

Research Article

In Vitro Biological Activity of Three Marine Sponges From *Theonella* and *Haliclona* Genera Collected From Bidong Island, Terengganu, Malaysia

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ABSTRACT

Marine sponges are primitive sessile animals that are rich sources of biologically active compounds. This paper aimed to assess the in-vitro biological activity of marine sponges from *Theonella* and *Haliclona* genera collected from Bidong Island, Terengganu, Malaysia. Biological activities such as antibacterial (discs diffusion assay), antioxidant (DPPH free-radical scavenging assay), and cytotoxicity activity (against cancerous HeLa, MCF-7, HepG-2 cell lines and the normal cell line Vero) were evaluated using MTT cytotoxicity assay. The bioassays were done on methanol extracts at different concentrations. Results indicate that *T. swinhoei*, and *T. cf cupola* showed low antibacterial capabilities ranging from 0 to 50 mg/mL and exhibited medium antioxidant activity with the IC₅₀ value of 23.25 ± 1.57 and 18.52 ± 0.86 mg/mL, respectively. Cytotoxicity activities indicate that both species of *T. swinhoei* and *T. cf cupola* possesses toxic capabilities to inhibit the proliferation of all cancer cell lines used and demonstrated no significant toxicity for the normal cell line used in this study. *Haliclona fascigera* showed medium antibacterial activity against all Gram-positive bacteria and low activity against Gram-negative bacteria used. *Haliclona fascigera* exhibited antioxidant activity with an IC₅₀ value of 1.80 ± 0.08 mg/mL and outcomes of the cytotoxicity activity assay against all cancer cells showed IC₅₀ below 30 µg/mL. Marine sponges evaluated in this study indicate promising bioactive compounds that can be an excellent candidate for drug discovery in prospecting novel antibiotics and anticancer. Despite showing low antibacterial and medium antioxidant activity, species from both *Theonella* can be further studied in other assays to explore other biological activities whilst marine sponge *H. fascigera* possesses excellent capabilities in antibacterial, antioxidant, and cytotoxicity activities that can be further studied its chemical compositions for future research.

Key words: Antibacterial activity, antioxidant activity, biological activities, cytotoxicity activity, marine sponges

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INTRODUCTION

Natural products have played major roles in the innovation of drug discoveries in past decades (Koparde *et al.*, 2019). However, due to the misuse of drugs and the evolution of diseases, currently available drugs are no longer adequate to treat certain illnesses such as bacterial infections and cancer. There has been increasing interest in the search for novel sources of secondary metabolites in the marine environment (Petersen *et al.*, 2020). A marine environment is a place full of a diversity of living organisms. The marine organisms offer abundant sources of novel bioactive compounds that lead to new drug discoveries (Hu *et al.*, 2015). The vast biological diversity of the marine ecosystem, such as coral reefs and marine sponges is extremely varied as compared to the tropical rainforest (Costello & Chaudhary, 2017; De *et al.*, 2018). Porifera is a group of multicellular primitive metazoans being reported as the source of numerous bioactive secondary metabolites by past research (Bhatnagar *et al.*, 2016). Due to the broad degree of chemical constituents and novel molecular structure, secondary metabolites isolated from marine natural products like marine sponges have been reported to be potential candidates for novel therapeutic drugs for critical diseases (Jimenez, 2018). The total number of secondary metabolites reported indicated the potential biological activities such as antioxidant, antibacterial, antifungal, anticancer, and anti-inflammatory properties. These biological activities are due to the rich chemical constituents' compounds in these sessile marine organisms and their symbiotic microorganisms such as alkaloids, terpenoids, sterols, flavonoids, phenolic compounds, organic acids, and sulfated polysaccharides (Sugappriya & Sudarsanam, 2016; El-Damhougy *et al.*, 2017).

Researchers have listed numerous compounds derived from marine sponges of *Theonella* and *Haliclona* species. *Theonella* spp. is one of the popular research materials with a variety of bioactivities such as antimicrobial (antibacterial, antifungal, antiviral) and cytotoxic

activities (Das *et al.*, 2022). Some compounds that have been isolated from the sponge, *Theonella* spp. from Japan have been reported to have moderate cytotoxicity against breast cancer cells, HeLa cells, and the P388 cell line (Fukuhara *et al.*, 2018). This *Theonella* species has been proven to possess various metabolites responsible for cytotoxic activity such as hurghadolide A, swinholides A-C, theonezolides A, and theonellamide F-G (Lai *et al.*, 2021; Varijakzhan *et al.*, 2021). *Haliclona* species are known to produce compounds with antibacterial, anti-malarial, anti-inflammatory, and cytotoxic bioactivity. Sponges from subgenus *Haliclona* are reported to produce powerful secondary metabolites from various classes including alkaloids, macrolides, steroids, and terpenoids (Bai *et al.*, 2021; Varijakzhan *et al.*, 2021; Das *et al.*, 2022).

