a specific compound present in the sample. These peaks are identified by comparing their retention time and mass spectra with known standards or existing databases. The presence of a particular compound is confirmed by a match of at least 70% with the standard or database.

Results and Discussion

Antifungal and antibacterial tests on marine plants and invertebrate animals. Samples of marine plants and invertebrates were collected from the waters of Jepara, Indonesia. Two types of seagrasses, *C. rotundata* and *E. acoroides*, one type of mangrove, *A. ilicifolius*, one type of sea cucumber, *H. atra*, and two types of mollusks, *T. setosus* and *T. fenestratus*, were found. Antifungal tests conducted on these samples showed no antifungal activity. Then, antibacterial activity tests were carried out on extracts from the seagrass (i.e., *C. rotundata* and *E. acoroides*), mangroves (i.e., *A. ilicifolius*), sea cucumbers (i.e., *H. atra*), and gastropods (*T. setosus* and *T. fenestratus*) against the *E. coli* bacteria at concentrations of 250 ppm (I), 500 ppm (II), 750 ppm (III), and 1,000 ppm (IV). The results of the antibacterial activity test are presented in Table 1. The test results on the *E. coli* bacteria indicated that the *C. rotundata* seagrass sample with a concentration of 500 ppm exhibited the most effective antibacterial activity compared to other samples, with a resulting clear zone diameter of 3.69 mm. Antibacterial activity was also observed in the *E. acoroides* seagrass samples, which had the largest clear zone area of 2.40 mm, and in the *A. ilicifolius* mangroves, with clear zone areas of 2.47 mm in leaf samples and 2.17 mm in root samples. No antibacterial activity against the *E. coli* bacteria was detected in samples of sea cucumbers *H. atra* and gastropods (*T. setosus* and *T. fenestratus*).

Table 1

Antibacterial activity test of samples on the *Escherichia coli* bacteria

Sample	*Concentration by disk (µL)*	*Fraction*	*Diameter of inhibition zone*		
			24 h	*48 h*	*72 h*
Seagrass - *Cymodocea rotundata*	25	I	0.96±0.07	2.33±0.32	2.20±0.27
		II	3.69±0.12	3.49±0.23	3.18±0.00
		III	2.63±0.12	2.59±0.37	2.49±0.43
		IV	2.99±0.08	3.02±0.19	2.88±0.30
		Control	15.99±0.33	16.68±1.23	17.48±1.37
Seagrass - *Enhalus acoroides*	25	I	2.02±0.08	2.01±0.34	1.97±0.68
		II	1.53±0.10	1.74±0.58	1.61±0.66
		III	1.21±0.23	1.52±0.78	1.39±0.80
		IV	2.40±0.08	2.28±0.01	2.25±0.18
		Control	19.04±0.21	17.74±0.19	17.47±0.13
Leaf of mangrove	25	I	1.95±0.28	1.70±0.77	0.98±0.19
		II	2.47±0.99	1.83±1.00	1.45±0.81
		III	2.36±0.33	1.68±0.68	0.96±0.61
		IV	2.22±0.42	1.32±0.17	0.60±0.00
		Control	16.41±0.50	15.67±0.67	16.40±0.35
Root of mangrove	25	I	1.32±0.63	1.12±0.45	1.07±0.31
		II	1.12±0.26	1.23±0.65	1.00±0.13
		III	1.46±0.37	1.15±0.00	1.18±0.26
		IV	2.17±2.08	1.31±0.68	1.50±0.56
		Control	16.83±0.19	17.67±0.60	17.15±0.63

