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

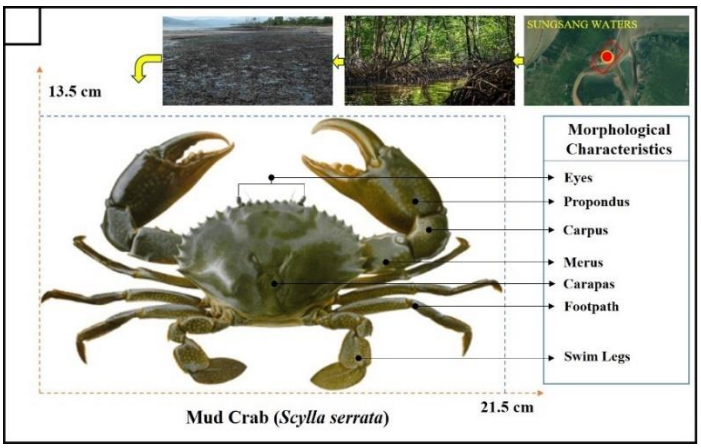

Figure 2. Crustacean species, *S. serrata*

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

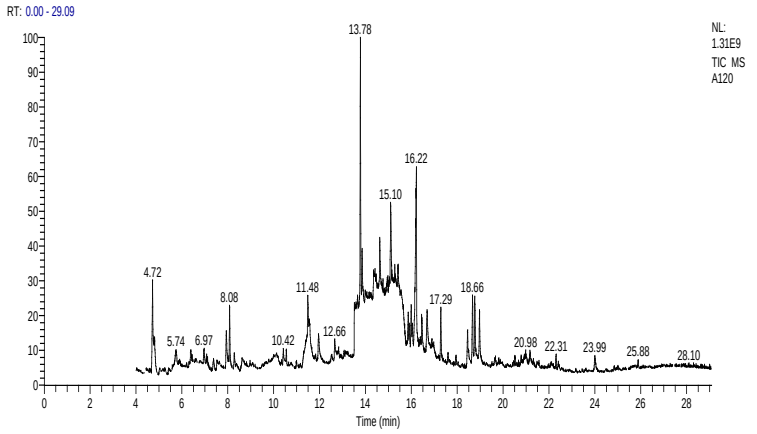

Figure 3. GC-MS chromatogram of ethanol extract *S. Serrata*

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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