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Antioxidant Activity and Chemical Profile of Fiddler Crabs as a Form of Self-Defense Against Various Anthropogenic Activities Along Urban Rivers

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ABSTRACT

Fiddler crabs utilize bioactive compounds for defense against environmental stressors. The study aimed to determine the antioxidant activity, bioactive compound profile of fiddler crab extracts as a form of self-defense against various anthropogenic activities along urban rivers. The fiddler crabs (*Uca spp*.) were collected from the Tanjung Api-Api Port area and Sungsang Village, located in Banyuasin Regency, South Sumatra, Indonesia. Fiddler crab samples were extracted by maceration in ethanol. The antioxidant activity of the extracts was evaluated using the 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay. Bioactive compounds profile was determined by qualitative phytochemical screening, followed by gas chromatography-mass spectrometry (GC-MS). Fiddler crab extracts of samples obtained from both locations exhibited weak antioxidant activity with IC_{50} values of 174.617 µg/mL and 190.786 µg/mL for crab extracts from Tanjung Api-Api area, and Sungsang village, respectively. Phytochemical screening of crab extract from Tanjung Api-Api area revealed the presence of alkaloid, triterpenoids, and saponins. GC-MS analysis identified hexadecanoic acid, trans-13-octadecenoic acid, (E)-9-octadecenoic acid ethyl ester, and cholesterol as the major bioactive compound groups in Tanjung Api-Api Fiddler crab extract. This findings suggest that fiddler crabs produce bioactive chemicals that could help combat oxidative stress caused by pollutants in urban rivers. Further research is needed to characterize these compounds in greater depth and explore their mechanisms of facilitating the adaptation of fiddler crabs to stressful environmental conditions.

Keywords: Antioxidant, Biochemical Profile, Fiddler Crabs, Anthropogenic, Urban Rivers.

Introduction

Increased urbanization and anthropogenic activity along urban rivers have led to a decline in environmental quality and aquatic ecosystem health.[1,2] Water pollution, industrial waste discharges, and other chemical contaminants pose a serious threat to the organisms living in these ecosystems.[3,4] Pollutants such as heavy metals, pesticides, and harmful organic compounds can enter aquatic systems.[5,6] These pollutants can accumulate in sediments and aquatic organisms, causing cellular damage, reproductive impairment, and even death.[7] Compounds like polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and other industrial chemicals have the potential to induce oxidative stress in living organisms. This can result in inflammation, tissue injury, and disruption of normal physiological functions.[8-10] Among aquatic organisms, crustaceans are important creatures exposed to environmental pollutants.[11-13] Crabs being one of the most dominant groups, tend to accumulate significant amounts of pollutants due to their close association with the substrate in contrast to pelagic fauna that live in the water column.[14-16]

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One genus that is often found in estuarine and estuary habitats is the fiddler crab.[17,18]Fiddler crabs a type of semi-terrestrial crustacean are commonly found along the coasts of tropical, subtropical, and warm temperate regions worldwide.[19,20] These crabs are resilient ecosystem components because they can adapt to a wide range of habitat conditions, polluted, brackish, and saline environments.[21,22] They play important ecological roles, including nutrient cycling, organic matter decomposition, and as a food source for various predators (birds, fish, and marine mammals).[23,24] Fiddler crabs are recognized as ecosystem engineers because of their influence on sediment composition and their impact on the biodiversity of mangrove ecosystems.[25] However, changes in water quality due to human activities have caused various challenges to their survival.[26,27] Fiddler crabs play important roles in coastal ecosystems, including inorganic matter decomposition and as a food source for various predators.[28,29] To survive in contaminated environments, fiddler crabs may have developed physiological and biochemical adaptations, including enhanced antioxidant responses and unique biochemical defense mechanisms.[30,31]
Antioxidants are molecules that can scavenge free radicals, thereby preventing cellular damage caused by oxidative stress.[32,33] In a polluted environment, the antioxidant defense system of fiddler crabs becomes very important to protect them from the damaging effects of chemical pollutants.[34,35] In addition, biochemical compounds, which are natural chemical components in organisms, can play a key role in biological defense mechanisms.[36,37] The biochemical profile of fiddler crabs, particularly their antioxidant and detoxification pathways, is crucial in mitigating oxidative stress and neutralizing pollutants. Flavonoids, terpenoids, and alkaloids are known for their antioxidant, anti-inflammatory, and other medicinal properties, which could help fiddler crabs adapt to polluted environments.[38-40]

