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Original Research Article

The Potential of Visceral Organs and Symbiotic Bacteria of Abalone (*Haliotis asinina*) as Antibacterial Ingredient

Nona Mu'minun[1], Delianis Pringgenies1*,Sri Sedjati[2], Ocky Karna Radjasa[3]

[1]*Student of Doctoral Program Marine Science, Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, Indonesia*
[2]*Department of Marine Science, Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, Indonesia*
[3]*National Research and Innovation Agency, Indonesia*

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ABSTRACT

Abalone (*Haliotis asinina*) is a marine organism with potential as a natural source of antibacterial compounds. This study explores its potential for treating diabetic ulcers. The aim of this research is to investigate the efficacy of abalone visceral organs and symbiotic bacteria as antibacterial agents for the treatment of diabetic ulcers. The methodology included the collection of abalone samples, isolation and identification of symbiotic bacteria, testing for antibacterial activity, extraction of visceral organs and GC-MS analysis of the extracted compounds. The results demonstrated significant antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* in various isolates of abalone symbiotic bacteria, specifically *Bacillus velezensis* and *Brevibacterium iodinum*. GC-MS analysis of the visceral organs revealed the presence of several active compounds, including nonadecanoic acid methyl ester, n-hexadecanoic acid, cholest-5-en-3-ol, eicosatetraenoic acid and 9-octadecenoic acid. These findings indicate the potential of abalone visceral organs and symbiotic bacteria as a natural source of antibacterial agents for the treatment of diabetic ulcers. This research can inform the development of an ointment for topical application, providing an effective treatment for diabetic ulcers while reducing the risk of antibiotic resistance. In conclusion, future research should focus on the isolation and identification of additional active compounds from abalone that may contribute to the treatment of diabetic ulcers.

Keywords: Active compounds, diabetic ulcers, GC-MS analysis, *Haliotis asinina*

Introduction

Diabetic foot ulcers represent a significant global health challenge, particularly among individuals with chronic diabetes, necessitating innovative and effective treatment alternatives. These ulcers are a common complication in individuals with diabetes, characterised by wounds that are difficult to heal and, in severe cases, can lead to amputation. This condition arises from damage to blood vessels and nerves in diabetic patients, resulting in tissue damage and hindering the wound-healing process. Current care management strategies have been developed and researched for their effectiveness in treating these ulcers; however, the recovery rate remains suboptimal. Standard treatment procedures often lead to prolonged healing times, frequent recurrences and a high risk of amputation, resulting in significant treatment costs, psychological and social burdens, and reduced productivity for patients and their families. Given the urgency of this issue, there is a pressing need for alternative or supplementary therapies that can enhance the effectiveness of diabetic foot ulcer treatment. One such potential therapy involves natural ingredients derived from marine life, particularly *Haliotis asinina* abalone, which is rich in nutraceutical content and possesses pharmacological effects, including antibacterial properties.

*Corresponding author. Email:pringganies@yahoo.com
Tel: 06281390800800

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[1]One study on the pharmacological effects of boiled abalone viscera found that it exhibits high antioxidant activity, which can inhibit free radicals, oxidative stress and DNA damage in H2O2-treated RAW 264.7 macrophage cells. Additionally, the study observed an antihypertensive effect in hypertensive rats following oral administration, based on time measurements. [2]

One natural ingredient that has been extensively studied for its antimicrobial potential is the visceral organ. Research shows that bioactive compounds found in symbionts often resemble those present in their hosts. [3] Abalone, a marine mollusc valued for its high nutritional content, has long been consumed as food and used in traditional medicine across Asia and America. Notably, the visceral organs of abalone – such as the liver, kidneys and intestines – have demonstrated significant antibacterial activity in multiple studies. Research findings indicate that abalone extract displays varying levels of antibacterial activity against both gram-positive and gram-negative bacteria, including Staphylococcus aureus and Escherichia coli, which are common pathogens in diabetic ulcer infections. [1] This evidence suggests that abalone contains active compounds capable of inhibiting the growth of pathogenic bacteria, even antibiotic-resistant strains. Consequently, the visceral organs of abalone could serve as a promising natural source of antibacterial agents for the treatment of diabetic ulcers. Additionally, a study from China found that polysaccharides with higher molecular weight and greater sulphate content exhibited stronger anticoagulant activity in *Haliotis discus* hannai. The mucus from Haliotis asinina has also been shown to support wound healing by reducing nitric oxide production during inflammation over a 24-hour incubation period. This mucus functions as an anti-inflammatory, antioxidant and antimicrobial agent. Researchers have further explored the anti-inflammatory potential of abalone. Similar findings were reported for *Haliotis rubra*, whose visceral extracts demonstrated significant in vitro anti-inflammatory activity in RAW 264.7 macrophage cells. [4] The aim of the present research is to investigate the

