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[IK.IJMS] Submission Acknowledgement

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[IK.IJMS] SCREENING AND PROFILING OF ANTIOXIDANT ACTIVITY IN MUD CRAB (Scylla serrata) FROM BANYUASIN WATERS

3 pesan

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Dear
Authors

Thank you for submitting your manuscript "SCREENING AND PROFILING OF ANTIOXIDANT ACTIVITY IN MUD CRAB (Scylla serrata) FROM BANYUASIN WATERS" to ILMU KELAUTAN: Indonesian Journal of Marine Sciences. The editorial team and a group of expert reviewers have assessed your submission and feel that it has potential for publication. We would like to invite you to revise the paper and resubmit it for further review.

Comments :
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[Kutipan teks disembunyikan]
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Rozirwan ROZIRWAN <rozirwan@unsri.ac.id> 23 Februari 2025 pukul 22.16
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Dear Editor,

We have carefully reviewed the comments and have made the necessary revisions as per reviewer's instructions. Here, we attach

the revision of our article "SCREENING AND PROFILING OF ANTIOXIDANT ACTIVITY IN MUD CRAB (Scylla serrata) FROM BANYUASIN WATERS".
We believe that these revisions address the issues raised, and we hope that they meet the requirements for publication in your journal. Please let me know if any further revisions are needed.

Thank you
Best regards
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2 lampiran

Response to Reviewer_3_IJMS.docx
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Response to Reviewers

Reviewer Comments	Response
Abstract must be 250 words	The abstract has been revised to ensure it contains exactly 250 words as requested.
Keywords must be five words	The keywords have been adjusted to include exactly five words.
Reference published in 2019-2024, 80% from international journals (minimum 25 references)	We have updated the references to ensure that at least 80% are from international journals published between 2019-2024. The total number of references now meets the required minimum of 25.
Check reference using Harvard Style (check format reference in manuscript published 2024)	The references have been formatted according to the Harvard Style, following the guidelines from a 2024 published manuscript.
Use SI unit (ex: $mg.L^{-1}$)	All measurement units have been revised to follow the SI unit system (e.g., $mg \cdot L^{-1}$).
Delete Table 6, we think it is not necessary	Table 6 has been removed as per the reviewer's suggestion.
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SCREENING AND PROFILING OF ANTIOXIDANT ACTIVITY IN MUD CRAB (*Scylla serrata*) FROM BANYUASIN WATERS

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Abstract

Mangrove crab (*Scylla serrata*) as one of the crustacean species, has a variety of bioactive compounds that can be utilized in the field of pharmacology. Antioxidant compounds act as therapeutic agents against degenerative diseases. Banyuasin waters have mangrove vegetation with associated marine organisms that have the potential to be studied for bioactive compounds. This study aims to identify the phytochemical profile quantitatively and qualitatively, samples were collected from mud flats near mangrove ecosystems in Banyuasin waters, South Sumatra. Samples were tested for antioxidant activity using the DPPH test, and IC50 values, qualitative phytochemical identification, and phytochemical profiles were calculated using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Based on the results of antioxidant testing, the IC50 value of *Scylla serrata* extract is 2.25 ppm, the sample is included in the category of very strong antioxidants. Phytochemical test results showed that the compound is thought to contain antioxidant activity from flavonoids and triterpenoids. GC-MS analysis detected major compound groups of alkaloids, purines, and vitamins. Minor compound groups detected amines, terpenoids, monosaccharides, amino acids, fatty acids, silanes, formamides, heterocycles, carboxylic acids, aminoglycosides, naphthalene derivatives, nitriles, amides, glycosides, and peptides. *Scylla serrata* extract shows very strong antioxidant activity, with major compounds such as alkaloids, purines, and vitamins. *Scylla serrata* extract detected compounds that have been reported as anti-inflammatory, anticancer, antimicrobial, and antiviral. These findings highlight the pharmaceutical potential of *Scylla serrata* as a source of bioactive compounds. The results of this study provide valuable information for the development of alternative medicines derived from marine organisms.

Keywords: Antioxidant, Bioactive compounds, DPPH, GC-MS, *Scylla serrata*

Introduction

Scylla serrata or mud crab, as one of the crustacean species, has great potential in the field of beauty and health (De Castro *et al.*, 2023), because of its diverse bioactive compounds (Yusof *et al.*, 2020; Beslin and Geni, 2021; Neelima *et al.*, 2022). The diversity of bioactive compounds triggers great potential in the search for alternative medicinal ingredients from marine organisms, including antioxidant compounds (Yogeshwaran *et al.*, 2020; Karnila and Ramadhani, 2021; Fajriaty *et al.*, 2024). Antioxidant compounds in mud crabs have a role as therapeutic agents (Wu *et al.*, 2021) in fighting degenerative diseases such as cancer (Nagarajan *et al.*, 2024), cardiovascular diseases (Nanda *et al.*, 2021), and neurodegenerative disorders (Galal-Khallaf *et al.*, 2024), caused by oxidative stress due to free radicals.

Antioxidant is the reaction of a compound in neutralizing free radicals (Delta *et al*., 2021; Rozirwan *et al*., 2023; Rozirwan *et al*., 2023). This process involves a highly efficient electron donation mechanism. Antioxidants work by donating electrons to free radicals (Frías-Espericueta *et al.*, 2022; Pati *et al*., 2022; Yang *et al*., 2023; Fajriaty *et al*., 2024). Free radicals, which have one or more unpaired electrons, are highly reactive and can cause oxidative damage to DNA, proteins, and cellular lipids (Alkadi, 2020; Di Meo and Venditti, 2020). Free radicals are often generated as by-products of various metabolic processes in the body or due to environmental exposures such as pollution and ultraviolet radiation (Martemucci *et al*., 2022; Sadiq, 2023). The damage caused by free radicals can contribute to the development of various degenerative diseases, including cancer, heart disease, and neurodegenerative disorders (Teleanu *et al*., 2022; Chaudhary *et al*., 2023). In inhibiting free radicals, antioxidant compounds such as carotenoids and polyphenols will interact with free radicals to enhance the activity of detoxification enzymes resulting in accelerated elimination of free radicals and strengthened antibodies (Pisoschi *et al*., 2021; Tumilaar *et al.*, 2024).

The analysis of antioxidant compounds in *Scylla serrata* requires an accurate technique to ensure identification and quantification are targeted (Baag and Mandal, 2023; Yao *et al*., 2023). The gas Chromatography-Mass Spectrometry (GC-MS) analysis method was chosen as an effective method in the analysis of bioactive compounds (Jabbar *et al*., 2022; Musa *et al*., 2022; Palma *et al*., 2023; Rozirwan *et al*., 2024). This method allows the separation and identification of compounds based on their density and chemical characteristics. This method is able to identify the diversity of antioxidant compounds in *Scylla serrata* meat with high accuracy while being able to measure the concentration of each compound analyzed. Antioxidant compounds such as carotenoids and polyphenols were identified through GC-MS analysis, showing significant therapeutic potential (Berwal *et al*., 2021; Vellapandian, 2022; Palaniyappan *et al*., 2023). The identified antioxidant content offers substantial health benefits to humans as an alternative medicine.

Previous studies have extracted various body parts of *Scylla serrata* and shown the content of antioxidant compounds such as flavonoids, carotenoids, and polyphenols (Yogeshwaran *et al.*, 2020; Karnila and Ramadhani, 2021; Neelima *et al*., 2022; Yang *et al*., 2023; Fajriaty *et al*., 2024). The results showed that some of these compounds are classified with high antioxidant characteristics. However, specific data on the type and quantity of antioxidant substances from this organism are still limited. Therefore, in-depth and comprehensive research is needed to reveal the potential of *Scylla serrata* as a source of antioxidants. This study aims to identify the profile of antioxidant compounds in *Scylla serrata*. Thus, the potential of *Scylla serrata* as a source of alternative medicinal ingredients can be further explored and optimally utilized.

Materials and Methods

Sampling Area

Mangrove crab samples were collected in January 2023 from Banyuasin Waters, South Sumatra, Indonesia (Figure 1). At this location, numerous crustacean and gastropod populations were found inhabiting the intertidal zone (Almaniar, Rozirwan and Herpandi, 2021; Rozirwan *et al*., 2021; Nugroho, Rozirwan and Fauziyah, 2022). The crab fishing area had a mud substrate with a depth of 1–2 m and was located within a mangrove vegetation zone directly connected to port and pond activities (Fitria *et al.*, 2023). Anthropogenic pollutants that accumulate in these waters are known to trigger an increase in the defense mechanisms of organisms, such as the production of antioxidant compounds derived from secondary metabolites (Rozirwan *et al*., 2023).

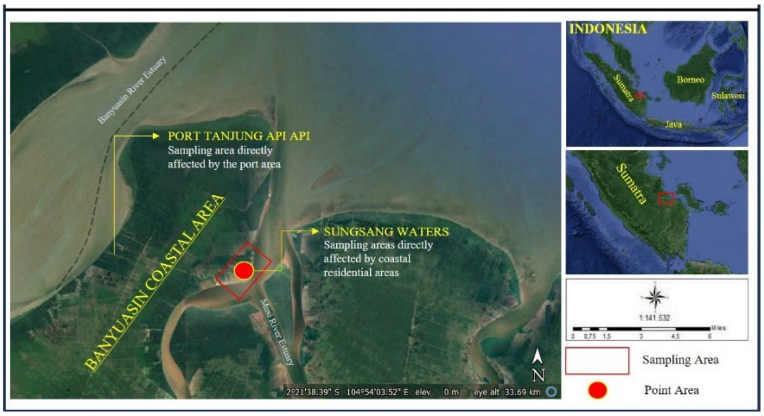

Figure 1. Map of Sampling Location in Banyuasin Waters Area

Samples Catch, Collage, Storage and Identification

Crustacean samples were taken using folding trawl gear. The samples were collected and stored in a cool box. The crab identification process was conducted based on the examination of morphological characteristics, such as body shape, color pattern, claw shape, and leg shape (Hidir *et al.*, 2021; Vermeiren, Lennard and Trave, 2021). Morphometric measurements were performed on the crab samples, and the identification process was completed in the laboratory. Taxon determination was carried out using data from the World Register of Marine Species (WoRMS) accessed in January 2023 (WoRMS, 2023).

Environmental Characteristics of Sampling Area

Environmental quality calculations were conducted to assess the condition of the sampling environment. Environmental parameter data were measured, including salinity, temperature, pH, and dissolved oxygen (DO) (Fitria *et al*., 2023; Rozirwan *et al*., 2024). Each parameter was measured in three repetitions to ensure consistency, and the results were then averaged. Environmental parameter measurements are typically used to evaluate habitat conditions, as they provide insights into the physical and chemical characteristics of the ecosystem. Repetition in measurements is a standard approach to improve data reliability.

Sample Preparation

The preparation method described by Ambekar *et al*. (2023), involves cleaning the crab to remove contaminants. In this study, the carapace and crab meat were separated and rinsed with distilled water to eliminate any remaining impurities. The wet weight of the crab meat was measured, and the samples were then dried in an oven at 40°C for 3 × 24 h. After drying, the samples were ground into powder using a blender. The dry weight of the crab meat was recorded for data analysis.

Sample Maceration and Extraction

The wet maceration method was used in this study. A total of 250 g of *Scylla serrata* meat powder was weighed and immersed in 1000 mL of 96% ethanol solvent at a ratio of 1:4 (b/v). The soaking process was conducted for 3 × 24 h, with stirring performed periodically to ensure optimal extraction. The maceration results were filtered using filter paper (No. 42, 125 mm). The extraction process was then carried out using a rotary evaporator at 40°C with a rotating speed of 3000 rpm (Hashim *et al.*, 2021). The resulting extract was stored as a stock solution. A total of 0.05 g of *Scylla serrata* extract was used as an additive for the stock solution (Habib *et al.*, 2022). Wet maceration is a commonly employed extraction method due to its ability to preserve heat-sensitive compounds. Stirring during the maceration process enhances solute dissolution, while rotary evaporation ensures efficient solvent removal under controlled temperature conditions.

Determination of Antioxidant Activity and IC50 Value

Antioxidant testing was conducted using the DPPH method (Vásquez, Cian and Drago, 2023). The stock solution of *Scylla serrata* extract was used as the test solution for antioxidant activity, while vitamin C solution served as a reference. The sample was dissolved in 96% ethanol. A total of 1 mL of sample solution was prepared for each concentration: 100 $mg.L^{-1}$, 150 $mg.L^{-1}$, 200 $mg.L^{-1}$, 250 $mg.L^{-1}$, and 300 $mg.L^{-1}$. Each concentration was mixed with 40 $\mu g.mL^{-1}$ DPPH solution and incubated in the dark for 30 min. The absorbance values were measured at 517 nm using UV-Vis spectrophotometry. The percentage of inhibition and IC50 value were calculated (Naveed *et al.*, 2022). The characterization of antioxidant activity is determined based on the IC50 value, which represents the concentration of the sample required to inhibit 50% of DPPH radicals. The strength of antioxidant activity is classified according to IC50 values, as shown in Table 1. The IC50 value is calculated using the following formula.

$$inhibisi = \frac{blank\ abs - sample\ abs.}{blank\ abs}\ x\ 100\ \%$$

The IC50 results were entered in the linear regression equation Y = AX + B. The sample concentration is the abscissa (X-axis), and the percentage of antioxidant inhibition is the ordinate (Y-axis) (Yuniarti *et al.*, 2020).

Table 1. Characteristic concentration value of IC50

Concentration Value ($\mu g.mL^{-1}$)	Characteristic
<50	Very Strong
50-100	Strong
100-150	Moderate
150-200	Low

Phytochemical analysis

Phytochemical Screening

Phytochemical tests of *Scylla serrata* meat extracts were conducted using qualitative methods to identify the presence of bioactive compounds. The analysis included steroid and triterpenoid tests, which were performed using the Liebermann-Burchard method; alkaloid tests using Mayer and Dragendorff reagents; flavonoid tests with the Shinoda staining method; tannin tests using the $FeCl_3$ reaction; and saponin tests using the foam test method. Each test was carried out following the procedures described in standard literature (Suwandi, Ula and Pertiwi, 2020; Dinesh *et al.*, 2022).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The identification of bioactive compound components in *Scylla serrata* meat extract was performed using the GC-MS analysis method. GC-MS analysis was conducted following the method described by Rozirwan *et al*. (2022). A total of 1 µL of extract was injected into the column (rt x 5 ms) with helium as the carrier gas and a split ratio of 1:50. The oven temperature was set at 50°C for 5 min, then gradually increased at a rate of 5°C per minute to a maximum temperature of 280°C, which was maintained for 5 min. Samples were injected 280°C. The Wiley 7 Library database was used as a reference for comparing the spectra of the analyzed compounds (Rafferty *et al.*, 2020).

Data Analysis

Further data processing was performed by conducting normality tests (Shapiro-Wilk) and homogeneity tests (Levene) (Gebruk *et al.*, 2021; Ebadzadeh, Shojaei and Seyfabadi, 2024). All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation. Statistical analysis was carried out using SPSS software. One-way analysis of variance (ANOVA), followed by Duncan's multiple range test for post hoc comparisons, was used to identify significant differences among groups. Statistical significance was determined at $p < 0.05$.

Result and Discussion

Samples Catch, Collage, Storage and Identification

Taxon determination was conducted using data from the World Register of Marine Species (WoRMS) accessed in January 2023 (WoRMS, 2023). Based on their morphological characteristics, the crustacean samples were identified as *Scylla serrata*.

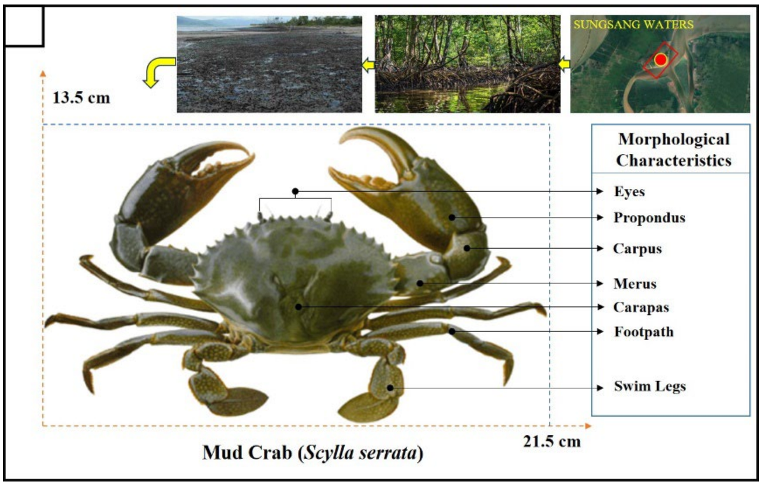

Figure 2. Crustacean species, *Scylla serrata*

Mangrove crabs of this species were caught using folding traps. The fishing process was carried out during low tide to facilitate crab capture. In this study, 2–5 crabs were used as stock samples. The crabs obtained were weighed, with weights ranging from 200 to 320 g, widths of approximately 21.5 cm, and lengths of around 13.5 cm. The crabs were then stored in a cool box filled with ice cubes for preservation.

The species identification of mangrove crabs is based on morphological characteristics, which include features such as the eyes, propound, carpus, merus, carapace, claws, walking legs, and swimming legs (Figure 2). Taxonomic data from databases such as WoRMS is widely used to confirm species classification accurately.

Environmental Characteristics of Sampling Area

Table 2. Observation of Environmental Parameters of the Research Site

Environment Parameter Quality	Station Sungsang Waters
Dissolved oxygen (mg.L^{-1})	4.2
pH	7
Temperature (°C)	28

Salinity (‰)	18

The results of environmental quality measurements in the *Scylla serrata* sampling areas in Banyuasin waters revealed diverse conditions. Measurements of water physicochemical parameters, including dissolved oxygen, pH, temperature, and salinity, were taken to assess the habitat suitability for *Scylla serrata* (Rozirwan *et al.*, 2021). The dissolved oxygen concentration was found to be 4.2 $mg.L^{-1}$, which is sufficient to support the respiration process of aquatic organisms (Ouyang *et al.*, 2021). The pH value of the water at the sampling location was 7, indicating neutral pH, which represents optimal ecological conditions (Chowdhury *et al.*, 2021). This is consistent with previous studies (Yusni and Haq, 2020; Muhtar and Lanuru, 2021; Putri *et al.*, 2022), which state that waters with a pH between 6.5 and 7.5 are ideal for the survival of mangrove crabs. The water temperature was measured at 28°C, indicating favorable conditions for mangrove crab growth (Indarjo *et al.*, 2020; Ren *et al.*, 2021) Water salinity was recorded at 18 ‰, reflecting typical conditions in estuarine areas or areas directly influenced by tidal movements (Wang *et al.*, 2021). *Scylla serrata* grows best in salinities between 15‰ and 25‰ but grows more slowly at salinities greater than 25 to 30‰ (Triajie *et al.*, 2020; Pati *et al.*, 2023; Adnan *et al.*, 2024).

Determination of Antioxidant Activity by DPPH Assay

Antioxidant analysis using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method on *Scylla serrata* meat extract showed promising results. The DPPH solution changed from purple to yellow, indicating the presence of antioxidants in the extract. The test results revealed that the *Scylla serrata* extract had an IC50 value of 2.25 ppm, while Vitamin C, used as a comparison solution, had an IC50 value of 2.16 ppm (Table 3). Both solutions demonstrated low IC50 values, categorizing them as very strong antioxidant compounds. The IC50 value, which is the concentration required to inhibit 50% of DPPH radical activity, is a critical parameter for assessing the antioxidant potential of a compound (Martinez-Morales *et al.*, 2020). The results indicate that *Scylla serrata* extract possesses an IC50 value comparable to that of Vitamin C. The percentage inhibition of DPPH free radicals by *Scylla serrata* meat extract increased as the extract concentration increased. This suggests that the extract has the ability to donate electrons or hydrogen to the DPPH radical, neutralizing it and converting it into a more stable form (Gulcin and Alwasel, 2023).

Table 3. Calculation results of antioxidant activity of *Scylla serrata* in Sungsang waters

Sample	Linear Regression			IC50 Value	Category
	a	b	R^2		
Scylla serrata	6.9429	52.808	0.9327	2.25 $mg.L^{-1}$	Very Strong
Asorbic Acid	6.7135	55.866	0.9435	2.16 $mg.L^{-1}$	Very Strong

The discovery of compounds such as flavonoids and triterpenoids, which were identified in the phytochemical analysis of *Scylla serrata* extracts, suggests that they may possess high antioxidant activity (Akinwumi *et al.*, 2022; Hajar-Azira *et al.*, 2023). Flavonoids are well known for their ability to capture free radicals and interrupt the chain of oxidative reactions. Triterpenoids are also recognized for their significant antioxidant activity through a similar mechanism. The combination of these compounds in *Scylla serrata* extract creates a synergistic effect, enhancing the overall capacity of the extract to neutralize free radicals.

Scylla serrata is known for its rich antioxidant system and strong enzymatic defense system, which help it survive in the dynamic and often challenging mangrove environment (Pati *et al.*, 2023). Mud crabs also possess defense enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, which work synergistically to detoxify reactive oxygen species (ROS) and maintain redox balance in the body (Jerome, Hassan and Chukwuka, 2020; Bal *et al.*, 2021; Costantini, Esposito and Ruocco, 2022; Zeng *et al.*, 2024). These enzymes play a crucial role in protecting the crab from oxidative stress generated by fluctuating environmental conditions, pollution, and pathogens.

This combination of antioxidant compounds and defense enzymes not only ensures the survival of mud crabs in their habitat but also positions them as a potential source for the development of natural health products that can harness their protective mechanisms.

Antioxidants and defense enzymes play a vital role in organisms' survival, especially in harsh environments. The synergy between these compounds and enzymes contributes significantly to mitigating oxidative stress and preserving cellular function, which can be explored for potential therapeutic applications.

Phytochemical Analysis

Phytochemical Screening

Phytochemical tests were carried out to identify the compounds present in gastropod and crustacean extracts using ethanol as the solvent (Fitria *et al*., 2023; Rozirwan *et al*., 2024) The phytochemical test aimed to determine the compounds in the test extract (Chen *et al.*, 2022), allowing for the identification of compounds that influence the strong or weak antioxidant activity of the extract (Baliyan *et al.*, 2022). The results of the phytochemical test, after UV-Vis spectrophotometric analysis of the extract, are presented in Table 4. The test results showed that only certain compounds were extracted by the ethanol solvent (Yuniarti *et al.*, 2020).

Table 4. Phytochemical screening results of *Scylla serrata*

No.	Parameters	Analysis Result	Analysis Type
1	Alkaloids	-	Qualitative
2	Flavonoids	+	Qualitative
3	Triterpenoids	+	Qualitative
4	Saponin	-	Qualitative
5	Tannins	-	Qualitative
6	Steroid	-	Qualitative

Qualitative phytochemical analysis of *Scylla serrata* extract showed significant results in identifying the content of bioactive compounds. Based on the test results, *Scylla serrata* extract tested positive for flavonoid and triterpenoid compounds. A similar finding was reported by Elshaarawy *et al*. (2023) for *Scylla olivacea* samples. These two compounds offer various health benefits and therapeutic potential, particularly due to their strong antioxidant properties. Flavonoids are a group of polyphenolic compounds that are widely recognized for their potent antioxidant activity (Shen *et al.*, 2022). These compounds can neutralize free radicals and prevent oxidative damage to cells and tissues. Additionally, flavonoids possess anti-inflammatory, anticancer, and cardioprotective properties (Mounika *et al.*, 2021; Jain *et al.*, 2024; Ullah *et al.*, 2024). The identification of flavonoid compounds in *Scylla serrata* indicates that mud crabs are not only valuable as food but also hold potential as an alternative source of medicine from marine organisms.

Triterpenoids are a group of terpenoid compounds that have potential biological activities (Mabou and Yossa, 2021; Zang *et al.*, 2022). These compounds are known to possess anti-inflammatory, antitumor, antimicrobial, and immunomodulatory properties (Harun *et al.*, 2020; Ahmad *et al.*, 2021). As antioxidant agents, triterpenoids have been used in pharmacology to treat inflammatory diseases and cancer. Similar to flavonoids, these compounds can neutralize free radicals caused by oxidative stress on body tissues. The discovery of triterpenoid compounds in *Scylla serrata* shows promising results, given their antioxidant potential that can be applied to address various diseases. Thus, the opportunity to explore alternative medicinal raw materials from *Scylla serrata* extract is increasingly attractive for further research. Overall, the phytochemical results focusing on flavonoid and triterpenoid compounds confirm the importance of *Scylla serrata* as a potential source of bioactive compounds. Further research is encouraged at the stage of isolation and purification of these compounds, so that alternative medicinal materials derived from this marine organism can contribute to the development of therapeutic agents from marine organisms.

Phytochemical Profile Screening

The antioxidant compound profile in *Scylla serrata* was determined using GC-MS (Gas Chromatography-Mass Spectrometry) analysis on the ethanol extract of mud crab. Figure 3 shows the chromatogram with 37 peaks identified in the extract. Each peak on the chromatogram represents a distinct chemical compound found in the extract, which was analyzed using GC-MS.

GC-MS analysis is a powerful technique for identifying and quantifying individual compounds in complex mixtures, providing insights into the chemical composition of the extract. This method is widely used for its ability to separate and identify volatile compounds, making it an essential tool for profiling bioactive compounds, such as antioxidants, in natural products.

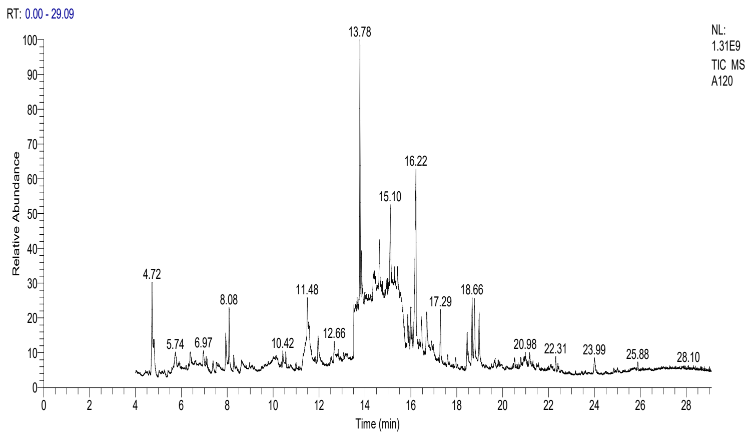

Figure 3. GC-MS chromatogram of ethanol extract *Scylla serrata*

Terpenoid, alkaloid, steroid, and tannin groups were among the pure compounds successfully detected using GC-MS. Based on the GC-MS analysis of the ethanol extract compound components presented in Table 5, the main components identified in the extract were Calycotomine, N-methyl-, with a value of 8.31% of the total area, and uric acid, with a value of 8.71% of the total area.

At a retention time of 4.72 minutes, the compound 2-Cyclohexylpiperidine was detected with an area of 3.45%, a probability of 6.07, and a chemical formula of C11H21N, which belongs to the alkaloid compound group. At a retention time of 5.74 minutes, the compound 2-Pyridinamine, 3,6-dimethyl was detected with an area of 1.69%, a probability of 7.02, and a chemical formula of C7H10N2, which belongs to the aminopyridine compound group. Furthermore, at a retention time of 6.97 minutes, the compound Pentanoic acid, dodec-9-ynyl ester was detected with an area of 1.00%, a probability of 8.47, and a chemical formula of C17H30O2, which belongs to the protein compound group.

At a retention time of 8.08 minutes, the compound L-Homoserine lactone, N, N-dimethyl was detected with an area of 1.97%, a probability of 82.23, and a chemical formula of C6H11NO2, which belongs to the amino acid compound group. At a retention time of 10.42 minutes, the compound Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetramethyl was detected with an area of 0.74%, a probability of 43.35, and a chemical formula of C11H14N2OS, which belongs to the EPA compound group. At a retention time of 11.48 minutes, the compound 1H-2-Indenol, 2,3,4,5,6,7-hexahydro-1-(2-hydroxy-2-methylpropyl) was detected with an area of 4.83%, a probability of 12.26, and a chemical formula of C13H22O2, which belongs to the lactone compound group.

At a retention time of 12.66 minutes, the compound dl-Lysine was detected with an area of 0.86%, a probability of 23.13, and a chemical formula of C6H14N2O2, which belongs to the amino acid compound group. At a retention time of 13.78 minutes, the compound Calycotomine, N-methyl- was detected with an area of 8.31%, a probability of 43.35, and a chemical formula of C13H19NO3, which belongs to the alkaloid compound group. At a retention time of 15.10 minutes, the compound Dasycarpidan-8(16H)-ethanol, 3,18-dihydro-1-(hydroxymethyl)-, (2.xi.,4.xi.)- was detected with an area of 4.14%, a probability of 12.00, and a chemical formula of C20H28N2O2, which belongs to the group of molport compounds.

At a retention time of 16.22 minutes, the uric acid compound was detected with an area of 8.71%, a probability of 55.93, and a chemical formula of C5H4N4O3, which belongs to the allantoin compound group. At a retention time of 17.29 minutes, the compound Actinomycin C2 was detected with an area of 1.49%, a probability of 30.73, and a chemical formula of C63H88N12O16, which belongs to a group of peptide compounds that are derivatives of peptide compounds.