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Research on the antioxidant activity and biochemical profile of fiddler crabs in urban rivers can provide important insights into how these organisms adapt to environmental stresses caused by human activities. This knowledge is not only beneficial for fiddler crab species conservation, but it can also provide indicators of ecosystem health and potential pollution mitigation strategies. This study aimed to explore the antioxidant activity and biochemical profile of fiddler crabs as a form of self-defense against various anthropogenic activities along urban rivers. Understanding their biochemical adaptations allows us to better appreciate the complexity of interactions between organisms and their environment and develop more effective approaches to protecting and managing urban aquatic ecosystems.

Materials and Methods

Sampling area

Fiddler crabs were collected in December 2022 around the mangrove ecosystem area of Tanjung Api-Api Port and Sungsang Village, Banyuasin, South Sumatra (Figure 1). Tanjung Api-Api is surrounded by an extensive mangrove ecosystem that provides important habitats for various types of flora and fauna (fiddler crabs).[32,41,42] In addition, Sungsang Village is surrounded by dense mangrove ecosystem, which are ideal habitats for fiddler crabs and various other marine species.[43-45] The mangrove ecosystem at these two sites are important habitats for fiddler crabs (*Uca spp*.), which are known for their unique behaviors, such as burrowing in mangrove mud, and their important role in the ecosystem as bioindicators of environmental health. According to Marochi *et al*. (2022)[46], fiddler crabs usually inhabit mangrove habitats. Due to the uneven distribution of mangroves, understanding their connectivity and identifying local endemism is essential.

Figure 1: Map of sampling locations in Tanjung Api-Api Port area and Sungsang Village, Banyuasin District, South Sumatra, Indonesia.[148]

Environmental quality measurement

The condition of the aquatic environment at the sampling site was measured using multiparameters, including dissolved oxygen (DO), salinity, pH, and temperature parameters.[47] Environmental parameters were assessed using a multiparameter device (Hanna HI 9829-01042, USA), and salinity was analyzed with a portable refractometer (ATC Portable, China).

Preparation of fiddler crabs

The sample preparation process was done according to the method described by Hamdi *et al*. (2020)[48] with modification. Fiddler crabs were cleaned from dirt using running water, followed by separation of the shell from the meat, and then oven-dried at 40ºC. The dried sample was thereafter pulverized with the aid of an electric blender.

Determination of weight loss on drying

Briefly, samples were weighed before and after drying in an oven at 40°C until a constant weight was achieved. The percentage of weight loss was then calculated.

Extraction of fiddler crabs

The dried powdered sample (500 g) was macerated in 2 L of ethanol (≥96% purity, Merck, Germany) at room temperature for 24 h. The extract solution was subsequently filtered through Whatman 40 filter paper (Whatman International Ltd, UK). The resulting extract was then subjected to evaporation using a rotary evaporator (DLAB RE100-Pro, China) at 40°C until the solvent has completely evaporated, yielding a concentrated paste. The percentage yield of the extract was calculated. This temperature is carefully selected to avoid degrading the compounds present in the extract[49].

Determination of antioxidant activity

The antioxidant activity of the Fiddler crab extract was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay as previously described with slight modification.[50-52] DPPH stock solution (0.1 mM) was made by dissolving 0.002 g of DPPH crystals (Merck, Germany) in 50 mL of ethanol. Different concentrations (2000, 1000, 500, 250, 125, and 62.5 ppm) of the crab extract were prepared in ethanol, and 1 mL of the extract solution was added to 1 mL of 0.1 mM DPPH solution, followed by the addition of 1 mL of 96% ethanol in a vial. The mixture was vortexed, then incubated at room temperature in the dark for 30 minutes. The absorbance of the reaction mixture was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-1900, Japan). Ascorbic acid at same concentrations as the extract was used as the reference standard. The percentage inhibition of DPPH radical was calculated using the formula below (Equation 1).

$$\%\,inhibition = \frac{blank\ abs - sample\ abs.}{blank\ abs} \text{ x } 100 \ldots\ldots\ldots\ldots \quad (1)$$

The antioxidant capacity of the extract was reported in terms of IC_{50}, which refers to the concentration in mg/mL of the extract required to reduce DPPH radical by 50%[53,54].