Table 2

Antibacterial activity test of samples on the *Staphylococcus aureus* bacteria

Sample	*Concentration by disk (µL)*	*Fraction*	*Diameter of inhibition zone*		
			24 h	*48 h*	*72 h*
Seagrass - *Cymodocea rotundata*	25	I	3.17±0.26	2.85±0.60	2.49±0.48
		II	2.93±0.45	2.70±0.53	2.50±0.11
		III	2.74±0.04	2.39±0.37	2.19±0.27
		IV	2.23±0.15	2.08±0.00	1.63±0.85
		Control	15.44±0.02	23.89±0.80	22.89±0.65
Seagrass - *Enhalus acoroides*	25	I	1.75±0.93	1.63±1.02	1.34±0.65
		II	1.95±0.77	2.13±1.09	2.06±0.80
		III	1.62±1.03	1.37±0.83	1.13±1.03
		IV	1.49±0.88	1.48±0.25	1.20±0.28
		Control	17.31±0.69	15.86±0.58	16.17±1.17
Leaf of mangrove	25	I	2.28±0.25	1.58±1.60	1.42±1.72
		II	2.46±0.14	2.56±0.19	2.23±0.90
		III	2.66±0.00	2.77±0.74	2.50±0.17
		IV	1.105±0.55	1.73±1.32	2.62±0.17
		Control	25.70±0.86	22.58±0.93	23.80±0.77
Root of mangrove	25	I	2.02±0.80	1.90±0.91	1.70±1.20
		II	1.68±0.30	2.11±0.65	1.47±0.63
		III	1.78±0.12	2.11±0.44	1.46±0.33
		IV	2.09±0.24	2.22±0.28	1.82±0.38
		Control	21.72±0.81	25.42±0.60	24.75±0.42

The test results for the *S. aureus* bacteria indicated that the *C. rotundata* seagrass sample with a concentration of 250 ppm exhibited the highest antibacterial activity among the samples tested, with a clear zone diameter of 3.17 mm. Additionally, antibacterial activity was observed in *E. acoroides* seagrass samples, with the largest clear zone area measuring 2.13 mm, and in *A. ilicifolius* mangroves, with clear zone

areas of 2.62 mm in leaf samples and 2.22 mm in root samples. No antibacterial activity was detected in the sea cucumber *H. atra* and the gastropods *T. setosus* and *T. fenestratus* against the *S. aureus* bacteria.

Phytochemical tests on marine plants and invertebrate animals. Phytochemical tests conducted on the extracts of plants and marine animals found that all samples contained flavonoid compounds. Samples containing alkaloid compounds included mangroves (*A. ilicifolius)*, sea cucumbers (*H. atra*), and gastropods (*T. setosus* and *T. fenestratus*). Steroid compounds were identified in the samples of seagrass (*E. acoroides*), sea cucumbers (*H. atra*), and gastropods (*T. setosus* and *T. fenestratus*). Saponin compounds were identified in samples of sea cucumbers *(H. atra)*, *while tannin* compounds were identified in the samples of mangroves (*A. ilicifolius).*

Gas chromatography–mass spectrometry (GC–MS) results of the extract. The GC–MS test conducted on the extract of the *C. rotundata* seagrass revealed the presence of 80 peaks, as shown in Figure 2a. The peak with the highest percentage was 9-Octadecenoic acid (Z)-, methyl ester at 29.73%. Conversely, the lowest peak detected was 9-octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis-, which was not identified in the analysis. Among the 80 peaks, there were 10 peaks with varying percentages, including hexadecanoic acid, methyl ester at 9.98%, 9,12-octadecadienoic acid (Z, Z)-, methyl ester at 9.14%, n-hexadecanoic acid at 7.36%, neophytadiene at 4.30%, methyl stearate at 3.38%, 9-octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester at 3.09%, 1-tetradecanol, 14-chloro- at 2.55%, bicyclo[9.3.1]pentadeca-3,7-dien-12-ol, 4,8,12,15,15-pentamethyl-, [1R-(1R*,3E,7E,11R*,12R*)]- at 2.23%, and trans-13-Octadecenoic acid at 1.96%.