Animals produce a diverse mixture of secondary metabolites such as phenols, alkaloids, flavonoids, tannins, and saponins. Several animal studies have shown the potential use of these metabolites as antibacterial agents due to the presence of abundant biomolecules. Synthetic drugs often have high secondary failure rates and severe side effects, while animal products contain a variety of free radical scavenging molecules with substantial antioxidant properties.

Research by Waluyo and Wahyuni (2021) on *Conus miles* bacteria identified three dominant compounds through GC-MS analysis, namely Acetic acid (CAS), Ethylic acid, Proanoic acid, 2-methyl-(CAS), and Isobutyric acid and Iso Valeric acid. Research on the ethanol extract of *Enhalus acoroides* conducted Mediarman *et al*., (2021) using GC-MS showed nine compounds dominated by alkaloids, flavonoids, terpenoids, and polyphenols. Some of the compounds found in *Enhalus acoroides* have similarities with the GC-MS results on *S. serrata*, but the percentage area of the alkaloid compound group in *S. serrata* was only 8.31%, compared to the ethanol extract of *Enhalus acoroides*, which reached 53.88%.

In the GC-MS results, the highest peak was identified as coming from the antioxidant compounds of the alkaloid group. However, in the phytochemical test, alkaloid compounds were not detected in the *S. serrata* sample. This could be due to differences in the analytical methods used. Phytochemical tests are qualitative, while GC-MS analysis is quantitative. Quantitative analysis methods are known to be more accurate than qualitative methods.

The difference in the results of phytochemical tests and GC-MS analysis can be influenced by the type of solvent used during the maceration process of mangrove crab (*S. serrata*) extracts. In this study, the solvent used was ethanol, which is polar and able to bind bioactive compounds quickly and is relatively safe for humans compared to methanol. Methanol solvent is more polar than ethanol but is more toxic. This is in accordance with research conducted by Rahmawati *et al*., (2023); Shofinita *et al*., (2024), which show that methanol is more polar but also more toxic than ethanol.

Table 5. Proposed peak order, retention time, probability, area, compound name, and molecular formula

Peak#	R. Time	Probability	Area%	Name	Molecular formula
1	4.72	6.07	3.45	2-Cyclohexylpiperidine	C11H21N
2	4.81	18.58	1.37	Edulan II	C13H20O
3	5.74	7.02	1.69	2-Pyridinamine, 3,6-dimethyl	C7H10N2
4	6.39	8.12	0.83	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	C16H28O3
5	6.61	11.07	0.66	d-Mannose	C6H12O6
6	6.81	50.91	0.89	Deoxyspergualin	C17H37N7O3
7	6.97	8.47	1.00	Pentanoic acid, dodec-9-ynyl ester	C17H30O2
8	7.93	51.03	1.42	trans-(2 Chlorovinyl)dimethylethoxysil ane	C6H13ClOSi
9	8.08	82.23	1.97	L-Homoserine lactone, N,N-dimethyl-	C6H11NO2
10	8.28	32.09	0.64	2-Propyl-tetrahydropyran-3-ol	C8H16O2
11	8.62	32.45	0.93	Imidazole	C3H4N2
12	10.00	11.83	0.76	Tertbutyloxyformamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-buty nyl]-	C14H24N2O2
13	10.42	15.27	0.74	Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetrameth yl	C11H14N2OS
14	11.48	12.26	4.83	1H-2-Indenol, 2,3,4,5,6,7-hexahydro-1-(2-hydroxy-2-methylpropyl)	C13H22O2
15	11.55	48.75	2.53	dl-Citrulline	C6H13N3O3
16	11.96	7.38	1.45	2-Pyridineacetic acid, hexahydro-	C7H13NO2
17	12.66	23.13	0.86	dl-Lysine	C6H14N2O2
18	13.51	60.28	1.62	D-Streptamine, O-6-amino-6-deoxy-à-D-glucopyranosy l-(1-4)-O-(3-deoxy-4-C-methyl-3-(meth ylamino)-á-L-arabinopyranosyl-(1-6))- 2-deoxy	C6H13NO2
19	13.78	43.35	8.31	Calycotomine, N-methyl-	C13H19NO3
20	13.99	17.84	2.38	4-[4-Diethylamino-1-methylbutylamino]-1,2-dimethoxy-6-bromonaphthalene	C21H31BrN2O2
21	14.35	32.83	1.22	Dasycarpidan-8(16H)-ethanol, 3,18-didehydro-1-(hydroxymethyl)-, (2.xi.,4.xi.)-	C20H28N2O2
22	14.62	12.2	2.67	4-[4-Diethylamino-1-methylbutylamino]-1,2-dimethoxy-6-bromonaphthalene	C21H31BrN2O2
23	15.10	12.00	4.14	Dasycarpidan-8(16H)-ethanol, 3,18-didehydro-1-(hydroxymethyl)-, (2.xi.,4.xi.)-	C20H28N2O2
24	15.21	32.49	0.87	Cystine	C6H12N2O4S2
25	15.65	33.28	1.26	3-[N-[2-Diethylaminoethyl]-1-cyclopes tenylamino]propionitrile	C14H25N3
26	15.86	16.52	0.92	á-Hydroxyquebrachamine	CH3CH(OH)COOH
27	15.99	14.23	1.02	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	C21H36O4
28	16.22	55.93	8.71	Uric acid	C5H4N403
29	16.45	8.30	1.33	Aminoacetamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-buty nyl]-	C5H4N4O3
30	16.69	32.32	2.06	Glucopyranuronamide, 1-(4-amino-2-oxo-1(2H)-pyrimidinyl)- 1,4-dideoxy-4-(D-2-(2-(methylamino) acetamido)hydracrylamido)-, á-D	C10H14N2O
31	16.90	29.78	0.80	1,2,4-Trioxolane-2-octanoic acid, 5-octyl-, methyl ester	C6H11NO6
32	17.29	30.73	1.49	Actinomycin C2	C19H36O5
33	18.45	37.85	2.00	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	C63H88N12O16
34	18.66	90.13	2.23	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	C63H88N12O16
35	18.76	60.11	2.62	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6 H-dipyrrolo[1,2-a:1',2'-d]pyrazine	C11H18N2O2
36	18.97	36	2.13	l-(+)-Ascorbic acid	C14H22
37	20.98	13.19	1.29	cis-13-Octadecenoic acid	C6H8O6
38	21.17	13.49	0.94	Ricinoleic acid	C18H34O2
39	23.99	31.48	0.89	Ergotaman-3',6',18-trione, 12'-hydroxy-2'-methyl-5'-(phenylmeth yl)-, (5'à)-	C18H34O3

The main group of compounds in the ethanol extract of mud crab (*S. serrata*) was represented by three peaks on the GC chromatogram, which had a higher percentage area than the others. These peaks were identified as Calycotomine, N-methyl- (8.31%), uric acid (8.71%), and Dasycarpidan-8(16H)-ethanol, 3,18-didehydro-1-(hydroxymethyl)-(2.xi.,4.xi.)- (4.14%). Antioxidant compounds detected in the GC-MS analysis included 2-Cyclohexylpiperidine with an area of 3.45%, which belongs to the alkaloid group. In addition, the Edullan II compound with an area of 1.37% belongs to the volatile compound group. The compound Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate, with an area of 0.83%, belongs to the terpenoid compound group, and Imidazole with an area of 0.93% also belongs to the alkaloid group.

In a study conducted by Rasyid (2016) on sea cucumber biota (*Bohadschia* sp.), steroid compounds consisting of cholest-2-ene (3.91%), stigmastan-3,5-diene (3.29%), cholest-5-en-3-yl nonanoate (4.89%), and cholest-5-ene, 3.beta.-chloro- (3.12%) were found. The compound Calycotomine, N-methyl- with an area of 8.31% belongs to the alkaloid group. The compound l-(+)-Ascorbic acid with an area of 2.13%, as well as 5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1',2'-d]pyrazine with an area of 2.62%, belong to the alkaloid group, and Ergotaman-3',6',18-trione, 12'-hydroxy-2'-

methyl-5'-(phenylmethyl)-, (5'à)- with an area of 0.89%, also belongs to the alkaloid group. Lalitha *et al*., (2021), in their research on antioxidants in mangrove *Avicennia officinalis* L. through GC-MS testing, identified a group of antioxidant compounds such as Trans-cinnamic acid, with a peak area percentage of 27.7%.

The group of compounds found in mud crabs (*S. serrata*) were alkaloids. Alkaloid compounds can also be found in marine plants such as seagrasses. This is consistent with the findings of Shaffai *et al*., (2023); Tjandrawinata and Nurkolis, (2024), who identified a group of alkaloid compounds in the ethanol extract of *Enhalus acoroides*, such as Phenyl-N-methylindole with a peak area of 53.88%, N-Methyldeacetylcolchicine with a peak area of 1.55%, and Phenyl-5-methylindole with a peak area of 28.98%

Conclusion

The results of antioxidant testing with the DPPH method on *Scylla serrata* extracts showed IC50 values at very strong antioxidant characteristics. Phytochemical identification shows the extract contains strong bioactive compounds in the flavonoid and triterpenoid compound groups. GC-MS compound profile identification showed GC-MS analysis detected major compound groups consisting of alkaloids, purines, and vitamins. Minor compound groups were detected from volatile compounds, amines, terpenoids, monosaccharides, amino acids, fatty acids, silanes, formamides, heterocycles, carboxylic acids, aminoglycosides, naphthalene derivatives, nitriles, amides, glycosides, and peptides. *Scylla serrata* extract has been reported to have potential as antioxidant, anti-inflammatory, anticancer, antimicrobial, and antiviral. The results of this study are expected to provide important information in the disclosure of alternative medicinal materials from marine organisms.

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Thank you for submitting the revision of manuscript, "SCREENING AND PROFILING OF ANTIOXIDANT ACTIVITY IN MUD CRAB (Scylla serrata) FROM BANYUASIN WATERS" to ILMU KELAUTAN: Indonesian Journal of Marine Sciences. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

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Dr Rozirwan M.Sc. Rozirwan:

We have reached a decision regarding your submission to ILMU KELAUTAN: Indonesian Journal of Marine Sciences, "SCREENING AND PROFILING OF ANTIOXIDANT ACTIVITY IN MUD CRAB (Scylla serrata) FROM BANYUASIN WATERS".

Our decision is to:
Revision

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Reviewer B:

1. Introduction
The introduction provides a good background on Scylla serrata as a source of bioactive compounds, particularly antioxidants, and their potential therapeutic roles. It includes relevant citations to recent studies and explains the importance of antioxidants in mitigating oxidative stress-related diseases. However, while it cites several previous works on antioxidant compounds in S. serrata, the “state of the art” — meaning the current research frontier and what is still unknown — could be presented more explicitly. The gap is mentioned (“specific data on the type and quantity… are still limited”), but it would be stronger if the authors clearly positioned their study in comparison to the latest findings and emphasized how their approach is novel compared to prior research.

2. Methods and Relation to the Results
The methods are described in detail, covering sampling, environmental parameter measurements, sample preparation, extraction, antioxidant activity determination (DPPH assay), phytochemical screening, and GC-MS analysis. The methodology appears appropriate for the objectives and is standard for antioxidant profiling. The inclusion of environmental parameters is useful for context. The described procedures align well with the results obtained, and the choice of ethanol as the extraction solvent is justified. However, while the methods are adequate for generating the reported data, the reproducibility could be further supported by specifying some operational parameters in GC-MS analysis (e.g., column dimensions, flow rate) and clarifying the statistical approach used for IC_{50} comparisons.

3. Results and Discussion Coherence
The results are presented clearly, with IC_{50} values showing very strong antioxidant activity, followed by qualitative and GC-MS-based compound identification. The discussion effectively connects the identified compounds (flavonoids, triterpenoids, alkaloids, purines, vitamins) with their known biological activities, citing relevant literature. However, there are some areas where the discussion could be more critical — for instance, interpreting why alkaloids were not detected in qualitative phytochemical screening but appeared in GC-MS, beyond the method differences. Additionally, more comparative analysis with similar studies (quantitative differences in compound abundance) would strengthen the interpretation.

4. Suitability of Results and Discussion
Overall, the results and discussion are consistent and support the conclusion that S. serrata extracts have strong antioxidant potential. The integration of phytochemical screening and GC-MS profiling provides a comprehensive view of the bioactive composition. Nonetheless, the discussion tends to be more descriptive than analytical in parts — stronger linkage between environmental conditions, extraction efficiency, and compound diversity could enhance the scientific depth.

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SCREENING AND PROFILING OF ANTIOXIDANT ACTIVITY IN MUD CRAB (*Scylla serrata*) FROM BANYUASIN WATERS

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Abstract

Mangrove crab (*Scylla serrata*) as one of the crustacean species, has a variety of bioactive compounds that can be utilized in the field of pharmacology. Antioxidant compounds act as therapeutic agents against degenerative diseases. Banyuasin waters have mangrove vegetation with associated marine organisms that have the potential to be studied for bioactive compounds. This study aims to identify the phytochemical profile quantitatively and qualitatively, samples were collected from mud flats near mangrove ecosystems in Banyuasin waters, South Sumatra. Samples were tested for antioxidant activity using the DPPH test, and IC50 values, qualitative phytochemical identification, and phytochemical profiles were calculated using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Based on the results of antioxidant testing, the IC50 value of *Scylla serrata* extract is 2.25 ppm, the sample is included in the category of very strong antioxidants. Phytochemical test results showed that the compound is thought to contain antioxidant activity from flavonoids and triterpenoids. GC-MS analysis detected major compound groups of alkaloids, purines, and vitamins. Minor compound groups detected amines, terpenoids, monosaccharides, amino acids, fatty acids, silanes, formamides, heterocycles, carboxylic acids, aminoglycosides, naphthalene derivatives, nitriles, amides, glycosides, and peptides. *Scylla serrata* extract shows very strong antioxidant activity, with major compounds such as alkaloids, purines, and vitamins. *Scylla serrata* extract detected compounds that have been reported as anti-inflammatory, anticancer, antimicrobial, and antiviral. These findings highlight the pharmaceutical potential of *Scylla serrata* as a source of bioactive compounds. The results of this study provide valuable information for the development of alternative medicines derived from marine organisms.

Keywords: Antioxidant, Bioactive compounds, DPPH, GC-MS, *Scylla serrata*

Introduction

Scylla serrata or mud crab, as one of the crustacean species, has great potential in the field of beauty and health (De Castro *et al.*, 2023), because of its diverse bioactive compounds (Yusof *et al.*, 2020; Beslin and Geni, 2021; Neelima *et al.*, 2022). The diversity of bioactive compounds triggers great potential in the search for alternative medicinal ingredients from marine organisms, including antioxidant compounds (Yogeshwaran *et al.*, 2020; Karnila and Ramadhani, 2021; Fajriaty *et al.*, 2024). Antioxidant compounds in mud crabs have a role as therapeutic agents (Wu *et al.*, 2021) in fighting degenerative diseases such as cancer (Nagarajan *et al.*, 2024), cardiovascular diseases (Nanda *et al.*, 2021), and neurodegenerative disorders (Galal-Khallaf *et al.*, 2024), caused by oxidative stress due to free radicals.

Antioxidant is the reaction of a compound in neutralizing free radicals (Delta *et al*., 2021; Rozirwan *et al*., 2023; Rozirwan *et al*., 2023). This process involves a highly efficient electron donation mechanism. Antioxidants work by donating electrons to free radicals (Frías-Espericueta *et al.*, 2022; Pati *et al.*, 2022; Yang *et al.*, 2023; Fajriaty *et al.*, 2024). Free radicals, which have one or more unpaired electrons, are highly reactive and can cause oxidative damage to DNA, proteins, and cellular lipids (Alkadi, 2020; Di Meo and Venditti, 2020). Free radicals are often generated as by-products of various metabolic processes in the body or due to environmental exposures such as pollution and ultraviolet radiation (Martemucci *et al.*, 2022; Sadiq, 2023). The damage caused by free radicals can contribute to the development of various degenerative diseases, including cancer, heart disease, and neurodegenerative disorders (Teleanu *et al.*, 2022; Chaudhary *et al.*, 2023). In inhibiting free radicals, antioxidant compounds such as carotenoids and polyphenols will interact with free radicals to enhance the activity of detoxification enzymes resulting in accelerated elimination of free radicals and strengthened antibodies (Pisoschi *et al.*, 2021; Tumilaar *et al.*, 2024).

The analysis of antioxidant compounds in *Scylla serrata* requires an accurate technique to ensure identification and quantification are targeted (Baag and Mandal, 2023; Yao *et al.*, 2023). The gas Chromatography-Mass Spectrometry (GC-MS) analysis method was chosen as an effective method in the analysis of bioactive compounds (Jabbar *et al.*, 2022; Musa *et al.*, 2022; Palma *et al*., 2023; Rozirwan *et al*., 2024). This method allows the separation and identification of compounds based on their density and chemical characteristics. This method is able to identify the diversity of antioxidant compounds in *Scylla serrata* meat with high accuracy while being able to measure the concentration of each compound analyzed. Antioxidant compounds such as carotenoids and polyphenols were identified through GC-MS analysis, showing significant therapeutic potential (Berwal *et al.*, 2021; Vellapandian, 2022; Palaniyappan *et al.*, 2023). The identified antioxidant content offers substantial health benefits to humans as an alternative medicine.

Previous studies have extracted various body parts of *Scylla serrata* and shown the content of antioxidant compounds such as flavonoids, carotenoids, and polyphenols (Yogeshwaran *et al.*, 2020; Karnila and Ramadhani, 2021; Neelima *et al.*, 2022; Yang *et al.*, 2023; Fajriaty *et al.*, 2024). The results showed that some of these compounds are classified with high antioxidant characteristics. However, specific data on the type and quantity of antioxidant substances from this organism are still limited. Therefore, in-depth and comprehensive research is needed to reveal the potential of *Scylla serrata* as a source of antioxidants. This study aims to identify the profile of antioxidant compounds in *Scylla serrata*. Thus, the potential of *Scylla serrata* as a source of alternative medicinal ingredients can be further explored and optimally utilized.

Materials and Methods

Sampling Area

Mangrove crab samples were collected in January 2023 from Banyuasin Waters, South Sumatra, Indonesia (Figure 1). At this location, numerous crustacean and gastropod populations were found inhabiting the intertidal zone (Almaniar, Rozirwan and Herpandi, 2021; Rozirwan *et al*., 2021; Nugroho, Rozirwan and Fauziyah, 2022). The crab fishing area had a mud substrate with a depth of 1–2 m and was located within a mangrove vegetation zone directly connected to port and pond activities (Fitria *et al.*, 2023). Anthropogenic pollutants that accumulate in these waters are known to trigger an increase in the defense mechanisms of organisms, such as the production of antioxidant compounds derived from secondary metabolites (Rozirwan *et al*., 2023).

Figure 1. Map of Sampling Location in Banyuasin Waters Area

Samples Catch, Collage, Storage and Identification

Crustacean samples were taken using folding trawl gear. The samples were collected and stored in a cool box. The crab identification process was conducted based on the examination of morphological characteristics, such as body shape, color pattern, claw shape, and leg shape (Hidir *et al.*, 2021; Vermeiren, Lennard and Trave, 2021). Morphometric measurements were performed on the crab samples, and the identification process was completed in the laboratory. Taxon determination was carried out using data from the World Register of Marine Species (WoRMS) accessed in January 2023 (WoRMS, 2023).

Environmental Characteristics of Sampling Area

Environmental quality calculations were conducted to assess the condition of the sampling environment. Environmental parameter data were measured, including salinity, temperature, pH, and dissolved oxygen (DO) (Fitria *et al*., 2023; Rozirwan *et al*., 2024). Each parameter was measured in three repetitions to ensure consistency, and the results were then averaged. Environmental parameter measurements are typically used to evaluate habitat conditions, as they provide insights into the physical and chemical characteristics of the ecosystem. Repetition in measurements is a standard approach to improve data reliability.

Sample Preparation

The preparation method described by Ambekar *et al*. (2023), involves cleaning the crab to remove contaminants. In this study, the carapace and crab meat were separated and rinsed with distilled water to eliminate any remaining impurities. The wet weight of the crab meat was measured, and the samples were then dried in an oven at 40°C for 3 × 24 h. After drying, the samples were ground into powder using a blender. The dry weight of the crab meat was recorded for data analysis.

Sample Maceration and Extraction

The wet maceration method was used in this study. A total of 250 g of *Scylla serrata* meat powder was weighed and immersed in 1000 mL of 96% ethanol solvent at a ratio of 1:4 (b/v). The soaking process was conducted for 3 × 24 h, with stirring performed periodically to ensure optimal extraction. The maceration results were filtered using filter paper (No. 42, 125 mm). The extraction process was then carried out using a rotary evaporator at 40°C with a rotating speed of 3000 rpm (Hashim *et al.*, 2021). The resulting extract was stored as a stock solution. A total of 0.05 g of *Scylla serrata* extract was used as an additive for the stock solution (Habib *et al*., 2022). Wet maceration is a commonly employed extraction method due to its ability to preserve heat-sensitive compounds. Stirring during the maceration process enhances solute dissolution, while rotary evaporation ensures efficient solvent removal under controlled temperature conditions.

Determination of Antioxidant Activity and IC50 Value

Antioxidant testing was conducted using the DPPH method (Vásquez, Cian and Drago, 2023). The stock solution of *Scylla serrata* extract was used as the test solution for antioxidant activity, while vitamin C solution served as a reference. The sample was dissolved in 96% ethanol. A total of 1 mL of sample solution was prepared for each concentration: 100 $mg.L^{-1}$, 150 $mg.L^{-1}$, 200 $mg.L^{-1}$, 250 $mg.L^{-1}$, and 300 $mg.L^{-1}$. Each concentration was mixed with 40 $\mu g.mL^{-1}$ DPPH solution and incubated in the dark for 30 min. The absorbance values were measured at 517 nm using UV-Vis spectrophotometry. The percentage of inhibition and IC50 value were calculated (Naveed *et al.*, 2022). The characterization of antioxidant activity is determined based on the IC50 value, which represents the concentration of the sample required to inhibit 50% of DPPH radicals. The strength of antioxidant activity is classified according to IC50 values, as shown in Table 1. The IC50 value is calculated using the following formula.

$$inhibi\cancel{si}tion = \frac{blank\ abs - sample\ abs.}{blank\ abs}\ x\ 100\ \%$$

The IC50 results were entered in the linear regression equation Y = AX + B. The sample concentration is the abscissa (X-axis), and the percentage of antioxidant inhibition is the ordinate (Y-axis) (Yuniarti *et al.*, 2020).

Table 1. Characteristic concentration value of IC50

Concentration Value ($\mu g.mL^{-1}$)	Characteristic
<50	Very Strong
50-100	Strong
100-150	Moderate
150-200	Low

Phytochemical analysis

Phytochemical Screening

Phytochemical tests of *Scylla serrata* meat extracts were conducted using qualitative methods to identify the presence of bioactive compounds. The analysis included steroid and triterpenoid tests, which were performed using the Liebermann-Burchard method; alkaloid tests using Mayer and Dragendorff reagents; flavonoid tests with the Shinoda staining method; tannin tests using the $FeCl_3$ reaction; and saponin tests using the foam test method. Each test was carried out following the procedures described in standard literature (Suwandi, Ula and Pertiwi, 2020; Dinesh *et al.*, 2022).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The identification of bioactive compound components in *Scylla serrata* meat extract was performed using the GC-MS analysis method. GC-MS analysis was conducted following the method described by Rozirwan *et al.* (2022). A total of 1 µL of extract was injected into the column (rt x 5 ms) with helium as the carrier gas and a split ratio of 1:50. The oven temperature was set at 50°C for 5 min, then gradually increased at a rate of 5°C per minute to a maximum temperature of 280°C, which was maintained for 5 min. Samples were injected 280°C. The Wiley 7 Library database was used as a reference for comparing the spectra of the analyzed compounds (Rafferty *et al.*, 2020).

Data Analysis

Further data processing was performed by conducting normality tests (Shapiro-Wilk) and homogeneity tests (Levene) (Gebruk *et al.*, 2021; Ebadzadeh, Shojaei and Seyfabadi, 2024). All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation. Statistical analysis was carried out using SPSS software. One-way analysis of variance (ANOVA), followed by Duncan's multiple range test for post hoc comparisons, was used to identify significant differences among groups. Statistical significance was determined at $p < 0.05$.

Result and Discussion

Samples Catch, Collage, Storage and Identification

Taxon determination was conducted using data from the World Register of Marine Species (WoRMS) accessed in January 2023 (WoRMS, 2023). Based on their morphological characteristics, the crustacean samples were identified as *Scylla serrata*.

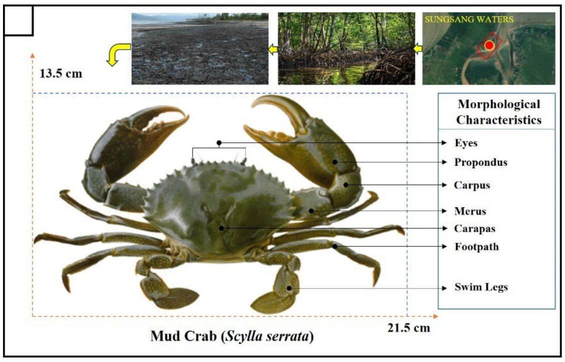

Figure 2. Crustacean species, *Scylla serrata*

Mangrove crabs of this species were caught using folding traps. The fishing process was carried out during low tide to facilitate crab capture. In this study, 2–5 crabs were used as stock samples. The crabs obtained were weighed, with weights ranging from 200 to 320 g, widths of approximately 21.5 cm, and lengths of around 13.5 cm. The crabs were then stored in a cool box filled with ice cubes for preservation.

The species identification of mangrove crabs is based on morphological characteristics, which include features such as the eyes, propound, carpus, merus, carapace, claws, walking legs, and swimming legs (Figure 2). Taxonomic data from databases such as WoRMS is widely used to confirm species classification accurately.

Environmental Characteristics of Sampling Area

Table 2. Observation of Environmental Parameters of the Research Site

	Station
Environment Parameter Quality	**Sungsang Waters**
Dissolved oxygen (mg.L^{-1})	4.2
pH	7
Temperature (°C)	28

Salinity (~~psu~~psu)	18

The results of environmental quality measurements in the *Scylla serrata* sampling areas in Banyuasin waters revealed diverse conditions. Measurements of water physicochemical parameters, including dissolved oxygen, pH, temperature, and salinity, were taken to assess the habitat suitability for *Scylla serrata* (Rozirwan *et al.*, 2021). The dissolved oxygen concentration was found to be 4.2 mg.L⁻¹, which is sufficient to support the respiration process of aquatic organisms (Ouyang *et al.*, 2021). The pH value of the water at the sampling location was 7, indicating neutral pH, which represents optimal ecological conditions (Chowdhury *et al.*, 2021). This is consistent with previous studies (Yusni and Haq, 2020; Muhtar and Lanuru, 2021; Putri *et al.*, 2022), which state that waters with a pH between 6.5 and 7.5 are ideal for the survival of mangrove crabs. The water temperature was measured at 28°C, indicating favorable conditions for mangrove crab growth (Indarjo *et al.*, 2020; Ren *et al.*, 2021) Water salinity was recorded at 18 ~~‰,~~ psu, reflecting typical conditions in estuarine areas or areas directly influenced by tidal movements (Wang *et al.*, 2021). *Scylla serrata* grows best in salinities between 15~~‰~~ psu and 25~~‰~~ psu but grows more slowly at salinities greater than 25 to 30~~‰~~ psu (Triajie *et al.*, 2020; Pati *et al.*, 2023; Adnan *et al.*, 2024).

Determination of Antioxidant Activity by DPPH Assay

Antioxidant analysis using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method on *Scylla serrata* meat extract showed promising results. The DPPH solution changed from purple to yellow, indicating the presence of antioxidants in the extract. The test results revealed that the *Scylla serrata* extract had an IC50 value of 2.25 ppm, while Vitamin C, used as a comparison solution, had an IC50 value of 2.16 ppm (Table 3). Both solutions demonstrated low IC50 values, categorizing them as very strong antioxidant compounds. The IC50 value, which is the concentration required to inhibit 50% of DPPH radical activity, is a critical parameter for assessing the antioxidant potential of a compound (Martinez-Morales *et al.*, 2020). The results indicate that *Scylla serrata* extract possesses an IC50 value comparable to that of Vitamin C. The percentage inhibition of DPPH free radicals by *Scylla serrata* meat extract increased as the extract concentration increased. This suggests that the extract has the ability to donate electrons or hydrogen to the DPPH radical, neutralizing it and converting it into a more stable form (Gulcin and Alwasel, 2023).

Table 3. Calculation results of antioxidant activity of *Scylla serrata* in Sungsang waters

Sample	Linear Regression			IC50 Value	Category
	a	b	R^2		
Scylla serrata	6.9429	52.808	0.9327	2.25 mg.L⁻¹	Very Strong
Asorbic Acid	6.7135	55.866	0.9435	2.16 mg.L⁻¹	Very Strong

The discovery of compounds such as flavonoids and triterpenoids, which were identified in the phytochemical analysis of *Scylla serrata* extracts, suggests that they may possess high antioxidant activity (Akinwumi *et al.*, 2022; Hajar-Azira *et al.*, 2023). Flavonoids are well known for their ability to capture free radicals and interrupt the chain of oxidative reactions. Triterpenoids are also recognized for their significant antioxidant activity through a similar mechanism. The combination of these compounds in *Scylla serrata* extract creates a synergistic effect, enhancing the overall capacity of the extract to neutralize free radicals.