The IC_{50} value was obtained from a linear regression equation (Equation 2) of the plot of sample concentration (x-axis) versus the percentage inhibion of DPPH radical (y-axis).[55]

$$Y = ax + b \ldots\ldots\ldots\ldots \quad (2)$$

Phytochemical analysis

Fiddler crab extract was subjected to qualitative phytochemical tests for alkaloids, flavonoids, saponins, tannins, and terpenoids/steroids according to standard procedures[56-58].

Gas chromatography-mass spectrometry (GC-MS) analysis

The chemical profile of fiddler crab extract was determined by GC-MS analysis as described previously.[59,60] The GC-MS analysis was conducted using a Shimadzu QP2010 Plus (Shimadzu Corp., Japan), equipped with a Rxi-5ms capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Briefly, a 1 µL sample was injected into the column, with helium used as the carrier gas at a flow rate of 1 mL/min, and the split ratio was 1:50. The temperature program for the oven started at 50°C for an initial 5 minutes, followed by a gradual increase of 5°C per minute until 280°C was reached, and held for 5 minutes. The MS transfer line was maintained at 200ºC. The ionization mode used was electron ionization at 70eV and source temperature of 280ºC. Compound identification was assessed using Total Ion Count (TIC). The spectra of the separated compounds were compared with the database of the NIST Reference Spectra Library. The relative percentage composition of the identified compounds was estimated from the GC peak area percentage.

Results and Discussion

Environmental quality

The growth and development of fiddler crabs in estuary waters are ecologically influenced by the quality of the aquatic environment. Study on antioxidant activity in fiddler crabs must consider water quality because these factors have a direct effect on the physiological and biochemical conditions of animals. Temperature, salinity, pH, and DO not only affect the health and growth of marine organisms, but they also

determine their ability to produce and store bioactive compounds.[61-63]
The aquatic environmental quality parameters including DO, pH, temperature, and salinity are presented in Table 1.

Table 1: Environmental quality of the sampling sites

Location	Environmental quality parameter			
	DO (mg/L)	pH	Temperature (°C)	Salinity (PSU)
Sungsang Village	6.83	7.13	31	12
Tanjung Api-Api Port	6.94	7.3	31.5	10

Based on environmental quality measurements, both sites showed DO levels > 5 mg/L. Most aquatic organisms depend on oxygen for survival and cannot endure for extended periods in water with DO levels below 5 mg/L.[64] Low DO levels significantly affect aquatic organisms, influencing respiration rates and overall survival.[65-67] DO is an important indicator for water quality, as aquatic organisms depend on oxygen for respiration.[68,69] DO values in Sungsang Village and Tanjung Api-Api Port area indicate an environment that supports the survival of aquatic organisms such as fish and fiddler crabs.
The pH levels at both sites suggest neutral to mildly alkaline water conditions, with a pH range between 6.5 and 8.5 which is generally considered safe for most aquatic organisms.[70] The pH values at Sungsang Village and Tanjung Api-Api Port are within the ideal range, indicating that there is no significant acidic or alkaline contaminants at these two sites. The causes of drastic changes in pH are pollution sources such as industrial waste, domestic waste, and agricultural watercourses that contain hazardous chemicals.[71-73] The temperature at both sites was around 31°C. This temperature is within the range generally found in tropical waters. A stable water temperature that matches the thermal tolerance of aquatic organisms is essential for their physiological processes, including metabolism and reproduction.[74,75] Extremely high or low temperatures can lead to thermal stress in aquatic organisms.[76,77] Temperature stability is essential for the physiological processes of aquatic organisms, including metabolism and reproduction.[78] Elevated temperatures can accelerate metabolism, reduce oxygen availability, and affect sexual maturity. Salinity of Sungsang Village water sample was slightly higher (12 PSU) compared to that of Tanjung Api-Api Port (10 PSU). Both values indicate slightly brackish water conditions, which are common in estuarine habitats. Salinity affects the distribution and adaptation of aquatic organisms.[79] Results from previous studies have shown that fiddler crabs exhibit higher physiological tolerance to increased salinity levels, supporting their osmoregulatory capabilities.[80,81]
Environmental quality is a key determinant of the survival and biological activities of fiddler crabs. Adequate DO enhances respiratory efficiency, a neutral to slightly alkaline pH stabilizes the chemical environment, temperature stability prevents thermal stress, and optimal salinity ensures osmoregulation. Monitoring these parameters is important because significant fluctuations could signal environmental changes due to anthropogenic activities.[41,82]