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potential of abalone symbiont bacteria as antibacterial agents and to identify the specific symbiont bacteria involved. In addition, the study seeks to evaluate the antibacterial properties of the visceral organs of abalone and to characterise the active compounds present in these organs.

Materials and Methods

Sample Collection

The samples used in this study were *Haliotis asinina* abalone collected from the waters of Tanjung Tiram, located at latitude -3.8918° and longitude 122.6346°, in Southeast Sulawesi, Indonesia. The tools employed for the antibacterial activity tests included digital scales, label paper, Petri dishes, Erlenmeyer flasks, paper discs, autoclaves, inoculating needles, Bunsen burners and incubators. The material tested was a sample of *Haliotis asinina* abalone that had been isolated at the UHO Laboratory of Halu Oleo University in Kendari, Southeast Sulawesi, for the purpose of assessing its antibacterial activity against Escherichia coli and Staphylococcus aureus. The materials used for preparing bacterial growth media included Nutrient Agar (NA), Mueller Hinton Agar (MHA), matches, spirit and 70% alcohol.

Production of MHA Media, Isolation of bacteria and Antibacterial Activity Test

MHA medium is used for testing antibacterial activity. It is prepared by dissolving 13.3 g of MHA in 280 ml (80%) sterile seawater and 70 ml (20%) distilled water. The mixture is heated on a hot plate with a magnetic stirrer to homogenise the medium, then sterilised in an autoclave at 121°C for 20 minutes. After sterilisation, approximately 10 ml of the medium is poured into each sterile petri dish.

Bacterial isolation was carried out on the visceral organs of abalone, following the described methodology. [5] The antibacterial activity test involved two bacterial species: the gram-positive *S. aureus* and *E. coli*. The antibacterial activity of each symbiotic bacterial isolate was evaluated over a period of 72 hours, with assessments conducted at 24-hour intervals. The disc diffusion method, as outlined by the referenced study [6], was employed for this test.

MHA (Mueller-Hinton Agar) was poured into petri dishes, and the medium was inoculated with the test bacteria by spreading them evenly over the surface. Paper discs were then placed onto the medium, with each petri dish divided into seven sections labelled with isolate codes: AL (abalone stomach symbiont isolate) and AU (abalone gut symbiont isolate), along with one control section. The test results were recorded at 24, 48 and 72 hours. After each observation period, the zones of inhibition around the bacterial colonies were measured using a calliper.

Molecular identification

A single isolate of the abalone symbiont bacterium was cultured on TSA medium and incubated at 30°C for 24 hours. Genomic DNA was extracted using a commercial kit, such as the GenElute™ Bacterial Genomic DNA Kit. The 16S rRNA gene was then amplified via PCR using the universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) (Martínez-Porchas et al., 2017). The PCR protocol consisted of an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 1 minute. A final extension was performed at 72°C for 7 minutes. Amplification was confirmed by electrophoresis on a 1% agarose gel, and the PCR products were subsequently sent for sequencing. The resulting sequence data were analysed using BLAST (NCBI), and a phylogenetic tree was constructed in MEGA11 employing the Neighbor-Joining method (Tamura et al., 2021).