Scylla serrata is known for its rich antioxidant system and strong enzymatic defense system, which help it survive in the dynamic and often challenging mangrove environment (Pati *et al.*, 2023). Mud crabs also possess defense enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, which work synergistically to detoxify reactive oxygen species (ROS) and maintain redox balance in the body (Jerome, Hassan and Chukwuka, 2020; Bal *et al.*, 2021; Costantini, Esposito and Ruocco, 2022; Zeng *et al.*, 2024). These enzymes play a crucial role in protecting the crab from oxidative stress generated by fluctuating environmental conditions, pollution, and pathogens.

This combination of antioxidant compounds and defense enzymes not only ensures the survival of mud crabs in their habitat but also positions them as a potential source for the development of natural health products that can harness their protective mechanisms.

Antioxidants and defense enzymes play a vital role in organisms' survival, especially in harsh environments. The synergy between these compounds and enzymes contributes significantly to mitigating oxidative stress and preserving cellular function, which can be explored for potential therapeutic applications.

Phytochemical Analysis

Phytochemical Screening

Phytochemical tests were carried out to identify the compounds present in gastropod and crustacean extracts using ethanol as the solvent (Fitria *et al*., 2023; Rozirwan *et al*., 2024) The phytochemical test aimed to determine the compounds in the test extract (Chen *et al.*, 2022), allowing for the identification of compounds that influence the strong or weak antioxidant activity of the extract (Baliyan *et al.*, 2022). The results of the phytochemical test, after UV-Vis spectrophotometric analysis of the extract, are presented in Table 4. The test results showed that only certain compounds were extracted by the ethanol solvent (Yuniarti *et al.*, 2020).

Table 4. Phytochemical screening results of *Scylla serrata*

No.	Parameters	Analysis Result	Analysis Type
1	Alkaloids	-	Qualitative
2	Flavonoids	+	Qualitative
3	Triterpenoids	+	Qualitative
4	Saponin	-	Qualitative
5	Tannins	-	Qualitative
6	Steroid	-	Qualitative

Qualitative phytochemical analysis of *Scylla serrata* extract showed significant results in identifying the content of bioactive compounds. Based on the test results, *Scylla serrata* extract tested positive for flavonoid and triterpenoid compounds. A similar finding was reported by Elshaarawy *et al*. (2023) for *Scylla olivacea* samples. These two compounds offer various health benefits and therapeutic potential, particularly due to their strong antioxidant properties. Flavonoids are a group of polyphenolic compounds that are widely recognized for their potent antioxidant activity (Shen *et al.*, 2022). These compounds can neutralize free radicals and prevent oxidative damage to cells and tissues. Additionally, flavonoids possess anti-inflammatory, anticancer, and cardioprotective properties (Mounika *et al.*, 2021; Jain *et al.*, 2024; Ullah *et al.*, 2024). The identification of flavonoid compounds in *Scylla serrata* indicates that mud crabs are not only valuable as food but also hold potential as an alternative source of medicine from marine organisms.

Triterpenoids are a group of terpenoid compounds that have potential biological activities (Mabou and Yossa, 2021; Zang *et al.*, 2022). These compounds are known to possess anti-inflammatory, antitumor, antimicrobial, and immunomodulatory properties (Harun *et al.*, 2020; Ahmad *et al.*, 2021). As antioxidant agents, triterpenoids have been used in pharmacology to treat inflammatory diseases and cancer. Similar to flavonoids, these compounds can neutralize free radicals caused by oxidative stress on body tissues. The discovery of triterpenoid compounds in *Scylla serrata* shows promising results, given their antioxidant potential that can be applied to address various diseases. Thus, the opportunity to explore alternative medicinal raw materials from *Scylla serrata* extract is increasingly attractive for further research. Overall, the phytochemical results focusing on flavonoid and triterpenoid compounds confirm the importance of *Scylla serrata* as a potential source of bioactive compounds. Further research is encouraged at the stage of isolation and purification of these compounds, so that alternative medicinal materials derived from this marine organism can contribute to the development of therapeutic agents from marine organisms.

Phytochemical Profile Screening

The antioxidant compound profile in *Scylla serrata* was determined using GC-MS (Gas Chromatography-Mass Spectrometry) analysis on the ethanol extract of mud crab. Figure 3 shows the chromatogram with 37 peaks identified in the extract. Each peak on the chromatogram represents a distinct chemical compound found in the extract, which was analyzed using GC-MS.

GC-MS analysis is a powerful technique for identifying and quantifying individual compounds in complex mixtures, providing insights into the chemical composition of the extract. This method is widely used for its ability to separate and identify volatile compounds, making it an essential tool for profiling bioactive compounds, such as antioxidants, in natural products.

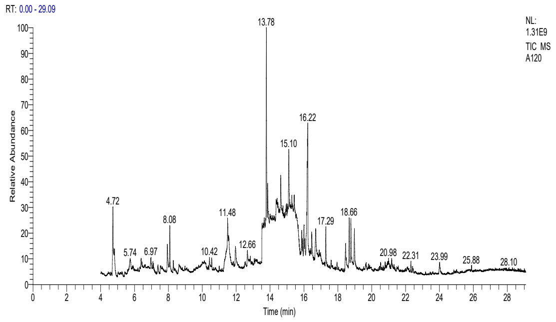

Figure 3. GC-MS chromatogram of ethanol extract *Scylla serrata*

Terpenoid, alkaloid, steroid, and tannin groups were among the pure compounds successfully detected using GC-MS. Based on the GC-MS analysis of the ethanol extract compound components presented in Table 5, the main components identified in the extract were Calycotomine, N-methyl-, with a value of 8.31% of the total area, and uric acid, with a value of 8.71% of the total area.

At a retention time of 4.72 minutes, the compound 2-Cyclohexylpiperidine was detected with an area of 3.45%, a probability of 6.07, and a chemical formula of $C_{11}H_{21}N$, which belongs to the alkaloid compound group. At a retention time of 5.74 minutes, the compound 2-Pyridinamine, 3,6-dimethyl was detected with an area of 1.69%, a probability of 7.02, and a chemical formula of $C_7H_{10}N_2$, which belongs to the aminopyridine compound group. Furthermore, at a retention time of 6.97 minutes, the compound Pentanoic acid, dodec-9-ynyl ester was detected with an area of 1.00%, a probability of 8.47, and a chemical formula of $C_{17}H_{30}O_2$, which belongs to the protein compound group.

At a retention time of 8.08 minutes, the compound L-Homoserine lactone, N, N-dimethyl was detected with an area of 1.97%, a probability of 82.23, and a chemical formula of $C_6H_{11}NO_2$, which belongs to the amino acid compound group. At a retention time of 10.42 minutes, the compound Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetramethyl was detected with an area of 0.74%, a probability of 43.35, and a chemical formula of $C_{11}H_{14}N_2OS$, which belongs to the EPA compound group. At a retention time of 11.48 minutes, the compound 1H-2-Indenol, 2,3,4,5,6,7-hexahydro-1-(2-hydroxy-2-methylpropyl) was detected with an area of 4.83%, a probability of 12.26, and a chemical formula of $C_{13}H_{22}O_2$, which belongs to the lactone compound group.

At a retention time of 12.66 minutes, the compound dl-Lysine was detected with an area of 0.86%, a probability of 23.13, and a chemical formula of $C_6H_{14}N_2O_2$, which belongs to the amino acid compound group. At a retention time of 13.78 minutes, the compound Calycotomine, N-methyl- was detected with an area of 8.31%, a probability of 43.35, and a chemical formula of $C_{13}H_{19}NO_3$, which belongs to the alkaloid compound group. At a retention time of 15.10 minutes, the compound Dasycarpidan-8(16H)-ethanol, 3,18-dihydro-1-(hydroxymethyl)-, (2.xi.,4.xi.)- was detected with an area of 4.14%, a probability of 12.00, and a chemical formula of $C_{20}H_{28}N_2O_2$, which belongs to the group of molport compounds.

At a retention time of 16.22 minutes, the uric acid compound was detected with an area of 8.71%, a probability of 55.93, and a chemical formula of $C_5H_4N_4O_3$, which belongs to the allantoin compound group. At a retention time of 17.29 minutes, the compound Actinomycin C2 was detected with an area of 1.49%, a probability of 30.73, and a chemical formula of $C_{63}H_{88}N_{12}O_{16}$, which belongs to a group of peptide compounds that are derivatives of peptide compounds.

Animals produce a diverse mixture of secondary metabolites such as phenols, alkaloids, flavonoids, tannins, and saponins. Several animal studies have shown the potential use of these metabolites as antibacterial agents due to the presence of abundant biomolecules. Synthetic drugs often have high secondary failure rates and severe side effects, while animal products contain a variety of free radical scavenging molecules with substantial antioxidant properties.

Research by Waluyo and Wahyuni (2021) on *Conus miles* bacteria identified three dominant compounds through GC-MS analysis, namely Acetic acid (CAS), Ethylic acid, Proanoic acid, 2-methyl-(CAS), and Isobutyric acid and Iso Valeric acid. Research on the ethanol extract of *Enhalus acoroides* conducted Mediarman *et al*., (2021) using GC-MS showed nine compounds dominated by alkaloids, flavonoids, terpenoids, and polyphenols. Some of the compounds found in *Enhalus acoroides* have similarities with the GC-MS results on *S. serrata*, but the percentage area of the alkaloid compound group in *S. serrata* was only 8.31%, compared to the ethanol extract of *Enhalus acoroides*, which reached 53.88%.

In the GC-MS results, the highest peak was identified as coming from the antioxidant compounds of the alkaloid group. However, in the phytochemical test, alkaloid compounds were not detected in the *S. serrata* sample. This could be due to differences in the analytical methods used. Phytochemical tests are qualitative, while GC-MS analysis is quantitative. Quantitative analysis methods are known to be more accurate than qualitative methods.

The difference in the results of phytochemical tests and GC-MS analysis can be influenced by the type of solvent used during the maceration process of mangrove crab (*S. serrata*) extracts. In this study, the solvent used was ethanol, which is polar and able to bind bioactive compounds quickly and is relatively safe for humans compared to methanol. Methanol solvent is more polar than ethanol but is more toxic. This is in accordance with research conducted by Rahmawati *et al*., (2023); Shofinita *et al*., (2024), which show that methanol is more polar but also more toxic than ethanol.

Table 5. Proposed peak order, retention time, probability, area, compound name, and molecular formula

Peak#	R. Time	Probability	Area%	Name	Molecular formula
1	4.72	6.07	3.45	2-Cyclohexylpiperidine	C11H21N
2	4.81	18.58	1.37	Edulan II	C13H20O
3	5.74	7.02	1.69	2-Pyridinamine, 3,6-dimethyl	C7H10N2
4	6.39	8.12	0.83	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	C16H28O3
5	6.61	11.07	0.66	d-Mannose	C6H12O6
6	6.81	50.91	0.89	Deoxyspergualin	C17H37N7O3
7	6.97	8.47	1.00	Pentanoic acid, dodec-9-ynyl ester	C17H30O2
8	7.93	51.03	1.42	trans-(2 Chlorovinyl)dimethylethoxysil ane	C6H13ClOSi
9	8.08	82.23	1.97	L-Homoserine lactone, N,N-dimethyl-	C6H11NO2
10	8.28	32.09	0.64	2-Propyl-tetrahydropyran-3-ol	C8H16O2
11	8.62	32.45	0.93	Imidazole	C3H4N2
12	10.00	11.83	0.76	Tertbutyloxyformamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-buty nyl]-	C14H24N2O2
13	10.42	15.27	0.74	Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetrameth yl	C11H14N2OS
14	11.48	12.26	4.83	1H-2-Indenol, 2,3,4,5,6,7-hexahydro-1-(2-hydroxy-2-methylpropyl)	C13H22O2
15	11.55	48.75	2.53	dl-Citrulline	C6H13N3O3
16	11.96	7.38	1.45	2-Pyridineacetic acid, hexahydro-	C7H13NO2
17	12.66	23.13	0.86	dl-Lysine	C6H14N2O2
18	13.51	60.28	1.62	D-Streptamine, O-6-amino-6-deoxy-à-D-glucopyranosy l-(1-4)-O-(3-deoxy-4-C-methyl-3-(meth ylamino)-á-L-arabinopyranosyl-(1-6))- 2-deoxy	C6H13NO2
19	13.78	43.35	8.31	Calycotomine, N-methyl-	C13H19NO3
20	13.99	17.84	2.38	4-[4-Diethylamino-1-methylbutylamino]-1,2-dimethoxy-6-bromonaphthalene	C21H31BrN2O2
21	14.35	32.83	1.22	Dasycarpidan-8(16H)-ethanol, 3,18-didehydro-1-(hydroxymethyl)-, (2.xi.,4.xi.)-	C20H28N2O2
22	14.62	12.2	2.67	4-[4-Diethylamino-1-methylbutylamino]-1,2-dimethoxy-6-bromonaphthalene	C21H31BrN2O2
23	15.10	12.00	4.14	Dasycarpidan-8(16H)-ethanol, 3,18-didehydro-1-(hydroxymethyl)-, (2.xi.,4.xi.)-	C20H28N2O2
24	15.21	32.49	0.87	Cystine	C6H12N2O4S2
25	15.65	33.28	1.26	3-[N-[2-Diethylaminoethyl]-1-cyclopes tenylamino]propionitrile	C14H25N3
26	15.86	16.52	0.92	á-Hydroxyquebrachamine	CH3CH(OH)COOH
27	15.99	14.23	1.02	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	C21H36O4
28	16.22	55.93	8.71	Uric acid	C5H4N403
29	16.45	8.30	1.33	Aminoacetamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-buty nyl]-	C5H4N4O3
30	16.69	32.32	2.06	Glucopyranuronamide, 1-(4-amino-2-oxo-1(2H)-pyrimidinyl)- 1,4-dideoxy-4-(D-2-(2-(methylamino) acetamido)hydracrylamido)-, á-D	C10H14N2O
31	16.90	29.78	0.80	1,2,4-Trioxolane-2-octanoic acid, 5-octyl-, methyl ester	C6H11NO6
32	17.29	30.73	1.49	Actinomycin C2	C19H36O5
33	18.45	37.85	2.00	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	C63H88N12O16
34	18.66	90.13	2.23	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	C63H88N12O16
35	18.76	60.11	2.62	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6 H-dipyrrolo[1,2-a:1',2'-d]pyrazine	C11H18N2O2
36	18.97	36	2.13	l-(+)-Ascorbic acid	C14H22
37	20.98	13.19	1.29	cis-13-Octadecenoic acid	C6H8O6
38	21.17	13.49	0.94	Ricinoleic acid	C18H34O2
39	23.99	31.48	0.89	Ergotaman-3',6',18-trione, 12'-hydroxy-2'-methyl-5'-(phenylmeth yl)-, (5'à)-	C18H34O3

The main group of compounds in the ethanol extract of mud crab (*S. serrata*) was represented by three peaks on the GC chromatogram, which had a higher percentage area than the others. These peaks were identified as Calycotomine, N-methyl- (8.31%), uric acid (8.71%), and Dasycarpidan-8(16H)-ethanol, 3,18-didehydro-1-(hydroxymethyl)-(2.xi.,4.xi.)- (4.14%). Antioxidant compounds detected in the GC-MS analysis included 2-Cyclohexylpiperidine with an area of 3.45%, which belongs to the alkaloid group. In addition, the Edullan II compound with an area of 1.37% belongs to the volatile compound group. The compound Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate, with an area of 0.83%, belongs to the terpenoid compound group, and Imidazole with an area of 0.93% also belongs to the alkaloid group.

In a study conducted by Rasyid (2016) on sea cucumber biota (*Bohadschia* sp.), steroid compounds consisting of cholest-2-ene (3.91%), stigmastan-3,5-diene (3.29%), cholest-5-en-3-yl nonanoate (4.89%), and cholest-5-ene, 3.beta.-chloro- (3.12%) were found. The compound Calycotomine, N-methyl- with an area of 8.31% belongs to the alkaloid group. The compound l-(+)-Ascorbic acid with an area of 2.13%, as well as 5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1',2'-d]pyrazine with an area of 2.62%, belong to the alkaloid group, and Ergotaman-3',6',18-trione, 12'-hydroxy-2'-

methyl-5'-(phenylmethyl)-, (5'à)- with an area of 0.89%, also belongs to the alkaloid group. Lalitha *et al.*, (2021), in their research on antioxidants in mangrove *Avicennia officinalis* L. through GC-MS testing, identified a group of antioxidant compounds such as Trans-cinnamic acid, with a peak area percentage of 27.7%.

The group of compounds found in mud crabs (*S. serrata*) were alkaloids. Alkaloid compounds can also be found in marine plants such as seagrasses. This is consistent with the findings of Shaffai *et al.*, (2023); Tjandrawinata and Nurkolis, (2024), who identified a group of alkaloid compounds in the ethanol extract of *Enhalus acoroides*, such as Phenyl-N-methylindole with a peak area of 53.88%, N-Methyldeacetylcolchicine with a peak area of 1.55%, and Phenyl-5-methylindole with a peak area of 28.98%

Conclusion

The results of antioxidant testing with the DPPH method on *Scylla serrata* extracts showed IC50 values at very strong antioxidant characteristics. Phytochemical identification shows the extract contains strong bioactive compounds in the flavonoid and triterpenoid compound groups. GC-MS compound profile identification showed GC-MS analysis detected major compound groups consisting of alkaloids, purines, and vitamins. Minor compound groups were detected from volatile compounds, amines, terpenoids, monosaccharides, amino acids, fatty acids, silanes, formamides, heterocycles, carboxylic acids, aminoglycosides, naphthalene derivatives, nitriles, amides, glycosides, and peptides. *Scylla serrata* extract has been reported to have potential as antioxidant, anti-inflammatory, anticancer, antimicrobial, and antiviral. The results of this study are expected to provide important information in the disclosure of alternative medicinal materials from marine organisms.

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Note

Response to Reviewers

Reviewer A

Reviewer Comments	**Response**
change the salinity unit from % to psu	Thank you for the suggestion. We have revised the salinity unit throughout the manuscript, changing from “%” to “psu” in accordance with the standard oceanographic unit usage.
For References Follow the IJMS Format See articles published in 2025	We appreciate your feedback. The references have been reformatted to fully comply with the IJMS referencing style, following the format of articles published in 2025.

Reviewer B

Reviewer Comments	**Response**
Introduction The introduction provides a good background on Scylla serrata as a source of bioactive compounds, particularly antioxidants, and their potential therapeutic roles. It includes relevant citations to recent studies and explains the importance of antioxidants in mitigating oxidative stress-related diseases. However, while it cites several previous works on antioxidant compounds in S. serrata, the “state of the art” — meaning the current research frontier and what is still unknown — could be presented more explicitly. The gap is mentioned (“specific data on the type and quantity… are still limited”), but it would be stronger if the authors clearly positioned their study in comparison to the latest findings and emphasized how their approach is novel compared to prior research.	We thank the reviewer for this valuable suggestion. In the revised version, we have clarified the state of the art by explicitly comparing our study with recent works. We now emphasize that most previous studies on Scylla serrata reported only general antioxidant activity using spectrophotometric assays (e.g., DPPH, ABTS, FRAP), without identifying specific compounds. To highlight the research gap, we added that there is no comprehensive GC-MS-based profiling available for S. serrata meat, which is crucial for understanding the diversity and quantity of bioactive antioxidants. Finally, we underscored the novelty of our approach, namely providing, for the first time, a detailed GC-MS profiling of antioxidant compounds in S. serrata.
Methods and Relation to the Results The methods are described in detail, covering sampling, environmental parameter measurements, sample preparation, extraction, antioxidant activity determination (DPPH assay), phytochemical screening, and GC-MS analysis. The methodology appears	We sincerely thank the reviewer for the constructive comment. In the revised manuscript, we have included additional operational parameters of the GC-MS system (column type and dimensions, carrier gas flow rate, ionization mode, source temperature, and scan range) to enhance reproducibility. Furthermore, we clarified the statistical

appropriate for the objectives and is standard for antioxidant profiling. The inclusion of environmental parameters is useful for context. The described procedures align well with the results obtained, and the choice of ethanol as the extraction solvent is justified. However, while the methods are adequate for generating the reported data, the reproducibility could be further supported by specifying some operational parameters in GC-MS analysis (e.g., column dimensions, flow rate) and clarifying the statistical approach used for IC_{50} comparisons.	approach used for IC_{50} determination and comparisons, including the use of linear regression for IC_{50} calculation and one-way ANOVA with Duncan's multiple range test for group comparisons. These revisions strengthen the methodological transparency and reproducibility of our study.
Results and Discussion Coherence The results are presented clearly, with IC_{50} values showing very strong antioxidant activity, followed by qualitative and GC-MS-based compound identification. The discussion effectively connects the identified compounds (flavonoids, triterpenoids, alkaloids, purines, vitamins) with their known biological activities, citing relevant literature. However, there are some areas where the discussion could be more critical — for instance, interpreting why alkaloids were not detected in qualitative phytochemical screening but appeared in GC-MS, beyond the method differences. Additionally, more comparative analysis with similar studies (quantitative differences in compound abundance) would strengthen the interpretation.	We thank the reviewer for this constructive comment. In the revised manuscript, we have expanded the discussion to provide a more critical interpretation of the observed differences between phytochemical screening and GC-MS results. Specifically, we now explain that the absence of alkaloids in qualitative screening is not only due to methodological differences but also to sensitivity limitations, as qualitative tests may overlook compounds present at low concentrations. On the other hand, GC-MS offers higher sensitivity, enabling the detection of minor constituents such as Calycotomine and Imidazole. Additionally, we have included comparative analyses with other studies. For example, our results show that alkaloids account for 8.31% of the total chromatogram area in S. serrata, compared to 53.88% in the seaweed Enhalus acoroides (Mediarman et al., 2021). This difference is discussed in relation to the ecological and physiological differences between crustaceans and flowering marine plants, which may explain the variation in secondary metabolite profiles. This addition strengthens the coherence and depth of the results and discussion sections. However, we have not found any specific studies addressing the alkaloid compound group from Scylla sp. extracts, so this data could represent a new breakthrough for future research.
Suitability of Results and Discussion Overall, the results and discussion are	We appreciate this insightful suggestion. In the revised version, we have strengthened the

consistent and support the conclusion that S. serrata extracts have strong antioxidant potential. The integration of phytochemical screening and GC-MS profiling provides a comprehensive view of the bioactive composition. Nonetheless, the discussion tends to be more descriptive than analytical in parts — stronger linkage between environmental conditions, extraction efficiency, and compound diversity could enhance the scientific depth.	analytical depth of the discussion by explicitly linking environmental conditions and extraction solvent choice to the observed metabolite profile. We highlighted that salinity (18 psu) and temperature (28°C) at the sampling site could influence secondary metabolite synthesis, as estuarine organisms often adjust their bioactive compound production for stress adaptation. Moreover, we acknowledged that ethanol, while safer than methanol, may reduce extraction efficiency for more polar compounds, possibly leading to underestimation of certain metabolites

SCREENING AND PROFILING OF ANTIOXIDANT ACTIVITY IN MUD CRAB (*Scylla serrata*~~*Scylla serrata*~~) FROM BANYUASIN WATERS

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Abstract

Mangrove crab (~~*Scylla serrata*~~*S. serrata*) as one of the crustacean species, has a variety of bioactive compounds that can be utilized in the field of pharmacology. Antioxidant compounds act as therapeutic agents against degenerative diseases. Banyuasin waters have mangrove vegetation with associated marine organisms that have the potential to be studied for bioactive compounds. This study aims to identify the phytochemical profile quantitatively and qualitatively, samples were collected from mud flats near mangrove ecosystems in Banyuasin waters, South Sumatra. Samples were tested for antioxidant activity using the DPPH test, and IC50 values, qualitative phytochemical identification, and phytochemical profiles were calculated using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Based on the results of antioxidant testing, the IC50 value of ~~*Scylla serrata*~~*S. serrata* extract is 2.25 ppm, the sample is included in the category of very strong antioxidants. Phytochemical test results showed that the compound is thought to contain antioxidant activity from flavonoids and triterpenoids. GC-MS analysis detected major compound groups of alkaloids, purines, and vitamins. Minor compound groups detected amines, terpenoids, monosaccharides, amino acids, fatty acids, silanes, formamides, heterocycles, carboxylic acids, aminoglycosides, naphthalene derivatives, nitriles, amides, glycosides, and peptides. ~~*Scylla serrata*~~*S. serrata* extract shows very strong antioxidant activity, with major compounds such as alkaloids, purines, and vitamins. ~~*Scylla serrata*~~*S. serrata* extract detected compounds that have been reported as anti-inflammatory, anticancer, antimicrobial, and antiviral. These findings highlight the pharmaceutical potential of ~~*Scylla serrata*~~*S. serrata* as a source of bioactive compounds. The results of this study provide valuable information for the development of alternative medicines derived from marine organisms.

Keywords: Antioxidant, Bioactive compounds, DPPH, GC-MS, ~~*Scylla serrata*~~*S. serrata*

Introduction

~~*Scylla serrata*~~*Scylla serrata*, or mud crab, as one of the crustacean species, has great potential in the field of beauty and health (De Castro ~~et al~~*et al*., 2023), because of its diverse bioactive compounds (Beslin and~~&~~ Geni, 2021; Neelima *et al*., 2022; Yusof *et al*., 2020). The diversity of bioactive compounds triggers great potential in the search for alternative medicinal ingredients from marine organisms, including antioxidant compounds (Fajriaty *et al*., 2024; Karnila and~~&~~ Ramadhan, 2021; Yogeshwaran *et al*., 2020). Antioxidant compounds in mud crabs have a role as therapeutic agents (Wu ~~et al~~*et al*., 2021) in fighting degenerative diseases such as cancer (Nagarajan ~~et al~~*et al*.,

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2024), cardiovascular diseases (Nanda ~~et al~~*et al*., 2021), and neurodegenerative disorders (Galal-Khallaf ~~et al~~*et al*., 2024), caused by oxidative stress due to free radicals.

~~Antioxidant~~ Antioxidants ~~is~~ are the reaction of a compound in neutralizing free radicals (Delta ~~*et al*~~*et al*., 2021; Rozirwan ~~*et al*~~*et al*., 2023; Rozirwan ~~*et al*~~*et al*., 2023). This process involves a highly efficient electron donation mechanism. Antioxidants work by donating electrons to free radicals (Fajriaty *et al*., 2024; Frías-Espericueta *et al*., 2022; Pati *et al*., 2022; Yang *et al*., 2023). Free radicals, which have one or more unpaired electrons, are highly reactive and can cause oxidative damage to DNA, proteins, and cellular lipids (Alkadi, 2020; Di Meo and~~&~~ Venditti, 2020). Free radicals are often generated as by-products of various metabolic processes in the body or due to environmental exposures such as pollution and ultraviolet radiation (Martemucci ~~et al~~*et al*., 2022; Sadiq, 2023). The damage caused by free radicals can contribute to the development of various degenerative diseases, including cancer, heart disease, and neurodegenerative disorders (Chaudhary *et al*., 2023; Teleanu *et al*., 2022). In inhibiting free radicals, antioxidant compounds such as carotenoids and polyphenols will interact with free radicals to enhance the activity of detoxification enzymes, resulting in accelerated elimination of free radicals and strengthened antibodies (Pisoschi ~~et al~~*et al*., 2021; Tumilaar ~~et al~~*et al*., 2024).