Description of fiddler crabs
The fiddler crab has an intact exoskeleton with a robust carapace, large chelipeds, and specialized legs adapted for sandy or muddy environments (Figure 2). Fiddler crab males are characterized by a large and strong body, which is adapted for various functions, such as attracting females and defense. The carapace surface is hardened, featuring distinct textures and colour patterns that enhance camouflage and provide mechanical protection from predators and environmental stressors.

Figure 2: Body shape of the fiddler crab (*Uca spp*.). **(A)** Full body, **(B)** Claws, and **(C)** Carapace

Based on the observations from the present study, the fiddler crabs discovered were *Uca spp*. Commonly found in estuarine environments like salt marshes and mangroves, play a significant role in ecosystem engineering and are recognized for their distinct sexual dimorphism.[83] Male crabs have one large cheliped, while females have two equally-sized chelipeds.[84-86] Male crabs use their large chelipeds to attract females and compete with fellow males, while female crabs invest more energy in reproduction than growth.[87]
Fiddler crabs construct above-ground sediment structures made of mud or sand, located within or slightly above the tidal zone.[88] Male crabs use their large chelipeds for visual signals and other vibratory behaviors to repel intruders or attract females.[83,89,90] Fiddler crab carapace and claw coloration have three main functions: thermoregulation, camouflage, and intraspecific communication.[91,92]
Feeding behavior analysis reveals that fiddler crabs primarily consume organic material, including bacteria and microalgae, obtained from mangrove sediments.[25] Mud fiddler crabs actively separate detritus from mineral particles, contributing to sediment aeration and organic matter decomposition.[93,94] Fiddler crabs face daily and seasonal variations in salinity, temperature, and environmental contamination, making them an attractive model for studying the biochemical mechanisms of environmental adaptation.[95-97] Due to their territorial nature, and lack of mobility, fiddler crabs are vulnerable to chronic environmental pollution.[98] Exposure of fiddler crabs to extreme tidal conditions and elevated temperatures can induce dehydration, oxidative stress, and metabolic impairment, leading to reduced survival rates.[99,100]

Characteristics of fiddler crab extract
The weight loss of fiddler crabs was higher in the samples collected from Tanjung Api-Api Port area with a percentage weight loss of 77.72%, while the samples from Sungsang Village had a percentage weight loss of 74.65% (Table 2).

Table 2: Weight loss of fiddler crabs

Location	Sample weight (g)		Weight loss (%)
	Wet	Dry	
Sungsang Village	200	50.7	74.65
Tanjung Api-Api Port	100	22.28	77.72

This weight loss is primarily due to the high water content of crabs. Environmental factors such as DO, pH, temperature, and salinity, as well as environmental stress from human activities, may have contributed to this difference. The lower salinity in Tanjung Api-Api Port may cause the crabs to absorb more water to maintain osmotic balance, resulting in higher weight shrinkage when dried. It has been opined that salinity fluctuations affect the physiology of aquatic organisms.[101] In addition, slightly higher temperatures can increase

metabolism and water evaporation from the crab's body.[99,102] Variations in pH and DO levels also influence crab adaptation and health, affecting physiological responses to environmental stress.[103]
The fiddler crab extraction process using ethanol resulted in a higher extract yield (15.75%) in the crab sample collected in Tanjung Api-api port area compared to that of Sungsang Village sample which produced an extract yield of 13.75% (Table 3).