Preparation of Abalone Visceral Extract:

The sample was soaked in a 96% ethanol solution at a simplicia-to-solvent ratio of 1:5. Maceration was carried out for 72 hours with occasional stirring. After each stirring, the vessel was sealed as tightly as possible and wrapped in a black cloth to protect light-sensitive compounds. Upon completion of the maceration process, the mixture was filtered through filter paper to obtain the final filtrate.[8]

GC-MS Analysis

The extracted sample is introduced into the separation column of the Shimadzu GCMS-QP2010S GC-MS system, where the compounds are separated based on their molecular weights. Subsequently, the compounds are evaporated using a high-temperature heater and directed into the GC-MS inlet. The evaporated compounds then travel through the separation column and enter the mass spectrometer. Inside the mass spectrometer, the compounds are fragmented into smaller ions, and their molecular weights are determined. Finally, the mass spectral data are presented graphically, displaying both the number and the molecular weights of the detected compounds.[5]

Results and Discussion

The samples were collected from the abalone *Haliotis asinina*, which is commonly found in the waters of Tanjung Tiram, Southeast Sulawesi, Indonesia (Figure 1a and 1b). Isolation procedures yielded 14 symbiotic bacterial isolates from the abalone. However, antibacterial activity assays against *E. coli* and *S. aureus* showed that only five of these isolates exhibited activity, as presented in Table 1.

Table 1: There are Inhibition Zones with *E.coli* and *S.aureus*

No	isolate code	Observation time					
		E.coli			*S.aureus*		
		24	48	72	24	48	72
1	AL 1	+	+	-	+	+	+
2	AL 5	+	+	+	-	-	-
3	AL 2	-	-	-	+	+	+
4	AU 8	-	-	-	+	+	+
5	AU 9.1	-	-	-	+	+	+

The research results indicated that five isolates were capable of forming an inhibition zone against the AL isolate. Among these, two isolates demonstrated activity against *E. coli* at both the 24-hour and 48-hour observations, while isolate AL 5 showed activity from 24 to 72 hours. The antibacterial activity test against *S. aureus* revealed that four isolates – specifically, two AL isolates and two AU isolates – exhibited activity during the 24-hour to 72-hour observation period. Morphological characteristics were assessed based on differences in elevation, margin, overall colony shape and colour. According to the test results, the inhibition zones produced by the AL isolate against *E. coli* were round, with a clear zone of inhibition, a flat surface and uniform edges.

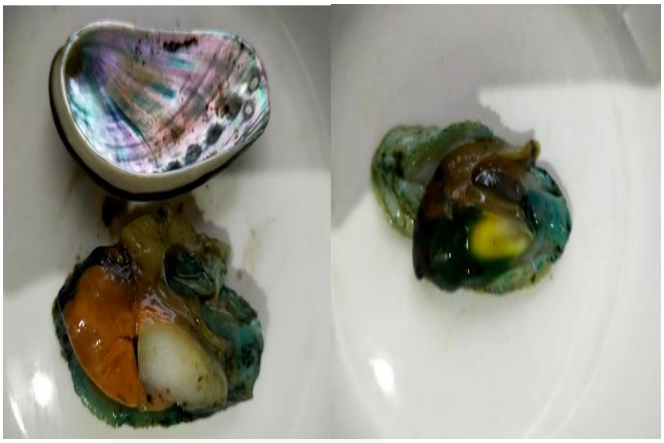

Figure 1: Abalone, sampling results in Tanjung Tiram waters (a), abalone *Haliotis asinina* (b)

The characteristics of the *S. aureus* inhibition zones were identified in four isolates, with two pairs of isolates sharing similar features. In the Gastric Abalone (AL), isolates AL1 and AL2 exhibited round inhibition zones that were clear in colour, with flat surfaces, well-defined edges

and small inhibition zone sizes. Similar characteristics were observed in the Intestinal Abalone (AU) isolates AU8 and AU9.1, which also had round, clear-coloured zones with flat surfaces and complete edges. However, they differed in the size of their inhibitory zones: AU8 displayed a medium-sized inhibition zone, while AU9.1 exhibited a larger zone.

