Antibacterial assay of crude extracts from marine sponge *Haliclona fascigera* in Badi Island of Spermonde Archipelago against shrimp pathogenic bacteria

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**Abstract.** The marine Sponge *Haliclona fascigera*, belonging to the genus Haliclona, Family Haliconidae, Order Haplosclerida, is a source of potentially valuable marine natural products, including diverse bioactive secondary metabolites. These bioactive secondary metabolites could be used as alternative antibiotics for managing pathogenic bacteria in aquaculture. The study aimed to determine the *in vitro* antibacterial activity of n-hexane, ethyl acetate, and acetone crude extracts against three shrimp pathogenic bacteria and to carry out phytochemical screening. The three crude extracts were produced from *H. fascigera* collected around Badi Island, Spermonde Archipelago, Indonesia. The crude extracts were screened for their activity against three shrimp pathogenic bacteria: *Vibrio harveyi* (M-120), *Vibrio alginolyticus* (B-425), and *Vibrio para-haemolyticus* (T-170). Antibacterial activity assays used the agar diffusion method; the paper discs were impregnated with extract concentrations of 2 mg/25μL. Phytochemical screening was carried out using standard protocols to provide supporting data. N-hexane and acetone crude extracts of *H. fascigera* were able to inhibit *V. parahaemolyticus* with inhibition zone diameters of 8.07 mm and 7.62 mm, respectively. The inhibition zone of ciprofloxacin (positive control) was 10.45 mm. The phytochemical analysis indicated that steroid, terpenoid, and alkaloid compounds were present in *H. fascigera*. Further studies are needed to reveal the compounds causing the observed antimicrobial effect.

**1. Introduction**

Indonesia is located at one of the centers of marine biodiversity covering 10.36 million km² of ocean and coastal waters [1,2]. Sponges, one of marine biodiversity in Indonesia, have raised the interest of natural product chemists due to the presence of structurally original and extremely diverse secondary metabolites. These secondary metabolites are produced by marine organisms, both macro, and microorganisms, as responses that are part of their defense strategies, responses to the food chain, or as communication signals with their environment [3,4].

Many natural product chemists have turned their attention to sponges, mainly in the discovery of new bioactive compounds as new drugs from the sea. More than 8000 sponge natural products have been reported [5,6]. Approximately 224 new marine compounds of sponges were reported in 2016, compared with 291 in 2015. While 222 new compounds were globally reported in 2018 [7-9]. Furthermore, Indonesian MNPs investigations specifically from sponges in Spermonde Archipelago have obtained some bioactive compounds [10-15].
Sponges produce interesting biologically active compounds that could be useful for animals, one of which was alternative antibiotics in aquaculture. In the last decades, antibiotic-resistant bacteria is a worldwide problem in aquaculture. Bacterial pathogens have evolved numerous defense mechanisms against antimicrobial agents. Moreover, infectious diseases have become the major causes of morbidity and mortality rates [16-18]. There is yet an immense untapped potential to determine bioactive secondary metabolites from sponges specifically *Haliclona fascigera*, which could be used as alternative antibiotics for managing pathogenic bacteria in aquaculture.

The marine sponge *H. fascigera*, belonging to the genus Haliclona, Family Haliclonidae, Order Haplosclerida, is a source of potentially valuable marine natural products, including diverse bioactive secondary metabolites with unique structures. It has proved to be a rich source of nitrogen-containing metabolites, most commonly bioactive alkaloids. Physically it had no special skin skeleton and took the form of an encrusting mass of cylindrical to volcano-shaped projections with oscula at the high end [10,19-20]. Several bioactive compounds from Haliclona, especially *H. fascigera*, are well known to contain chemically diverse secondary metabolites with interesting antibacterial activities [15,21-24].

This study looks at some of the reports that *H. fascigera* has been determined to enable the application of antibacterial agents. Thus, this study will be investigating the antibacterial activities of crude extracts of *H. fascigera* from Spermonde Archipelago against three shrimp pathogenic bacteria. Information on the antibacterial activities can be used as supporting data for further isolated chemicals.