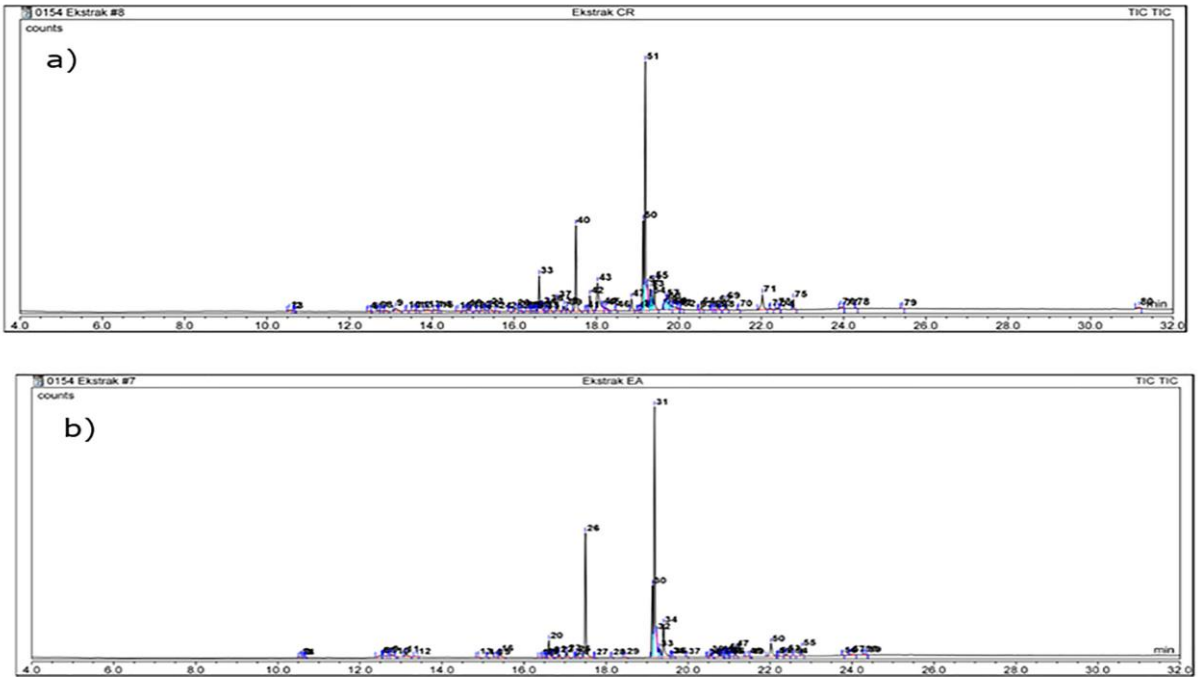

Figure 2. GC–MS chromatography of the seagrass extract *(a) Cymodocea rotundata* and (b) *Enhalus acoroides.*

The GC–MS test conducted on the *E. acoroides* seagrass extract revealed 59 peaks, as shown in Figure 2. The highest peak identified was 9-octadecenoic acid (Z)-, methyl ester, with a percentage of 40.71%. Conversely, the lowest peak detected was 13,16-octadecadiynoic acid, methyl ester, with a percentage of 0.00%. Among all the peaks, the top 10 peaks were determined based on their respective percentages. These peaks include 9-octadecenoic acid (Z)-, methyl ester at 40.71%, hexadecanoic acid, methyl

ester at 19.10%, 9,12-octadecadienoic acid (Z, Z)-, methyl ester at 9.99%, methyl stearate at 5.95%, oleic acid, 3-hydroxypropyl ester at 3.74%, neophytadiene at 2.74%, cis-13-octadecenoic acid at 1.70%, docosanoic acid, methyl ester at 1.16%, eicosanoic acid, methyl ester at 1.08%, and undecanoic acid, 10-methyl-, methyl ester at 1.00%.

The GC–MS chromatography test conducted on the *A. ilicifolius* mangrove leaf extract revealed 63 peaks in the spectrum shown in Figure 2b. The highest peak, trans-13-Octadecenoic acid, was observed at peak 19, with a total percentage of 33.02% as indicated in Table 1. Conversely, the lowest peaks were identified at peak numbers 2, 62, and 1, corresponding to acetamide, N-methyl-N-[4-[4-fluoro-1-hexahydropyridyl]-2-butynyl]-, 3-pyridinecarboxylic acid, 2,7,10-tris(acetyloxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro-1,1,3,6,9-pentamethyl-4-oxo-4a,7a-epoxy-5H-cyclopenta[a]cyclopropa[f]cycl oundecen-11-yl ester, [1aR-(1aR*,2R*,3S*,4aR*,6S*,7S*,7aS*,8E,10R*,11R*,11aS*)]-, and ethyl iso-allocholate, with a total percentage of 0.09% as shown in Figure 2b.