Antibacterial activity test with E. coli test bacteria
Observations indicated that an inhibition zone formed around the abalone shell isolate, specifically in AL 1, when tested against E. coli at the 24th and 48th hours. While an inhibition zone was present at both 24 and 48 hours, it was absent by the 72nd hour. Similarly, inhibition zones were observed around the balloon mussel isolates, but only in AL 5 with E. coli at 24, 48 and 72 hours. Notably, no inhibition zone was detected at 24 hours; however, clear zones were visible at both 48 and 72 hours (Figure 2).

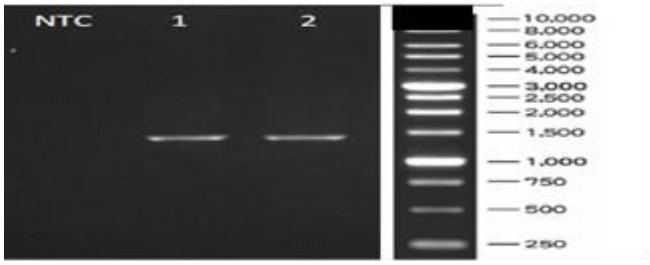

Figure 2: Results of DNA Electrophoresis i 16S rRNA gene fragment of symbiont

Antibacterial activity test with S. aureus Test Bacteria
In the antibacterial activity test using *S. aureus* as the test bacterium, inhibitory zones were observed in abalone isolates AL 1 and AL 2 after 24, 48 and 72 hours. Additionally, some inhibition zones were noted in isolates AU 8 and AU 9.1 during the same observation periods. The results indicated that isolates AL 1, AU 8 and AU 9.1 exhibited significant inhibitory zones against *S. aureus.*

Molecular identification
Based on the observations, the formation of inhibitory zones in abalone isolates was evident in AL 1 and AL 2 against S. aureus at 24, 48 and 72 hours. Several inhibition zones were also observed in AU 8 and AU 9.1 during the same observation periods. Furthermore, the results demonstrated that two isolates, namely AL 1, AU 8 and AU 9.1 exhibited quite significant inhibition zones against *S. aureus.*

Visceral Abalone GCMS Results
The GCMS chromatography test conducted on the H. asinina abalone viscera extract yielded 89 peaks, as illustrated in the spectrum in Figure 4. The highest peak, at number 32, corresponds to the biomolecular compound Nonadecanoic acid, methyl ester, followed by peak number 36, which is n-Hexadecanoic acid, peak number 86, Cholest-5-en-3-ol, 6-methyl-, peak number 53, Eicosatetraenoic acid, methyl ester, (all-Z)-, peak number 43, 9-Octadecenoic acid, methyl ester, (E)- (CAS) and peak number 48, 9-Octadecenoic acid, (E). Conversely, the lowest peak is at number 1, corresponding to the compound 5-(1',1'-Bicycloprop-2'-yl)pentanoic acid, followed by peak number 2, which is Cyclopentane, Ethyl-, and peak number 6, Ethyl 3-(ethoxycarbonyl)-6,6-dimethylheptanoate. The next lowest peak is number 7, corresponding to Cysteine-homocysteine disulphide, and the final lowest peak is number 8, which is Methionine, 2-methyl.

In summary, the formation of inhibitory zones in abalone isolates was evident in AL 1 and AL 2 against the S. aureus test bacteria at 24, 48 and 72 hours. Several inhibition zones were also visible in AU 8 and AU 9.1 during the same observation periods. The results regarding S. aureus indicated that isolates AL 1, AU 8 and AU 9.1, exhibited notably significant inhibition zones.
Based on observations, the formation of an inhibitory zone in abalone isolates was only evident in AL 1 and AL 2 against the S. aureus test bacteria at 24, 48 and 72 hours. Several inhibition zones were also observed in AU 8 and AU 9.1 during the same time intervals. Notably, the results indicated that two isolates, namely AL 1, AU 8 and AU 9.1, exhibited significant inhibition zones against S. aureus.
Bacteria are microorganisms capable of infecting humans. Two common pathogenic bacteria that frequently cause infections in humans are *E. coli* and *S. aureus.* To address this issue, research was conducted to identify bacterial isolates capable of forming an inhibitory zone against these two bacteria. The findings revealed five isolates with antibacterial activity: two isolates demonstrated activity against E. coli, while four isolates exhibited activity against *S. aureus.* Additionally, morphological characteristics were examined to identify each isolate.
Diabetic ulcers are a common complication of diabetes mellitus and can lead to wound infections due to elevated blood sugar levels and diabetic neuropathy. Infections in diabetic ulcers can exacerbate the patient's condition and may even necessitate amputation of the affected area. Therefore, it is crucial to treat diabetic ulcers to prevent more serious complications.

