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First Report Molecular Identification and Bioactivity Screening of Razor Clam (*Solen* sp.) from Merauke, Indonesia

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Abstract

Marine bivalves are increasingly recognized as reservoirs of natural metabolites that may support biotechnological development and functional food innovation. Nevertheless, information on the taxonomic status and biological properties of bamboo clams from eastern Indonesia is still scarce. This research examined the genetic affiliation and preliminary bioactive characteristics of *Solen sarawakensis* obtained from the coastal area of Merauke, Indonesia. Specimens were collected from the intertidal habitat, followed by molecular characterization based on the mitochondrial cytochrome oxidase I marker. The crude extract was subsequently evaluated for antibacterial properties using an agar disk assay against *Staphylococcus aureus* and *Escherichia coli*. Free-radical scavenging capacity was determined using DPPH analysis, while major secondary-metabolite groups were assessed through qualitative phytochemical tests. Sequence comparison and phylogenetic reconstruction placed the Merauke specimens within the *S. sarawakensis* lineage. The extract inhibited the growth of both tested bacterial species, suggesting activity across Gram-positive and Gram-negative targets. However, its radical-scavenging performance was relatively low, producing an IC_{50} of 353.52 ppm. Alkaloids, steroids, and saponins were detected in the extract, whereas flavonoids and tannins were absent. These findings document the genetic characterization and initial bioactivity profile of *S. sarawakensis* from Merauke. Despite modest antioxidant performance, its antibacterial response and detected metabolite classes indicate that this bivalve may warrant further investigation as a natural source of antimicrobial compounds. Purification of active fractions, compound elucidation, and mechanistic evaluation should be pursued in subsequent work.

Keywords: antibacterial, antioxidant, bamboo clam, bivalvia, phytochemical screening

1. Introduction

Tropical seas support exceptionally rich biological communities, with the Coral Triangle widely regarded as a central region for global marine species richness. Its diverse coastal habitats contain intricate ecological assemblages in which benthic fauna, including bivalve molluscs, forms a major component (Stark and Johnson, 2025; Silva et al., 2025). Bivalves perform multiple ecological roles in these habitats. Their interactions with surrounding organisms and their recruitment processes help maintain coastal ecosystem processes and population persistence. They also provide an accessible dietary protein resource for many coastal populations (Meyer-Kaiser et al., 2019).

In addition to these ecological and nutritional roles, marine bivalves are being explored as natural reservoirs of secondary metabolites. Reported activities of bivalve-derived compounds

include free-radical scavenging, inhibition of microbial growth, and modulation of inflammatory responses. Such properties support their prospective use in biotechnology and the development of functional food products (Chakraborty and Joy, 2020; Izzati et al., 2021).

Although bivalves have considerable potential, evidence concerning their biochemical composition and bioactive properties is not yet distributed evenly across taxa and geographic areas. Investigations of marine-derived natural compounds have predominantly emphasized organisms such as sponges and octocorals. By comparison, molluscan groups remain less extensively examined, particularly in poorly documented areas of eastern Indonesia (Liang et al., 2020; Izzati et al., 2021).

This imbalance leaves important gaps in the baseline information required for evidence-based development of marine biological resources. The issue is particularly relevant to geographically isolated Indonesian locations that lie far from established research institutions. Merauke waters, for example, have received limited scientific attention, especially regarding the biological activities of marine organisms occurring in this area.

In this context, the razor clam (*Solen* sp.) represents a relevant bivalve group for further study, as it is widely distributed in coastal and estuarine soft-bottom sediments and exhibits a characteristic infaunal lifestyle, burrowing within muddy substrates (Saeedi et al., 2016). It has also long been utilized by coastal communities as a valuable food resource. However, compared with other bivalve groups, scientific information regarding the biochemical composition and bioactive potential of *Solen* sp. remains limited. Consequently, its utilization has largely relied on traditional knowledge rather than structured scientific evidence.