Table 3: Percentage yield of fiddler crab ethanol extract

Location	Sample weight (g)		Percentage yield (%)
	Dry powder	Crude extract	
Sungsang Village	20	2.75	13.75
Tanjung Api-Api Port	20	3.15	15.75

This difference is likely due to variations in environmental factors, such as water quality and food availability. Solvent type, extraction conditions (temperature, duration, solvent-to-sample ratio), and crab biochemical composition has been shown to significantly influence extraction efficiency.[104] For instance, ethanol as a polar solvent is widely used to extract bioactive compounds,[105,106] and has been shown to be effectiveness in isolating various biochemicals in good yield.[107] Optimized extraction conditions enhance bioactive compound recovery, improving extract yield and chemical quality.[108,109]

Antioxidant activity in fiddler crabs

The results of the antioxidant activity test on fiddler crabs from two different areas, namely Sungsang Village and Tanjung Api-api Port are presented in Figure 3. The crab samples from both locations were found to possess weak antioxidant activity based on their IC_{50} values. The IC_{50} values of the crab extracts from Sungsang village and Tanjung Api-Api Port area were 190.786 µg/mL and 174.617 µg/mL.

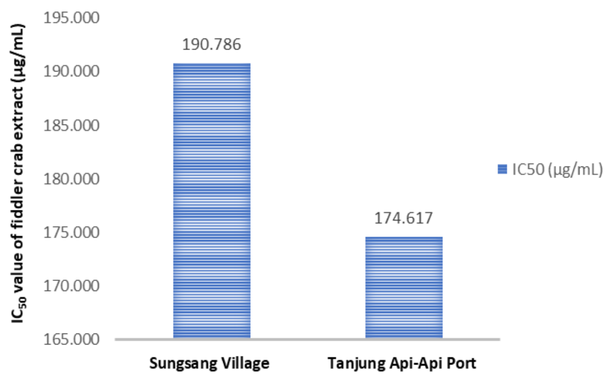

Figure 3: Antioxidant activity of fiddler crab ethanol extract

High IC_{50} values suggest that a higher concentration of the extract is needed to inhibit 50% of DPPH free radicals, implying a lower level of antioxidant activity. These results may have been influenced by environmental factors such as pollution levels, pollutants, and habitat conditions. A previous report on the antioxidant activity of *Anadara granosa* from Sungsang village area revealed a strong activity with IC_{50} value of 85 µg/mL.[53] As for the Tanjung Api-Api Port area, a weak antioxidant activity was found for mangrove species *Avicennia marina* with IC_{50} value of 171.160 µg/mL, a moderate activity for *Bruguiera gymnorrizha* with IC_{50} of 105.090 µg/mL, and a strong activity for *Sonneratia alba* with IC_{50} of 28.064 µg/mL.[32] The difference may be due to different species and changing environmental conditions.
The marked difference between the two areas may be due to variations in pollution levels and the types of pollutants present. Tanjung Api-Api Port is likely to have higher pollution levels due to intense port activities and vessel traffic, which can produce various pollutants, including heavy metals and toxic organic compounds.[44,110] Changes in an organism's antioxidant capacity have a significant impact and have been regarded as a marker for assessing environmental pollution in aquatic ecosystems resulting from human activities.[111]
Fiddler crabs living in an area may be exposed to various pollutants that can induce oxidative stress and damage their natural antioxidant capacity. Previous research has reported that there has been heavy metal pollution in the Tanjung Api-api Port area, with lead (Pb) concentration in sediment ranging from 7.0104 to 11.8186 mg/kg, and copper (Cu) concentration ranging from 3.7127 to 4.5347 mg/kg.[27] Heavy metal contamination in biological and environmental samples has been widely studied due to its potential health risks. A study on RUZU bitters, a polyherbal extract with antidiabetic properties, reported the presence of heavy metals such as Cr (1.09 mg/L), Zn (0.10 mg/L), Fe (2.10 mg/L), Mn (0.23 mg/L), Cu (0.20 mg/L), Cd (0.06 mg/L), and others, but Pb and Cd were found to be absent.[112] Various studies have highlighted that oxidative stress in organisms, caused by pollutant exposure, can impair the immune function of mud crabs by damaging the hepatopancreas, triggering antioxidant enzyme activity, and affecting immune gene expression.[113] A research report explained that exposure to heavy metals leads to the accumulation of reactive oxygen species (ROS) in marine decapods, surpassing the capacity of antioxidant enzymes to neutralize them.[114] It has also been reported that the effects of persistent organic pollutants in the cellular defense mechanisms and physiological functions in crustaceans differ based on the compound, tissue, and species involved.[12] In addition to pollutant-induced factors, variations in diet and the nutrient content of their habitat may also play a role in low antioxidant activity.[115-117]

Phytochemical constituents of fiddler crab extract

The crude ethanol extracts of fiddler crabs with the stronger antioxidant activity from both areas were subjected to phytochemical tests. From the results of the phytochemical screening, fiddler crabs from the Tanjung Api-api harbor contained a group of bioactive compounds, including saponins, terpenoids, and alkaloids (Table 4).