Bidong archipelago is located on the east coast of Peninsular Malaysia. Being slowly explored by scientists and researchers from the nearby Universiti Malaysia Terengganu, this archipelago has recorded abundant and diverse marine organisms. Until today, only infrequent reports on the biological activities of the Bidong's marine sponges have been published (Mohamad *et al.*, 2009; Annuar, 2013; Baharuddin & Zakaria, 2018). If this archipelago remains unexplored by the world, it could be a waste of potential high sources of natural products that could be handy for future uses. In this report, the biological effect of the methanol extracts of marine sponges collected from Terengganu islands was investigated with the hope of discovering potential promising marine metabolites from the marine environment. This study reports the initial findings on the antibacterial, antioxidant, and cytotoxicity activity of methanol extracts of identified marine sponges collected within the area of the Bidong archipelago in Terengganu, Malaysia.

MATERIALS AND METHODS

Materials

Müller-Hilton agar was purchased from Difco Thermo Scientific (Waltham Massachusetts, U.S), DPPH (2,2-diphenyl-1-picrylhydrazyl radical), quercetin and butylated hydroxyl toluene (BHT), methanol was purchased from Merck (Germany). Dulbecco's Medium Essential Media (DMEM), Fetal Bovine Serum (FBS) was purchased from Gibco, Thermo Scientific (Waltham Massachusetts, U.S).

Bacteria and cell lines

The bacteria used in this study were four Gram-positive bacteria: *Bacillus cereus* (ATCC 11778), *Micrococcus luteus* (ATCC 10240), *Staphylococcus aureus* (ATCC 25923), and *Streptococcus uberis* (ATCC 19436) and four Gram-negative bacteria: *Salmonella enterica* (WDCM 00030), *Vibrio parahaemolyticus* (ATCC 17802), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922). Cell lines used in this study were breast cancer (MCF-7) (ATCC HTB-22), cervical cancer (HeLa) (ATCC CCL-2), liver cancer (HepG2) (ATCC HB-8065), and normal Vero cell lines (ATCC CCL-8). All bacteria and cell lines used in this study were obtained from the Microbiology and Animal Cell Culture Laboratory, Institute of Marine Biotechnology, Universiti Malaysia Terengganu.

Sponge material

Marine sponges (Figure 1) used in this study were collected on October 2017 at the coastal area of Bidong Island at 5°36' 16.7"N 103°03' 42.6" E, via scuba diving at depth of 16 to 20 m. Marine sponges collected were stored at 4 °C in the ice box until they reached the laboratory. Afterward, they were cleaned to remove any epibiont and small animals. The whole sponge was cut into small pieces and lyophilized to eliminate the water from the sample.



UMTSpon 00429:
Theonella swinhoei



UMTSpon 00426:
Theonella cf cupola



UMTSpon 00406:
Haliclona fascigera

Fig. 1. Photographs of marine sponge used in this study taken during sample collection.

The identity of sponges was confirmed by the presence of spicules that matched the literature in conjunction with molecular identification via cytochrome oxidase subunit 1 (CO1). Specimens were deposited at the South China Sea Repository and Reference Centre (RRC), Institute of Oceanography and Environment, Universiti Malaysia Terengganu. The voucher numbers were UMTSpon 00429 for *T. swinhoei*, UMTSpon 00426 for *T. cf cupola*, and UMTSpon 00406 for *H. fascigera*.

Extraction

Five hundred gram of lyophilized specimens were extracted by maceration technique with 100% methanol at a 1:1 w/v ratio at room temperature for three days and filtered through Whatman No. 2 filter paper. The solvent in the filtrate was removed using a rotary evaporator at 37 °C. The extraction process was repeated three times. Extracted samples are labeled and stored at 4 °C until usage in biological assays.

Phytochemical screening

Phytochemical screening was conducted for the extracts by using the standard procedure adopted by Harborne (1988) for identifying alkaloids, terpenoids, flavonoids, coumarins, steroids, phenolic and tannins, saponin, total phenolic glycosides, cardiac glycosides, and resin (Elghobashy *et al.*, 2019).