### 2. Materials and Methods

#### 2.1. Sampling method

*Haliclona fascigera* was collected from Badi Island, South Sulawesi, Indonesia, using hand with scuba diving in the depth of 10-18 m at the latitude 4°58’21.976224, and longitude 119°17’4.923946. The sponge soon after collection was put into a sterile plastic bag and stored in the icebox and transported to the laboratory. The sample was identified at the Faculty of Marine Science and Fisheries, Hasanuddin University, South Sulawesi Province, Indonesia.

#### 2.2. Preparation of extraction

Samples (800 g) were cut into small pieces and extracted in various solvents based on the level of polarity. The extraction of secondary metabolites was carried out using n-hexane, ethyl acetate, and acetone. The combined n-hexane extracts were concentrated in vacuo, and the resultant oil was soaked with ethyl acetate and acetone.

#### 2.3. Antibacterial analysis

Bacterial species (*Vibrio harveyi* (M-120), *V. alginolyticus* (B-425), and *V. parahaemolyticus* (T-170) (shrimp pathogenic bacteria)) were donated by The Center for Brackish Water Aquaculture, Takalar, and Maros, South Sulawesi Province, Indonesia. The turbidity of the suspension was standardized against 0.5 McFarland using a spectrophotometer at 600 nm wavelength. The bacterial inoculum was 10<sup>6</sup> cfu mL<sup>-1</sup> [25].

Antibacterial activity tests included positive, negative control, and antibacterial activity tests for sponge extracts. The positive control test was carried out using ciprofloxacin antibiotics, and the negative control test used solvents (n-hexane, ethyl acetate, acetone, dimethyl sulfoxide (DMSO)). The method applied in this test is the agar diffusion method with slight modification [26,27]. Sterile paper discs with a diameter of 6 mm were impregnated with extract concentrations of 2 mg (25 μL /disc). The samples were incubated for 24 hours at 30°C. The distinct zone around the paper disc is a sign of bacterial activity. Every experiment was conducted three times. Inhibition zones 15-20 mm were declared as strong, >11 - <15 mm as moderate, and ≤10 mm as weak activities [27].
2.4. Phytochemical test
Phytochemical tests were determined by the Harborne method. This method was carried out using Wagner, Meyer, Dragendorf reagents to test the content of alkaloids, Liebermann-Burchard reagent for steroids/terpenoids, and FeCl$_3$ reagent for phenols [28].

3. Result and Discussion
Results of the antibacterial activities of *Haliclona fascigera* crude extracts were summarized in Table 1. The results showed that sponge *H. fascigera* was effective in inhibiting the growth of *V. parahaemolyticus*. The clear zone (Figure 1) showed crude n-hexane and acetone extract containing compounds that can inhibit the growth of pathogenic bacteria. However, none of the crude extract tested with *Vibrio harveyi*, *V. alginolyticus*, and *V. parahaemolyticus* can exceed the inhibition zone of positive control.

![Table 1. Antibacterial activities of *Haliclona fascigera* crude extracts.](image)

| Name of Bacteria             | Zone of Inhibition (mm) (mean ± SD)$^\text{(*)}$ | Control (+) ciprofloxacin |
|------------------------------|--------------------------------------------------|---------------------------|
|                              | N-hexane  Ethyl acetate  Acetone                 |                           |
| *Vibrio harveyi*             | 6.0 ± 0    6.0 ± 0     6.0 ± 0     | 16.24 ± 0.78              |
| *Vibrio parahaemolyticus*    | 8.07 ± 0.51 6.0 ± 0 7.62 ± 0.78 | 8.90 ± 0.45              |
| *Vibrio alginolyticus*       | 6.0 ± 0    6.0 ± 0     6.0 ± 0     | 10.45 ± 0.69              |