Table 4: Phytochemical compounds of fiddler crab ethanol extract from Tanjung api-api port area

Phytochemical	Inference
Alkaloids	+
Flavonoids	-
Terpenoids	+
Steroids	-
Saponins	+
Tannins	-

'+' indicates presence of pytoconstituents;
'-' indicates absence of pytoconstituents

These compounds are known to have various beneficial biological activities. Alkaloids are known to have various pharmacological effects including antimicrobial, anticancer, and antioxidant activities.[118,119] Terpenoids are also recognized for their anti-inflammatory and anticancer activities.[120-122] On the other hand, saponins are reported to have antimicrobial, anti-inflammatory, and antifungal activities.[123-125] The presence of these compounds in fiddler crabs indicates their potential as a source of biologically active compounds with applications in various medical and industrial fields.
However, the preliminary phytochemical tests were unable to detect the presence of flavonoids, steroids, and tannins in the crab extract from Tanjung Api-api harbor. Flavonoids are commonly present in a range of plants and are known to have strong antioxidant activity.[126,127] The absence of flavonoids may be due to environmental factors or the type of diet of fiddler crabs in the Tanjung Api-Api Port area, which may not support flavonoid biosynthesis. Steroids and tannins were also not detected, although previous studies reported them to have important roles in various biological activities (anti-inflammatory and antimicrobial), this could be due to species-specific metabolic

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variations or differences in extraction efficiency using ethanol as a solvent.[128-130]

Compounds identified from the GC-MS analysis of fiddler crab extract
GC-MS analysis was conducted on fiddler crab samples collected from the Tanjung Api-Api Port area, which demonstrated higher antioxidant activity compared to that of Sungsang Village. The following classes of compounds were detected; alcohols, aliphatic amines, lactones, alkaloids, sugars, esters, steroids, ketones, and organic fatty acids. The compounds identified were determined by comparing the height of the chromatogram peaks and the mass spectra data of the chromatogram peaks with those in the WILEY 7 database library. The GC-MS analysis of the fiddler crab extract identified 13 secondary metabolites, with hexadecanoic acid and its derivatives, as well as unsaturated fatty acids such as trans-13-octadecenoic acid and (E)-9-octadecenoic acid ethyl ester, and cholesterol constituting the major compounds (Figure 4, Table 5). The presence of these compounds suggests that fiddler crabs from this pollution-exposed area have developed biochemical adaptations to harsh environmental conditions. In addition, these compounds have shown potentially significant biological activities. The presence of the compound 2-Piperidinone, which belongs to the alkaloid group,[131,132] suggests that these crabs may have developed chemical defense mechanisms to fight predators or pathogens in polluted environments. Alkaloids are known to have antimicrobial and antiparasitic properties,[133,134] which may help the crab defend itself in environmental conditions that may be rich in pathogenic microorganisms.

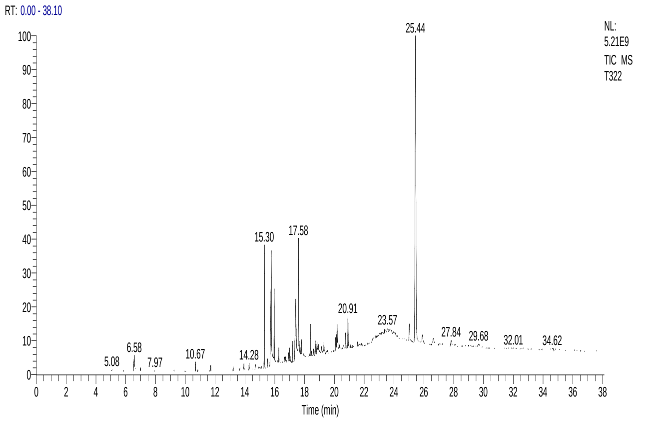

Figure 4: GC-MS chromatogram of fiddler crab extract from Tanjung Api-Api Port area