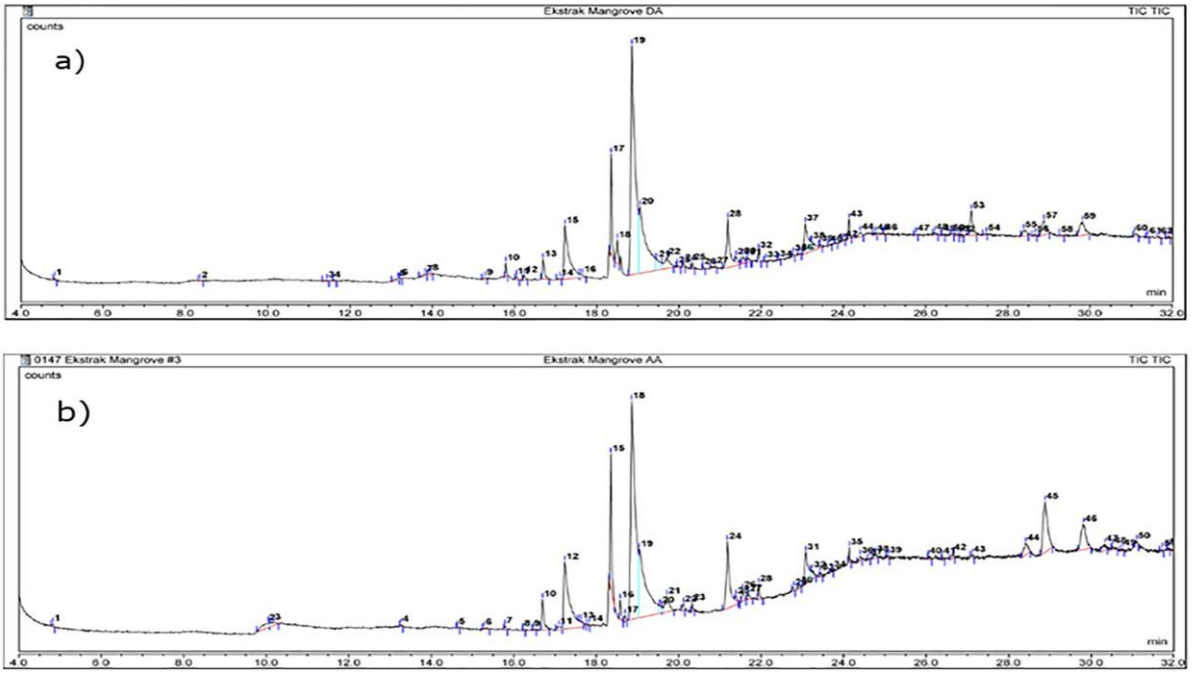

Figure 3. GC–MS chromatography from the *Acanthus ilicifolius* (a) mangrove leaf and (b) root.

During the GC–MS chromatography test conducted on the *A. ilicifolius* mangrove leaf extract, 63 peaks were observed in the spectrum shown in Figure 3a. The peak with the highest intensity was identified as trans-13-octadecenoic acid at peak 19, accounting for 33.02% of the total. Conversely, the lowest peaks were detected at peak numbers 2, 62, and 1, corresponding to acetamide, N-methyl-N-[4-[4-fluoro-1-hexahydropyridyl]-2-butynyl], 3-pyridinecarboxylic acid, and ethyl iso-allocholate, collectively amounting to 0.09% as depicted in Figure 3b.

The GC–MS chromatogram test conducted on the gastropod (*T. fenestratus*) extract revealed 51 visible peaks in the spectrum in Figure 4a. The peak with the highest intensity is peak number 34, identified as n-hexadecanoic acid, with a percentage of 12.72%. The gastropod (*T. setosus*) extract underwent a GCMS chromatogram test, revealing 38 peaks in Figure 3a. The highest peak, peak 34, is n-hexadecanoic acid, representing 12.72% of the total. In contrast, the lowest peak is the first peak, l-Gala-l-ido-octose, with a percentage of 0.1%. There is a significant difference of 21.91% between the highest and lowest peaks. Additionally, in terms of retention time, the 51st peak, 17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a] phenanthren-3-ol, had the highest retention time value of 28.79 minutes, while the lowest retention time value was found in l-Gala-l-ido-octose at 4.02

minutes. After conducting a GC–MS chromatogram test on the gastropod (*T. setosus*) extract, 38 peaks were observed in the spectrum shown in Figure 4b. The peak with the highest intensity was peak 34, identified as n-hexadecanoic acid, accounting for 12.72% of the total. In contrast, the lowest peak was the first peak, identified as l-Gala-l-ido-octose, representing only 0.1% of the total. The difference between the highest and lowest peaks is significant, amounting to 21.91%. The compound 17-(1,5-dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren -3-ol, found in peak 38, has the highest retention time of 28.8 minutes. On the other hand, acetamide, N-methyl-N-[4-[4-fluoro-1-hexahydropyridyl]-2-butynyl]- has the lowest retention time of 8.01 minutes.