The analysis of antioxidant compounds in *S. serrata* requires an accurate technique to ensure that identification and quantification are targeted (Baag and~~&~~ Mandal, 2023; Yao *et al*., 2023). Previous studies have mainly reported the antioxidant potential of *S. serrata* using spectrophotometric or colorimetric assays such as DPPH, ABTS, and FRAP, which provide only general antioxidant capacity without revealing the identity of specific compounds (Karnila and~~&~~ Ramadhani, 2021; Neelima *et al*., 2022; Yogeshwaran *et al*., 2020). In contrast, Gas Chromatography-Mass Spectrometry (GC-MS) offers higher resolution in profiling bioactive compounds by separating and identifying molecules based on their mass and chemical characteristics (Jabbar *et al*., 2022; Musa *et al*., 2022; Palma *et al*., 2023; Rozirwan~~, Khotimah,~~ *et al*., 2024). This advanced method not only allows the detection of a broader spectrum of antioxidant molecules but also quantifies their abundance, thereby providing a more comprehensive understanding of their therapeutic potential. ~~The analysis of antioxidant compounds in *Scylla serrata* requires an accurate technique to ensure identification and quantification are targeted (Baag and Mandal, 2023; Yao et al., 2023). The gas Chromatography-Mass Spectrometry (GC-MS) analysis method was chosen as an effective method in the analysis of bioactive compounds (Jabbar *et al*., 2022; Musa *et al*., 2022; Palma *et al*., 2023; Rozirwan *et al*., 2024). This method allows the separation and identification of compounds based on their density and chemical characteristics. This method is able to identify the diversity of antioxidant compounds in *Scylla serrata* meat with high accuracy while being able to measure the concentration of each compound analyzed. Antioxidant compounds such as carotenoids and polyphenols were identified through GC-MS analysis, showing significant therapeutic potential (Berwal et al., 2021; Vellapandian, 2022; Palaniyappan et al., 2023). The identified antioxidant content offers substantial health benefits to humans as an alternative medicine.~~

Although antioxidant compounds such as flavonoids, carotenoids, and polyphenols have been reported from different body parts of *S. serrata* (Fajriaty *et al*., 2024; Karnila and~~&~~ Ramadhani, 2021; Neelima *et al*., 2022; Yang *et al*., 2023; Yogeshwaran *et al*., 2020), most of these studies remain descriptive and do not provide detailed profiles of the specific types and relative quantities of individual compounds. To date, there is still no comprehensive GC-MS based profiling that systematically characterizes the antioxidant repertoire of *S. serrata* meat. This represents a critical knowledge gap, since understanding the diversity and concentration of specific compounds is essential to support its therapeutic application. Therefore, the present study aims to provide, for the first time, a detailed GC-MS profiling of antioxidant compounds in *S. serrata*. By doing so, this work contributes novel insights into its biochemical composition and strengthens its potential utilization as a sustainable source of natural antioxidants for pharmaceutical and nutraceutical development.~~Previous studies have extracted various body parts of *Scylla serrata* and shown the~~

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~~content of antioxidant compounds such as flavonoids, carotenoids, and polyphenols (Fajriaty et al., 2024; Karnila & Ramadhani, 2021; Neelima et al., 2022; Yang et al., 2023; Yogeshwaran et al., 2020). The results showed that some of these compounds are classified with high antioxidant characteristics. However, specific data on the type and quantity of antioxidant substances from this organism are still limited. Therefore, in-depth and comprehensive research is needed to reveal the potential of *Scylla serrata* as a source of antioxidants. This study aims to identify the profile of antioxidant compounds in *Scylla serrata*. Thus, the potential of *Scylla serrata* as a source of alternative medicinal ingredients can be further explored and optimally utilized.~~

Materials and Methods

Sampling Area

Mangrove crab samples were collected in January 2023 from Banyuasin Waters, South Sumatra, Indonesia (Figure 1). At this location, numerous crustacean and gastropod populations were found inhabiting the intertidal zone (~~Rozirwan, Almaniar et al., 2021; Saputra et al., 2021)~~ (Rozirwan~~, Almaniar~~ ~~et al~~*et al*., 2021; Rozirwan~~, Melki~~ ~~et al~~*et al*., 2021; Rozirwan~~, Nugroho and Fauziyah,~~*et al*~~,~~ 2022). The crab fishing area had a mud substrate with a depth of 1~~-~~–2 m and was located within a mangrove vegetation zone directly connected to port and pond activities (Fitria ~~et al~~*et al*., 2023). Anthropogenic pollutants that accumulate in these waters are known to trigger an increase in the defense mechanisms of organisms, such as the production of antioxidant compounds derived from secondary metabolites (Rozirwan ~~et al~~*et al*., 2023).

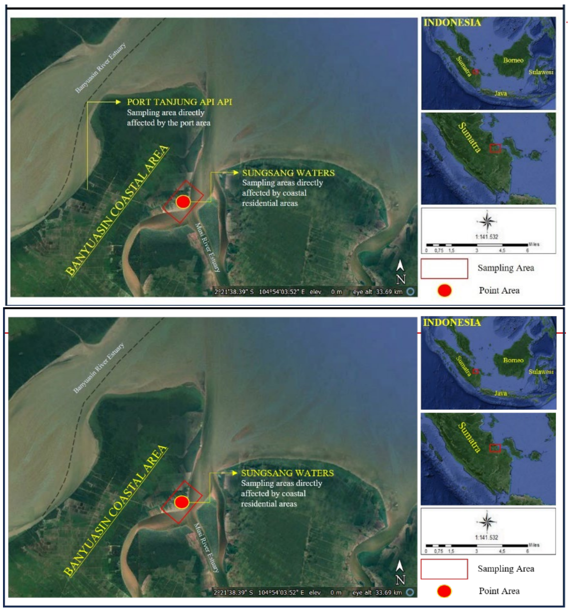

Figure 1. Map of Sampling Location in Banyuasin Waters Area

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Sample Identification and Collection ~~Samples Catch, Collage, Storage and Identification~~

Crustacean samples were taken using folding trawl gear. The samples were collected and stored in a cool box. The crab identification process was conducted based on the examination of morphological characteristics, such as body shape, color pattern, claw shape, and leg shape (Hidir ~~et al~~*et al*., 2021; Vermeiren ~~et al~~*et al*., 2021). Morphometric measurements were performed on the crab samples, and the identification process was completed in the laboratory. Taxon determination was carried out using data from the World Register of Marine Species (WoRMS) accessed in January 2023 (WoRMS, 2023).

Environmental Characteristics of Sampling Area

Environmental quality calculations were conducted to assess the condition of the sampling environment. Environmental parameter data were measured, including salinity, temperature, pH, and dissolved oxygen (DO) (Fitria ~~*et al*~~*et al*., 2023; Rozirwan ~~*et al*~~*et al*., 2024). Each parameter was measured in three repetitions to ensure consistency, and the results were then averaged. Environmental parameter measurements are typically used to evaluate habitat conditions, as they provide insights into the physical and chemical characteristics of the ecosystem. Repetition in measurements is a standard approach to improve data reliability.

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Sample Preparation

The preparation method described by Ambekar ~~*et al*~~*et al*. (2023), involves cleaning the crab to remove contaminants. In this study, the carapace and crab meat were separated and rinsed with distilled water to eliminate any remaining impurities. The wet weight of the crab meat was measured, and the samples were then dried in an oven at 40°C for 3 × 24 h. After drying, the samples were ground into powder using a blender. The dry weight of the crab meat was recorded for data analysis.

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Sample Maceration and Extraction

The wet maceration method was used in this study. A total of 250 g of ~~*Scylla serrata*~~*S. serrata* meat powder was weighed and immersed in 1000 mL of 96% ethanol solvent at a ratio of 1:4 (b/v). The soaking process was conducted for 3 × 24 h, with stirring performed periodically to ensure optimal extraction. The maceration results were filtered using filter paper (No. 42, 125 mm). The extraction process was then carried out using a rotary evaporator at 40°C with a rotating speed of 3000 rpm (Hashim ~~et al~~*et al*., 2021). The resulting extract was stored as a stock solution. A total of 0.05 g of ~~*Scylla serrata*~~*S. serrata* extract was used as an additive for the stock solution (Habib ~~et al~~*et al*., 2022). Wet maceration is a commonly employed extraction method due to its ability to preserve heat-sensitive compounds. Stirring during the maceration process enhances solute dissolution, while rotary evaporation ensures efficient solvent removal under controlled temperature conditions.

Determination of Antioxidant Activity and IC50 Value

Antioxidant testing was conducted using the DPPH method (Vásquez *et al*., 2023). The stock solution of *S. serrata* extract was used as the test solution, while vitamin C served as a reference. The sample was dissolved in 96% ethanol. A total of 1 mL of sample solution was prepared for each concentration: 100, 150, 200, 250, and 300 mg/L. Each concentration was mixed with 40 µg/mL DPPH solution and incubated in the dark for 30 min. Absorbance was measured at 517 nm using UV-Vis spectrophotometry. The strength of antioxidant activity is classified according to IC50 values, as shown in Table 1.~~Antioxidant testing was conducted using the DPPH method (Vásquez et al., 2023). The stock solution of *Scylla serrata* extract was used as the test solution for antioxidant~~

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~~activity, while vitamin C solution served as a reference. The sample was dissolved in 96% ethanol. A total of 1 mL of sample solution was prepared for each concentration: 100 mg.L⁻¹, 150 mg.L⁻¹, 200 mg.L⁻¹, 250 mg.L⁻¹, and 300 mg.L⁻¹. Each concentration was mixed with 40 µg.mL⁻¹ DPPH solution and incubated in the dark for 30 min. The absorbance values were measured at 517 nm using UV-Vis spectrophotometry. The percentage of inhibition and IC50 value were calculated (Naveed et al., 2022). The characterization of antioxidant activity is determined based on the IC50 value, which represents the concentration of the sample required to inhibit 50% of DPPH radicals. The strength of antioxidant activity is classified according to IC50 values, as shown in Table 1.~~ The IC50 value is calculated using the following formula.

$$\cancel{inhibisi}\,inhibition = \frac{blank\ abs - sample\ abs.}{blank\ abs}\ x\ 100\ \%$$

The IC50 results were entered in the linear regression equation y= ax + b~~Y = AX + B~~. The sample concentration is the abscissa (X-axis), and the percentage of antioxidant inhibition is the ordinate (Y-axis) (Yuniarti ~~et al~~*et al*., 2020). The concentration corresponding to 50% inhibition was interpolated from the regression equation. All determinations were conducted in triplicate, and the mean IC_{50} values were statistically compared using one-way ANOVA, followed by Duncan's multiple range test ($p < 0.05$).

Table 1. Characteristic concentration value of IC50

Concentration Value (µg.mL^{-1})	Characteristic
<50	Very Strong
50-100	Strong
100-150	Moderate
150-200	Low

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Phytochemical analysis

Phytochemical Screening

Phytochemical tests of ~~*Scylla serrata*~~*S. serrata* meat extracts were conducted using qualitative methods to identify the presence of bioactive compounds. The analysis included steroid and triterpenoid tests, which were performed using the Liebermann-Burchard method,~~;~~ alkaloid tests using Mayer and Dragendorff reagents, ~~;~~ flavonoid tests with the Shinoda staining method,~~;~~ tannin tests using the $FeCl_3$ reaction,~~;~~ and saponin tests using the foam test method. Each test was carried out following the procedures described in standard literature (Dinesh *et al*., 2022; Suwandi *et al*., 2020).

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Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The identification of bioactive compound components in *S. serrata* meat extract was performed using the GC-MS analysis method, following (Rozirwan~~, Nugroho, Hendri,~~ *et al*.~~,~~ (2022) with modifications. A total of 1 µL of extract was injected into an RTX-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) using helium as the carrier gas at a constant flow rate of 1.0 mL/min and a split ratio of 1:50. The oven temperature was initially set at 50°C for 5 min, then increased at a rate of 5°C/min to 280°C, and held for 5 min. The injector and ion source temperatures were 280°C and 230°C, respectively, with an electron ionization (EI) energy of 70 eV. The mass spectrometer was operated in scan mode with a mass range of 40–550 m/z. The Wiley 7 Library database was used as a reference for spectral comparison (Rafferty *et al*., 2020).~~The identification of bioactive compound components in *Scylla serrata* meat extract was performed using the GC-MS analysis method. GC-MS analysis was conducted following the method described by Rozirwan *et al*. (2022).~~

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~~A total of 1 μL of extract was injected into the column (rt x 5 ms) with helium as the carrier gas and a split ratio of 1:50. The oven temperature was set at 50°C for 5 min, then gradually increased at a rate of 5°C per minute to a maximum temperature of 280°C, which was maintained for 5 min. Samples were injected 280°C. The Wiley 7 Library database was used as a reference for comparing the spectra of the analyzed compounds (Rafferty et al., 2020).~~

~~***Data Analysis***~~

~~Further data processing was performed by conducting normality tests (Shapiro-Wilk) and homogeneity tests (Levene) (Ebadzadeh et al., 2024; Gebruk et al., 2021). All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation. Statistical analysis was carried out using SPSS software. One-way analysis of variance (ANOVA), followed by Duncan's multiple range test for post hoc comparisons, was used to identify significant differences among groups. Statistical significance was determined at $p < 0.05$.~~

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Result and Discussion

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Sample Identification and Collection

~~***Samples Catch, Collage, Storage and Identification***~~

Taxon determination was conducted using data from the World Register of Marine Species (WoRMS) accessed in January 2023 (WoRMS, 2023). Based on their morphological characteristics, the crustacean samples were identified as ~~*Scylla serrata*~~*S. serrata*.

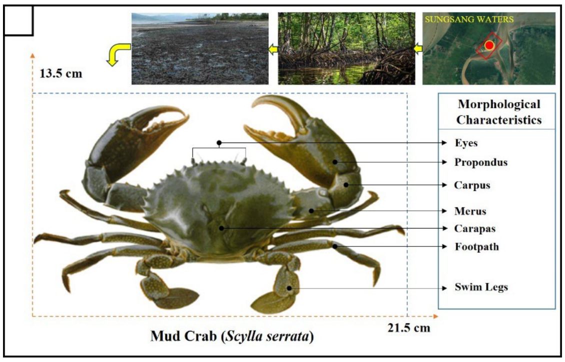

Figure 2. Crustacean species, ~~*Scylla serrata*~~*S. serrata*

Mangrove crabs of this species were caught using folding traps. The fishing process was carried out during low tide to facilitate crab capture. In this study, 2~~-~~5 crabs were used as stock samples. The crabs obtained were weighed, with weights ranging from 200 to 320 g, widths of approximately 21.5 cm, and lengths of around 13.5 cm. The crabs were then stored in a cool box filled with ice cubes for preservation.

The species identification of mangrove crabs is based on morphological characteristics, which include features such as the eyes, ~~propound~~propodus, carpus, merus, carapace, claws, walking legs, and swimming legs (Figure 2). Taxonomic data from databases such as WoRMS is widely used to confirm species classification accurately.

Environmental Characteristics of Sampling Area

Table 2. Observation of Environmental Parameters of the Research Site

Environment Parameter Quality	**Station**
	Sungsang Waters
Dissolved oxygen (~~mg.L⁻¹~~mg/L)	4.2
pH	7
Temperature (°C)	28
Salinity (psu~~‰~~)	18

The results of environmental quality measurements in the ~~*Scylla serrata*~~*S. serrata* sampling areas in Banyuasin waters revealed diverse conditions. Measurements of water physicochemical parameters, including dissolved oxygen, pH, temperature, and salinity, were taken to assess the habitat suitability for ~~*Scylla serrata*~~*S. serrata* (Rozirwan ~~, Melki et al~~*et al*., 2021). The dissolved oxygen concentration was found to be 4.2 ~~mg.L⁻¹~~mg/L, which is sufficient to support the respiration process of aquatic organisms (Ouyang ~~et al~~*et al*., 2021). The pH value of the water at the sampling location was 7, indicating neutral pH, which represents optimal ecological conditions (Chowdhury ~~et al~~*et al*., 2021). This is consistent with previous studies (Muhtar and~~&~~ Lanuru, 2021; Putri *et al*., 2022; Yusni and~~&~~ Haq, 2020), which state that waters with a pH between 6.5 and 7.5 are ideal for the survival of mangrove crabs. The water temperature was measured at 28°C, indicating favorable conditions for mangrove crab growth (Indarjo ~~et al~~*et al*., 2020; Ren ~~et al~~*et al*., 2021) Water salinity was recorded at 18 psu~~‰~~, reflecting typical conditions in estuarine areas or areas directly influenced by tidal movements (Wang ~~et al~~*et al*., 2021). ~~*Scylla serrata*~~*S. serrata* grows best in salinities between 15 psu~~‰~~ and 25 psu~~‰~~ but grows more slowly at salinities greater than 25 to 30 psu~~‰~~ (Adnan *et al*., 2024; Pati *et al*., 2023; Triajie *et al*., 2020).

Environmental conditions at the sampling site, particularly salinity (18 psu) and temperature (28°C), could also influence the diversity and concentration of secondary metabolites in *S. serrata*. Estuarine environments are dynamic, and organisms inhabiting them may prioritize the synthesis of specific bioactive compounds for stress adaptation. Previous studies reported that salinity stress can regulate secondary metabolite production, including alkaloids and flavonoids, in marine organisms (Pati *et al*., 2023). Furthermore, the choice of ethanol as the extraction solvent, although safer and less toxic than methanol, may have influenced the recovery efficiency of certain compounds, possibly underestimating the abundance of more polar metabolites.

Determination of Antioxidant Activity by DPPH Assay

Antioxidant analysis using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method on Rozirwan *et al*. (2023) ~~*Scylla serrata*~~*S. serrata* meat extract showed promising results. The DPPH solution changed from purple to yellow, indicating the presence of antioxidants in the extract. The test results revealed that the ~~*Scylla serrata*~~*S. serrata* extract had an IC50 value of 2.25 ppm, while Vitamin C, used as a comparison solution, had an IC50 value of 2.16 ppm (Table 3). Both solutions demonstrated low IC50 values, categorizing them as very strong antioxidant compounds. The IC50 value, which is the concentration required to inhibit 50% of DPPH radical activity, is a critical parameter for assessing the antioxidant potential of a compound (Martinez-Morales ~~et al~~*et al*., 2020). The results indicate that ~~*Scylla serrata*~~*S. serrata* extract possesses an IC50 value comparable to that of Vitamin C. The percentage inhibition of DPPH free radicals by ~~*Scylla serrata*~~*S. serrata* meat extract increased as

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the extract concentration increased. This suggests that the extract has the ability to donate electrons or hydrogen to the DPPH radical, neutralizing it and converting it into a more stable form (Gulcin and~~&~~ Alwasel, 2023).

Table 3. Calculation results of antioxidant activity of ~~*Scylla serrata*~~*S. serrata* in Sungsang waters

Sample	Linear Regression			IC50 Value	Category
	a	b	R^2		
~~*Scylla serrata*~~*S. serrata*	6.9429	52.808	0.9327	2.25 ~~$mg.L^{-1}$~~mg/L	Very Strong
~~Asorbic~~ Ascorbic Acid	6.7135	55.866	0.9435	2.16 ~~$mg.L^{-1}$~~mg/L	Very Strong

The discovery of compounds such as flavonoids and triterpenoids, which were identified in the phytochemical analysis of ~~*Scylla serrata*~~*S. serrata* extracts, suggests that they may possess high antioxidant activity (Akinwumi ~~et al~~*et al*., 2022; Hajar-Azira ~~et al~~*et al*., 2023). Flavonoids are well known for their ability to capture free radicals and interrupt the chain of oxidative reactions. Triterpenoids are also recognized for their significant antioxidant activity through a similar mechanism. The combination of these compounds in ~~*Scylla serrata*~~*S. serrata* extract creates a synergistic effect, enhancing the overall capacity of the extract to neutralize free radicals.

~~*Scylla serrata*~~*S. serrata* is known for its rich antioxidant system and strong enzymatic defense system, which help it survive in the dynamic and often challenging mangrove environment (Pati ~~et al~~*et al*., 2023). Mud crabs also possess defense enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, which work synergistically to detoxify reactive oxygen species (ROS) and maintain redox balance in the body (Bal *et al*., 2021; Costantini *et al*., 2022; Jerome *et al*., 2020; Zeng *et al*., 2024). These enzymes play a crucial role in protecting the crab from oxidative stress generated by fluctuating environmental conditions, pollution, and pathogens.

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This combination of antioxidant compounds and defense enzymes not only ensures the survival of mud crabs in their habitat but also positions them as a potential source for the development of natural health products that can harness their protective mechanisms.

Antioxidants and defense enzymes play a vital role in organisms' survival, especially in harsh environments. The synergy between these compounds and enzymes contributes significantly to mitigating oxidative stress and preserving cellular function, which can be explored for potential therapeutic applications.

Phytochemical Analysis

Phytochemical Screening

Phytochemical tests were carried out to identify the compounds present in gastropod and crustacean extracts using ethanol as the solvent (Fitria ~~et al~~*et al*., 2023; Rozirwan ~~et al~~*et al*., 2024) The phytochemical test aimed to determine the compounds in the test extract (Chen ~~et al~~*et al*., 2022), allowing for the identification of compounds that influence the strong or weak antioxidant activity of the extract (Baliyan ~~et al~~*et al*., 2022). The results of the phytochemical test, after UV-Vis spectrophotometric analysis of the extract, are presented in Table 4. The test results showed that only certain compounds were extracted by the ethanol solvent (Yuniarti ~~et al~~*et al*., 2020).

Table 4. Phytochemical screening results of ~~*Scylla serrata*~~*S. serrata*

No.	Parameters	Analysis Result	Analysis Type
1	Alkaloids	-	Qualitative
2	Flavonoids	+	Qualitative
3	Triterpenoids	+	Qualitative
4	Saponin	-	Qualitative
5	Tannins	-	Qualitative
6	Steroid	-	Qualitative

Qualitative phytochemical analysis of ~~*Scylla serrata*~~*S. serrata* extract showed significant results in identifying the content of bioactive compounds. Based on the test results, ~~*Scylla serrata*~~*S. serrata* extract tested positive for flavonoid and triterpenoid compounds. A similar finding was reported by Elshaarawy ~~*et al*~~*et al*. (2023) for *Scylla olivacea* samples. These two compounds offer various health benefits and therapeutic potential, particularly due to their strong antioxidant properties. Flavonoids are a group of polyphenolic compounds that are widely recognized for their potent antioxidant activity (Shen ~~et al~~*et al*., 2022). These compounds can neutralize free radicals and prevent oxidative damage to cells and tissues. Additionally, flavonoids possess anti-inflammatory, anticancer, and cardioprotective properties (Jain *et al*., 2024; Mounika *et al*., 2021; Ullah *et al*., 2024). The identification of flavonoid compounds in ~~*Scylla serrata*~~*S. serrata* indicates that mud crabs are not only valuable as food but also hold potential as an alternative source of medicine from marine organisms.

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Triterpenoids are a group of terpenoid compounds that have potential biological activities (Mabou and~~&~~ Yossa, 2021; Zang *et al*., 2022). These compounds are known to possess anti-inflammatory, antitumor, antimicrobial, and immunomodulatory properties (Ahmad *et al*., 2021; Harun *et al*., 2020). As antioxidant agents, triterpenoids have been used in pharmacology to treat inflammatory diseases and cancer. Similar to flavonoids, these compounds can neutralize free radicals caused by oxidative stress on body tissues. The discovery of triterpenoid compounds in ~~*Scylla serrata*~~*S. serrata* shows promising results, given their antioxidant potential that can be applied to address various diseases. Thus, the opportunity to explore alternative medicinal raw materials from ~~*Scylla serrata*~~*S. serrata* extract is increasingly attractive for further research. Overall, the phytochemical results focusing on flavonoid and triterpenoid compounds confirm the importance of ~~*Scylla serrata*~~*S. serrata* as a potential source of bioactive compounds. Further research is encouraged at the stage of isolation and purification of these compounds, so that alternative medicinal materials derived from this marine organism can contribute to the development of therapeutic agents from marine organisms.

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Phytochemical Profile Screening

The antioxidant compound profile in ~~*Scylla serrata*~~*S. serrata* was determined using GC-MS (Gas Chromatography-Mass Spectrometry) analysis on the ethanol extract of mud crab. Figure 3 shows the chromatogram with 37 peaks identified in the extract. Each peak on the chromatogram represents a distinct chemical compound found in the extract, which was analyzed using GC-MS.

GC-MS analysis is a powerful technique for identifying and quantifying individual compounds in complex mixtures, providing insights into the chemical composition of the extract. This method is widely used for its ability to separate and identify volatile compounds, making it an essential tool for profiling bioactive compounds, such as antioxidants, in natural products.

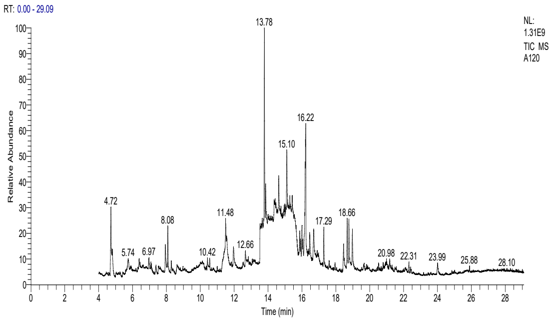

Figure 3. GC-MS chromatogram of ethanol extract ~~*Scylla serrata*~~*S. serrata*

Terpenoid, alkaloid, steroid, and tannin groups were among the pure compounds successfully detected using GC-MS. Based on the GC-MS analysis of the ethanol extract compound components presented in Table 5, the main components identified in the extract were Calycotomine, N-methyl-, with a value of 8.31% of the total area, and uric acid, with a value of 8.71% of the total area.

At a retention time of 4.72 ~~minutes~~min, the compound 2-Cyclohexylpiperidine was detected with an area of 3.45%, a probability of 6.07, and a chemical formula of C11H21N, which belongs to the alkaloid compound group. At a retention time of 5.74 ~~minutes~~min, the compound 2-Pyridinamine, 3,6-dimethyl was detected with an area of 1.69%, a probability of 7.02, and a chemical formula of C7H10N2, which belongs to the aminopyridine compound group. Furthermore, at a retention time of 6.97 ~~minutes~~min, the compound Pentanoic acid, dodec-9-ynyl ester was detected with an area of 1.00%, a probability of 8.47, and a chemical formula of C17H30O2, which belongs to the protein compound group.

At a retention time of 8.08 ~~minutes~~min, the compound L-Homoserine lactone, N, N-dimethyl was detected with an area of 1.97%, a probability of 82.23, and a chemical formula of C6H11NO2, which belongs to the amino acid compound group. At a retention time of 10.42 ~~minutes~~min, the compound Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetramethyl was detected with an area of 0.74%, a probability of 43.35, and a chemical formula of C11H14N2OS, which belongs to the EPA compound group. At a retention time of 11.48 ~~minutes~~min, the compound 1H-2-Indenol, 2,3,4,5,6,7-hexahydro-1-(2-hydroxy-2-methylpropyl) was detected with an area of 4.83%, a probability of 12.26, and a chemical formula of C13H22O2, which belongs to the lactone compound group.

At a retention time of 12.66 ~~minutes~~min, the compound dl-Lysine was detected with an area of 0.86%, a probability of 23.13, and a chemical formula of C6H14N2O2, which belongs to the amino acid compound group. At a retention time of 13.78 ~~minutes~~min, the compound Calycotomine, N-methyl- was detected with an area of 8.31%, a probability of 43.35, and a chemical formula of C13H19NO3, which belongs to the alkaloid compound group. At a retention time of 15.10 ~~minutes~~min, the compound Dasycarpidan-8(16H)-ethanol, 3,18-dihydro-1-(hydroxymethyl)-, (2.xi.,4.xi.)- was detected with an area of 4.14%, a probability of 12.00, and a chemical formula of C20H28N2O2, which belongs to the group of molport compounds.

At a retention time of 16.22 ~~minutes~~min, the uric acid compound was detected with an area of 8.71%, a probability of 55.93, and a chemical formula of C5H4N4O3, which belongs to the allantoin compound group. At a retention time of 17.29 ~~minutes~~min, the compound Actinomycin C2 was detected with an area of 1.49%, a probability of 30.73, and a chemical formula of C63H88N12O16, which belongs to a group of peptide compounds that are derivatives of peptide compounds.

Animals produce a diverse mixture of secondary metabolites such as phenols, alkaloids, flavonoids, tannins, and saponins. Several animal studies have shown the potential use of these metabolites as antibacterial agents due to the presence of abundant biomolecules. Synthetic drugs often have high secondary failure rates and severe side effects, while animal products contain a variety of free radical scavenging molecules with substantial antioxidant properties.

As a more taxonomically relevant comparison, Yao *et al*. (2020) conducted a GC-MS-based metabolomic study on Scylla paramamosain experiencing acute salinity reduction (from 23 psu to 3 psu). This study identified 519 metabolites (mainly lipids), with 13 significantly enriched metabolic pathways ($P < 0.05$), related to signaling, lipid metabolism, and transport. Additionally, in that study, combining LC-MS and GC-MS data revealed 28 significant metabolic pathways, dominated by amino acid and energy metabolism, with lipid metabolism playing a supporting role. In comparison, in our study, Scylla serrata ethanol extract showed only 8.31% alkaloid content among the dominant compounds. This difference suggests that environmental stress factors such as sudden salinity reduction can significantly modulate the metabolite profile among related *Scylla* species, especially under similar polar extraction conditions.

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Interestingly, alkaloid compounds were not detected in the qualitative phytochemical screening, but several alkaloids such as Calycotomine and Imidazole were identified through GC-MS analysis. This discrepancy is not only due to methodological differences but may also be related to the detection sensitivity. Qualitative tests often fail to detect compounds present at low concentrations, whereas GC-MS has a higher sensitivity and can identify minor constituents. In addition, the polarity of ethanol as a solvent might have selectively extracted certain alkaloids in low yield, which escaped detection in qualitative assays but were quantifiable in GC-MS analysis. Similar observations were reported in marine-derived extracts where alkaloids were inconsistently detected depending on the analytical method (Rahmawati *et al*., 2023; Shofinita *et al*., 2024).