Sample preparation

Crude extract of the sponge sample was prepared by dissolving 50 mg of crude extract in 1 mL 100% complete DMSO to serve as master stock (50 mg/mL) during the biological assays. Two-fold dilution was done to create different concentrations to be used. The negative control used in this study was 100% DMSO in different volumes based on the biological assays.

DPPH radical scavenging assay

A modified method in 96 well plates DPPH free radical assay by Chang *et al.* was used in this study (Chang *et al.*, 2001; Oogarah *et al.*, 2020). In brief, 20 μ L of extracts at different concentrations was aliquoted into a 96-well plate according to the designed template. Then, 200 μ L of DPPH solution that was prepared beforehand was mixed with the sample. After 30 min of incubation at room temperature, the absorbance at different concentrations (0 - 50 mg/mL) of crude extracts was measured at 490 nm using a THERMO Multiskan Ascent 354 Microplate Reader (Waltham Massachusetts, U.S). The sample was tested in three replicates. The percentage of DPPH antioxidant activity was calculated by using the following equation:

$$\text{DPPH antioxidant activity (\%)} = \frac{OD_B - OD_T}{OD_B} \times 100$$

OD_T represents the absorbance of the sample while OD_B represents the absorbance of the blank. The concentration of extract that results in 50% antioxidant activity is defined as IC₅₀.

Antibacterial disk diffusion assay

The antibacterial assay for the methanol extracts was evaluated using the disc diffusion method on Müller-Hilton agar. The inhibition zone diameter was measured in millimeters (mm) (Cita *et al.*, 2017). In brief, 10 μ L of 10 mg sponge extract dissolved in 1 mL 100% DMSO was dropped to sterile filter paper discs of 6 mm diameter each at different concentrations (1.5625, 3.125, 6.25, 12.5, 25, 50 mg/mL) and placed on the inoculated Petri dishes containing 100 μ L of the bacterial suspension. Streptomycin and chloramphenicol pre-dosed at (50 μ g/mL) per disc were used as controls whereas discs with 10 μ L of 100% absolute dimethyl sulfoxide (DMSO) were used as negative control. Three replicates were experimented with, and the mean was calculated with standard deviation. The zones of inhibition including the diameter of extract infused disc were compared with those of control after incubation at 37 °C for 24 h.

MTT cytotoxicity assay

The cytotoxic bioactivity of samples on MCF-7, HeLa, HepG-2, and Vero cells was determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay. Two-fold dilutions were done for the various concentrations (1.5625, 3.125, 6.25, 12.5, 50, 100 μ g/mL) for each cell line following 72 h of incubation in three replications (Bashari *et al.*, 2019). The absorbance was read at 520 nm using a Tecan spectrophotometer (Switzerland). Data generated were used to plot a dose-response curve in which the concentration of extract required to kill 50% of the cell population (IC₅₀) was determined using the equation:

$$\text{Sample inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{treatment}}}{A_{\text{control}}} \times 100$$

Where A_{treatment} is the sample and A_{control} is the control without the sample.

Statistical analysis

The data collected were statistically and analyzed on GraphPad PRISM version 9.0.1 software for antioxidant activity and cell cytotoxicity. The values of results are the mean of three independent determinations ($n=3$) \pm standard deviation.

RESULTS AND DISCUSSION

The result of the phytochemical screening analysis are shown in Table 1. In this qualitative analysis, all extracts showed positive results for several classes of chemical compounds. The methanol extracts were rich in phenolic glycosides, alkaloids, flavonoids, coumarins, saponins, and resins. Results from this study have shown that extracts from *Theonella* species are poor in terpenoids and steroids which are present in *Haliclona* species. Both genera showed no presence of cardiac glycosides, phenolic, and tannins. The results of these findings are parallel to the reported papers that study other marine sponges (Andriani *et al.*, 2017; Latifah *et al.*, 2021).

The antioxidant activity of marine sponges *Theonella swinhoei*, *Theonella cf cupola*, and *Haliclona fascigera* methanolic extracts was assessed by the scavenging capacity of the free DPPH radicals. The results expressed as IC₅₀ values are shown in Table 2.