Table 2: Molecular identification results

Code	Relative Similarity	Scientific Name	Query Cover	E Value	Percent Identify	Acc Number
AU121	*Bacillus subtilis* strain MK722404.1 16S ribosomal RNA, partial sequence	*Bacillus subtilis*	100%	0	100.00%	MT111088.1
AL11	*Brevibacterium iodinum* strain BUS 3 16S ribosomal RNA, partial sequence	*Brevibacterium iodinum*	100%	0	99.63%	PP.140678.1

Antibiotics are commonly employed to treat infections in diabetic ulcers. However, the overuse of antibiotics can lead to bacterial resistance, complicating the treatment of these ulcers. Consequently, there is a pressing need for alternative treatments that possess strong antibacterial properties and are safe for use, thereby reducing reliance on antibiotics. Various studies have demonstrated that several symbiotic bacteria found in marine animals exhibit antibacterial properties beneficial for health. For instance, symbiotic bacteria in the digestive systems of sea cucumbers have potential applications in hand sanitisers. Additionally, due to their antibacterial activity, these symbiotic bacteria can be utilised as fish preservatives. Furthermore, symbiotic bacteria in marine animals such as molluscs not only possess antibacterial potential but also exhibit strong enzyme activity, making them suitable for bio industrial applications. [3] The robust antibacterial properties, tissue regeneration capabilities and potential to prevent antibiotic resistance render symbiotic bacteria a promising alternative for treating diabetic ulcers. However, further extensive research is necessary to ensure their safety and effectiveness for human use.
Further research indicated that the formation of an inhibitory zone in abalone shell isolates was only observed in AL 1 and AL 2 against S. aureus test bacteria at 24, 48 and 72 hours. Some inhibition zones were also noted in AU 8 and AU 9.1 during these observations. This suggests that the E. coli test bacteria did not exhibit significant formation of inhibition zones after 72 hours, indicating resistance to the antibacterial compounds produced by abalone shell isolates, most of which are gram-negative bacteria. This difference can be attributed to the ability of *E. coli* to protect itself, as the biofilm makes it challenging for antibacterial compounds to penetrate these bacteria. In contrast, observations on *S.*

aureus showed that two isolates, AL 1, AU 8 and AU 9.1, exhibited significant inhibition zones, indicating that *S. aureus* is highly sensitive to the antibacterial tests conducted. Based on these observations, the formation of an inhibitory zone in abalone isolates was only evident in AL 1 and AL 2 against the S. aureus test bacteria at 24, 48 and 72 hours. Several inhibition zones were also observed in AU 8 and AU 9.1 during the same time intervals. This indicates that the E. coli test bacteria did not exhibit a high percentage of inhibition zone formation after 72 hours, suggesting that this bacterium is resistant to the antibacterial compounds produced by abalone shell isolates, most of which are gram-negative bacteria. In contrast, the results for *S. aureus* demonstrated that two isolates, AL 1, AU 8 and AU 9.1 exhibited significant inhibition zones. Abalone (*Haliotis asinina*) is a commercially valuable shellfish and a popular dish in seafood restaurants. In addition to its high nutritional value, the potential antibacterial properties of its symbiotic bacteria make abalone meat particularly beneficial for health, acting as an antibacterial agent and aiding in the reduction of blood sugar levels. [1] The findings of this research suggest that the bioactive potential in the host is comparable to that of the symbiont, which is highly advantageous for abalone conservation. Research indicates that bacteria living symbiotically with abalone possess antibacterial properties that can be harnessed to develop an ointment for treating diabetic ulcers. The bacteria found in *Haliotis asinina* have demonstrated strong antibacterial activity against both E. coli and S. aureus. Furthermore, this ointment is safer than excessive use of antibiotics, as it does not produce side effects. Symbiotic bacteria with potential antibacterial properties include *Bacillus velezensis* and *Brevibacterium iodinum*. The results of the phylogenetic tree reconstruction consistently show that sample AU121 (Figure 3) forms a clade with the genus Bacillus sp. and a more distant clade with Bacillus velezensis This indicates that sample AU121 is closely related to Bacillus sp. and has a more distant relationship with Bacillus velezensis , supported by a high similarity value and a small genetic distance between the sample and the reference bacteria. Sample AL11 forms a clade with Brevibacterium lines EU660, indicating a close phylogenetic relationship with *Brevibacterium iodinum.*