~~Research by Waluyo and Wahyuni (2021) on *Conus miles* bacteria identified three dominant compounds through GC-MS analysis, namely Acetic acid (CAS), Ethylic acid, Proanoic acid, 2-methyl (CAS), and Isobutyric acid and Iso Valeric acid. Research on the ethanol extract of *Enhalus acoroides* conducted Mediarman *et al*., (2021) using GC-MS showed nine compounds dominated by alkaloids, flavonoids, terpenoids, and polyphenols. Some of the compounds found in *Enhalus acoroides* have similarities with the GC-MS results on *S. serrata*, but the percentage area of the alkaloid compound group in *S. serrata* was only 8.31%, compared to the ethanol extract of *Enhalus acoroides*, which reached 53.88%.~~

~~In the GC-MS results, the highest peak was identified as coming from the antioxidant compounds of the alkaloid group. However, in the phytochemical test, alkaloid compounds were not detected in the *S. serrata* sample. This could be due to differences in the analytical methods used. Phytochemical tests are qualitative, while GC-MS analysis is quantitative. Quantitative analysis methods are known to be more accurate than qualitative methods.~~

~~The difference in the results of phytochemical tests and GC-MS analysis can be influenced by the type of solvent used during the maceration process of mangrove crab (*S. serrata*) extracts. In this study, the solvent used was ethanol, which is polar and able to bind bioactive compounds quickly and is relatively safe for humans compared to methanol. Methanol solvent is more polar than ethanol but is more toxic. This is in accordance with research conducted by Rahmawati *et al*., (2023); Shofinita *et al*., (2024), which show that methanol is more polar but also more toxic than ethanol.~~

Table 5. Proposed peak order, retention time, probability, area, compound name, and molecular formula

Peak#	R. Time	Probability	Area%	Name	Molecular formula
1	4.72	6.07	3.45	2-Cyclohexylpiperidine	C11H21N
2	4.81	18.58	1.37	Edulan II	C13H20O
3	5.74	7.02	1.69	2-Pyridinamine, 3,6-dimethyl	C7H10N2
4	6.39	8.12	0.83	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	C16H28O3
5	6.61	11.07	0.66	d-Mannose	C6H12O6
6	6.81	50.91	0.89	Deoxyspergualin	C17H37N7O3
7	6.97	8.47	1.00	Pentanoic acid, dodec-9-ynyl ester	C17H30O2
8	7.93	51.03	1.42	trans-(2 Chlorovinyl)dimethylethoxysil ane	C6H13ClOSi
9	8.08	82.23	1.97	L-Homoserine lactone, N,N-dimethyl-	C6H11NO2
10	8.28	32.09	0.64	2-Propyl-tetrahydropyran-3-ol	C8H16O2
11	8.62	32.45	0.93	Imidazole	C3H4N2
12	10.00	11.83	0.76	Tertbutyloxyformamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-buty nyl]-	C14H24N2O2
13	10.42	15.27	0.74	Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetrameth yl	C11H14N2OS
14	11.48	12.26	4.83	1H-2-Indenol, 2,3,4,5,6,7-hexahydro-1-(2-hydroxy-2-methylpropyl)	C13H22O2
15	11.55	48.75	2.53	dl-Citrulline	C6H13N3O3
16	11.96	7.38	1.45	2-Pyridineacetic acid, hexahydro-	C7H13NO2
17	12.66	23.13	0.86	dl-Lysine	C6H14N2O2
18	13.51	60.28	1.62	D-Streptamine, O-6-amino-6-deoxy-à-D-glucopyranosy l-(1-4)-O-(3-deoxy-4-C-methyl-3-(meth ylamino)-á-L-arabinopyranosyl-(1-6))- 2-deoxy	C6H13NO2
19	13.78	43.35	8.31	Calycotomine, N-methyl-	C13H19NO3
20	13.99	17.84	2.38	4-[4-Diethylamino-1-methylbutylamino]-1,2-dimethoxy-6-bromonaphthalene	C21H31BrN2O2
21	14.35	32.83	1.22	Dasycarpidan-8(16H)-ethanol, 3,18-didehydro-1-(hydroxymethyl)-, (2.xi.,4.xi.)-	C20H28N2O2
22	14.62	12.2	2.67	4-[4-Diethylamino-1-methylbutylamino]-1,2-dimethoxy-6-bromonaphthalene	C21H31BrN2O2
23	15.10	12.00	4.14	Dasycarpidan-8(16H)-ethanol, 3,18-didehydro-1-(hydroxymethyl)-, (2.xi.,4.xi.)-	C20H28N2O2
24	15.21	32.49	0.87	Cystine	C6H12N2O4S2
25	15.65	33.28	1.26	3-[N-[2-Diethylaminoethyl]-1-cyclopes tenylamino]propionitrile	C14H25N3
26	15.86	16.52	0.92	á-Hydroxyquebrachamine	CH3CH(OH)COOH
27	15.99	14.23	1.02	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	C21H36O4
28	16.22	55.93	8.71	Uric acid	C5H4N403
29	16.45	8.30	1.33	Aminoacetamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-buty nyl]-	C5H4N4O3
30	16.69	32.32	2.06	Glucopyranuronamide, 1-(4-amino-2-oxo-1(2H)-pyrimidinyl)- 1,4-dideoxy-4-(D-2-(2-(methylamino) acetamido)hydracrylamido)-, á-D	C10H14N2O
31	16.90	29.78	0.80	1,2,4-Trioxolane-2-octanoic acid, 5-octyl-, methyl ester	C6H11NO6
32	17.29	30.73	1.49	Actinomycin C2	C19H36O5
33	18.45	37.85	2.00	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	C63H88N12O16
34	18.66	90.13	2.23	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	C63H88N12O16
35	18.76	60.11	2.62	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6 H-dipyrrolo[1,2-a:1',2'-d]pyrazine	C11H18N2O2
36	18.97	36	2.13	l-(+)-Ascorbic acid	C14H22
37	20.98	13.19	1.29	cis-13-Octadecenoic acid	C6H8O6
38	21.17	13.49	0.94	Ricinoleic acid	C18H34O2
39	23.99	31.48	0.89	Ergotaman-3',6',18-trione, 12'-hydroxy-2'-methyl-5'-(phenylmeth yl)-, (5'à)-	C18H34O3

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The main group of compounds in the ethanol extract of mud crab (*S. serrata*) was represented by three peaks on the GC chromatogram, which had a higher percentage area than the others. These peaks were identified as Calycotomine, N-methyl- (8.31%), uric acid (8.71%), and Dasycarpidan-8(16H)-ethanol, 3,18-didehydro-1-(hydroxymethyl)-(2.xi.,4.xi.)- (4.14%). Antioxidant compounds detected in the GC-MS analysis included 2-Cyclohexylpiperidine with an area of 3.45%, which belongs to the alkaloid group. In addition, the Edullan II compound with an area of 1.37% belongs to the volatile compound group. The compound Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate, with an area of 0.83%, belongs to the terpenoid compound group, and Imidazole with an area of 0.93% also belongs to the alkaloid group.

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In a study conducted by Karnila *et al.* (2021) on mud crab (*S. serrata*), antioxidant compounds such as astaxanthin with a peak area of 18.6% and β-carotene with a peak area of 7.9% were identified through GC-MS analysis, both of which belong to the carotenoid group and contribute to antioxidant activity. Similarly, a study by Thiruvengadarajan *et al.* (2024) on the nutritional composition of *S. serrata* reported the presence of unsaturated fatty acids such as eicosapentaenoic acid (EPA, 5.42%) and docosahexaenoic acid (DHA, 4.87%), which are categorized as bioactive lipids and known for their health-promoting effects.

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~~In a study conducted by Rasyid (2016) on sea cucumber biota (*Bohadschia* sp.), steroid compounds consisting of cholest-2-ene (3.91%), stigmastan-3,5-diene (3.29%), cholest-5-en-3-yl nonanoate (4.89%), and cholest-5-ene, 3.beta.-chloro- (3.12%) were found. The compound Calycotomine, N-methyl- with an area of 8.31% belongs to the alkaloid group. The compound l-(+)-Ascorbic acid with~~

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~~an area of 2.13%, as well as 5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1',2'-d]pyrazine with an area of 2.62%, belong to the alkaloid group, and Ergotaman-3',6',18-trione, 12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'à)- with an area of 0.89%, also belongs to the alkaloid group. Lalitha *et al*., (2021), in their research on antioxidants in mangrove *Avicennia officinalis* L. through GC-MS testing, identified a group of antioxidant compounds such as Trans-cinnamic acid, with a peak area percentage of 27.7%.~~

The group of compounds identified in Scylla serrata are alkaloids. Based on research by Karim *et al*. (2024) analyzing muscle and hepatopancreas extracts of *S. olivacea*, they reported a lipid profile including EPA and DHA content, as well as significant antioxidant capacity measured through the DPPH and ferric-reducing tests. Similarly, Taufik *et al*. (2020) applied GC–MS to *S. olivacea* tissues, revealing a predominance of monounsaturated and polyunsaturated fatty acids, including long-chain polyunsaturated fatty acids (PUFAs) in the gonads and hepatopancreas. Although specific alkaloid compounds have not been reported for *Scylla*, these lipid-based metabolites provide relevant biological context within the genus *Scylla*. Therefore, comparisons of the alkaloid profiles of *S. serrata* should emphasize the same metabolic pathways within this genus and highlight the need for targeted alkaloid profile studies on *Scylla* species.

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~~The group of compounds found in mud crabs (*S. serrata*) were alkaloids. Alkaloid compounds can also be found in marine plants such as seagrasses. This is consistent with the findings of Shaffai *et al*., (2023); Tjandrawinata and Nurkolis, (2024), who identified a group of alkaloid compounds in the ethanol extract of *Enhalus acoroides*, such as Phenyl-N-methylindole with a peak area of 53.88%, N-Methyldeacetylcolchicine with a peak area of 1.55%, and Phenyl-5-methylindole with a peak area of 28.98%~~

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Conclusion

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This study successfully demonstrated that *S. serrata* extract possesses very strong antioxidant activity, supported by the presence of flavonoids, triterpenoids, and various bioactive compounds identified through phytochemical and GC-MS analyses. These findings confirm that the research objective to explore and characterize the antioxidant potential and compound profile of *S. serrata* has been achieved. The extract's bioactive profile, comprising both major and minor compounds, reinforces its potential as a promising source of natural antioxidants with additional pharmacological properties, including anti-inflammatory, anticancer, antimicrobial, and antiviral activities. Overall, this study contributes to the growing evidence that marine organisms represent valuable resources for the development of alternative medicinal agents.~~The results of antioxidant testing with the DPPH method on *Scylla serrata* extracts showed IC50 values at very strong antioxidant characteristics. Phytochemical identification shows the extract contains strong bioactive compounds in the flavonoid and triterpenoid compound groups. GC-MS compound profile identification showed GC-MS analysis detected major compound groups consisting of alkaloids, purines, and vitamins. Minor compound groups were detected from volatile compounds, amines, terpenoids, monosaccharides, amino acids, fatty acids, silanes, formamides, heterocycles, carboxylic acids, aminoglycosides, naphthalene derivatives, nitriles, amides, glycosides, and peptides. *Scylla serrata* extract has been reported to have potential as antioxidant, anti-inflammatory, anticancer, antimicrobial, and antiviral. The results of this study are expected to provide important information in the disclosure of alternative medicinal materials from marine organisms.~~

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Screening and Profiling of Antioxidant Activity in Mud Crab (*Scylla Serrata*) from Banyuasin Waters

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Abstract

Mangrove crab (Scylla serrata) as one of the crustacean species, has a variety of bioactive compounds that can be utilized in the field of pharmacology. Antioxidant compounds act as therapeutic agents against degenerative diseases. Banyuasin waters have mangrove vegetation with associated marine organisms that have the potential to be studied for bioactive compounds. This study aims to identify the phytochemical profile quantitatively and qualitatively, samples were collected from mud flats near mangrove ecosystems in Banyuasin waters, South Sumatra. Samples were tested for antioxidant activity using the DPPH test, and IC_{50} values, qualitative phytochemical identification, and phytochemical profiles were calculated using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Based on the results of antioxidant testing, the IC_{50} value of S. serrata extract is 2.25 ppm, the sample is included in the category of very strong antioxidants. Phytochemical test results showed that the compound is thought to contain antioxidant activity from flavonoids and triterpenoids. GC-MS analysis detected major compound groups of alkaloids, purines, and vitamins. Minor compound groups detected amines, terpenoids, monosaccharides, amino acids, fatty acids, silanes, formamides, heterocycles, carboxylic acids, aminoglycosides, naphthalene derivatives, nitriles, amides, glycosides, and peptides. S. serrata extract shows very strong antioxidant activity, with major compounds such as alkaloids, purines, and vitamins. S. serrata extract detected compounds that have been reported as anti-inflammatory, anticancer, antimicrobial, and antiviral. These findings highlight the pharmaceutical potential of S. serrata as a source of bioactive compounds. The results of this study provide valuable information for the development of alternative medicines derived from marine organisms.

Keywords: *Antioxidant, Bioactive compounds, DPPH, GC-MS, S. serrata*

Introduction

Scylla serrata, or mud crab, as one of the crustacean species, has great potential in the field of beauty and health (De Castro *et al*., 2023), because of its diverse bioactive compounds (Yusof *et al*., 2020; Beslin and Geni, 2021; Neelima *et al*., 2022). The diversity of bioactive compounds triggers great potential in the search for alternative medicinal ingredients from marine organisms, including antioxidant compounds (Yogeshwaran *et al*., 2020; Karnila and Ramadhani, 2021; Fajriaty *et al*., 2024). Antioxidant compounds in mud crabs have a role as therapeutic agents (Wu *et al*., 2021) in fighting degenerative diseases such as cancer (Nagarajan *et al*., 2024), cardiovascular diseases (Nanda *et al*., 2021), and neurodegenerative disorders (Galal-Khallaf *et al*., 2024), caused by oxidative stress due to free radicals.

Antioxidants are the reaction of a compound in neutralizing free radicals (Delta *et al*., 2021; Rozirwan *et al*., 2023a; 2023b). This process involves a highly efficient electron donation mechanism. Antioxidants work by donating electrons to free radicals (Fajriaty *et al*., 2024; Frías-Espericueta *et al*., 2022; Pati *et al*., 2022; Yang *et al*., 2023). Free radicals, which have one or more unpaired electrons, are highly reactive and can cause oxidative damage to DNA, proteins, and cellular lipids (Alkadi, 2020; Di Meo and Venditti, 2020). Free radicals are often generated as by-products of various metabolic processes in the body or due to environmental exposures such as pollution and ultraviolet radiation (Martemucci *et al*., 2022; Sadiq, 2023). The damage caused by free radicals can contribute to the development of various degenerative diseases, including cancer, heart disease, and neurodegenerative disorders (Teleanu *et al*., 2022; Chaudhary *et al*., 2023). In inhibiting free radicals, antioxidant compounds such as carotenoids and polyphenols will interact with free radicals to enhance the activity of detoxification enzymes, resulting in accelerated elimination of free radicals and strengthened antibodies (Pisoschi *et al*., 2021; Tumilaar *et al*., 2024).

The analysis of antioxidant compounds in *S. serrata* requires an accurate technique to ensure that identification and quantification are targeted (Baag and Mandal, 2023; Yao *et al*., 2023). Previous studies have mainly reported the antioxidant potential of *S. serrata* using spectrophotometric or colorimetric assays such as DPPH, ABTS, and FRAP, which provide only general antioxidant capacity without revealing the identity of specific compounds (Yogeshwaran *et al*., 2020; Karnila and Ramadhani, 2021; Neelima *et al*., 2022). In contrast, Gas Chromatography-Mass Spectrometry (GC-MS) offers higher resolution in profiling bioactive compounds by separating and identifying molecules based on their mass and chemical characteristics (Jabbar *et al*., 2022; Musa *et al*., 2022; Palma *et al*., 2023; Rozirwan *et al*., 2024). This advanced method not only allows the detection of a broader spectrum of antioxidant molecules but also quantifies their abundance, thereby providing a more comprehensive understanding of their therapeutic potential.

Although antioxidant compounds such as flavonoids, carotenoids, and polyphenols have been reported from different body parts of *S. serrata* (Yogeshwaran *et al*., 2020; Karnila and Ramadhani, 2021; Neelima *et al*., 2022; Yang *et al*., 2023; Fajriaty *et al*., 2024), most of these studies remain descriptive and do not provide detailed profiles of the specific types and relative quantities of individual compounds. To date, there is still no comprehensive GC-MS based profiling that systematically characterizes the antioxidant repertoire of *S. serrata* meat. This represents a critical knowledge gap, since understanding the diversity and concentration of specific compounds is essential to support its therapeutic application. The present study aims to provide a detailed GC-MS profiling of antioxidant compounds in *S. serrata*. This work contributes novel insights into its biochemical composition and strengthens its potential utilization as a sustainable source of natural antioxidants for pharmaceutical and nutraceutical development.

Materials and Methods

Mangrove crab samples were collected in January 2023 from Banyuasin Waters, South Sumatra, Indonesia (Figure 1). At this location, numerous crustacean and gastropod populations were found inhabiting the intertidal zone ((Rozirwan *et al*., 2021; Rozirwan *et al*., 2021; Rozirwan *et al*., 2022). The crab fishing area had a mud substrate with a depth of 1-2 m and was located within a mangrove vegetation zone directly connected to port and pond activities (Fitria *et al*., 2023). Anthropogenic pollutants that accumulate in these waters are known to trigger an increase in the defense mechanisms of organisms, such as the production of antioxidant compounds derived from secondary metabolites (Rozirwan *et al*., 2023).

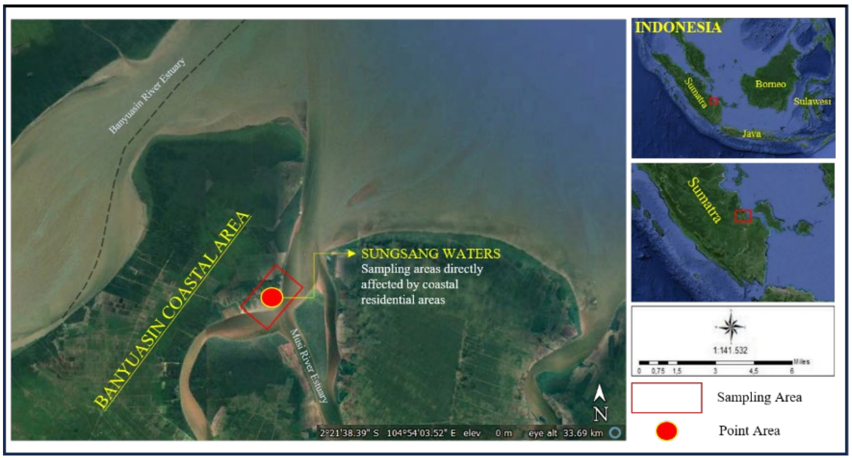

Figure 1. Map of Sampling Location in Banyuasin Waters Area

Sample identification and collection

Crustacean samples were taken using folding trawl gear. The samples were collected and stored in a cool box. The crab identification process was conducted based on the examination of morphological characteristics, such as body shape, color pattern, claw shape, and leg shape (Hidir *et al*., 2021; Vermeiren *et al*., 2021). Morphometric measurements were performed on the crab samples, and the identification process was completed in the laboratory. Taxon determination was carried out using data from the World Register of Marine Species (WoRMS) accessed in January 2023 (WoRMS, 2023).

Environmental characteristics of sampling area

Environmental quality calculations were conducted to assess the condition of the sampling environment. Environmental parameter data were measured, including salinity, temperature, pH, and dissolved oxygen (DO) (Fitria *et al*., 2023; Rozirwan *et al*., 2024). Each parameter was measured in three repetitions to ensure consistency, and the results were then averaged. Environmental parameter measurements are typically used to evaluate habitat conditions, as they provide insights into the physical and chemical characteristics of the ecosystem. Repetition in measurements is a standard approach to improve data reliability.

Sample preparation

The preparation method described by Ambekar *et al*. (2023), involves cleaning the crab to remove contaminants. In this study, the carapace and crab meat were separated and rinsed with distilled water to eliminate any remaining impurities. The wet weight of the crab meat was measured, and the samples were then dried in an oven at 40°C for 3 × 24 h. After drying, the samples were ground into powder using a blender. The dry weight of the crab meat was recorded for data analysis.

Sample maceration and extraction

The wet maceration method was used in this study. A total of 250 g of S. *serrata* meat powder was weighed and immersed in 1000 mL of 96% ethanol solvent at a ratio of 1:4 (b/v). The soaking process was conducted for 3 × 24 h, with stirring performed periodically to ensure optimal extraction. The maceration results were filtered using filter paper (No. 42, 125 mm). The extraction process was then carried out using a rotary evaporator at 40°C with a rotating speed of 3000 rpm (Hashim *et al*., 2021). The resulting extract was stored as a stock solution. A total of 0.05 g of *S. serrata* extract was used as an additive for the stock solution (Habib *et al*., 2022). Wet maceration is a commonly employed extraction method due to its ability to preserve heat-sensitive compounds. Stirring during the maceration process enhances solute dissolution, while rotary evaporation ensures efficient solvent removal under controlled temperature conditions.

Determination of antioxidant activity and IC_{50} value

Antioxidant testing was conducted using the DPPH method (Vásquez *et al*., 2023). The stock solution of S. *serrata* extract was used as the test solution, while vitamin C served as a reference. The sample was dissolved in 96% ethanol. A total of 1 mL of sample solution was prepared for each concentration: 100, 150, 200, 250, and 300 $mg.L^{-1}$. Each concentration was mixed with 40 $\mu g.mL^{-1}$ DPPH solution and incubated in the dark for 30 min. Absorbance was measured at 517 nm using UV-Vis spectrophotometry. The strength of antioxidant activity is classified according to IC_{50} values, as shown in Table 1. The IC_{50} value is calculated using the following formula.

$$inhibition = \frac{blank\ abs - sample\ abs.}{blank\ abs}\ x\ 100\ \%$$

The IC_{50} results were entered in the linear regression equation y= ax + b. The sample concentration is the abscissa (X-axis), and the percentage of antioxidant inhibition is the ordinate (Y-axis) (Yuniarti *et al*., 2020). The concentration corresponding to 50% inhibition was interpolated from the regression equation. All determinations were conducted in triplicate, and the mean IC_{50} values were statistically compared using one-way ANOVA, followed by Duncan's multiple range test (*P*< 0.05).

Table 1. Characteristic concentration value of IC_{50}

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Concentration Value ($\mu g.mL^{-1}$)	Characteristic
<50	Very Strong
50-100	Strong
100-150	Moderate
150-200	Low

Phytochemical screening

Phytochemical tests of *S. serrata* meat extracts were conducted using qualitative methods to identify the presence of bioactive compounds. The analysis included steroid and triterpenoid tests, which were performed using the Liebermann-Burchard method, alkaloid tests using Mayer and Dragendorff reagents, flavonoid tests with the Shinoda staining method, tannin tests using the $FeCl_3$ reaction, and saponin tests using the foam test method. Each test was carried out following the procedures described in standard literature (Suwandi *et al.*, 2020; Dinesh *et al*., 2022).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The identification of bioactive compound components in *S. serrata* meat extract was performed using the GC-MS analysis method, following Rozirwan *et al*. (2022) with modifications. A total of 1 µL of extract was injected into an RTX-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) using helium as the carrier gas at a constant flow rate of 1.0 $mL.min^{-1}$ and a split ratio of 1:50. The oven temperature was initially set at 50°C for 5 min, then increased at a rate of 5°C/min to 280°C, and held for 5 min. The injector and ion source temperatures were 280°C and 230°C, respectively, with an electron ionization (EI) energy of 70 eV. The mass spectrometer was operated in scan mode with a mass range of 40–550 m/z. The Wiley 7 Library database was used as a reference for spectral comparison (Rafferty *et al*., 2020).

Result and Discussion

Sample identification and collection

Taxon determination was conducted using data from the World Register of Marine Species (WoRMS) accessed in January 2023 (WoRMS, 2023). Based on their morphological characteristics, the crustacean samples were identified as *S. serrata*.

Mangrove crabs of this species were caught using folding traps. The fishing process was carried out during low tide to facilitate crab capture. In this study, 2-5 crabs were used as stock samples. The crabs obtained were weighed, with weights ranging from 200 to 320 g, widths of approximately 21.5 cm, and lengths of around 13.5 cm. The crabs were then stored in a cool box filled with ice cubes for preservation. The species identification of mangrove crabs is based on morphological characteristics, which include features such as the eyes, propodus, carpus, merus, carapace, claws, walking legs, and swimming legs (Figure 2). Taxonomic data from databases such as WoRMS is widely used to confirm species classification accurately.

Environmental Characteristics of Sampling Area

The results of environmental quality measurements in the *S. serrata* sampling areas in Banyuasin waters revealed diverse conditions. Measurements of water physicochemical parameters, including dissolved oxygen, pH, temperature, and salinity, were taken to assess the habitat suitability for *S. serrata* (Rozirwan *et al*., 2021). The dissolved oxygen concentration was found to be 4.2 mg/L, which is sufficient to support the respiration process of aquatic organisms (Ouyang *et al*., 2021). The pH value of the water at the sampling location was 7, indicating neutral pH, which represents optimal ecological conditions (Chowdhury *et al*., 2021). This is consistent with previous studies (Yusni and Haq, 2020; Muhtar and Lanuru, 2021; Putri *et al*., 2022), which state that waters with a pH between 6.5 and 7.5 are ideal for the survival of mangrove crabs. The water temperature was measured at 28°C, indicating favorable conditions for mangrove crab growth (Indarjo *et al*., 2020; Ren *et al*., 2021) Water salinity was recorded at 18 psu, reflecting typical conditions in estuarine areas or areas directly influenced by tidal movements (Wang *et al*., 2021). *S. serrata* grows best in salinities between 15 psu and 25 psu but grows more slowly at salinities greater than 25 to 30 psu (Triajie *et al*., 2020; Pati *et al*., 2023; Adnan *et al*., 2024).

Environmental conditions at the sampling site, particularly salinity (18 psu) and temperature (28°C), could also influence the diversity and concentration of secondary metabolites in *S. serrata*. Estuarine environments are dynamic, and organisms inhabiting them may prioritize the synthesis of specific bioactive compounds for stress adaptation.

Table 2. Observation of Environmental Parameters of the Research Site

	Station
Environment Parameter Quality	Sungsang Waters
Dissolved oxygen ($mg.L^{-1}$)	4.2
pH	7
Temperature (°C)	28
Salinity (psu)	18

Previous studies reported that salinity stress can regulate secondary metabolite production, including alkaloids and flavonoids, in marine organisms (Pati *et al*., 2023). Furthermore, the choice of ethanol as the extraction solvent, although safer and less toxic than methanol, may have influenced the recovery efficiency of certain compounds, possibly underestimating the abundance of more polar metabolites.

Determination of antioxidant activity by DPPH assay

Antioxidant analysis using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method on Rozirwan *et al*. (2023) *S. serrata* meat extract showed promising results. The DPPH solution changed from purple to yellow, indicating the presence of antioxidants in the extract. The test results revealed that the *S. serrata* extract had an IC_{50} value of 2.25 ppm, while Vitamin C, used as a comparison solution, had an IC50 value of 2.16 ppm (Table 3). Both solutions demonstrated low IC_{50} values, categorizing them as very strong antioxidant compounds. The IC50 value, which is the concentration required to inhibit 50% of DPPH radical activity, is a critical parameter for assessing the antioxidant potential of a compound (Martinez-Morales *et al*., 2020). The results indicate that *S. serrata* extract possesses an IC_{50} value comparable to that of Vitamin C. The percentage inhibition of DPPH free radicals by *S. serrata* meat extract increased as the extract concentration increased. This suggests that the extract has the ability to donate electrons or hydrogen to the DPPH radical, neutralizing it and converting it into a more stable form (Gulcin and Alwasel, 2023).

The discovery of compounds such as flavonoids and triterpenoids, which were identified in the phytochemical analysis of *S. serrata* extracts, suggests that they may possess high antioxidant activity (Akinwumi *et al*., 2022; Hajar-Azira *et al*., 2023). Flavonoids are well known for their ability to capture free radicals and interrupt the chain of oxidative reactions. Triterpenoids are also recognized for their significant antioxidant activity through a similar mechanism. The combination of these compounds in *S. serrata* extract creates a synergistic effect, enhancing the overall capacity of the extract to neutralize free radicals.

S. serrata is known for its rich antioxidant system and strong enzymatic defense system, which help it survive in the dynamic and often challenging mangrove environment (Pati *et al*., 2023). Mud crabs also possess defense enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, which work synergistically to detoxify reactive oxygen species (ROS) and maintain redox balance in the body (Jerome *et al*., 2020; Bal *et al*., 2021; Costantini *et al*., 2022; Zeng *et al*., 2024). These enzymes play a crucial role in protecting the crab from oxidative stress generated by fluctuating environmental conditions, pollution, and pathogens.

Table 3. Calculation results of antioxidant activity of *S. serrata* in Sungsang waters

Sample	Linear Regression			IC_{50} Value	Category
	a	b	R^2		
S. serrata	6.9429	52.808	0.9327	2.25 mg.L^{-1}	Very Strong
Ascorbic Acid	6.7135	55.866	0.9435	2.16 mg.L^{-1}	Very Strong

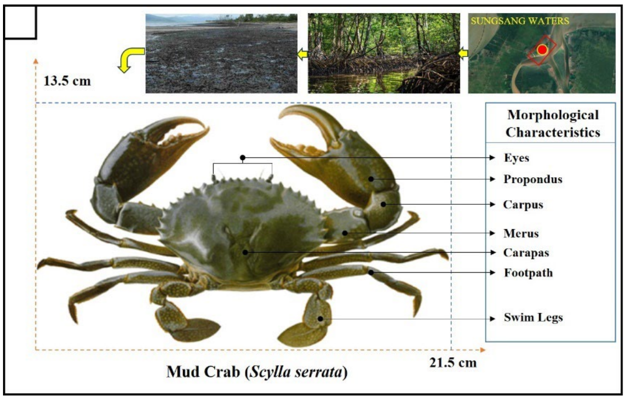

Figure 2. Crustacean species, *S. Serrata*

This combination of antioxidant compounds and defence enzymes not only ensures the survival of mud crabs in their habitat but also positions them as a potential source for the development of natural health products that can harness their protective mechanisms. Antioxidants and defence enzymes play a vital role in organisms' survival, especially in harsh environments. The synergy between these compounds and enzymes contributes significantly to mitigating oxidative stress and preserving cellular function, which can be explored for potential therapeutic applications.