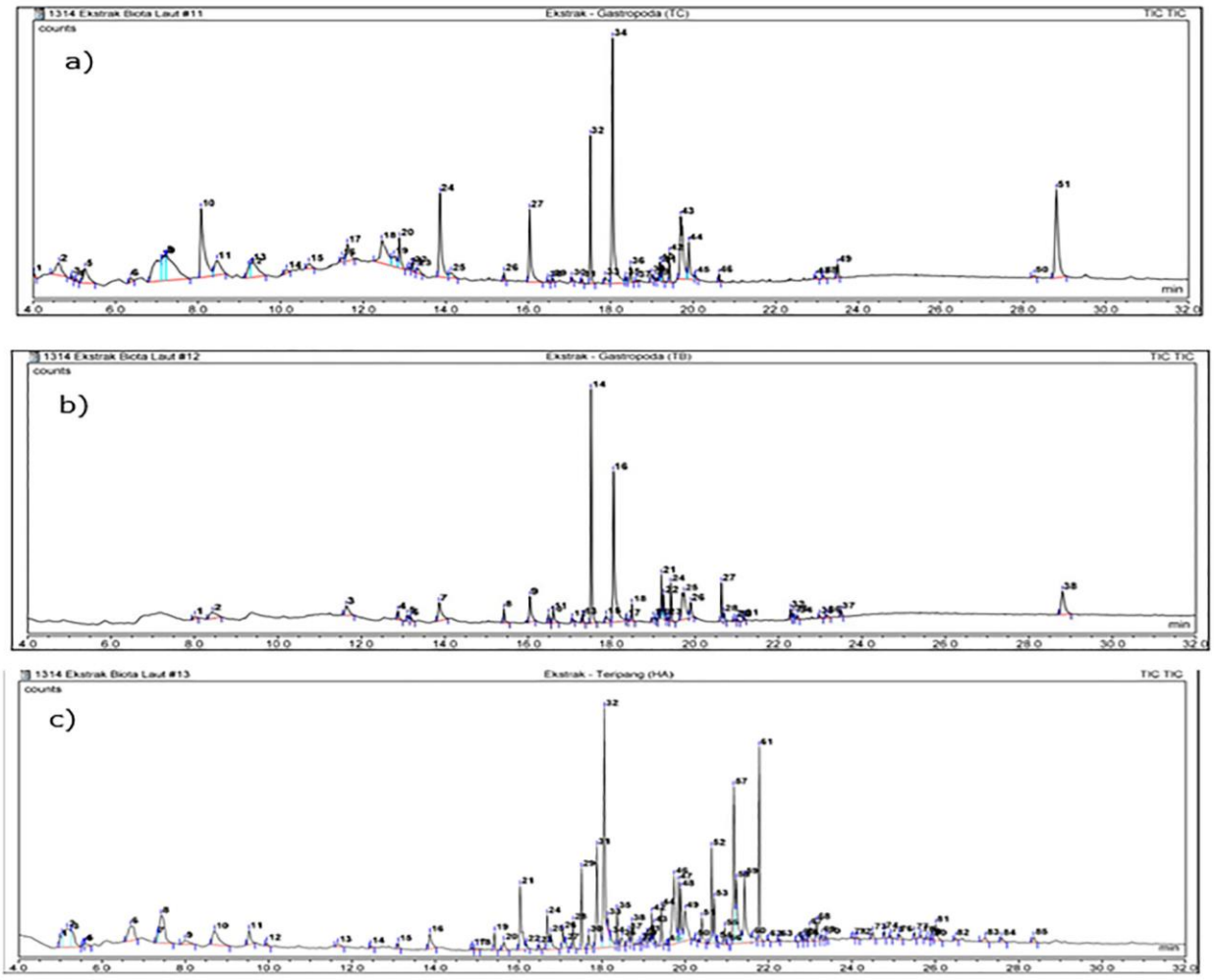

Figure 4. GC–MS chromatography of the sea cucumber and gastropod extract (a) *Tectus fenestratus*, (b) *Turbo setosus*, and (c) *Holothuria atra.*