Marine organisms respond to the conditions of their habitats. For estuarine bivalves, salinity regimes, tidal cycles, and substrate characteristics can shape physiological performance and the composition of metabolites they produce (Mckeon and Barshis, 2015; Dildar et al., 2025). Consequently, populations inhabiting different coastal settings may exhibit distinct biochemical compositions and levels of biological activity. Assessing organisms within their specific environmental setting is therefore important for interpreting their chemical and biological attributes appropriately. Merauke waters constitute a relatively little-studied part of eastern Indonesia and provide a valuable setting for investigating marine organisms whose bioactive properties have not yet been comprehensively documented. Research from this area may expand the evidence base for functional-food development and marine biotechnology while contributing new records to regional biodiversity and bioactivity databases (Centella et al., 2017; Pertiwi et al., 2025).

Reliable taxonomic assignment is fundamental to biological research, particularly for bivalves, whose external morphology may vary considerably in response to environmental conditions and may conceal cryptic taxa (Gomes-dos-Santos et al., 2020; Bouchet et al., 2023). DNA-based methods can reduce uncertainty in species delimitation. Among the markers commonly applied, the mitochondrial cytochrome oxidase I region has been extensively used for molecular identification and DNA barcoding (Hebert et al., 2003). In addition to supporting species-level classification, sequence data from this marker can be used to examine population-level variation and evolutionary relatedness (Hebert et al., 2003; Deiner et al., 2017). Nevertheless, Indonesian investigations of Solen spp. have mainly addressed taxonomic identification or genetic variation, whereas their possible bioactive properties have received comparatively little attention.

Preliminary bioactivity assays are commonly used in marine natural-product studies to determine whether an extract warrants more detailed chemical and biological investigation (Blunt et al., 2009). Radical-scavenging capacity is generally measured using DPPH or ABTS assays, whereas antibacterial testing examines whether an extract can suppress the growth of selected microorganisms (Brand-Williams et al., 1995; Re et al., 1999; Sharma et al., 2023). Although these assays do not identify the specific compounds responsible for the observed effects, they provide an initial basis for selecting promising extracts for fractionation, compound characterization, and subsequent bioactivity studies (Atanasov et al., 2021).

Accordingly, this research examined the razor clam *Solen sarawakensis,* locally known as a bamboo clam, collected from the coastal waters of Merauke, Indonesia. The study combined molecular characterization with an initial assessment of biological activity. Taxonomic placement was evaluated through analysis of the mitochondrial COI marker, while the crude extract was tested for its capacity to inhibit selected bacterial indicators and to scavenge DPPH radicals. Qualitative phytochemical tests were also conducted to identify broad metabolite groups that could be associated with the recorded biological responses. Through this integrated approach, the study provides baseline information on the molecular affiliation and bioactive profile of *S. sarawakensis* from Merauke, supporting future investigation of its potential applications in marine biotechnology and functional-food development.

2. Materials and Methods

2.1. Sample Collection and Preparation

A total of 30 bamboo clam specimens (*Solen* sp.), with shell lengths ranging from 16 to 19 cm, were collected from the coastal waters of Semangga District, Merauke, South Papua, Indonesia, during low tide, The specimens were collected manually by digging the sediment substrate using a small shovel or by hand, particularly in areas characterized by small openings on the sediment surface indicating the presence of clam burrows. A traditional collection technique was also applied by introducing limewater (calcium hydroxide solution) into the clam burrows to induce the clams to emerge from the sediment, thereby facilitating their collection.

2.2. Sample Extraction

Methanol was selected as the extraction solvent owing to its broad polarity range, which allows efficient solubilization of secondary metabolites such as phenolic compounds, flavonoids, and alkaloids (Blunt et al., 2009; Qin and Xi, 2021). The dried plant material, previously ground into fine powder, was left to soak at ambient temperature for 24–72 hours. During this period, the mixture was intermittently agitated to facilitate better mass transfer and compound diffusion (Zhang et al., 2018). After maceration, the liquid phase was separated and subsequently subjected to solvent removal using a rotary evaporator operated under reduced pressure, thereby minimizing thermal degradation of heat-sensitive metabolites (Abubakar and Haque, 2020). The resulting crude extract was then weighed to calculate yield and preserved in airtight dark containers at approximately 4°C until further analysis.