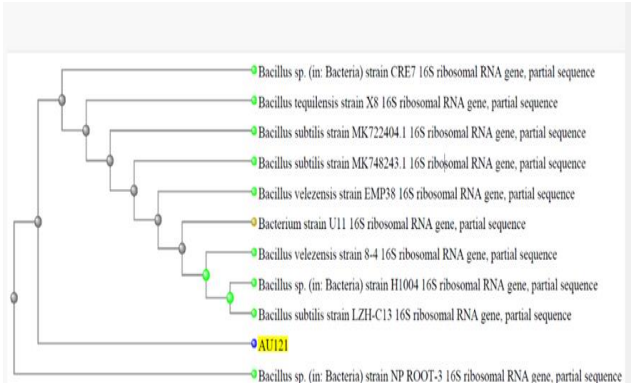

Figure 3: Phylogenetic tree of bacterial isolate Bacillus subtilis strain MK722404.1

Based on the identification results, the symbiotic bacteria found in the visceral organs of abalone (*Haliotis asinina*) are Bacillus velezensis and Brevibacterium iodinum. These bacteria can thrive and reproduce in the nutrient-rich environment of the abalone's body. *Bacillus velezensis* is a Gram-positive bacterium capable of forming endospores when environmental conditions are unfavourable for growth and reproduction. This bacterium offers several health benefits, including boosting the immune system, inhibiting the growth of harmful bacteria and producing antibacterial compounds that effectively combat infections and potentially act as antifungal agents. *Bacillus velezensis* is also capable of producing indole-3-acetic acid (IAA), forming robust biofilms and fixing nitrogen and iron.[11, 12].

Moreover, *B. velezensis* produces enzymes that support the digestion and breakdown of complex nutrients, allowing it to work synergistically with the abalone's digestive system. Its presence in the visceral organs of abalone can significantly enhance their health and growth. Additionally, previous research has demonstrated that this bacterium is effective as an antibacterial agent.