An analysis using a GC–MS spectrometer was conducted on an extract from the sea cucumber *H. atra*, resulting in 85 peaks. The highest peak identified was n-Hexadecanoic acid, accounting for 10.78% of the total peaks, while the lowest peak was cis-13-eicosenoic acid, representing 0.04%. The top ten peaks, listed from highest to lowest, are n-hexadecanoic acid, arachidonic acid, palmitoleic acid, trans-13-octadecenoic acid, hexanedioic acid, bis(2-ethylhexyl) ester, 1-nonene, cis-5,8,11,14,17-eicosapentaenoic acid, cis-13-eicosenoic acid, tetradecanoic acid, and octane, 1-chloro-. The percentages of various acids in these peaks are as follows: arachidonic acid is 5.98%, palmitoleic acid is 5.52%, trans-13-octadecenoic acid is 5.18%, hexanedioic acid bis(2-ethylhexyl) ester is 5.13%, 1-nonene is 3.97%, cis-5,8,11,14,17-eicosapentaenoic acid is 3.94%, cis-13-eicosenoic acid is 3.92%,

tetradecanoic acid is 3.69%, and octane, 1-chloro- is 3.60%. This information is depicted in Figure 4c.

This research explored the health benefits of bioactive compounds derived from marine plants and invertebrates such as seagrass, mangroves, sea cucumbers, and mollusks. One notable finding was the presence of antibacterial properties in the *C. rotundata* seagrass samples at a concentration of 500 ppm (Table 1). These antibacterial properties are believed to be attributed to alkaloid compounds and tannins found in the seagrass. The study revealed that seagrass, mangroves, sea cucumbers, and gastropods contain alkaloids, terpenoids, steroids, flavonoids, saponins, and tannins, which are compounds with potential antibacterial effects. Similar to previous research findings, these compounds have been shown to possess potent antibacterial properties and can effectively inhibit bacterial growth. These compounds include alkaloids, terpenoids, steroids, anthraquinones, flavonoids, saponins, and tannins (Shamsudin et al 2022). The research findings also indicate that the bioactive compounds found in seagrass have demonstrated varying antibacterial properties against harmful bacteria. For instance, the extract from *Halodule pinifolia* seagrass using n-hexane solvent can act as an antibacterial agent against multi-drug resistant (MDR) *E. coli* strains (Kusuma et al 2023). Therefore, seagrass is a valuable source of antibacterial compounds that could be further developed.

The testing results of the mangrove *A. ilicifolius* also showed significant antibacterial activity against the *E. coli* bacteria. Chemical content testing of the mangrove revealed that flavonoid compounds are present in *A. ilicifolius* and are known to have strong antibacterial activity. However, the surprising result was the absence of antibacterial activity in the samples of sea cucumber *H. atra* and the gastropods *T. setosus* and *T. fenestratus*.

Sea cucumbers and gastropods have been used in traditional medicine for a long time. They contain bioactive compounds such as holothurin and conchalexin in sea cucumbers and alkaloids and flavonoids in sea cucumbers and gastropods, which have health benefits. However, factors like inappropriate extraction processes or differences in the composition of bioactive compounds can affect these results and their antibacterial activity. In addition, the environmental conditions in which marine plants and invertebrates live can also affect the bioactive compounds they produce. For instance, marine plants living in polluted or chemically contaminated areas may produce different compounds than those living in unpolluted environments (Figure 1, Figure 2, and Figure 3). Furthermore, the extraction process of bioactive compounds from marine plants and invertebrates can impact their ability to combat bacteria and fungi. Certain bioactive compounds may not dissolve well in the solvent, leading to ineffective extraction. Research in the pharmaceutical and health fields has long focused on the potential of bioactive compounds in marine plants and invertebrates. These organisms, which live in brackish and marine environments under specific ecological conditions, possess various compounds with biological activities such as antibacterial and antifungal properties. They have an effective defence system against pathogens and microorganisms. Studies have demonstrated that these compounds could serve as effective antibacterials for treating bacterial infections, with sea cucumbers being one example (Pringgenies et al 2017; Wodi et al 2024).