Phytochemical screening

Phytochemical tests were carried out to identify the compounds present in gastropod and crustacean extracts using ethanol as the solvent (Fitria *et al*., 2023; Rozirwan *et al*., 2024) The phytochemical test aimed to determine the compounds in the test extract (Chen *et al*., 2022), allowing for the identification of compounds that influence the strong or weak antioxidant activity of the extract (Baliyan *et al*., 2022). The results of the phytochemical test, after UV-Vis spectrophotometric analysis of the extract, are presented in Table 4. The test results showed that only certain compounds were extracted by the ethanol solvent (Yuniarti *et al*., 2020).

Qualitative phytochemical analysis of *S. serrata* extract showed significant results in identifying the content of bioactive compounds. Based on the test results, *S. serrata* extract tested positive for flavonoid and triterpenoid compounds. A similar finding was reported by Elshaarawy *et al*. (2023) for *Scylla olivacea* samples. These two compounds offer various health benefits and therapeutic potential, particularly due to their strong antioxidant properties. Flavonoids are a group of polyphenolic compounds that are widely recognized for their potent antioxidant activity (Shen *et al*., 2022). These compounds can neutralize free radicals and prevent oxidative damage to cells and tissues. Additionally, flavonoids possess anti-inflammatory, anticancer, and cardioprotective properties (Mounika *et al*., 2021; Jain *et al*., 2024; Ullah *et al*., 2024). The identification of flavonoid compounds in *S. serrata* indicates that mud crabs are not only valuable as food but also hold potential as an alternative source of medicine from marine organisms.

Triterpenoids are a group of terpenoid compounds that have potential biological activities (Mabou and Yossa, 2021; Zang *et al*., 2022). These compounds are known to possess anti-inflammatory, antitumor, antimicrobial, and immunomodulatory properties (Harun *et al*., 2020; Ahmad *et al*., 2021). As antioxidant agents, triterpenoids have been used in pharmacology to treat inflammatory diseases and cancer. Similar to flavonoids, these compounds can neutralize free radicals caused by oxidative stress on body tissues. The discovery of triterpenoid compounds in *S. serrata* shows promising results, given their antioxidant potential that can be applied to address various diseases. Thus, the opportunity to explore alternative medicinal raw materials from *S. serrata* extract is increasingly attractive for further research. Overall, the phytochemical results focusing on flavonoid and triterpenoid compounds confirm the importance of *S. serrata* as a potential source of bioactive compounds. Further research is encouraged at the stage of isolation and purification of these compounds, so that alternative medicinal materials derived from this marine organism can contribute to the development of therapeutic agents from marine organisms.

Phytochemical profile screening

The antioxidant compound profile in *S. serrata* was determined using GC-MS (Gas Chromatography-Mass Spectrometry) analysis on the ethanol extract of mud crab. Figure 3 shows the chromatogram with 37 peaks identified in the extract. Each peak on the chromatogram represents a distinct chemical compound found in the extract, which was analyzed using GC-MS.

GC-MS analysis is a powerful technique for identifying and quantifying individual compounds in complex mixtures, providing insights into the chemical composition of the extract. This method is widely used for its ability to separate and identify volatile compounds, making it an essential tool for profiling bioactive compounds, such as antioxidants, in natural products.

Table 4. Phytochemical screening results of *S. Serrata*

Parameters	Analysis Result	Analysis Type
Alkaloids	-	Qualitative
Flavonoids	+	Qualitative
Triterpenoids	+	Qualitative
Saponin	-	Qualitative
Tannins	-	Qualitative
Steroid	-	Qualitative

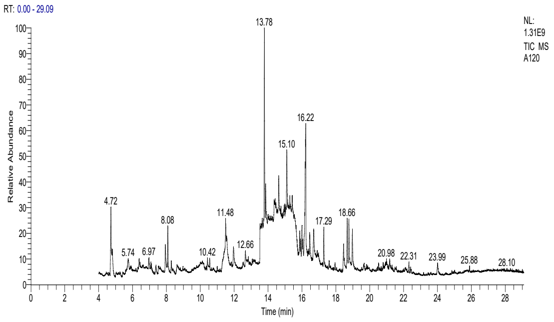

Figure 3. GC-MS chromatogram of ethanol extract *S. Serrata*

Terpenoid, alkaloid, steroid, and tannin groups were among the pure compounds successfully detected using GC-MS. Based on the GC-MS analysis of the ethanol extract compound components presented in Table 5, the main components identified in the extract were Calycotomine, N-methyl-, with a value of 8.31% of the total area, and uric acid, with a value of 8.71% of the total area.

At a retention time of 4.72 min, the compound 2-Cyclohexylpiperidine was detected with an area of 3.45%, a probability of 6.07, and a chemical formula of $C_{11}H_{21}N$, which belongs to the alkaloid compound group. At a retention time of 5.74 min, the compound 2-Pyridinamine, 3,6-dimethyl was detected with an area of 1.69%, a probability of 7.02, and a chemical formula of $C_7H_{10}N_2$, which belongs to the aminopyridine compound group. Furthermore, at a retention time of 6.97 min, the compound Pentanoic acid, dodec-9-ynyl ester was detected with an area of 1.00%, a probability of 8.47, and a chemical formula of $C_{17}H_{30}O_2$, which belongs to the protein compound group.

At a retention time of 8.08 min, the compound L-Homoserine lactone, N, N-dimethyl was detected with an area of 1.97%, a probability of 82.23, and a chemical formula of $C_6H_{11}NO_2$, which belongs to the amino acid compound group. At a retention time of 10.42 min, the compound Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetramethyl was detected with an area of 0.74%, a probability of 43.35, and a chemical formula of $C_{11}H_{14}N_2OS$, which belongs to the EPA compound group. At a retention time of 11.48 min, the compound 1H-2-Indenol, 2,3,4,5,6,7- hexahydro -1-(2-hydroxy-2-methylpropyl) was detected with an area of 4.83%, a probability of 12.26, and a chemical formula of $C_{13}H_{22}O_2$, which belongs to the lactone compound group.

At a retention time of 12.66 min, the compound dl-Lysine was detected with an area of 0.86%, a probability of 23.13, and a chemical formula of $C_6H_{14}N_2O_2$, which belongs to the amino acid compound group. At a retention time of 13.78 min, the compound Calycotomine, N-methyl- was detected with an area of 8.31%, a probability of 43.35, and a chemical formula of $C_{13}H_{19}NO_3$, which belongs to the alkaloid compound group. At a retention time of 15.10 min, the compound Dasycarpidan-8(16H)-ethanol, 3,18-dihydro-1-(hydroxymethyl)-, (2.xi.,4.xi.)- was detected with an area of 4.14%, a probability of 12.00, and a chemical formula of $C_{20}H_{28}N_2O_2$, which belongs to the group of molport compounds.

At a retention time of 16.22 min, the uric acid compound was detected with an area of 8.71%, a probability of 55.93, and a chemical formula of $C_5H_4N_4O_3$, which belongs to the allantoin compound group. At a retention time of 17.29 min, the compound Actinomycin C2 was detected with an area of 1.49%, a probability of 30.73, and a chemical formula of $C_{63}H_{88}N_{12}O_{16}$, which belongs to a group of peptide compounds that are derivatives of peptide compounds.

Animals produce a diverse mixture of secondary metabolites such as phenols, alkaloids, flavonoids, tannins, and saponins. Several animal studies have shown the potential use of these metabolites as antibacterial agents due to the presence of abundant biomolecules. Synthetic drugs often have high secondary failure rates and severe side effects, while animal products contain a variety of free radical scavenging molecules with substantial antioxidant properties.

As a more taxonomically relevant comparison, Yao *et al*. (2020) conducted a GC-MS-based metabolomic study on Scylla paramamosain experiencing acute salinity reduction (from 23 psu to 3 psu). This study identified 519 metabolites (mainly lipids), with 13 significantly enriched metabolic pathways ($P < 0.05$), related to signaling, lipid metabolism, and transport. Additionally, in that study, combining LC-MS and GC-MS data revealed 28 significant metabolic pathways, dominated by amino

Table 5. Proposed peak order, retention time, probability, area, compound name, and molecular formula

Peak#	R. Time	Probability	Area%	Name	Molecular formula
1	4.72	6.07	3.45	2-Cyclohexylpiperidine	C11H21N
2	4.81	18.58	1.37	Edulan II	C13H20O
3	5.74	7.02	1.69	2-Pyridinamine, 3,6-dimethyl	C7H10N2
4	6.39	8.12	0.83	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	C16H28O3
5	6.61	11.07	0.66	d-Mannose	C6H12O6
6	6.81	50.91	0.89	Deoxyspergualin	C17H37N7O3
7	6.97	8.47	1.00	Pentanoic acid, dodec-9-ynyl ester	C17H30O2
8	7.93	51.03	1.42	trans-(2 Chlorovinyl)dimethylethoxysil ane	C6H13ClOSi
9	8.08	82.23	1.97	L-Homoserine lactone, N,N-dimethyl-	C6H11NO2
10	8.28	32.09	0.64	2-Propyl-tetrahydropyran-3-ol	C8H16O2
11	8.62	32.45	0.93	Imidazole	C3H4N2
12	10.00	11.83	0.76	Tertbutyloxyformamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-buty nyl]-	C14H24N2O2
13	10.42	15.27	0.74	Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetrameth yl	C11H14N2OS
14	11.48	12.26	4.83	1H-2-Indenol, 2,3,4,5,6,7-hexahydro-1-(2-hydroxy-2-methylpropyl)	C13H22O2
15	11.55	48.75	2.53	dl-Citrulline	C6H13N3O3
16	11.96	7.38	1.45	2-Pyridineacetic acid, hexahydro-	C7H13NO2
17	12.66	23.13	0.86	dl-Lysine	C6H14N2O2
18	13.51	60.28	1.62	D-Streptamine, O-6-amino-6-deoxy-à-D-glucopyranosy l-(1-4)-O-(3-deoxy-4-C-methyl-3-(meth ylamino)-á-L-arabinopyranosyl-(1-6))- 2-deoxy	C6H13NO2
19	13.78	43.35	8.31	Calycotomine, N-methyl-	C13H19NO3
20	13.99	17.84	2.38	4-[4-Diethylamino-1-methylbutylamino]-1,2-dimethoxy-6-bromonaphthalene	C21H31BrN2O2
21	14.35	32.83	1.22	Dasycarpidan-8(16H)-ethanol, 3,18-didehydro-1-(hydroxymethyl)-, (2.xi.,4.xi.)-	C20H28N2O2
22	14.62	12.2	2.67	4-[4-Diethylamino-1-methylbutylamino]-1,2-dimethoxy-6-bromonaphthalene	C21H31BrN2O2
23	15.10	12.00	4.14	Dasycarpidan-8(16H)-ethanol, 3,18-didehydro-1-(hydroxymethyl)-, (2.xi.,4.xi.)-	C20H28N2O2
24	15.21	32.49	0.87	Cystine	C6H12N2O4S2
25	15.65	33.28	1.26	3-[N-[2-Diethylaminoethyl]-1-cyclopes tenylamino]propionitrile	C14H25N3
26	15.86	16.52	0.92	á-Hydroxyquebrachamine	CH3CH(OH)COOH
27	15.99	14.23	1.02	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	C21H36O4
28	16.22	55.93	8.71	Uric acid	C5H4N4O3
29	16.45	8.30	1.33	Aminoacetamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-buty nyl]-	C5H4N4O3
30	16.69	32.32	2.06	Glucopyranuronamide, 1-(4-amino-2-oxo-1(2H)-pyrimidinyl)-1,4-dideoxy-4-(D-2-(2-(methylamino) acetamido)hydracrylamido)-, á-D	C10H14N2O
31	16.90	29.78	0.80	1,2,4-Trioxolane-2-octanoic acid, 5-octyl-, methyl ester	C6H11NO6
32	17.29	30.73	1.49	Actinomycin C2	C19H36O5
33	18.45	37.85	2.00	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	C63H88N12O16
34	18.66	90.13	2.23	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	C63H88N12O16
35	18.76	60.11	2.62	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6 H-dipyrrolo[1,2-a:1',2'-d]pyrazine	C11H18N2O2
36	18.97	36	2.13	l-(+)-Ascorbic acid	C14H22
37	20.98	13.19	1.29	cis-13-Octadecenoic acid	C6H8O6
38	21.17	13.49	0.94	Ricinoleic acid	C18H34O2
39	23.99	31.48	0.89	Ergotaman-3',6',18-trione, 12'-hydroxy-2'-methyl-5'-(phenylmeth yl)-, (5'à)-	C18H34O3

acid and energy metabolism, with lipid metabolism playing a supporting role. In comparison, in our study, *Scylla serrata* ethanol extract showed only 8.31% alkaloid content among the dominant compounds. This difference suggests that environmental stress factors such as sudden salinity reduction can significantly modulate the metabolite profile among related *Scylla* species, especially under similar polar extraction conditions.

Interestingly, alkaloid compounds were not detected in the qualitative phytochemical screening, but several alkaloids such as Calycotomine and Imidazole were identified through GC-MS analysis.

This discrepancy is not only due to methodological differences but may also be related to the detection sensitivity. Qualitative tests often fail to detect compounds present at low concentrations, whereas GC-MS has a higher sensitivity and can identify minor constituents. In addition, the polarity of ethanol as a solvent might have selectively extracted certain alkaloids in low yield, which escaped detection in qualitative assays but were quantifiable in GC-MS analysis. Similar observations were reported in marine-derived extracts where alkaloids were inconsistently detected depending on the analytical method (Rahmawati *et al*., 2023; Shofinita *et al*., 2024).

The main group of compounds in the ethanol extract of mud crab (*S. serrata*) was represented by three peaks on the GC chromatogram, which had a higher percentage area than the others. These peaks were identified as Calycotomine, N-methyl- (8.31%), uric acid (8.71%), and Dasycarpidan-8(16H)-ethanol, 3,18-didehydro-1-(hydroxymethyl)-(2.xi.,4.xi.)-4.14%). Antioxidant compounds detected in the GC-MS analysis included 2-Cyclohexylpiperidine with an area of 3.45%, which belongs to the alkaloid group. In addition, the Edullan II compound with an area of 1.37% belongs to the volatile compound group. The compound Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate, with an area of 0.83%, belongs to the terpenoid compound group, and Imidazole with an area of 0.93% also belongs to the alkaloid group.

In a study conducted by Karnila *et al*. (2021) on mud crab (*S. serrata*), antioxidant compounds such as astaxanthin with a peak area of 18.6% and β-carotene with a peak area of 7.9% were identified through GC-MS analysis, both of which belong to the carotenoid group and contribute to antioxidant activity. Similarly, a study by Thiruvengadarajan *et al*. (2024) on the nutritional composition of *S. serrata* reported the presence of unsaturated fatty acids such as eicosapentaenoic acid (EPA, 5.42%) and docosahexaenoic acid (DHA, 4.87%), which are categorized as bioactive lipids and known for their health-promoting effects.

The group of compounds identified in Scylla serrata are alkaloids. Based on research by Karim *et al*. (2024) analyzing muscle and hepatopancreas extracts of *S. olivacea*, they reported a lipid profile including EPA and DHA content, as well as significant antioxidant capacity measured through the DPPH and ferric-reducing tests. Similarly, Taufik *et al*. (2020) applied GC–MS to *S. olivacea* tissues, revealing a predominance of monounsaturated and polyunsaturated fatty acids, including long-chain polyunsaturated fatty acids (PUFAs) in the gonads and hepatopancreas. Although specific alkaloid compounds have not been reported for *Scylla*, these lipid-based metabolites provide relevant biological context within the genus *Scylla*. Therefore, comparisons of the alkaloid profiles of *S. serrata* should emphasize the same metabolic pathways within this genus and highlight the need for targeted alkaloid profile studies on *Scylla* species.

Conclusion

This study successfully demonstrated that *S. serrata* extract possesses very strong antioxidant activity, supported by the presence of flavonoids, triterpenoids, and various bioactive compounds identified through phytochemical and GC-MS analyses. These findings confirm that the research objective to explore and characterize the antioxidant potential and compound profile of *S. serrata* has been achieved. The extract's bioactive profile, comprising both major and minor compounds, reinforces its potential as a promising source of natural antioxidants with additional pharmacological properties, including anti-inflammatory, anticancer, antimicrobial, and antiviral activities. Overall, this study contributes to the growing evidence that marine organisms represent valuable resources for the development of alternative medicinal agents.

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Response to Reviewers

Reviewer

<table>
<tr><th>Reviewer Comments</th><th>Response</th></tr>
<tr><td>Source?Reference?
Table 1. Characteristic concentration value of IC50</td><td>We thank the reviewer for this valuable comment. We have carefully revised Table 1 and added the appropriate references to support the IC_{50} values presented. Each concentration value is now accompanied by its corresponding citation to ensure clarity and traceability.</td></tr>
<tr><td>References
Too many self-citations, please reduce them. References in red need to be completed. Several information missing. Check format reference in article publish 2025</td><td>We greatly appreciate this observation. In the revised manuscript:
We have reduced the number of self-citations to avoid potential bias and to align with the journal's standards.
All references highlighted in red have been verified and completed with missing information (authors, year, volume, issue, page numbers, and DOIs where available).
The reference list has been reformatted according to the journal's latest 2025 guidelines to ensure consistency and accuracy.
We believe these revisions improve the overall quality and readability of the manuscript.</td></tr>
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Screening and Profiling of Antioxidant Activity in Mud Crab (*Scylla Serrata*) from Banyuasin Waters

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Abstract

Mangrove crab (Scylla serrata) as one of the crustacean species, has a variety of bioactive compounds that can be utilized in the field of pharmacology. Antioxidant compounds act as therapeutic agents against degenerative diseases. Banyuasin waters have mangrove vegetation with associated marine organisms that have the potential to be studied for bioactive compounds. This study aims to identify the phytochemical profile quantitatively and qualitatively, samples were collected from mud flats near mangrove ecosystems in Banyuasin waters, South Sumatra. Samples were tested for antioxidant activity using the DPPH test, and IC_{50} values, qualitative phytochemical identification, and phytochemical profiles were calculated using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Based on the results of antioxidant testing, the IC_{50} value of S. serrata extract is 2.25 ppm, the sample is included in the category of very strong antioxidants. Phytochemical test results showed that the compound is thought to contain antioxidant activity from flavonoids and triterpenoids. GC-MS analysis detected major compound groups of alkaloids, purines, and vitamins. Minor compound groups detected amines, terpenoids, monosaccharides, amino acids, fatty acids, silanes, formamides, heterocycles, carboxylic acids, aminoglycosides, naphthalene derivatives, nitriles, amides, glycosides, and peptides. S. serrata extract shows very strong antioxidant activity, with major compounds such as alkaloids, purines, and vitamins. S. serrata extract detected compounds that have been reported as anti-inflammatory, anticancer, antimicrobial, and antiviral. These findings highlight the pharmaceutical potential of S. serrata as a source of bioactive compounds. The results of this study provide valuable information for the development of alternative medicines derived from marine organisms.

Keywords: *Antioxidant, Bioactive compounds, DPPH, GC-MS, S. serrata*

Introduction

Scylla serrata, or mud crab, as one of the crustacean species, has great potential in the field of beauty and health ~~(De Castro *et al*., 2023)~~, because of its diverse bioactive compounds (Yusof *et al*., 2020; Beslin and Geni, 2021; Neelima *et al*., 2022). The diversity of bioactive compounds triggers great potential in the search for alternative medicinal ingredients from marine organisms, including antioxidant compounds (Yogeshwaran *et al*., 2020; Karnila and Ramadhani, 2021; Fajriaty *et al*., 2024). Antioxidant compounds in mud crabs have a role as therapeutic agents (Wu *et al*., 2021) in fighting degenerative diseases such as cancer (Nagarajan *et al*., 2024), cardiovascular diseases (Nanda *et al*., 2021), and neurodegenerative disorders (Galal-Khallaf *et al*., 2024), caused by oxidative stress due to free radicals.

Antioxidants are the reaction of a compound in neutralizing free radicals (Delta *et al*., 2021; Rozirwan *et al*., 2023a; 2023b). This process involves a highly efficient electron donation mechanism. Antioxidants work by donating electrons to free radicals (Fajriaty *et al*., 2024; Frías-Espericueta *et al*., 2022; Pati *et al*., 2022; Yang *et al*., 2023). Free radicals, which have one or more unpaired electrons, are highly reactive and can cause oxidative damage to DNA, proteins, and cellular lipids (Alkadi, 2020; Di Meo and Venditti, 2020). Free radicals are often generated as by-products of various metabolic processes in the body or due to environmental exposures such as pollution and ultraviolet radiation (Martemucci *et al*., 2022; Sadiq, 2023). The damage caused by free radicals can contribute to the development of various degenerative diseases, including cancer, heart disease, and neurodegenerative disorders (Teleanu *et al*., 2022; Chaudhary *et al*., 2023). In inhibiting free radicals, antioxidant compounds such as carotenoids and polyphenols will interact with free radicals to enhance the activity of detoxification enzymes, resulting in accelerated elimination of free radicals and strengthened antibodies (Pisoschi *et al*., 2021; Tumilaar *et al*., 2024).

The analysis of antioxidant compounds in *S. serrata* requires an accurate technique to ensure that identification and quantification are targeted (Baag and Mandal, 2023; Yao *et al*., 2023). Previous studies have mainly reported the antioxidant potential of *S. serrata* using spectrophotometric or colorimetric assays such as DPPH, ABTS, and FRAP, which provide only general antioxidant capacity without revealing the identity of specific compounds (Yogeshwaran *et al*., 2020; Karnila and Ramadhani, 2021; Neelima *et al*., 2022). In contrast, Gas Chromatography-Mass Spectrometry (GC-MS) offers higher resolution in profiling bioactive compounds by separating and identifying molecules based on their mass and chemical characteristics (Jabbar *et al*., 2022; Musa *et al*., 2022; Palma *et al*., 2023; Rozirwan *et al*., 2024). This advanced method not only allows the detection of a broader spectrum of antioxidant molecules but also quantifies their abundance, thereby providing a more comprehensive understanding of their therapeutic potential.

Although antioxidant compounds such as flavonoids, carotenoids, and polyphenols have been reported from different body parts of *S. serrata* (Yogeshwaran *et al*., 2020; Karnila and Ramadhani, 2021; Neelima *et al*., 2022; Yang *et al*., 2023; Fajriaty *et al*., 2024), most of these studies remain descriptive and do not provide detailed profiles of the specific types and relative quantities of individual compounds. To date, there is still no comprehensive GC-MS based profiling that systematically characterizes the antioxidant repertoire of *S. serrata* meat. This represents a critical knowledge gap, since understanding the diversity and concentration of specific compounds is essential to support its therapeutic application. The present study aims to provide a detailed GC-MS profiling of antioxidant compounds in *S. serrata*. This work contributes novel insights into its biochemical composition and strengthens its potential utilization as a sustainable source of natural antioxidants for pharmaceutical and nutraceutical development.

Materials and Methods

Mangrove crab samples were collected in January 2023 from Banyuasin Waters, South Sumatra, Indonesia (Figure 1). At this location, numerous crustacean and gastropod populations were found inhabiting the intertidal zone ~~(~~(Rozirwan *et al*., 2021; Rozirwan *et al*., 2021~~; Rozirwan *et al*., 2022~~). The crab fishing area had a mud substrate with a depth of 1-2 m and was locasted within a mangrove vegetation zone directly connected to port and pond activities (Fitria *et al*., 2023). Anthropogenic pollutants that accumulate in these waters are known to trigger an increase in the defense mechanisms of organisms, such as the production of antioxidant compounds derived from secondary metabolites (Rozirwan *et al*., 2023).

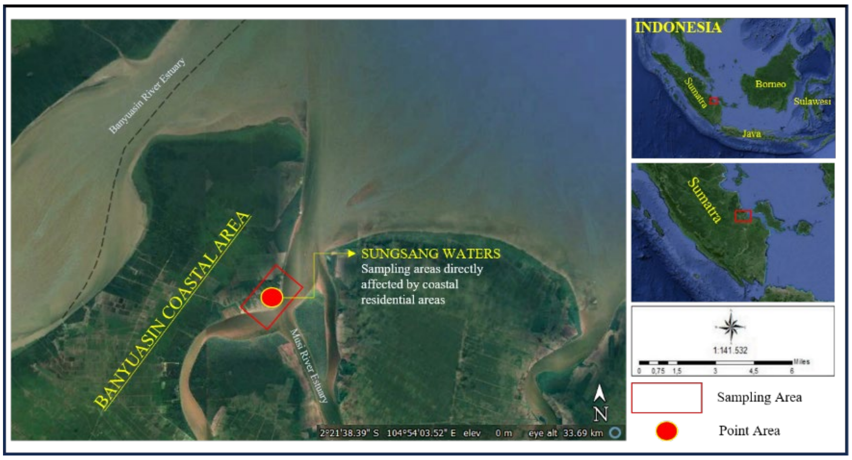

Figure 1. Map of Sampling Location in Banyuasin Waters Area

Sample identification and collection

Crustacean samples were taken using folding trawl gear. The samples were collected and stored in a cool box. The crab identification process was conducted based on the examination of morphological characteristics, such as body shape, color pattern, claw shape, and leg shape (Hidir *et al*., 2021; Vermeiren *et al*., 2021). Morphometric measurements were performed on the crab samples, and the identification process was completed in the laboratory. Taxon determination was carried out using data from the World Register of Marine Species (WoRMS) accessed in January 2023 (WoRMS, 2023).

Environmental characteristics of sampling area

Environmental quality calculations were conducted to assess the condition of the sampling environment. Environmental parameter data were measured, including salinity, temperature, pH, and dissolved oxygen (DO) (Fitria *et al*., 2023; Rozirwan *et al*., 2024). Each parameter was measured in three repetitions to ensure consistency, and the results were then averaged. Environmental parameter measurements are typically used to evaluate habitat conditions, as they provide insights into the physical and chemical characteristics of the ecosystem. Repetition in measurements is a standard approach to improve data reliability.

Sample preparation

The preparation method described by Ambekar *et al*. (2023), involves cleaning the crab to remove contaminants. In this study, the carapace and crab meat were separated and rinsed with distilled water to eliminate any remaining impurities. The wet weight of the crab meat was measured, and the samples were then dried in an oven at 40°C for 3 × 24 h. After drying, the samples were ground into powder using a blender. The dry weight of the crab meat was recorded for data analysis.

Sample maceration and extraction

The wet maceration method was used in this study. A total of 250 g of S. *serrata* meat powder was weighed and immersed in 1000 mL of 96% ethanol solvent at a ratio of 1:4 (b/v). The soaking process was conducted for 3 × 24 h, with stirring performed periodically to ensure optimal extraction. The maceration results were filtered using filter paper (No. 42, 125 mm). The extraction process was then carried out using a rotary evaporator at 40°C with a rotating speed of 3000 rpm (Hashim *et al*., 2021). The resulting extract was stored as a stock solution. A total of 0.05 g of *S. serrata* extract was used as an additive for the stock solution (Habib *et al*., 2022). Wet maceration is a commonly employed extraction method due to its ability to preserve heat-sensitive compounds. Stirring during the maceration process enhances solute dissolution, while rotary evaporation ensures efficient solvent removal under controlled temperature conditions.

Determination of antioxidant activity and IC_{50} value

Antioxidant testing was conducted using the DPPH method (Vásquez *et al*., 2023). The stock solution of *S. serrata* extract was used as the test solution, while vitamin C served as a reference. The sample was dissolved in 96% ethanol. A total of 1 mL of sample solution was prepared for each concentration: 100, 150, 200, 250, and 300 $mg.L^{-1}$. Each concentration was mixed with 40 $\mu g.mL^{-1}$ DPPH solution and incubated in the dark for 30 min. Absorbance was measured at 517 nm using UV-Vis spectrophotometry. The strength of antioxidant activity is classified according to IC_{50} values, as shown in Table 1. The IC_{50} value is calculated using the following formula.

$$inhibition = \frac{blank\ abs - sample\ abs.}{blank\ abs}\ x\ 100\ \%$$

The IC_{50} results were entered in the linear regression equation y= ax + b. The sample concentration is the abscissa (X-axis), and the percentage of antioxidant inhibition is the ordinate (Y-axis) (Yuniarti *et al*., 2020). The concentration corresponding to 50% inhibition was interpolated from the regression equation. All determinations were conducted in triplicate, and the mean IC_{50} values were statistically compared using one-way ANOVA, followed by Duncan's multiple range test (*P*< 0.05).