2.3. Antibacterial Activity Assay

The antibacterial activity of the *Solen* sp. extract was evaluated against *Staphylococcus aureus* and *Escherichia coli* using the agar diffusion method (Andini et al., 2022). Bacterial suspensions were adjusted to a turbidity equivalent to 0.5 McFarland and evenly spread onto Mueller–Hinton agar plates. Paper discs containing 20 µg/mL of the extract were placed on the agar surface. An antibiotic disc was used as the reference treatment, while methanol served as the negative control. The plates were incubated at 37°C for 24 h, after which antibacterial activity was evaluated by measuring the diameter of the inhibition zones (mm) surrounding the discs. The antibacterial activity assay was performed as a preliminary qualitative screening, and the results were recorded based on the presence (+) or absence (−) of inhibition zones rather than quantitative measurements of inhibition zone diameter.

2.4. Antioxidant Activity Assay

Antioxidant potential of the extract was evaluated through a DPPH radical-scavenging assay (Zhang et al., 2018). Different concentrations of the extract were prepared in methanol and allowed to react with DPPH solution under dark conditions (Putri et al., 2025). Following incubation, optical density was recorded at 517 nm using a UV–Vis spectrophotometer. Radical-scavenging capacity was expressed as the percentage inhibition of DPPH radicals relative to the control, with a standard antioxidant used as a reference compound.

2.5. Phytochemical Analysis

Screening of secondary metabolites was performed to determine the presence of major chemical constituents in the extract, namely alkaloids, flavonoids, phenolic compounds, saponins, terpenoids, and steroids (Harborne, 1998; Sarker and Nahar, 2012). Detection of each metabolite group was carried out using established colorimetric and precipitation assays. Dragendorff's and Mayer's reagents were applied for alkaloid detection, whereas flavonoids and phenolics were examined using Mg–HCl and ferric chloride reagents, respectively. Saponins were identified based on their foam-producing ability, while terpenoids and steroids were evaluated through the Liebermann–Burchard reaction (Zhang et al., 2018; Indrayanto, 2018). Observations were expressed as either presence (+) or absence (−) of the corresponding metabolites and were further considered in explaining the biological activities of the extract (Dubale and Zeynudin, 2023).

2.6. Molecular Identification

DNA barcoding of marine molluscs (Saleky and Merly, 2021; Shi et al., 2022). Genomic DNA was extracted from approximately 10 g of tissue from each of five selected bamboo clam specimens following the Qiagen protocol. The mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using the Folmer primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994). PCR was performed following the BIONESIA laboratory protocol in a 26 µL reaction mixture containing 2 µL template DNA, 1.25 µL of each primer (10 mM), 9 µL ddH_2O, and 12.5 µL Ready Mix, using an Applied Biosystems™ 2720 Thermal Cycler. The PCR conditions consisted of an initial denaturation at 94°C for 3 min, followed by 38 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 60 s, with a final extension at 72°C for 2 min.

Amplified fragments were examined by electrophoretic separation on 1% agarose gel according to established procedures (Green and Sambrook, 2019). PCR products were visualized on 1% agarose gel stained with GelRed®, and successfully amplified products were subjected to Sanger sequencing at PT Genetika Science, Jakarta, Indonesia. The resulting AB1 sequences were edited and aligned using ClustalW implemented in MEGA XI, manually checked for sequence quality, and compared with reference sequences in the NCBI GenBank database using BLAST (Hung & Weng, 2016). Phylogenetic relationships were evaluated using the Neighbor-Joining (NJ) method with 1,000 bootstrap replicates in MEGA XI. Taxonomic assignment was further evaluated by constructing a phylogenetic tree based on the Neighbor-Joining algorithm with 1,000 bootstrap iterations (Kumar et al., 2018). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT) for post hoc comparisons, using RStudio version 4.3.2.

3. Results and Discussion

3.1. Molecular Identification of *Solen* sp.

Morphologically, the *Solen* sp. specimens collected from the coastal waters of Merauke exhibited elongated, narrow, and bamboo-like shells with relatively open ends, consistent with the general morphological characteristics of the genus *Solen* sp. (**Figure 1**). PCR amplification of the mitochondrial cytochrome c oxidase subunit I (COI) gene produced a clear DNA band of approximately **660 bp**, confirming successful amplification of the target fragment (**Figure 2**). BLAST analysis of the COI sequence against the NCBI GenBank database showed that the closest available reference sequence was *Solen sarawakensis* (GenBank accession no. FJ662782.1), with a query coverage of 97% and a sequence identity of 89.42% (**Table 1**).