The GC–MS test conducted on the *C. rotundata* seagrass extract revealed the presence of 80 peaks. Among these peaks, 9-octadecenoic acid (Z)-, methyl ester was the highest, accounting for 29.73% of the total. This compound is a fatty acid known for inhibiting bacterial growth. This finding aligns with previous research indicating that fatty acid compounds exhibit strong antibacterial properties against various bacteria (Tafti et al 2019). Moreover, there are nine other compounds present in the extract with notable percentages, such as hexadecanoic acid, methyl ester (9.98%). Hexadecanoic acid, methyl ester is an organic compound with a carbon chain that is 16 atoms long and a bonded ester group. This compound possesses strong antibacterial and antifungal properties due to the natural preservative properties of hexadecanoic acid methyl ester, which stem from its lengthy carbon chain. The carbon chain disrupts the permeability of cell membranes and the metabolic processes of microorganisms, thereby halting the

growth of bacteria and fungi. Furthermore, methyl ester compounds are easily soluble in fats and oils, allowing them to penetrate the fat layer on the cell walls of bacteria and fungi. This characteristic makes the compound effective in inhibiting the growth and reproduction of lipophilic microorganisms. The compound n-hexadecanoic acid is both antibacterial and antifungal due to its antimicrobial properties, which can hinder the growth of bacteria and fungi. The chemical structure of these fatty acids can break down the lipid layer on bacterial and fungal cell membranes, leading to damage and death of these microorganisms. Additionally, n-hexadecanoic acid can impact the metabolism of microorganisms and inhibit the production of enzymes necessary for cell growth and development. Therefore, n-hexadecanoic acid can serve as an active ingredient in cleaning products and natural preservatives to prevent the growth of bacteria and fungi in cosmetic and food products.

The GC–MS test results on the *E. acoroides* seagrass extract indicate that it contains natural compounds that have the potential to act as antibacterial or antifungal agents. The main compound identified in the extract is 9-octadecenoic acid (Z)-, methyl ester, which makes up 40.71% of the total content. This compound has been extensively researched and shown to exhibit strong antibacterial activity against various bacteria, including *S. aureus* and *E. coli* (Table 1 and Table 2). Besides, it has a notable antifungal effect on the fungi Malassezia furfur and *C. albicans* (Pringgenies & Setyati 2023). Another ingredient with a high percentage in seagrass extract is hexadecanoic acid, methyl ester at 19.10%. This compound has been proven to have antibacterial activity against many types of bacteria, including bacteria resistant to antibiotics. This compound has also been proven effective as an antifungal agent, particularly in inhibiting the growth of fungi. Several other compounds found in seagrass extracts also have the potential for antibacterial or antifungal properties. For instance, methyl stearate has been shown to effectively inhibit the growth of Salmonella and E. coli. Furthermore, neophytadiene and cis-13-octadecenoic acid have been demonstrated to possess antifungal activity.

The results of the GC–MS chromatography test indicate that *A. ilicifolius* mangrove leaves contain various compounds that have the potential to act as antibacterial or antifungal agents. Among these compounds, trans-13-octadecenoic acid stands out at 33.02%. This compound, an unsaturated fatty acid, is known for its ability to combat bacteria and fungi by penetrating cell membranes and disrupting enzyme function. Extensive research has shown its effectiveness against various bacteria and fungi, including *S. aureus*, *E. coli*, and *C. albicans* (Pringgenies & Setyati 2023). In addition, the test results revealed the presence of the compound acetamide, N-methyl-N-[4-[4-fluoro-1-hexahydropyridyl]-2-butynyl]- with a concentration of 0.09%. This compound is a derivative of amine amide known to possess potential antibacterial and antifungal properties. It hinders protein synthesis and interferes with lipid metabolism in bacteria and fungi. Previous research has demonstrated that this compound can impede the growth of the *S. aureus* and *E. coli* bacteria.