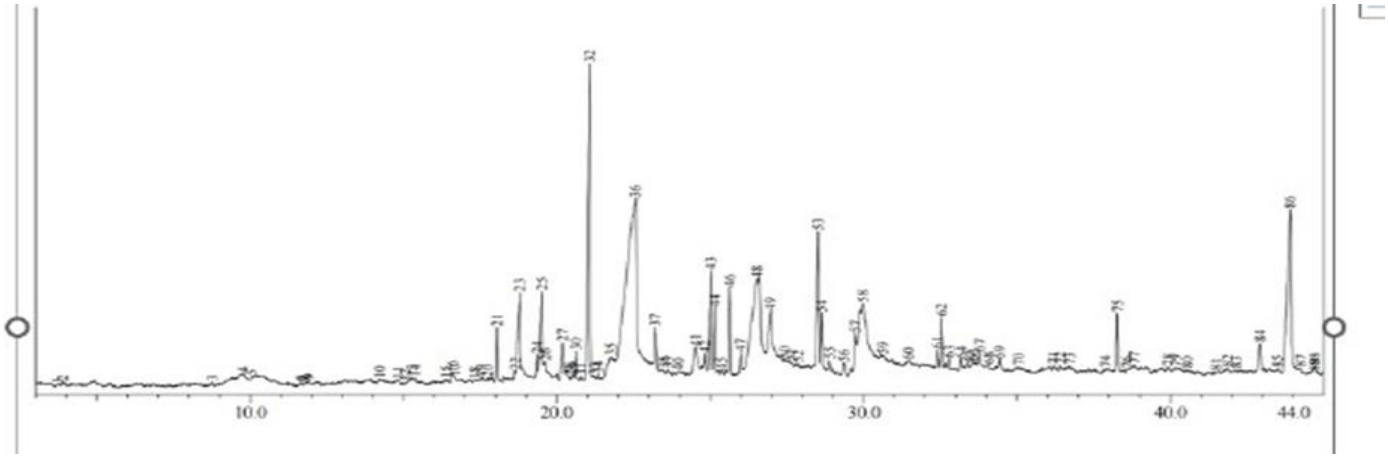

Figure 4: GC-MS chromatography test results on *H. asinina* abalone viscera

Brevibacterium iodinum is a Gram-positive bacterium capable of forming endospores under unfavourable conditions. It is commonly found in marine environments and is considered one of the dominant bacterial species in abalone [13]. Research indicates that *Brevibacterium iodinum* can produce potent antibacterial compounds, as well as vitamins, amino acids and other substances that promote the growth of abalone. Its presence in the visceral organs of abalone is believed to help maintain a balanced microbiota and prevent the proliferation of pathogenic bacteria that could cause health issues. Consequently, the two symbiotic bacterial species found in the internal organs of abalone – *Bacillus velezensis* and *Brevibacterium iodinum* – hold significant potential both as antibacterial agents and as contributors to the growth and health of abalone.

The research findings indicate that the viscera of Haliotis *asinina* abalone hold significant potential as a source of effective antibacterial compounds. GC-MS chromatographic analysis revealed 89 peaks, each corresponding to biomolecular compounds with potential antibacterial activity. Among these, nonadecanoic acid methyl ester is believed to exert antibacterial effects through its carboxylic acid group, which can increase bacterial cell membrane permeability and thereby inhibit bacterial growth and reproduction. Moreover, this compound has been shown to possess strong anti-inflammatory and analgesic properties. [14,15].

The biomolecular compound n-Hexadecanoic acid is believed to exhibit antibacterial activity due to the presence of alcohol and carboxylic acid groups, which can interact with essential components of bacterial cells and inhibit their growth. Additionally, its hydrophobic nature can disrupt bacterial membrane structures, leading to cellular damage and death. [16] It may also increase the permeability of bacterial cell

membranes, further hindering bacterial growth and reproduction. Moreover, studies have demonstrated that this compound possesses strong anti-inflammatory and analgesic properties. [14,15].

Cholest-5-en-3-ol, 6-methyl and Eicosatetraenoic acid, methyl ester are compounds believed to possess antibacterial properties. Cholest-5-en-3-ol, 6-methyl has demonstrated strong antimicrobial activity, particularly against Gram-positive bacteria. [17] In contrast, Eicosatetraenoic acid, methyl ester has been shown to exhibit effective antioxidant and anti-inflammatory properties. [18] Due to their different chemical structures, these two compounds may act synergistically to combat bacteria.

In addition to these compounds, 9-Octadecenoic acid, methyl ester and 9-Octadecenoic acid (E) are also believed to possess significant antibacterial properties. Research has shown that 9-Octadecenoic acid, methyl ester exhibits strong antimicrobial activity against both Gram-positive and Gram-negative bacteria. Similarly, 9-Octadecenoic acid (E) has demonstrated effective antibacterial activity, particularly against Gram-negative bacteria. Both compounds contain carboxylic acid groups, which play a crucial role in inhibiting bacterial growth and reproduction. [19]