Although the high query coverage indicates substantial alignment between the sequences, the relatively low sequence identity does not provide sufficient support for confident species-level assignment. Therefore, the specimen is conservatively retained as *Solen* sp. rather than assigned unequivocally to *S. sarawakensis*.

Figure 1. Morphological characteristics of razor clams of the genus *Solen*, showing an elongated, bamboo-like shell structure.

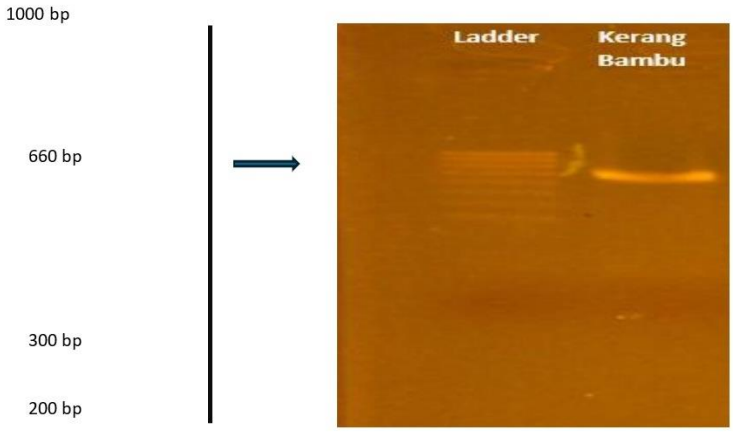

Figure 2. PCR amplification results of razor clam samples, showing a target band of approximately 660 bp.

These values indicate a close genetic relationship with *Solen* sp. However, the sequence identity, which did not reach the commonly accepted species-level threshold (≥98%), suggests the possibility of genetic divergence or indicates that the specimen should currently be retained as *Solen* sp. To further evaluate its taxonomic position, phylogenetic analysis was performed using the Neighbor-Joining method with 1,000 bootstrap replicates. The resulting phylogenetic tree showed that the Merauke specimen clustered within the same clade as *S. sarawakensis*, although it exhibited a relatively distinct genetic distance from the available reference sequences (Figure 3). Therefore, clade association alone is insufficient to confirm species-level identity, particularly given the relatively low BLAST sequence identity. Pairwise genetic distance analysis was subsequently performed to quantitatively assess the genetic divergence between the study sequence and its closest reference sequences.

Table 1. BLAST-based DNA barcoding results of the razor clam sample.

Field ID	Lab ID	Species	bp	Gene	Accession Number	Query Cover (%)	Identity (%)
Bamboo-like shell	BIOSUB272.001	*Solen sarawakensis*	660	COI	FJ662782.1	97	89.42