*paper discs 6 mm

![Figure 1. Result of antibacterial activity: (A) n-hexane extract, (B) negative control (C) acetone extract, (D) positive control, (E) ethyl acetate extract against *Vibrio parahaemolyticus*.](image)

The crude n-hexane and acetone extract can inhibit *V. parahaemolyticus* (Figure 2). The highest inhibition value was shown in n-hexane extract (8.07 mm) followed by acetone extract (7.62 mm). The value of the inhibition zone belonged to the category of weak antibacterial activities [27]. However, crude ethyl acetate extract has no antibacterial activities (6.0 mm) against three Gram-negative bacteria. It is probably caused by the low concentration and the resistance of pathogenic bacteria to the extracts. Besides, the bacteria was made possible bacteriostatic, when it can inhibit bacterial growth but do not kill bacteria until within 48 hours [21,29].

Some studies reported that the ethyl acetate extract sponge *H. fascigera* has potential antimicrobial activities against *Staphylococcus aureus*, *Escherichia coli*, and multidrug-resistant organisms. Eight extracts can inhibit the growth of *S. aureus* with inhibition zone between 11 and 16.5 mm. All extracts were inactive against Gram-negative bacteria *E. coli*. Other studies determined twelve extracts that can
inhibit the growth of MRSA with inhibition zone between 11.1±0.17 to 15.17±0.76, and nine ethyl acetate extracts that are considered active to Vancomycin-resistant Enterococcus sp (VRE), and extended-spectrum-beta (β)-lactamase gram-negative organisms (ESBL) [22-24]. Not only polar extracts but also nonpolar extract showed to have some antibacterial activities [15]. Altogether, it would appear that sponge can exhibit a broad spectrum of antibacterial activity against bacterial pathogens.

![Figure 2](image-url) Mean values (mm +/- SE) of clear zone measurement in antibacterial activity test.

Analysis of the chemical reaction of crude extracts isolated from H. fascigera was conducted to determine the chemical groups of secondary metabolites. The compound belongs to different chemical groups. In this study, the compounds of the crude extract were performed by the phytochemical test. Based on the results of the phytochemical test (Table 2), most of the extracts contain steroid, terpenoid, and alkaloid compounds. This result confirms the possible use of steroid, terpenoid, or alkaloid compounds from H. fascigera as a source of antimicrobial compounds.

| No | Phytochemical         | N-hexane | Ethyl acetate | Acetone |
|----|-----------------------|----------|---------------|---------|
| 1  | Wegner                | -        | +             | +       |
| 2  | Meyer                 | -        | -             | -       |
| 3  | Dragendorf            | -        | -             | -       |
| 4  | FeCl₃                 | -        | -             | -       |
| 5  | Lieberman-Burchard    | ++       | +             | -       |

(-) Negative, (+) Weak positive, (++) Strong positive

Several compounds of Haliclona sp. have been investigated. An earlier report described the presence haliclonadiamine (alkaloid), polyhydroxylated sterols (halicrasterols) (Figure 3), and cyclostellettamine (alkaloid) as antimicrobial agents isolated from the sponge Haliclona sp. [30-32].
Data of antibacterial activities and phytochemical tests can be used as supporting data for further fractionation and elucidate structures. Moreover, the possible use of active *H. fascigera* as alternative antibiotics for managing or preventing pathogenic bacteria in aquaculture should be discussed. Further studies are needed to reveal the compounds causing the observed antimicrobial effect.

4. Conclusion

Based on this research, it can be concluded that the crude n-hexane and acetone extract of *Haliclona fascigera* have great potential to inactivate *Vibrio parahaemolyticus* compared with another crude extract. Phytochemical screening was carried out using standard protocols to provide supporting data. The presence of antibacterial activities from crude n-hexane and acetone extract is supported by steroids, terpenoids, and alkaloids. More specifically, separation and structure elucidation is needed to be able to reveal the compounds causing the observed antimicrobial effect.

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