Table 1. Result for phytochemical screening

Marine Sponge extracts	<i>Theonella swinhoei</i>	<i>Theonella cf cupola</i>	<i>Haliclona fascigera</i>
Alkaloids	+	+	++
Terpenoids	-	-	+
Flavonoids	+	+	+
Coumarins	+	+	+
Steroids	-	-	+
Phenolic and tannins	-	-	-
Saponin	+	+	+
Total phenolic glycosides	++	++	++
Cardiac glycosides	-	-	-
Resin	+	++	+

(-) =negative; (+) =weak positive; (++) =strong positive

Table 2. Antioxidant activity of *Theonella swinhoei*, *Theonella cf cupola*, and *Haliclona fascigera* in DPPH assay

Marine sponge	Percentage of inhibition activity at 10 mg/mL
<i>Theonella swinhoei</i>	46.98 ± 1.10
<i>Theonella cf cupola</i>	42.24 ± 0.66
<i>Haliclona fascigera</i>	81.07 ± 0.71
Butylated Hydroxytoluene (BHT)	83.03 ± 0.52
Quercetin	85.21 ± 0.35

DPPH is a stable free radical with maximum absorbance at 517 nm (Seradj *et al.*, 2012). When there are compounds capable of donating H-atom or an electron, there is a decrease in the absorbance at 517 nm. The change of color from purple to light yellow indicates the formation of a stable diamagnetic molecule from a scavenging potential of an extract or compound. Low absorbance of the reaction mixture indicates high free radical scavenging activity in which a stable diamagnetic molecule is formed. In Table 2, the methanol extracts from *Theonella* species displayed a low percentage of inhibition activity at 10 mg/mL compared to *Haliclona* species. The methanol extract of *H. fascigera* exhibited a low value of IC₅₀, indicating high radical scavenging activity compared to both *T. swinhoei* and *T. cf cupola*. There was no significant difference between the methanol extract of *T. swinhoei* and *T. cf cupola* ($p > 0.05$) in the DPPH radical scavenging activity assay (figure 2). From the observed tabulated data, *T. cf cupola* possessed more antioxidant activity compared to *T. swinhoei* by DPPH assay with IC₅₀ of 18.52 ± 0.86 mg/mL and 23.25 ± 1.57 mg/mL, respectively, whilst *H. fascigera* exhibit strong antioxidant activity from the DPPH assay in which the IC₅₀ recorded 1.80 ± 0.07 mg/mL and high percentage of DPPH scavenging activity at 81.07 ± 0.71. Despite showing strong antioxidant activity, *H. fascigera* did not surpass the standard BHT which was 0.48 ± 0.01 mg/mL. However, the percentage of BHT and *H. fascigera* at 10 mg/mL are quite similar which is 81.07 ± 0.71 and 83.04 ± 0.51, respectively. A previous study conducted in other countries has reported that *H. fascigera* has low antioxidant activity that differs from the marine sponge that has been collected in Malaysia (Francisco & Uy, 2016) (Figure 2). This may be due to the amount of quantity crude extract used which hypothesized that the percentage of activity is affected by the dosage used. In addition, in the different locations of *Haliclona* species in Terengganu, Malaysia, and other places, the organisms might also have been adapted to different ecosystems with different temperatures, light exposure, depth, and pressure. These parameters could affect the biological activities of the secondary metabolites. Antioxidant activity from secondary metabolites of marine sponges has been continuously reported over the years (Chairman *et al.*, 2012; Abdillah *et al.*, 2013; Balakrishnan *et al.*, 2014) however, there are currently no studies regarding the antioxidant secondary metabolites of *T. swinhoei* and *T. cf cupola*. Based on the percentage of scavenging activity in Table 2, these marine sponges have potential antioxidant capacities that play a role in sponge defense (Balakrishnan *et al.*, 2014). The percentage of DPPH radical scavenging activity of all extracts was dose dependent where increasing the dose concentration resulted in higher scavenging activity.

In comparison to the scavenging activity of the standard; Quercetin and BHT used, marine species from the genus *Theonella* exhibited low antioxidant activity. This indicates that *Theonella* species did not produce secondary metabolites with relevant and adequate capacity for antioxidant properties. The results support similar marine sponges *Cliona celata* and *Cliona viridis* from the same family of sponges reported in 2021 and 2016 (Bary *et al.*, 2016, Alves *et al.*, 2021). Despite showing strong antioxidant activity, *H. fascigera* did not surpass the standard BHT and quercetin. Despite low antioxidant activity reported in this report, there are other reports about the potential biological activities of genus *Theonella* such as antimalarial (Aguiar *et al.*, 2021), antibacterial (Muhammad Sulaiman *et al.*, 2018) and anti-protease activity (Zaporozhets & Besednova, 2020). The study was limited to testing crude extract, thus, there is a possibility that the pure compounds of the extracts have a stronger free-radical muffling activity.