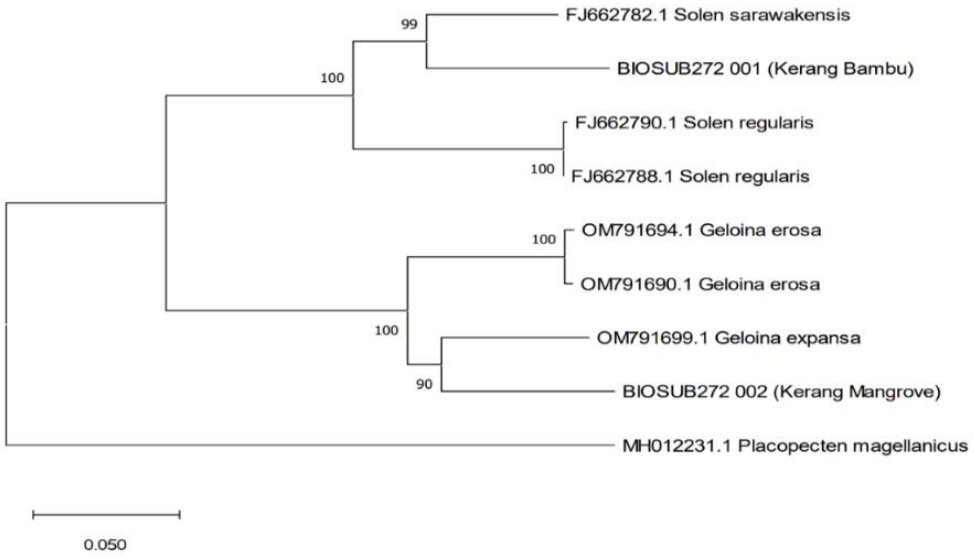

Figure 3. Phylogenetic tree of *S. sarawakensis* based on the COI gene.

3.2. Antibacterial Activity

The antibacterial activity assay demonstrated that the extract of the razor clam *Solen* sp. inhibited the growth of the test bacteria, *S. aureus* and *E. coli* (**Table 2**). This activity was indicated by the formation of inhibition zones around the discs containing the extract, whereas the negative control showed no inhibition zone. A limitation of this study is that antibacterial activity was assessed qualitatively without measuring inhibition zone diameters. Therefore, future studies should include quantitative disc diffusion measurements or minimum inhibitory concentration (MIC) assays to better evaluate the antibacterial potency of the extract.

Qualitatively, these results indicate that the extract has antibacterial potential against both Gram-positive and Gram-negative bacteria. The observed differences in inhibition responses suggest that the effectiveness of the extract may vary depending on the type of test bacterium.

Table 2. Antibacterial activity of ***Solen* sp.**, extract against *S. aureus* and *E. coli*

Treatment / Sample	*S. aureus*	*E. coli*	Interpretation
***Solen* sp.**, extract	+	+	Exhibited antibacterial activity against both Gram-positive and Gram-negative bacteria
Negative control	–	–	No antibacterial activity observed

3.3. Antioxidant Activity

The antioxidant activity of the *Solen* sp. extract was evaluated using the DPPH radical scavenging assay at concentrations ranging from 125 to 1,000 ppm. The extract showed a concentration-dependent increase in radical scavenging activity (%RSA). The lowest %RSA was observed at 125 ppm (16.47%), whereas the highest activity was recorded at 1,000 ppm (82.75%) (Table 3).

Regression analysis of the DPPH assay data yielded an IC_{50} value of 353.52 ppm for the *Solen* sp. extract, indicating relatively weak antioxidant activity. The relationship between extract concentration and %RSA is presented in **Figure 4**, showing an overall increase in radical scavenging activity with increasing extract concentration. One-way ANOVA revealed significant differences in %RSA among the tested extract concentrations ($F = 31.55$, $p < 0.05$), indicating that extract concentration significantly influenced the DPPH radical scavenging response.

Table 3. DPPH radical scavenging activity of *Solen* sp. extract at different concentrations.

Concentration (ppm)	Mean Absorbance	% RSA	Log_{10} Concentration [log_{10}(ppm)]	Probit
1,000	0.030± 0.036[b]	83	3.00	5.95
500	0.060±0.006 [b]	66	2.70	5.39
250	0.120±0.002 [a]	28	2.40	4.42
125	0.140±0.001 [a]	16	2.10	4.01
62.5	0.150±0.002 [a]	12	1.80	3.82

Note: Values are expressed as mean ± SD (n = 3). %RSA, percentage radical scavenging activity