Furthermore, the GC-MS analysis of the visceral organs of H. asinina abalone revealed the presence of a compound, 5-(1′,1′-Bicycloprop-2′-yl)pentanoic acid, identified at peak number 1 and detected at the lowest concentration. This compound, commonly found in various fish oils, has demonstrated significant antimicrobial activity against pathogenic bacteria. Additionally, studies indicate that it possesses anti-inflammatory properties and promotes collagen production, which may aid wound healing. These properties support its potential use as an ingredient in ointments for treating diabetic ulcers. Moreover, peak number 2 revealed the presence of Cyclopentane, Ethyl-, another compound known for its antimicrobial activity. This compound, found in the essential oils of various plants, can inhibit the growth of both gram-positive and gram-negative bacteria. Therefore, it may serve as an effective ingredient in ointments for treating infections associated with diabetic ulcer wounds. Peak number 6 corresponds to Ethyl 3-(ethoxycarbonyl)-6,6-dimethylheptanoate, which demonstrates strong antibacterial activity. This compound, a derivative of heptanoic acid, is present in plants such as rosemary and basil. It has been shown to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*, two bacteria commonly responsible for infections in diabetic ulcer wounds.

The results of the GC-MS analysis indicate that the visceral organs of abalone contain the compound cysteine-homocysteine disulphide, detected at peak number 7. This compound has been shown to possess strong antimicrobial activity against pathogenic bacteria commonly responsible for wound infections, thereby providing an antibiotic effect that can aid the wound healing process. Additionally, cysteine-homocysteine disulphide and related compounds serve as biomarkers for various diseases. Using cysteine and homocysteine as biomarkers can help in the prevention and diagnosis of many conditions. [20] At peak number 8, the compound 2-methylmethionine was identified, which exhibits antioxidant and anti-inflammatory properties. This compound can enhance collagen production and reduce inflammation, both of which are crucial for wound healing. Furthermore, it can protect the skin from free radicals that might otherwise slow the wound healing process.

The results of the GC-MS analysis indicate that the visceral organs and symbiotic bacteria of abalone possess potential antibacterial properties that could be utilised in the treatment of diabetic ulcers. Incorporating these natural compounds into ointment formulations may help accelerate wound healing and prevent infections that could worsen diabetic ulcers.

Based on the references, it is evident that the compounds found in the viscera of the abalone *H. asinina* have been extensively studied for their potent antibacterial properties. Consequently, the viscera of *H. asinina* show significant promise as an ingredient in ointments for treating diabetic ulcers. Beyond the antibacterial compounds present in the viscera, symbiotic bacteria also reside within the abalone, living in harmony with their host. These bacteria play a crucial role in maintaining the balance of the abalone's microbiota, thereby strengthening its immune system and protecting it against infections caused by pathogenic bacteria. Therefore, the symbiotic bacteria of abalone likewise hold potential as a source of antibacterial compounds and merit further research and development.

Therefore, it is essential to maximise the therapeutic potential of both viscera-derived compounds and the symbiotic bacteria of *H. asinina*. [21] Their research highlights the importance of evaluating natural extracts not only for their bioactivity but also for their safety, anti-inflammatory effects and healing properties through in vivo testing. Integrating these aspects – including compound characterisation, assessment of biological efficacy and formulation for topical use – will strengthen the development of abalone-based antibacterial agents into clinically relevant treatments for diabetic ulcers.

Conclusion

Symbiotic bacteria from the abalone *Haliotis asinina* exhibited inhibition zones against *E. coli* in isolates AL 1 and AL 5. Additionally, inhibition zones against *S. aureus* were observed in isolates AL 1, AL 2, AU 8 and AU 9.1, indicating that several isolates were effective in combating these pathogenic bacteria. The identified symbiotic bacteria included *Bacillus velezensis* and *Brevibacterium iodinum.* GC-MS analysis of the visceral organs revealed that the most prevalent compound was nonadecanoic acid methyl ester, which is involved in lipid metabolism. Other compounds identified include n-hexadecanoic acid, cholest-5-en-3-ol and eicosatetraenoic acid, which are products of cholesterol and fatty acid metabolism, as well as 9-octadecenoic acid, known for its various health benefits. The visceral organs and symbiotic bacteria of abalone (Haliotis asinina) hold significant potential due to their antibacterial activity, which could be harnessed in ointments for treating diabetic ulcers. Future research should focus on the formulation and clinical testing of these bioactive extracts in topical wound therapies, encompassing stability assessments, safety evaluations and efficacy trials in diabetic ulcer models, to support their development into effective therapeutic products.

Conflict of Interest

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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