Table 1. Characteristic concentration value of IC_{50} (Molyneux, 2004)

Concentration Value ($\mu g.mL^{-1}$)	Characteristic
<50	Very Strong
50-100	Strong
100-150	Moderate
150-200	Low

Phytochemical screening

Phytochemical tests of *S. serrata* meat extracts were conducted using qualitative methods to identify the presence of bioactive compounds. The analysis included steroid and triterpenoid tests, which were performed using the Liebermann-Burchard method, alkaloid tests using Mayer and Dragendorff reagents, flavonoid tests with the Shinoda staining method, tannin tests using the $FeCl_3$ reaction, and saponin tests using the foam test method. Each test was carried out following the procedures described in standard literature (Suwandi *et al.*, 2020; Dinesh *et al*., 2022).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The identification of bioactive compound components in *S. serrata* meat extract was performed using the GC-MS analysis method, following Rozirwan *et al*. (2022) with modifications. A total of 1 μL of extract was injected into an RTX-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) using helium as the carrier gas at a constant flow rate of 1.0 $mL.min^{-1}$ and a split ratio of 1:50. The oven temperature was initially set at 50°C for 5 min, then increased at a rate of 5°C/min to 280°C, and held for 5 min. The injector and ion source temperatures were 280°C and 230°C, respectively, with an electron ionization (EI) energy of 70 eV. The mass spectrometer was operated in scan mode with a mass range of 40–550 m/z. The Wiley 7 Library database was used as a reference for spectral comparison (Rafferty *et al*., 2020).

Result and Discussion

Sample identification and collection

Taxon determination was conducted using data from the World Register of Marine Species (WoRMS) accessed in January 2023 (WoRMS, 2023). Based on their morphological characteristics, the crustacean samples were identified as *S. serrata*.

Mangrove crabs of this species were caught using folding traps. The fishing process was carried out during low tide to facilitate crab capture. In this study, 2-5 crabs were used as stock samples. The crabs obtained were weighed, with weights ranging from 200 to 320 g, widths of approximately 21.5 cm, and lengths of around 13.5 cm. The crabs were then stored in a cool box filled with ice cubes for preservation. The species identification of mangrove crabs is based on morphological characteristics, which include features such as the eyes, propodus, carpus, merus, carapace, claws, walking legs, and swimming legs (Figure 2). Taxonomic data from databases such as WoRMS is widely used to confirm species classification accurately.

Environmental Characteristics of Sampling Area

The results of environmental quality measurements in the *S. serrata* sampling areas in Banyuasin waters revealed diverse conditions. Measurements of water physicochemical parameters, including dissolved oxygen, pH, temperature, and salinity, were taken to assess the habitat suitability for *S. serrata* (Rozirwan *et al*., 2021). The dissolved oxygen concentration was found to be 4.2 $mg.L^{-1}$, which is sufficient to support the respiration process of aquatic organisms (Ouyang *et al*., 2021). The pH value of the water at the sampling location was 7, indicating neutral pH, which represents optimal ecological conditions (Chowdhury *et al*., 2021). This is consistent with previous studies (Yusni and Haq, 2020; Muhtar and Lanuru, 2021; Putri *et al*., 2022), which state that waters with a pH between 6.5 and 7.5 are ideal for the survival of mangrove crabs. The water temperature was measured at 28°C, indicating favorable conditions for mangrove crab growth (Indarjo *et al*., 2020; Ren *et al*., 2021) Water salinity was recorded at 18 psu, reflecting typical conditions in estuarine areas or areas directly influenced by tidal movements (Wang *et al*., 2021). *S. serrata* grows best in salinities between 15 psu and 25 psu but grows more slowly at salinities greater than 25 to 30 psu (Triajie *et al*., 2020; Pati *et al*., 2023; Adnan *et al*., 2024).

Environmental conditions at the sampling site, particularly salinity (18 psu) and temperature (28°C), could also influence the diversity and concentration of secondary metabolites in *S. serrata*. Estuarine environments are dynamic, and organisms inhabiting them may prioritize the synthesis of specific bioactive compounds for stress adaptation.

Table 2. Observation of Environmental Parameters of the Research Site

	Station
Environment Parameter Quality	Sungsang Waters
Dissolved oxygen ($mg.L^{-1}$)	4.2
pH	7
Temperature (°C)	28
Salinity (psu)	18

Previous studies reported that salinity stress can regulate secondary metabolite production, including alkaloids and flavonoids, in marine organisms (Pati *et al*., 2023). Furthermore, the choice of ethanol as the extraction solvent, although safer and less toxic than methanol, may have influenced the recovery efficiency of certain compounds, possibly underestimating the abundance of more polar metabolites.

Determination of antioxidant activity by DPPH assay

Antioxidant analysis using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method on Rozirwan *et al*. (2023) *S. serrata* meat extract showed promising results. The DPPH solution changed from purple to yellow, indicating the presence of antioxidants in the extract. The test results revealed that the *S. serrata* extract had an IC_{50} value of 2.25 ppm, while Vitamin C, used as a comparison solution, had an IC50 value of 2.16 ppm (Table 3). Both solutions demonstrated low IC_{50} values, categorizing them as very strong antioxidant compounds. The IC50 value, which is the concentration required to inhibit 50% of DPPH radical activity, is a critical parameter for assessing the antioxidant potential of a compound (Martinez-Morales *et al*., 2020). The results indicate that *S. serrata* extract possesses an IC_{50} value comparable to that of Vitamin C. The percentage inhibition of DPPH free radicals by *S. serrata* meat extract increased as the extract concentration increased. This suggests that the extract has the ability to donate electrons or hydrogen to the DPPH radical, neutralizing it and converting it into a more stable form (Gulcin and Alwasel, 2023).

The discovery of compounds such as flavonoids and triterpenoids, which were identified in the phytochemical analysis of *S. serrata* extracts, suggests that they may possess high antioxidant activity (Akinwumi *et al*., 2022; Hajar-Azira *et al*., 2023). Flavonoids are well known for their ability to capture free radicals and interrupt the chain of oxidative reactions. Triterpenoids are also recognized for their significant antioxidant activity through a similar mechanism. The combination of these compounds in *S. serrata* extract creates a synergistic effect, enhancing the overall capacity of the extract to neutralize free radicals.

S. serrata is known for its rich antioxidant system and strong enzymatic defense system, which help it survive in the dynamic and often challenging mangrove environment (Pati *et al*., 2023). Mud crabs also possess defense enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, which work synergistically to detoxify reactive oxygen species (ROS) and maintain redox balance in the body (Jerome *et al*., 2020; Bal *et al*., 2021; Costantini *et al*., 2022; Zeng *et al*., 2024). These enzymes play a crucial role in protecting the crab from oxidative stress generated by fluctuating environmental conditions, pollution, and pathogens.

Table 3. Calculation results of antioxidant activity of *S. serrata* in Sungsang waters

Sample	Linear Regression			IC_{50} Value	Category
	a	b	R^2		
S. serrata	6.9429	52.808	0.9327	2.25 $mg.L^{-1}$	Very Strong
Ascorbic Acid	6.7135	55.866	0.9435	2.16 $mg.L^{-1}$	Very Strong

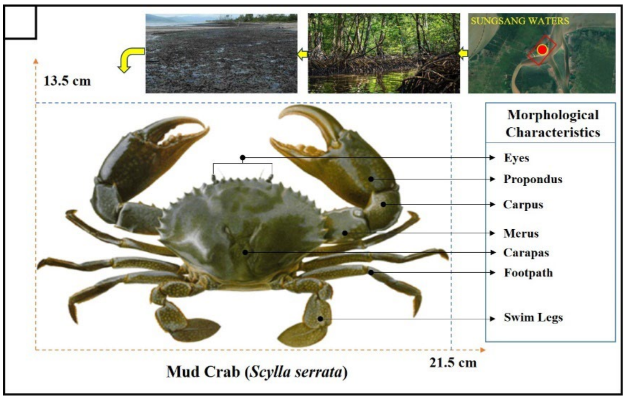

Figure 2. Crustacean species, *S. Serrata*

This combination of antioxidant compounds and defence enzymes not only ensures the survival of mud crabs in their habitat but also positions them as a potential source for the development of natural health products that can harness their protective mechanisms. Antioxidants and defence enzymes play a vital role in organisms' survival, especially in harsh environments. The synergy between these compounds and enzymes contributes significantly to mitigating oxidative stress and preserving cellular function, which can be explored for potential therapeutic applications.

Phytochemical screening

Phytochemical tests were carried out to identify the compounds present in gastropod and crustacean extracts using ethanol as the solvent (Fitria *et al*., 2023; Rozirwan *et al*., 2024) The phytochemical test aimed to determine the compounds in the test extract (Chen *et al*., 2022), allowing for the identification of compounds that influence the strong or weak antioxidant activity of the extract (Baliyan *et al*., 2022). The results of the phytochemical test, after UV-Vis spectrophotometric analysis of the extract, are presented in Table 4. The test results showed that only certain compounds were extracted by the ethanol solvent (Yuniarti *et al*., 2020).

Qualitative phytochemical analysis of *S. serrata* extract showed significant results in identifying the content of bioactive compounds. Based on the test results, *S. serrata* extract tested positive for flavonoid and triterpenoid compounds. A similar finding was reported by Elshaarawy *et al*. (2023) for *Scylla olivacea* samples. These two compounds offer various health benefits and therapeutic potential, particularly due to their strong antioxidant properties. Flavonoids are a group of polyphenolic compounds that are widely recognized for their potent antioxidant activity (Shen *et al*., 2022). These compounds can neutralize free radicals and prevent oxidative damage to cells and tissues. Additionally, flavonoids possess anti-inflammatory, anticancer, and cardioprotective properties (Mounika *et al*., 2021; Jain *et al*., 2024; Ullah *et al*., 2024). The identification of flavonoid compounds in *S. serrata* indicates that mud crabs are not only valuable as food but also hold potential as an alternative source of medicine from marine organisms.

Triterpenoids are a group of terpenoid compounds that have potential biological activities (Mabou and Yossa, 2021; Zang *et al*., 2022). These compounds are known to possess anti-inflammatory, antitumor, antimicrobial, and immunomodulatory properties (Harun *et al*., 2020; Ahmad *et al*., 2021). As antioxidant agents, triterpenoids have been used in pharmacology to treat inflammatory diseases and cancer. Similar to flavonoids, these compounds can neutralize free radicals caused by oxidative stress on body tissues. The discovery of triterpenoid compounds in *S. serrata* shows promising results, given their antioxidant potential that can be applied to address various diseases. Thus, the opportunity to explore alternative medicinal raw materials from *S. serrata* extract is increasingly attractive for further research. Overall, the phytochemical results focusing on flavonoid and triterpenoid compounds confirm the importance of *S. serrata* as a potential source of bioactive compounds. Further research is encouraged at the stage of isolation and purification of these compounds, so that alternative medicinal materials derived from this marine organism can contribute to the development of therapeutic agents from marine organisms.

Phytochemical profile screening

The antioxidant compound profile in *S. serrata* was determined using GC-MS (Gas Chromatography-Mass Spectrometry) analysis on the ethanol extract of mud crab. Figure 3 shows the chromatogram with 37 peaks identified in the extract. Each peak on the chromatogram represents a distinct chemical compound found in the extract, which was analyzed using GC-MS.

GC-MS analysis is a powerful technique for identifying and quantifying individual compounds in complex mixtures, providing insights into the chemical composition of the extract. This method is widely used for its ability to separate and identify volatile compounds, making it an essential tool for profiling bioactive compounds, such as antioxidants, in natural products.

Table 4. Phytochemical screening results of *S. Serrata*

Parameters	Analysis Result	Analysis Type
Alkaloids	-	Qualitative
Flavonoids	+	Qualitative
Triterpenoids	+	Qualitative
Saponin	-	Qualitative
Tannins	-	Qualitative
Steroid	-	Qualitative

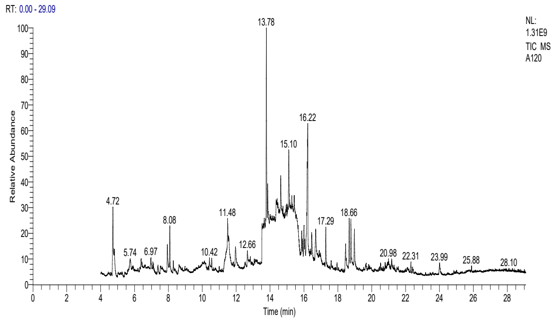

Figure 3. GC-MS chromatogram of ethanol extract *S. Serrata*

Terpenoid, alkaloid, steroid, and tannin groups were among the pure compounds successfully detected using GC-MS. Based on the GC-MS analysis of the ethanol extract compound components presented in Table 5, the main components identified in the extract were Calycotomine, N-methyl-, with a value of 8.31% of the total area, and uric acid, with a value of 8.71% of the total area.

At a retention time of 4.72 min, the compound 2-Cyclohexylpiperidine was detected with an area of 3.45%, a probability of 6.07, and a chemical formula of C11H21N, which belongs to the alkaloid compound group. At a retention time of 5.74 min, the compound 2-Pyridinamine, 3,6-dimethyl was detected with an area of 1.69%, a probability of 7.02, and a chemical formula of C7H10N2, which belongs to the aminopyridine compound group. Furthermore, at a retention time of 6.97 min, the compound Pentanoic acid, dodec-9-ynyl ester was detected with an area of 1.00%, a probability of 8.47, and a chemical formula of C17H30O2, which belongs to the protein compound group.

At a retention time of 8.08 min, the compound L-Homoserine lactone, N, N-dimethyl was detected with an area of 1.97%, a probability of 82.23, and a chemical formula of C6H11NO2, which belongs to the amino acid compound group. At a retention time of 10.42 min, the compound Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetramethyl was detected with an area of 0.74%, a probability of 43.35, and a chemical formula of C11H14N2OS, which belongs to the EPA compound group. At a retention time of 11.48 min, the compound 1H-2-Indenol, 2,3,4,5,6,7- hexahydro -1-(2-hydroxy-2-methylpropyl) was detected with an area of 4.83%, a probability of 12.26, and a chemical formula of C13H22O2, which belongs to the lactone compound group.

At a retention time of 12.66 min, the compound dl-Lysine was detected with an area of 0.86%, a probability of 23.13, and a chemical formula of C6H14N2O2, which belongs to the amino acid compound group. At a retention time of 13.78 min, the compound Calycotomine, N-methyl- was detected with an area of 8.31%, a probability of 43.35, and a chemical formula of C13H19NO3, which belongs to the alkaloid compound group. At a retention time of 15.10 min, the compound Dasycarpidan-8(16H)-ethanol, 3,18-dihydro-1-(hydroxymethyl)-, (2.xi.,4.xi.)- was detected with an area of 4.14%, a probability of 12.00, and a chemical formula of C20H28N2O2, which belongs to the group of molport compounds.

At a retention time of 16.22 min, the uric acid compound was detected with an area of 8.71%, a probability of 55.93, and a chemical formula of C5H4N4O3, which belongs to the allantoin compound group. At a retention time of 17.29 min, the compound Actinomycin C2 was detected with an area of 1.49%, a probability of 30.73, and a chemical formula of C63H88N12O16, which belongs to a group of peptide compounds that are derivatives of peptide compounds.

Animals produce a diverse mixture of secondary metabolites such as phenols, alkaloids, flavonoids, tannins, and saponins. Several animal studies have shown the potential use of these metabolites as antibacterial agents due to the presence of abundant biomolecules. Synthetic drugs often have high secondary failure rates and severe side effects, while animal products contain a variety of free radical scavenging molecules with substantial antioxidant properties.

As a more taxonomically relevant comparison, Yao *et al*. (2020) conducted a GC-MS-based metabolomic study on Scylla paramamosain experiencing acute salinity reduction (from 23 psu to 3 psu). This study identified 519 metabolites (mainly lipids), with 13 significantly enriched metabolic pathways ($P< 0.05$), related to signaling, lipid metabolism, and transport. Additionally, in that study, combining LC-MS and GC-MS data revealed 28 significant metabolic pathways, dominated by amino

Table 5. Proposed peak order, retention time, probability, area, compound name, and molecular formula

Peak#	R. Time	Probability	Area%	Name	Molecular formula
1	4.72	6.07	3.45	2-Cyclohexylpiperidine	C11H21N
2	4.81	18.58	1.37	Edulan II	C13H20O
3	5.74	7.02	1.69	2-Pyridinamine, 3,6-dimethyl	C7H10N2
4	6.39	8.12	0.83	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	C16H28O3
5	6.61	11.07	0.66	d-Mannose	C6H12O6
6	6.81	50.91	0.89	Deoxyspergualin	C17H37N7O3
7	6.97	8.47	1.00	Pentanoic acid, dodec-9-ynyl ester	C17H30O2
8	7.93	51.03	1.42	trans-(2 Chlorovinyl)dimethylethoxysil ane	C6H13ClOSi
9	8.08	82.23	1.97	L-Homoserine lactone, N,N-dimethyl-	C6H11NO2
10	8.28	32.09	0.64	2-Propyl-tetrahydropyran-3-ol	C8H16O2
11	8.62	32.45	0.93	Imidazole	C3H4N2
12	10.00	11.83	0.76	Tertbutyloxyformamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-buty nyl]-	C14H24N2O2
13	10.42	15.27	0.74	Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetrameth yl	C11H14N2OS
14	11.48	12.26	4.83	1H-2-Indenol, 2,3,4,5,6,7-hexahydro-1-(2-hydroxy-2-methylpropyl)	C13H22O2
15	11.55	48.75	2.53	dl-Citrulline	C6H13N3O3
16	11.96	7.38	1.45	2-Pyridineacetic acid, hexahydro-	C7H13NO2
17	12.66	23.13	0.86	dl-Lysine	C6H14N2O2
18	13.51	60.28	1.62	D-Streptamine, O-6-amino-6-deoxy-à-D-glucopyranosy l-(1-4)-O-(3-deoxy-4-C-methyl-3-(meth ylamino)-á-L-arabinopyranosyl-(1-6))- 2-deoxy	C6H13NO2
19	13.78	43.35	8.31	Calycotomine, N-methyl-	C13H19NO3
20	13.99	17.84	2.38	4-[4-Diethylamino-1-methylbutylamino]-1,2-dimethoxy-6-bromonaphthalene	C21H31BrN2O2
21	14.35	32.83	1.22	Dasycarpidan-8(16H)-ethanol, 3,18-didehydro-1-(hydroxymethyl)-, (2.xi.,4.xi.)-	C20H28N2O2
22	14.62	12.2	2.67	4-[4-Diethylamino-1-methylbutylamino]-1,2-dimethoxy-6-bromonaphthalene	C21H31BrN2O2
23	15.10	12.00	4.14	Dasycarpidan-8(16H)-ethanol, 3,18-didehydro-1-(hydroxymethyl)-, (2.xi.,4.xi.)-	C20H28N2O2
24	15.21	32.49	0.87	Cystine	C6H12N2O4S2
25	15.65	33.28	1.26	3-[N-[2-Diethylaminoethyl]-1-cyclopes tenylamino]propionitrile	C14H25N3
26	15.86	16.52	0.92	á-Hydroxyquebrachamine	CH3CH(OH)COOH
27	15.99	14.23	1.02	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	C21H36O4
28	16.22	55.93	8.71	Uric acid	C5H4N4O3
29	16.45	8.30	1.33	Aminoacetamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-buty nyl]-	C5H4N4O3
30	16.69	32.32	2.06	Glucopyranuronamide, 1-(4-amino-2-oxo-1(2H)-pyrimidinyl)-1,4-dideoxy-4-(D-2-(2-(methylamino) acetamido)hydracrylamido)-, á-D	C10H14N2O
31	16.90	29.78	0.80	1,2,4-Trioxolane-2-octanoic acid, 5-octyl-, methyl ester	C6H11NO6
32	17.29	30.73	1.49	Actinomycin C2	C19H36O5
33	18.45	37.85	2.00	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	C63H88N12O16
34	18.66	90.13	2.23	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	C63H88N12O16
35	18.76	60.11	2.62	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6 H-dipyrrolo[1,2-a:1',2'-d]pyrazine	C11H18N2O2
36	18.97	36	2.13	l-(+)-Ascorbic acid	C14H22
37	20.98	13.19	1.29	cis-13-Octadecenoic acid	C6H8O6
38	21.17	13.49	0.94	Ricinoleic acid	C18H34O2
39	23.99	31.48	0.89	Ergotaman-3',6',18-trione, 12'-hydroxy-2'-methyl-5'-(phenylmeth yl)-, (5'à)-	C18H34O3

acid and energy metabolism, with lipid metabolism playing a supporting role. In comparison, in our study, *Scylla serrata* ethanol extract showed only 8.31% alkaloid content among the dominant compounds. This difference suggests that environmental stress factors such as sudden salinity reduction can significantly modulate the metabolite profile among related *Scylla* species, especially under similar polar extraction conditions.

Interestingly, alkaloid compounds were not detected in the qualitative phytochemical screening, but several alkaloids such as Calycotomine and Imidazole were identified through GC-MS analysis.

This discrepancy is not only due to methodological differences but may also be related to the detection sensitivity. Qualitative tests often fail to detect compounds present at low concentrations, whereas GC-MS has a higher sensitivity and can identify minor constituents. In addition, the polarity of ethanol as a solvent might have selectively extracted certain alkaloids in low yield, which escaped detection in qualitative assays but were quantifiable in GC-MS analysis. Similar observations were reported in marine-derived extracts where alkaloids were inconsistently detected depending on the analytical method (Rahmawati *et al*., 2023; Shofinita *et al*., 2024).

The main group of compounds in the ethanol extract of mud crab (*S. serrata*) was represented by three peaks on the GC chromatogram, which had a higher percentage area than the others. These peaks were identified as Calycotomine, N-methyl- (8.31%), uric acid (8.71%), and Dasycarpidan-8(16H)-ethanol, 3,18-didehydro-1-(hydroxymethyl)-(2.xi.,4.xi.)-4.14%). Antioxidant compounds detected in the GC-MS analysis included 2-Cyclohexylpiperidine with an area of 3.45%, which belongs to the alkaloid group. In addition, the Edullan II compound with an area of 1.37% belongs to the volatile compound group. The compound Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate, with an area of 0.83%, belongs to the terpenoid compound group, and Imidazole with an area of 0.93% also belongs to the alkaloid group.

In a study conducted by Karnila *et al*. (2021) on mud crab (*S. serrata*), antioxidant compounds such as astaxanthin with a peak area of 18.6% and β-carotene with a peak area of 7.9% were identified through GC-MS analysis, both of which belong to the carotenoid group and contribute to antioxidant activity. Similarly, a study by (Surrete, 2013)~~Thiruvengadarajan *et al*. (2024)~~ on the nutritional composition of *S. serrata* reported the presence of unsaturated fatty acids such as eicosapentaenoic acid (EPA, 5.42%) and docosahexaenoic acid (DHA, 4.87%), which are categorized as bioactive lipids and known for their health-promoting effects.

The group of compounds identified in Scylla serrata are alkaloids. Based on research by Karim *et al*. (2024) analyzing muscle and hepatopancreas extracts of *S. olivacea*, they reported a lipid profile including EPA and DHA content, as well as significant antioxidant capacity measured through the DPPH and ferric-reducing tests. Similarly, Taufik *et al*. (2020) applied GC–MS to *S. olivacea* tissues, revealing a predominance of monounsaturated and polyunsaturated fatty acids, including long-chain polyunsaturated fatty acids (PUFAs) in the gonads and hepatopancreas. Although specific alkaloid compounds have not been reported for *Scylla*, these lipid-based metabolites provide relevant biological context within the genus *Scylla*. Therefore, comparisons of the alkaloid profiles of *S. serrata* should emphasize the same metabolic pathways within this genus and highlight the need for targeted alkaloid profile studies on *Scylla* species.

Conclusion

This study successfully demonstrated that *S. serrata* extract possesses very strong antioxidant activity, supported by the presence of flavonoids, triterpenoids, and various bioactive compounds identified through phytochemical and GC-MS analyses. These findings confirm that the research objective to explore and characterize the antioxidant potential and compound profile of *S. serrata* has been achieved. The extract's bioactive profile, comprising both major and minor compounds, reinforces its potential as a promising source of natural antioxidants with additional pharmacological properties, including anti-inflammatory, anticancer, antimicrobial, and antiviral activities. Overall, this study contributes to the growing evidence that marine organisms represent valuable resources for the development of alternative medicinal agents.

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Screening and Profiling of Antioxidant Activity in Mud Crab (*Scylla Serrata*) from Banyuasin Waters

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Abstract

Mangrove crab (Scylla serrata) as one of the crustacean species, has a variety of bioactive compounds that can be utilized in the field of pharmacology. Antioxidant compounds act as therapeutic agents against degenerative diseases. Banyuasin waters have mangrove vegetation with associated marine organisms that have the potential to be studied for bioactive compounds. This study aims to identify the phytochemical profile quantitatively and qualitatively, samples were collected from mud flats near mangrove ecosystems in Banyuasin waters, South Sumatra. Samples were tested for antioxidant activity using the DPPH test, and IC_{50} values, qualitative phytochemical identification, and phytochemical profiles were calculated using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Based on the results of antioxidant testing, the IC_{50} value of S. serrata extract is 2.25 ppm, the sample is included in the category of very strong antioxidants. Phytochemical test results showed that the compound is thought to contain antioxidant activity from flavonoids and triterpenoids. GC-MS analysis detected major compound groups of alkaloids, purines, and vitamins. Minor compound groups detected amines, terpenoids, monosaccharides, amino acids, fatty acids, silanes, formamides, heterocycles, carboxylic acids, aminoglycosides, naphthalene derivatives, nitriles, amides, glycosides, and peptides. S. serrata extract shows very strong antioxidant activity, with major compounds such as alkaloids, purines, and vitamins. S. serrata extract detected compounds that have been reported as anti-inflammatory, anticancer, antimicrobial, and antiviral. These findings highlight the pharmaceutical potential of S. serrata as a source of bioactive compounds. The results of this study provide valuable information for the development of alternative medicines derived from marine organisms.

Keywords: *Antioxidant, Bioactive compounds, DPPH, GC-MS, S. serrata*

Introduction

Scylla serrata, or mud crab, as one of the crustacean species, has great potential in the field of beauty and health, because of its diverse bioactive compounds (Yusof *et al*., 2020; Beslin and Geni, 2021; Neelima *et al*., 2022). The diversity of bioactive compounds triggers great potential in the search for alternative medicinal ingredients from marine organisms, including antioxidant compounds (Yogeshwaran *et al*., 2020; Karnila and Ramadhani, 2021; Fajriaty *et al*., 2024). Antioxidant compounds in mud crabs have a role as therapeutic agents (Wu *et al*., 2021) in fighting degenerative diseases such as cancer (Nagarajan *et al*., 2024), cardiovascular diseases (Nanda *et al*., 2021), and neurodegenerative disorders (Galal-Khallaf *et al*., 2024), caused by oxidative stress due to free radicals.

Antioxidants are the reaction of a compound in neutralizing free radicals (Delta *et al*., 2021; Rozirwan *et al*., 2023a; 2023b). This process involves a highly efficient electron donation mechanism. Antioxidants work by donating electrons to free radicals (Fajriaty *et al*., 2024; Frías-Espericueta *et al*., 2022; Pati *et al*., 2022; Yang *et al*., 2023). Free radicals, which have one or more unpaired electrons, are highly reactive and can cause oxidative damage to DNA, proteins, and cellular lipids (Alkadi, 2020; Di Meo and Venditti, 2020). Free radicals are often generated as by-products of various metabolic processes in the body or due to environmental exposures such as pollution and ultraviolet radiation (Martemucci *et al*., 2022; Sadiq, 2023). The damage caused by free radicals can contribute to the development of various degenerative diseases, including cancer, heart disease, and neurodegenerative disorders (Teleanu *et al*., 2022; Chaudhary *et al*., 2023). In inhibiting free

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radicals, antioxidant compounds such as carotenoids and polyphenols will interact with free radicals to enhance the activity of detoxification enzymes, resulting in accelerated elimination of free radicals and strengthened antibodies (Pisoschi *et al*., 2021; Tumilaar *et al*., 2024).

The analysis of antioxidant compounds in *S. serrata* requires an accurate technique to ensure that identification and quantification are targeted (Baag and Mandal, 2023; Yao *et al*., 2023). Previous studies have mainly reported the antioxidant potential of *S. serrata* using spectrophotometric or colorimetric assays such as DPPH, ABTS, and FRAP, which provide only general antioxidant capacity without revealing the identity of specific compounds (Yogeshwaran *et al*., 2020; Karnila and Ramadhani, 2021; Neelima *et al*., 2022). In contrast, Gas Chromatography-Mass Spectrometry (GC-MS) offers higher resolution in profiling bioactive compounds by separating and identifying molecules based on their mass and chemical characteristics (Jabbar *et al*., 2022; Musa *et al*., 2022; Palma *et al*., 2023; Rozirwan *et al*., 2024). This advanced method not only allows the detection of a broader spectrum of antioxidant molecules but also quantifies their abundance, thereby providing a more comprehensive understanding of their therapeutic potential.

Although antioxidant compounds such as flavonoids, carotenoids, and polyphenols have been reported from different body parts of *S. serrata* (Yogeshwaran *et al*., 2020; Karnila and Ramadhani, 2021; Neelima *et al*., 2022; Yang *et al*., 2023; Fajriaty *et al*., 2024), most of these studies remain descriptive and do not provide detailed profiles of the specific types and relative quantities of individual compounds. To date, there is still no comprehensive GC-MS based profiling that systematically characterizes the antioxidant repertoire of *S. serrata* meat. This represents a critical knowledge gap, since understanding the diversity and concentration of specific compounds is essential to support its therapeutic application. The present study aims to provide a detailed GC-MS profiling of antioxidant compounds in *S. serrata*. This work contributes novel insights into its biochemical composition and strengthens its potential utilization as a sustainable source of natural antioxidants for pharmaceutical and nutraceutical development.