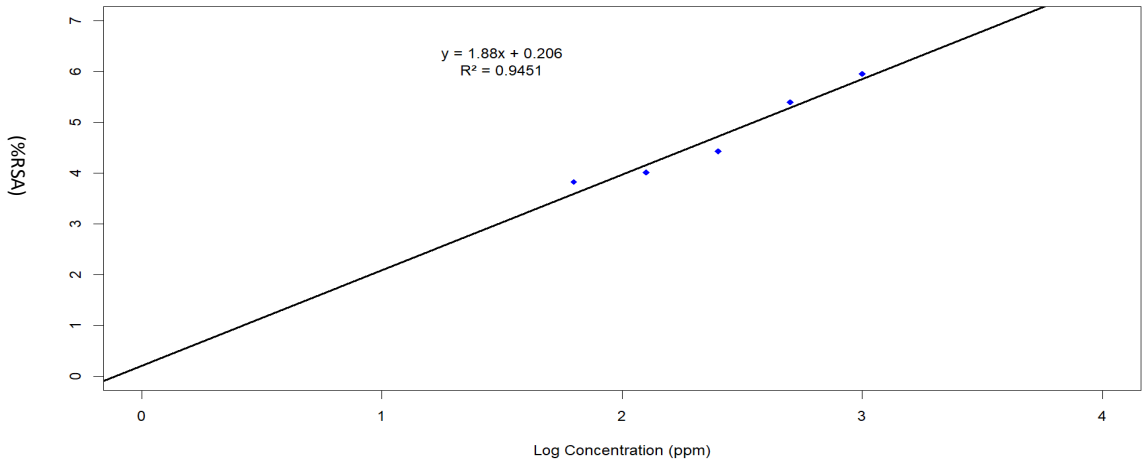

Figure 4. Regression analysis for IC_{50} determination of Harborne (1998) and Sarker Nahar (2011) *Solen* sp. extract using the DPPH assay.

3.4. Phytochemical Profile of Razor Clam (*Solen* sp.) Extract

Phytochemical screening of the razor clam (*Solen* sp.) extract revealed the presence of several classes of bioactive compounds (**Table 4**). The extract tested positive for alkaloids, steroids, and saponins, while flavonoids and tannins were not detected in this analysis. The presence of alkaloids, steroids, and saponins suggests that the razor clam extract has biological potential associated with bioactive properties, including antibacterial and antioxidant activities. In contrast, the absence of flavonoids and tannins indicates that the observed antioxidant activity is likely not dominated by phenolic compounds, but rather by other classes of bioactive constituents. These findings provide an initial overview of the chemical composition of the extract and support the biological activity results obtained from the previous assays.

Table 4. Phytochemical profile of *Solen* sp. extract

Phytochemical group	Result
Alkaloids	+
Steroids	+
Saponins	+
Flavonoids	–
Tannins	–

3.5. Discussion

The molecular evidence indicates that the Merauke bamboo clam specimen (BIOSUB272.001) is genetically affiliated with the *Solen* lineage, with *Solen* sp. representing the closest reference sequence identified in GenBank. Although the COI sequence showed a high query coverage of 97%, its sequence identity was only 89.42%, indicating substantial genetic divergence from the available *S. sarawakensis* reference. Therefore, the phylogenetic clustering of the specimen with *S. sarawakensis* should be interpreted cautiously and cannot, by itself, support definitive

species-level identification. The specimen is consequently retained as *Solen* sp. This genetic divergence may reflect geographic differentiation of populations, limited representation of *Solen* diversity in public databases, or the possibility that the Merauke specimen represents a closely related taxon that is currently underrepresented in reference datasets.

Among molecular markers available for species discrimination, the mitochondrial cytochrome c oxidase subunit I (COI) region remains one of the most reliable tools for identifying molluscan taxa due to its high discriminatory power among morphologically similar species (Xu et al., 2022).

The mitochondrial cytochrome c oxidase subunit I (COI) region is widely used for DNA barcoding because of its ability to discriminate among closely related animal taxa. However, the relatively low sequence identity obtained in the present study highlights the limitations of relying on a single barcode marker and incomplete reference databases for species-level identification. The eastern Indonesian region, including Merauke, remains comparatively underrepresented in molecular reference datasets, which may limit the accuracy of sequence-based taxonomic assignment. Thus, the present finding is important not because it definitively identifies the specimen as *Solen* sp., but because it provides molecular evidence of genetic affinity within the Solen lineage and indicates the need for broader sampling and additional molecular markers to resolve its taxonomic status. Bioactivity screening revealed that the *Solen* sp. extract was capable of suppressing the growth of both *S. aureus* and *E. coli*. Such activity highlights the prospect of this species as a source of antimicrobial compounds effective against different bacterial groups. The response observed in both bacterial models suggests that the active constituents may interact with cellular targets shared across distinct bacterial types rather than acting exclusively on a specific cell-wall structure. Similar observations have been reported for numerous marine benthic organisms, including mollusks and their symbiotic communities, which are recognized as reservoirs of chemically diverse secondary metabolites exhibiting antibacterial properties (Carroll et al., 2019; Liu et al., 2019).