The chromatography test also indicated the presence of the compound 3-pyridinecarboxylic acid, 2,7,10-tris(acetyloxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro-1,1,3,6,9-pentamethyl-4-oxo-4a,7a-epoxy-5H-cyclopenta[a]cyclopropa[f]cycloundecen-11-yl ester, [1aR-(1aR*,2R*,3S*,4aR*,6S*,7S*,7aS*,8E,10R*,11R*,11aS*)]- with a percentage of 0.09%. This compound is an ester compound, which has been shown to possess antimicrobial activity against various types of bacteria, including *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa* (Song et al 2023). Similarly, the *A. ilicifolius* mangrove root extract contains 52 peaks, with the highest peak being number 18, identified as trans-13-octadecenoic acid, comprising 28.89% of the total. This compound shows potential as an antibacterial and antifungal agent.

The results of the GC–MS chromatogram test on the gastropod (*T. fenestratus*) extract revealed the presence of several compounds that may have antibacterial or anti-fungal properties. Previous studies have demonstrated that compounds present in gastropods exhibit antimicrobial effects. One such example is the compound n-hexadecanoic acid, which is the most prominent peak in the chromatogram results (Pringgenies & Setyati 2023). This compound has been shown to exhibit antibacterial and antifungal properties, with different mechanisms of action depending on the targeted

microorganism. Additionally, the compound 17-(1,5-dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol, which has the longest retention time in the chromatogram results, is also recognized for its antimicrobial activity. The presence of these two compounds suggests that the *T. fenestratus* extract has the potential to serve as an antibacterial or antifungal agent for the treatment of bacterial or fungal infections (Babar 2016). Furthermore, this potential can also be used in the development of more effective medicines and aims to prevent microorganisms from developing resistance to antibiotics, a problem that is currently increasing. The results of the GC–MS chromatogram test on the *T. setosus* extract revealed several peaks, including n-hexadecanoic acid and 9-octadecenoic acid, which are compounds known to possess strong antibacterial and antifungal properties against various types of microorganisms. Compounds like 7-hydroxycolestene-3-one and 17-octadecynoic acid also show potential as antibacterial and antifungal agents derived from the body of *T. setosus* (Figure 4). Aquatic invertebrates are the primary source of biomaterials and bioactive natural products that can be utilized in various fields such as pharmaceuticals, nutraceuticals, cosmetics, antibiotics, antifouling products, and biomaterials. These compounds are unsaturated fatty acid compounds, many of which have the potential to exhibit antibacterial and antifungal properties (Guimarães & Venâncio 2022; Setyati et al 2023b).

The results of the GC–MS chromatogram test on the gastropod extract revealed 51 peaks, with the highest peak belonging to the compound n-hexadecanoic acid, accounting for 12.72% of the total. This compound is recognized for its antibacterial and antifungal properties (Idris et al 2022), indicating that *T. fenestratus* extract contains compounds that could serve as antimicrobial agents. Sea cucumbers of the species *H. atra* contain several active compounds that exhibit antibacterial and antifungal properties. Some of these compounds include n-hexadecanoic acid, arachidonic acid, palmitoleic acid, trans-13-octadecenoic acid, cis-13-eicosenoic acid, and cis-5,8,11,14,17-eicosapentaenoic acid. These compounds are present in the symbiotic bacteria of the sea cucumber *H. atra*. For example, palmitoleic acid has the potential to act as an antibacterial agent (Ningsih et al 2020). Research has shown that the symbiotic bacteria of sea cucumbers can serve as antibacterial agents that are resistant to multiple drugs (Pringgenies et al 2017). The bioactive compounds of the symbiont are similar to those of its host. Therefore, it can be concluded that the compounds found in the *H. atra* sea cucumber have the potential to be antimicrobial and useful as a functional food (Wodi et al 2024).

Conclusions. Marine plants and invertebrate animals, such as seagrass, mangroves, sea cucumbers, and gastropods, have the potential to be sources of bioactive compounds that can be utilized as antibacterials and antifungals. Analysis conducted using the GC–MS technique indicates that these samples contain compounds with antibacterial and antifungal properties. Flavonoid compounds were present in all samples examined, with alkaloids, steroids, and tannins also being identified in several samples. On the other hand, saponin compounds, which can act as antimicrobial substances, were found only in sea cucumber samples. This discovery suggests that sea cucumbers can be used as a natural alternative for maintaining health. The results also indicate that marine plants and invertebrate animals have significant potential as sources of bioactive compounds that could be utilized in clinical and pharmaceutical applications for health maintenance.

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