The presence of fatty acids such as hexadecanoic acid and its derivatives, as well as unsaturated fatty acids such as trans-13-octadecenoic acid and (E)-9-octadecenoic acid ethyl ester may suggest an adaptation of the crab to oxidative stress that may be caused by pollutants. These compounds are recognized for their anti-inflammatory and antioxidant activities,[135-137] and may help reduce cellular damage due to chemical and heavy metal pollutants that may be present in the harbor area. In addition, compounds such as arachidonic acid and eicosapentaenoic acid (EPA), which belong to the omega-3 fatty acid group, can help reduce cellular damage due to chemical pollution and heavy metals that may be present in the harbor area.[138,139] These fatty acids also have an important role in reducing inflammation and maintaining cell membrane integrity,[140,141] especially for crabs living in habitats that may be exposed to oil pollution and other chemicals.
The high cholesterol content (40.32%) in this extract suggests an important adaptation in maintaining the composition and role of cell membranes under environmental stress conditions. Cholesterol helps to maintain cell membranes and protect cells from damage caused by pollutants and drastic environmental changes.[142,143] Environmental conditions with high pollution levels due to port activities and ship traffic,[144,145] may be the main factor that encourages fiddler crabs to produce these bioactive compounds. Contaminants like heavy metals, petroleum, and industrial chemicals can cause oxidative stress and damage crab cells, so crabs must develop chemical defense mechanisms to survive.[114,146,147] Overall, these results suggest that fiddler crabs in the Tanjung Api-Api Port area have developed complex biochemical adaptations to deal with challenging environmental conditions. Further research is needed to characterize these compounds in greater depth and explore their mechanisms of facilitating the adaptation of fiddler crabs to stressful environmental conditions.

Table 5: Compounds identified from the GC-MS analysis of fiddler crab extract from Tanjung Api-Api Port area

Ret. time	Peak area %	Name of compound	Compound group
6.57	2.36	2-Piperidinone	Alkaloid
15.3	5.94	Hexadecanoic acid, methyl ester	Methyl palmitate
15.76	13.76	n-Hexadecanoic acid	Palmitic acid
15.96	4.1	Hexadecanoic acid, ethyl ester	Ethyl palmitate
17.4	9.11	trans-13-Octadecenoic acid	Fatty acid
17.58	7.92	(E)-9-Octadecenoic acid ethyl ester	Fatty acid ester
18.41	2.14	Arachidonic acid	Polyunsaturated fatty acid
18.83	1.66	cis-5,8,11,14,17-Eicosapentaenoic acid	Omega-3 fatty acid
23.57	1.44	9-Octadecenoid acid, 1,2,3-propanetriyl ester, (E,E,E)-	Triglyceride
23.74	2.37	Hexadecenoid acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	Glyceride
25.03	2.14	1-Heptatriacotanol	Alcohol
25.44	40.32	Cholesterol	Sterol
26.65	1.61	Ethyl iso-allocholate	Bile acid derivative

Conclusion

Fiddler crabs collected from Tanjung Api-api Port and Sungsang village were found to possess weak antioxidant activity. Phytochemical screening of the crab extract from Tanjung Api-api harbor revealed the presence of alkaloids, terpenoids, and saponins. GC-MS analysis further revealed the presence of hexadecanoic acid, trans-13-octadecenoic acid, (E)-9-octadecenoic acid ethyl ester, and cholesterol as major compounds in fiddler crab extract from Tanjung Api-api harbor. These findings suggest that fiddler crabs have developed complex biochemical adaptations as a defense mechanism against various anthropogenic activities along urban rivers. The presence of these bioactive compounds may also have helped the crabs mitigate oxidative stress caused by pollutants, underscoring the significant impact of human activities on aquatic ecosystems and the adaptive responses of their inhabitants. Additional studies are required to thoroughly investigate the biological potentials of these compounds and their mechanisms of action in safeguarding against environmental stress.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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