Materials and Methods

Mangrove crab samples were collected in January 2023 from Banyuasin Waters, South Sumatra, Indonesia (Figure 1). At this location, numerous crustacean and gastropod populations were found inhabiting the intertidal zone (Rozirwan *et al*., 2021; Rozirwan *et al*., 2021). The crab fishing area had a mud substrate with a depth of 1-2 m and was located within a mangrove vegetation zone directly connected to port and pond activities (Fitria *et al*., 2023). Anthropogenic pollutants that accumulate in these waters are known to trigger an increase in the defense mechanisms of organisms, such as the production of antioxidant compounds derived from secondary metabolites (Rozirwan *et al*., 2023).

Figure 1. Map of sampling location in Banyuasin Waters area

Sample identification and collection

Crustacean samples were taken using folding trawl gear. The samples were collected and stored in a cool box. The crab identification process was conducted based on the examination of morphological characteristics, such as body shape, color pattern, claw shape, and leg shape (Hidir *et al*., 2021; Vermeiren *et al*., 2021). Morphometric measurements were performed on the crab samples, and the identification process was completed in the laboratory. Taxon determination was carried out using data from the World Register of Marine Species (WoRMS) accessed in January 2023 (WoRMS, 2023).

Environmental characteristics of sampling area

Environmental quality calculations were conducted to assess the condition of the sampling environment. Environmental parameter data were measured, including salinity, temperature, pH, and dissolved oxygen (DO) (Fitria *et al*., 2023; Rozirwan *et al*., 2024). Each parameter was measured in three repetitions to ensure consistency, and the results were then averaged. Environmental parameter measurements are typically used to evaluate habitat conditions, as they provide insights into the physical and chemical characteristics of the ecosystem. Repetition in measurements is a standard approach to improve data reliability.

Sample preparation

The preparation method described by Ambekar *et al*. (2023), involves cleaning the crab to remove contaminants. In this study, the carapace and crab meat were separated and rinsed with distilled water to eliminate any remaining impurities. The wet weight of the crab meat was measured, and the samples were then dried in an oven at 40°C for 3 × 24 h. After drying, the samples were ground into powder using a blender. The dry weight of the crab meat was recorded for data analysis.

Sample maceration and extraction

The wet maceration method was used in this study. A total of 250 g of *S. serrata* meat powder was weighed and immersed in 1000 mL of 96% ethanol solvent at a ratio of 1:4 (b/v). The soaking process was conducted for 3 × 24 h, with stirring performed periodically to ensure optimal extraction. The maceration results were filtered using filter paper (No. 42, 125 mm). The extraction process was then carried out using a rotary evaporator at 40°C with a rotating speed of 3000 rpm (Hashim *et al*., 2021). The resulting extract was stored as a stock solution. A total of 0.05 g of *S. serrata* extract was used as an additive for the stock solution (Habib *et al*., 2022). Wet maceration is a commonly employed extraction method due to its ability to preserve heat-sensitive compounds. Stirring during the maceration process enhances solute dissolution, while rotary evaporation ensures efficient solvent removal under controlled temperature conditions.

Determination of antioxidant activity and IC_{50} value

Antioxidant testing was conducted using the DPPH method (Vásquez *et al*., 2023). The stock solution of *S. serrata* extract was used as the test solution, while vitamin C served as a reference. The sample was dissolved in 96% ethanol. A total of 1 mL of sample solution was prepared for each concentration: 100, 150, 200, 250, and 300 mg.L^{-1}. Each concentration was mixed with 40 µg.mL^{-1} DPPH solution and incubated in the dark for 30 min. Absorbance was measured at 517 nm using UV-Vis spectrophotometry. The strength of antioxidant activity is classified according to IC_{50} values, as shown in Table 1. The IC_{50} value is calculated using the following formula.

$$inhibition = \frac{blank\ abs - sample\ abs.}{blank\ abs}\ x\ 100\ \%$$

The IC_{50} results were entered in the linear regression equation y= ax + b. The sample concentration is the abscissa (X-axis), and the percentage of antioxidant inhibition is the ordinate (Y-axis) (Yuniarti *et al*., 2020). The concentration corresponding to 50% inhibition was interpolated from the regression equation. All determinations were conducted in triplicate, and the mean IC_{50} values were statistically compared using one-way ANOVA, followed by Duncan's multiple range test (P< 0.05).

Table 1. Characteristic concentration value of IC_{50} (Molyneux, 2004)

Concentration Value (µg.mL^{-1})	Characteristic
<50	Very Strong
50-100	Strong
100-150	Moderate
150-200	Low

Phytochemical screening

Phytochemical tests of *S. serrata* meat extracts were conducted using qualitative methods to identify the presence of bioactive compounds. The analysis included steroid and triterpenoid tests, which were performed using the Liebermann-Burchard method, alkaloid tests using Mayer and Dragendorff reagents, flavonoid tests with the Shinoda staining method, tannin tests using the $FeCl_3$ reaction, and saponin tests using the foam test method. Each test was carried out following the procedures described in standard literature (Suwandi *et al*., 2020; Dinesh *et al*., 2022).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The identification of bioactive compound components in *S. serrata* meat extract was performed using the GC-MS analysis method, following Rozirwan *et al*. (2022) with modifications. A total of 1 μL of extract was injected into an RTX-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) using helium as the carrier gas at a constant flow rate of 1.0 $mL.min^{-1}$ and a split ratio of 1:50. The oven temperature was initially set at 50°C for 5 min, then increased at a rate of 5°C/min to 280°C, and held for 5 min. The injector and ion source temperatures were 280°C and 230°C, respectively, with an electron ionization (EI) energy of 70 eV. The mass spectrometer was operated in scan mode with a mass range of 40–550 m/z. The Wiley 7 Library database was used as a reference for spectral comparison (Rafferty *et al*., 2020).

Result and Discussion

Sample identification and collection

Taxon determination was conducted using data from the World Register of Marine Species (WoRMS) accessed in January 2023 (WoRMS, 2023). Based on their morphological characteristics, the crustacean samples were identified as *S. serrata*.

Mangrove crabs of this species were caught using folding traps. The fishing process was carried out during low tide to facilitate crab capture. In this study, 2-5 crabs were used as stock samples. The crabs obtained were weighed, with weights ranging from 200 to 320 g, widths of approximately 21.5 cm, and lengths of around 13.5 cm. The crabs were then stored in a cool box filled with ice cubes for preservation. The species identification of mangrove crabs is based on morphological characteristics, which include features such as the eyes, propodus, carpus, merus, carapace, claws, walking legs, and swimming legs (Figure 2). Taxonomic data from databases such as WoRMS is widely used to confirm species classification accurately.

Environmental characteristics of sampling area

The results of environmental quality measurements in the *S. serrata* sampling areas in Banyuasin waters revealed diverse conditions. Measurements of water physicochemical parameters, including dissolved oxygen, pH, temperature, and salinity, were taken to assess the habitat suitability for *S. serrata* (Rozirwan *et al*., 2021). The dissolved oxygen concentration was found to be 4.2 $mg.L^{-1}$, which is sufficient to support the respiration process of aquatic organisms (Ouyang *et al*., 2021). The pH value of the water at the sampling location was 7, indicating neutral pH, which represents optimal ecological conditions (Chowdhury *et al*., 2021). This is consistent with previous studies (Yusni and Haq, 2020; Muhtar and Lanuru, 2021; Putri *et al*., 2022), which state that waters with a pH between 6.5 and 7.5 are ideal for the survival of mangrove crabs. The water temperature was measured at 28°C, indicating favorable conditions for mangrove crab growth (Indarjo *et al*., 2020; Ren *et al*., 2021) Water salinity was recorded at 18 psu, reflecting typical conditions in estuarine areas or areas directly influenced by tidal movements (Wang *et al*., 2021). *S. serrata* grows best in salinities between 15 psu and 25 psu but grows more slowly at salinities greater than 25 to 30 psu (Triajie *et al*., 2020; Pati *et al*., 2023; Adnan *et al*., 2024).

Environmental conditions at the sampling site, particularly salinity (18 psu) and temperature (28°C), could also influence the diversity and concentration of secondary metabolites in *S. serrata*. Estuarine environments are dynamic, and organisms inhabiting them may prioritize the synthesis of specific bioactive compounds for stress adaptation.

Table 2. Observation of Environmental Parameters of the Research Site

	Station
Environment Parameter Quality	Sungsang Waters
Dissolved oxygen ($mg.L^{-1}$)	4.2
pH	7
Temperature (°C)	28
Salinity (psu)	18

Previous studies reported that salinity stress can regulate secondary metabolite production, including alkaloids and flavonoids, in marine organisms (Pati *et al*., 2023). Furthermore, the choice of ethanol as the extraction solvent, although safer and less toxic than methanol, may have influenced the recovery efficiency of certain compounds, possibly underestimating the abundance of more polar metabolites.

Determination of antioxidant activity by DPPH assay

Antioxidant analysis using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method on Rozirwan *et al*. (2023) *S. serrata* meat extract showed promising results. The DPPH solution changed from purple to yellow, indicating the presence of antioxidants in the extract. The test results revealed that the *S. serrata* extract had an IC_{50} value of 2.25 ppm, while Vitamin C, used as a comparison solution, had an IC_{50} value of 2.16 ppm (Table 3). Both solutions demonstrated low IC_{50} values, categorizing them as very strong antioxidant compounds. The IC_{50} value, which is the concentration required to inhibit 50% of DPPH radical activity, is a critical parameter for assessing the antioxidant potential of a compound (Martinez-Morales *et al*., 2020). The results indicate that *S. serrata* extract possesses an IC_{50} value comparable to that of Vitamin C. The percentage inhibition of DPPH free radicals by *S. serrata* meat extract increased as the extract concentration increased. This suggests that the extract has the ability to donate electrons or hydrogen to the DPPH radical, neutralizing it and converting it into a more stable form (Gulcin and Alwasel, 2023).

The discovery of compounds such as flavonoids and triterpenoids, which were identified in the phytochemical analysis of *S. serrata* extracts, suggests that they may possess high antioxidant activity (Akinwumi *et al*., 2022; Hajar-Azira *et al*., 2023). Flavonoids are well known for their ability to capture free radicals and interrupt the chain of oxidative reactions. Triterpenoids are also recognized for their significant antioxidant activity through a similar mechanism. The combination of these compounds in *S. serrata* extract creates a synergistic effect, enhancing the overall capacity of the extract to neutralize free radicals.

S. serrata is known for its rich antioxidant system and strong enzymatic defense system, which help it survive in the dynamic and often challenging mangrove environment (Pati *et al*., 2023). Mud crabs also possess defense enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, which work synergistically to detoxify reactive oxygen species (ROS) and maintain redox balance in the body (Jerome *et al*., 2020; Bal *et al*., 2021; Costantini *et al*., 2022; Zeng *et al*., 2024). These enzymes play a crucial role in protecting the crab from oxidative stress generated by fluctuating environmental conditions, pollution, and pathogens.

Table 3. Calculation results of antioxidant activity of *S. serrata* in Sungsang waters

Sample	Linear Regression			IC_{50} Value	Category
	a	b	R^2		
S. serrata	6.9429	52.808	0.9327	2.25 $mg.L^{-1}$	Very Strong
Ascorbic Acid	6.7135	55.866	0.9435	2.16 $mg.L^{-1}$	Very Strong

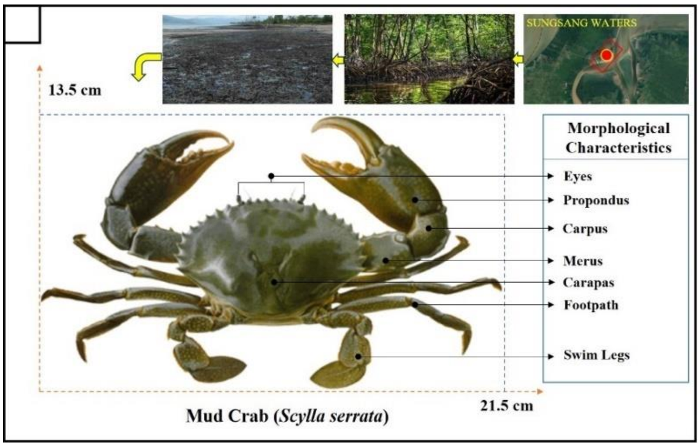

Figure 2. Crustacean species, *S. serrata*

This combination of antioxidant compounds and defence enzymes not only ensures the survival of mud crabs in their habitat but also positions them as a potential source for the development of natural health products that can harness their protective mechanisms. Antioxidants and defence enzymes play a vital role in organisms' survival, especially in harsh environments. The synergy between these compounds and enzymes contributes significantly to mitigating oxidative stress and preserving cellular function, which can be explored for potential therapeutic applications.

Phytochemical screening

Phytochemical tests were carried out to identify the compounds present in gastropod and crustacean extracts using ethanol as the solvent (Fitria *et al*., 2023; Rozirwan *et al*., 2024) The phytochemical test aimed to determine the compounds in the test extract (Chen *et al*., 2022), allowing for the identification of compounds that influence the strong or weak antioxidant activity of the extract (Baliyan *et al*., 2022). The results of the phytochemical test, after UV-Vis spectrophotometric analysis of the extract, are presented in Table 4. The test results showed that only certain compounds were extracted by the ethanol solvent (Yuniarti *et al*., 2020).

Qualitative phytochemical analysis of *S. serrata* extract showed significant results in identifying the content of bioactive compounds. Based on the test results, *S. serrata* extract tested positive for flavonoid and triterpenoid compounds. A similar finding was reported by Elshaarawy *et al*. (2023) for *Scylla olivacea* samples. These two compounds offer various health benefits and therapeutic potential, particularly due to their strong antioxidant properties. Flavonoids are a group of polyphenolic compounds that are widely recognized for their potent antioxidant activity (Shen *et al*., 2022). These compounds can neutralize free radicals and prevent oxidative damage to cells and tissues. Additionally, flavonoids possess anti-inflammatory, anticancer, and cardioprotective properties (Mounika *et al*., 2021; Jain *et al*., 2024; Ullah *et al*., 2024). The identification of flavonoid compounds in *S. serrata* indicates that mud crabs are not only valuable as food but also hold potential as an alternative source of medicine from marine organisms.

Triterpenoids are a group of terpenoid compounds that have potential biological activities (Mabou and Yossa, 2021; Zang *et al*., 2022). These compounds are known to possess anti-inflammatory, antitumor, antimicrobial, and immunomodulatory properties (Harun *et al*., 2020; Ahmad *et al*., 2021). As antioxidant agents, triterpenoids have been used in pharmacology to treat inflammatory diseases and cancer. Similar to flavonoids, these compounds can neutralize free radicals caused by oxidative stress on body tissues. The discovery of triterpenoid compounds in *S. serrata* shows promising results, given their antioxidant potential that can be applied to address various diseases. Thus, the opportunity to explore alternative medicinal raw materials from *S. serrata* extract is increasingly attractive for further research. Overall, the phytochemical results focusing on flavonoid and triterpenoid compounds confirm the importance of *S. serrata* as a potential source of bioactive compounds. Further research is encouraged at the stage of isolation and purification of these compounds, so that alternative medicinal materials derived from this marine organism can contribute to the development of therapeutic agents from marine organisms.

Phytochemical profile screening

The antioxidant compound profile in *S. serrata* was determined using GC-MS (Gas Chromatography-Mass Spectrometry) analysis on the ethanol extract of mud crab. Figure 3 shows the chromatogram with 37 peaks identified in the extract. Each peak on the chromatogram represents a distinct chemical compound found in the extract, which was analyzed using GC-MS.

GC-MS analysis is a powerful technique for identifying and quantifying individual compounds in complex mixtures, providing insights into the chemical composition of the extract. This method is widely used for its ability to separate and identify volatile compounds, making it an essential tool for profiling bioactive compounds, such as antioxidants, in natural products.

Table 4. Phytochemical screening results of *S. Serrata*

Parameters	Analysis Result	Analysis Type
Alkaloids	-	Qualitative
Flavonoids	+	Qualitative
Triterpenoids	+	Qualitative
Saponin	-	Qualitative
Tannins	-	Qualitative
Steroid	-	Qualitative

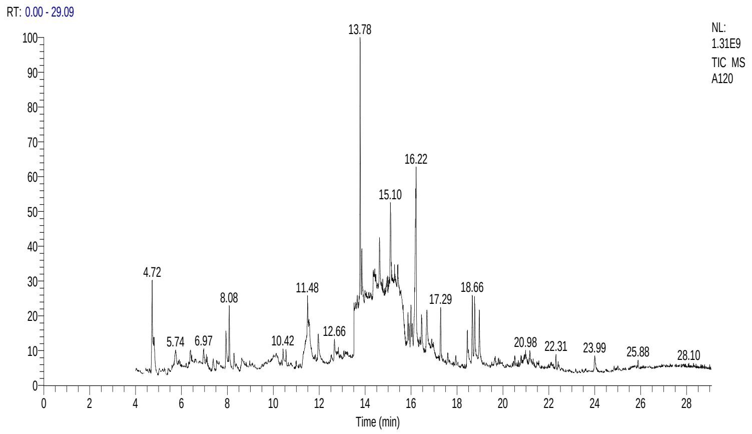

Figure 3. GC-MS chromatogram of ethanol extract *S. Serrata*

Terpenoid, alkaloid, steroid, and tannin groups were among the pure compounds successfully detected using GC-MS. Based on the GC-MS analysis of the ethanol extract compound components presented in Table 5, the main components identified in the extract were Calycotomine, N-methyl-, with a value of 8.31% of the total area, and uric acid, with a value of 8.71% of the total area.

At a retention time of 4.72 min, the compound 2-Cyclohexylpiperidine was detected with an area of 3.45%, a probability of 6.07, and a chemical formula of C11H21N, which belongs to the alkaloid compound group. At a retention time of 5.74 min, the compound 2-Pyridinamine, 3,6-dimethyl was detected with an area of 1.69%, a probability of 7.02, and a chemical formula of C7H10N2, which belongs to the aminopyridine compound group. Furthermore, at a retention time of 6.97 min, the compound Pentanoic acid, dodec-9-ynyl ester was detected with an area of 1.00%, a probability of 8.47, and a chemical formula of C17H30O2, which belongs to the protein compound group.

At a retention time of 8.08 min, the compound L-Homoserine lactone, N, N-dimethyl was detected with an area of 1.97%, a probability of 82.23, and a chemical formula of C6H11NO2, which belongs to the amino acid compound group. At a retention time of 10.42 min, the compound Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetramethyl was detected with an area of 0.74%, a probability of 43.35, and a chemical formula of C11H14N2OS, which belongs to the EPA compound group. At a retention time of 11.48 min, the compound 1H-2-Indenol, 2,3,4,5,6,7- hexahydro -1-(2-hydroxy-2-methylpropyl) was detected with an area of 4.83%, a probability of 12.26, and a chemical formula of C13H22O2, which belongs to the lactone compound group.

At a retention time of 12.66 min, the compound dl-Lysine was detected with an area of 0.86%, a probability of 23.13, and a chemical formula of C6H14N2O2, which belongs to the amino acid compound group. At a retention time of 13.78 min, the compound Calycotomine, N-methyl- was detected with an area of 8.31%, a probability of 43.35, and a chemical formula of C13H19NO3, which belongs to the alkaloid compound group. At a retention time of 15.10 min, the compound Dasycarpidan-8(16H)-ethanol, 3,18-dihydro-1-(hydroxymethyl)-, (2.xi.,4.xi.)- was detected with an area of 4.14%, a probability of 12.00, and a chemical formula of C20H28N2O2, which belongs to the group of molport compounds.

At a retention time of 16.22 min, the uric acid compound was detected with an area of 8.71%, a probability of 55.93, and a chemical formula of C5H4N4O3, which belongs to the allantoin compound group. At a retention time of 17.29 min, the compound Actinomycin C2 was detected with an area of 1.49%, a probability of 30.73, and a chemical formula of C63H88N12O16, which belongs to a group of peptide compounds that are derivatives of peptide compounds.

Animals produce a diverse mixture of secondary metabolites such as phenols, alkaloids, flavonoids, tannins, and saponins. Several animal studies have shown the potential use of these metabolites as antibacterial agents due to the presence of abundant biomolecules. Synthetic drugs often have high secondary failure rates and severe side effects, while animal products contain a variety of free radical scavenging molecules with substantial antioxidant properties.

As a more taxonomically relevant comparison, Yao *et al*. (2020) conducted a GC-MS-based metabolomic study on Scylla paramamosain experiencing acute salinity reduction (from 23 psu to 3 psu). This study identified 519 metabolites (mainly lipids), with 13 significantly enriched metabolic pathways ($P < 0.05$), related to signaling, lipid metabolism, and transport. Additionally, in that study, combining LC-MS and GC-MS data revealed 28 significant metabolic pathways, dominated by amino acid and energy metabolism, with lipid metabolism playing a supporting role. In comparison, in our study, *Scylla serrata* ethanol extract showed only 8.31% alkaloid content among the dominant compounds.

Table 5. Proposed peak order, retention time, probability, area, compound name, and molecular formula

Peak#	R. Time	Probability	Area%	Name	Molecular formula
1	4.72	6.07	3.45	2-Cyclohexylpiperidine	C11H21N
2	4.81	18.58	1.37	Edulan II	C13H20O
3	5.74	7.02	1.69	2-Pyridinamine, 3,6-dimethyl	C7H10N2
4	6.39	8.12	0.83	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	C16H28O3
5	6.61	11.07	0.66	d-Mannose	C6H12O6
6	6.81	50.91	0.89	Deoxyspergualin	C17H37N7O3
7	6.97	8.47	1.00	Pentanoic acid, dodec-9-ynyl ester	C17H30O2
8	7.93	51.03	1.42	trans-(2 Chlorovinyl)dimethylethoxysil ane	C6H13ClOSi
9	8.08	82.23	1.97	L-Homoserine lactone, N,N-dimethyl-	C6H11NO2
10	8.28	32.09	0.64	2-Propyl-tetrahydropyran-3-ol	C8H16O2
11	8.62	32.45	0.93	Imidazole	C3H4N2
12	10.00	11.83	0.76	Tertbutyloxyformamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-buty nyl]-	C14H24N2O2
13	10.42	15.27	0.74	Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetrameth yl	C11H14N2OS
14	11.48	12.26	4.83	1H-2-Indenol, 2,3,4,5,6,7-hexahydro-1-(2-hydroxy-2-methylpropyl)	C13H22O2
15	11.55	48.75	2.53	dl-Citrulline	C6H13N3O3
16	11.96	7.38	1.45	2-Pyridineacetic acid, hexahydro-	C7H13NO2
17	12.66	23.13	0.86	dl-Lysine	C6H14N2O2
18	13.51	60.28	1.62	D-Streptamine, O-6-amino-6-deoxy-à-D-glucopyranosy l-(1-4)-O-(3-deoxy-4-C-methyl-3-(meth ylamino)-á-L-arabinopyranosyl-(1-6))- 2-deoxy	C6H13NO2
19	13.78	43.35	8.31	Calycotomine, N-methyl-	C13H19NO3
20	13.99	17.84	2.38	4-[4-Diethylamino-1-methylbutylamino]-1,2-dimethoxy-6-bromonaphthalene	C21H31BrN2O2
21	14.35	32.83	1.22	Dasycarpidan-8(16H)-ethanol, 3,18-didehydro-1-(hydroxymethyl)-, (2.xi.,4.xi.)-	C20H28N2O2
22	14.62	12.2	2.67	4-[4-Diethylamino-1-methylbutylamino]-1,2-dimethoxy-6-bromonaphthalene	C21H31BrN2O2
23	15.10	12.00	4.14	Dasycarpidan-8(16H)-ethanol, 3,18-didehydro-1-(hydroxymethyl)-, (2.xi.,4.xi.)-	C20H28N2O2
24	15.21	32.49	0.87	Cystine	C6H12N2O4S2
25	15.65	33.28	1.26	3-[N-[2-Diethylaminoethyl]-1-cyclopes tenylamino]propionitrile	C14H25N3
26	15.86	16.52	0.92	á-Hydroxyquebrachamine	CH3CH(OH)COOH
27	15.99	14.23	1.02	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	C21H36O4
28	16.22	55.93	8.71	Uric acid	C5H4N4O3
29	16.45	8.30	1.33	Aminoacetamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-buty nyl]-	C5H4N4O3
30	16.69	32.32	2.06	Glucopyranuronamide, 1-(4-amino-2-oxo-1(2H)-pyrimidinyl)-1,4-dideoxy-4-(D-2-(2-(methylamino) acetamido)hydracrylamido)-, á-D	C10H14N2O
31	16.90	29.78	0.80	1,2,4-Trioxolane-2-octanoic acid, 5-octyl-, methyl ester	C6H11NO6
32	17.29	30.73	1.49	Actinomycin C2	C19H36O5
33	18.45	37.85	2.00	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	C63H88N12O16
34	18.66	90.13	2.23	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	C63H88N12O16
35	18.76	60.11	2.62	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6 H-dipyrrolo[1,2-a:1',2'-d]pyrazine	C11H18N2O2
36	18.97	36	2.13	l-(+)-Ascorbic acid	C14H22
37	20.98	13.19	1.29	cis-13-Octadecenoic acid	C6H8O6

38	21.17	13.49	0.94	Ricinoleic acid		C18H34O2
39	23.99	31.48	0.89	Ergotaman-3',6',18-trione, (phenylmeth yl)-, (5'à)-	12'-hydroxy-2'-methyl-5'-	C18H34O3

This difference suggests that environmental stress factors such as sudden salinity reduction can significantly modulate the metabolite profile among related *Scylla* species, especially under similar polar extraction conditions.

Interestingly, alkaloid compounds were not detected in the qualitative phytochemical screening, but several alkaloids such as Calycotomine and Imidazole were identified through GC-MS analysis. This discrepancy is not only due to methodological differences but may also be related to the detection sensitivity. Qualitative tests often fail to detect compounds present at low concentrations, whereas GC-MS has a higher sensitivity and can identify minor constituents. In addition, the polarity of ethanol as a solvent might have selectively extracted certain alkaloids in low yield, which escaped detection in qualitative assays but were quantifiable in GC-MS analysis. Similar observations were reported in marine-derived extracts where alkaloids were inconsistently detected depending on the analytical method (Rahmawati *et al*., 2023; Shofinita *et al*., 2024).

The main group of compounds in the ethanol extract of mud crab (*S. serrata*) was represented by three peaks on the GC chromatogram, which had a higher percentage area than the others. These peaks were identified as Calycotomine, N-methyl- (8.31%), uric acid (8.71%), and Dasycarpidan-8(16H)-ethanol, 3,18-didehydro-1-(hydroxymethyl)-(2.xi.,4.xi.)-4.14%). Antioxidant compounds detected in the GC-MS analysis included 2-Cyclohexylpiperidine with an area of 3.45%, which belongs to the alkaloid group. In addition, the Edullan II compound with an area of 1.37% belongs to the volatile compound group. The compound Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate, with an area of 0.83%, belongs to the terpenoid compound group, and Imidazole with an area of 0.93% also belongs to the alkaloid group.

In a study conducted by Karnila *et al*. (2021) on mud crab (*S. serrata*), antioxidant compounds such as astaxanthin with a peak area of 18.6% and β-carotene with a peak area of 7.9% were identified through GC-MS analysis, both of which belong to the carotenoid group and contribute to antioxidant activity. Similarly, a study by (Surrete, 2013) on the nutritional composition of *S. serrata* reported the presence of unsaturated fatty acids such as eicosapentaenoic acid (EPA, 5.42%) and docosahexaenoic acid (DHA, 4.87%), which are categorized as bioactive lipids and known for their health-promoting effects.

The group of compounds identified in Scylla serrata are alkaloids. Based on research by Karim *et al*. (2024) analyzing muscle and hepatopancreas extracts of *S. olivacea*, they reported a lipid profile including EPA and DHA content, as well as significant antioxidant capacity measured through the DPPH and ferric-reducing tests. Similarly, Taufik *et al*. (2020) applied GC–MS to *S. olivacea* tissues, revealing a predominance of monounsaturated and polyunsaturated fatty acids, including long-chain polyunsaturated fatty acids (PUFAs) in the gonads and hepatopancreas. Although specific alkaloid compounds have not been reported for *Scylla*, these lipid-based metabolites provide relevant biological context within the genus *Scylla*. Therefore, comparisons of the alkaloid profiles of *S. serrata* should emphasize the same metabolic pathways within this genus and highlight the need for targeted alkaloid profile studies on *Scylla* species.

Conclusion

This study successfully demonstrated that *S. serrata* extract possesses very strong antioxidant activity, supported by the presence of flavonoids, triterpenoids, and various bioactive compounds identified through phytochemical and GC-MS analyses. These findings confirm that the research objective to explore and characterize the antioxidant potential and compound profile of *S. serrata* has been achieved. The extract's bioactive profile, comprising both major and minor compounds, reinforces its potential as a promising source of natural antioxidants with additional pharmacological properties, including anti-inflammatory, anticancer, antimicrobial, and antiviral activities. Overall, this study contributes to the growing evidence that marine organisms represent valuable resources for the development of alternative medicinal agents.

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