The antibacterial activity observed in this study agrees with earlier reports describing bioactive metabolites from marine fauna. Investigations on polychaetes and several other marine invertebrates have shown inhibitory effects on pathogenic bacteria, including *S. aureus* and *E. coli*, suggesting the production of compounds with antimicrobial properties (Pringgenies et al., 2021). Comparable evidence from marine natural product research further identifies marine invertebrates as valuable reservoirs of antibacterial substances (Carroll et al., 2019). Taken together, these observations indicate that the antimicrobial response detected in *S. sarawakensis* is likely part of a broader ecological adaptation that enables marine organisms to withstand continuous microbial challenges in their habitat.

Unlike its antibacterial performance, the extract displayed only limited free radical-scavenging capacity. The IC_{50} obtained in this study falls within the weak antioxidant range, suggesting a low ability to neutralize reactive radicals. This observation may be explained by the phytochemical composition of the extract, which lacked prominent antioxidant constituents, particularly flavonoids and tannins. Such metabolites are widely recognized as major contributors to antioxidant activity due to their electron-donating properties and their capacity to stabilize reactive species (Zhang et al., 2018). Consequently, the phytochemical profile obtained here is consistent with the modest antioxidant response observed.

Nevertheless, chemical screening detected alkaloids, steroids, and saponins in the extract. These metabolite groups have frequently been linked to antimicrobial effects. Alkaloids may inhibit bacterial growth through interference with nucleic acid synthesis and cellular enzymes, whereas saponins can alter membrane permeability and compromise cell integrity. Steroidal compounds have likewise been reported to affect the structural stability of microbial membranes (Yan et al., 2021). Similar relationships between metabolite composition and antibacterial properties have been documented in marine organisms, including Indonesian marine biota examined by Pringgenies et al. (2023).

Evidence from previous investigations has demonstrated that microbial symbionts associated with molluscs are capable of producing metabolites with antibacterial properties through complex host–microbe interactions (Carroll et al., 2019; Liu et al., 2019). Consequently, the

inhibitory effects recorded here may result not only from compounds synthesized by the clam itself but also from metabolites derived from its associated microbial community.

Taken together, the findings presented here expand current knowledge regarding the biological properties of *Solen* sp. from Merauke waters. Although free radical-scavenging performance was limited, the extract consistently suppressed bacterial growth and contained several metabolite groups commonly linked to antimicrobial effects. These characteristics suggest that *S. sarawakensis* represents a promising candidate for future natural-product development. The combination of molecular characterization, biological assays, and chemical profiling employed in this work provides a broader perspective on the applied value of marine biodiversity, particularly from regions that have received relatively little scientific attention.

The present findings also emphasize the scientific importance of eastern Indonesian marine ecosystems as reservoirs of chemically diverse metabolites. Future investigations should focus on purification of the active constituents, structural characterization of the responsible compounds, and clarification of the biological pathways underlying their observed activities.

4. Conclusions

The This study represents a preliminary report on the molecular identification and bioactivity screening of the razor clam *Solen* sp. from the waters of Merauke. COI gene analysis confirmed its close relationship with *Solen* sp., while also indicating the potential presence of local genetic variation that remains poorly documented. The extract of *Solen* sp. exhibited antibacterial activity against *S. aureus* and *E. coli*, suggesting its potential as a source of antimicrobial agents. In contrast, the observed antioxidant activity was relatively weak, which is likely associated with the absence of major phenolic compounds, such as flavonoids and tannins. Phytochemical analysis revealed the presence of alkaloids, steroids, and saponins, which may contribute to the observed antibacterial activity. Overall, this study provides important baseline data for exploring the biotechnological potential of marine organisms from underexplored regions. Further studies are required to isolate and characterize specific active compounds and to evaluate their mechanisms of action in order to support the development of *S. sarawakensis* as a source of functional natural products.

Conflicts of Interest

There are no conflicts to declare.

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AI Writing Statement

The authors did not use any artificial intelligence-assisted technologies in the writing process.

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