The antibacterial activity of the marine sponge extracts was determined by the disc diffusion method against different bacteria. The strains used are frequently encountered in transmissible infections. The effectiveness of the marine sponge crude extracts was assessed by evaluating the inhibition zone capable of inhibiting the growth of bacteria used at different concentrations. Disc diffusion assay was performed on a crude extract that showed inhibition on the tested bacterial strains. The diameter of the inhibition zones formed is shown in Table 3. The extract showed a wide range of activity.

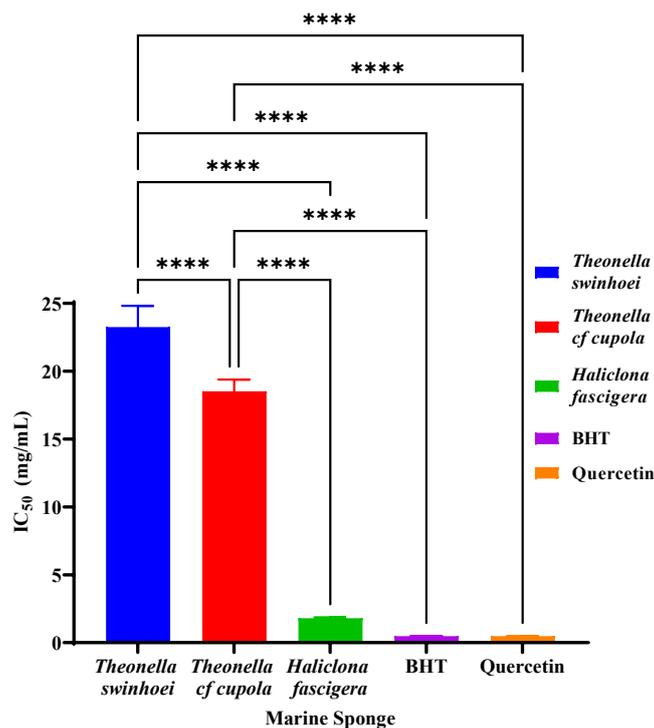


Fig. 2. DPPH scavenging IC₅₀ value of *Theonella swinhoei*, *Theonella cf cupola* and *Haliclona fascigera*.

The sponge extracts showed varying results on antibacterial activity against the pathogenic bacteria (Table 3). Extracts from *H. fascigera* showed antibacterial activities on all Gram-positive bacteria while extracts from both genus *Theonella* showed low antibacterial activities to at least one to two Gram-positive bacteria. Reports from previous studies showed bacteria associated with marine sponge *T. swinhoei* indicate high antimicrobial activity, this study reports that extract from both *T. swinhoei* and *T. cf cupola* exhibited low to no activities, indicating that the extract has no similar compounds to the bacteria associated with the marine sponges as mentioned (Kuo *et al.*, 2019). *Haliclona fascigera* methanol extract showed inhibition against all bacteria strains except for *Pseudomonas aeruginosa* with activity at only higher concentration and *Salmonella enterica* with no activity. Despite showing activity to all bacteria strains used, the result for inhibition of *H. fascigera* on Gram-negative bacteria was considered low ($7 \leq$ diameter of zone inhibition). Low bacterial inhibition on Gram-negative bacteria could be due to the speciality of Gram-negative bacteria cell walls that have large molecules like lipoprotein, exo-membrane, and lipopolysaccharides (Nazemi *et al.*, 2014).

According to the above results, the extract showed better activity towards Gram-positive bacteria (*Bacillus cereus*, *Micrococcus luteus*, *Streptococcus uberis*, & *Staphylococcus aureus*) compared to Gram-negative bacteria. Findings showed that there is agreement among researchers that Gram-positive bacteria are susceptible to sponge extracts whilst Gram-negative are not sensitive to them (Qaralleh *et al.*, 2010; Nazemi *et al.*, 2014). *Haliclona fascigera* has a broad range of active secondary metabolites such as steroids, polyketides, enamines, substituted macrolides, terpenes, and complex structures of alkaloids. In a previous study, it has been reported that this species has a high potential antibacterial activity by the alkaloid compound named haliclondiamine and papuamide that was first isolated in Palau (Yamazaki *et al.* 2013). Cyclostelletamine class of alkaloids isolated from *Haliclona* sp. from Korea are also reported to inhibit the growth of Gram-positive bacteria and to be cytotoxic against human cancer cells (Lee *et al.*, 2012; Yamazaki *et al.*, 2013). Other reports of *Haliclona* species show polyhydroxylated sterols and steroids found in *H. crassiloba* from China which are responsible for the antibacterial activity of this species (Cheng *et al.*, 2013).

The quality of extracts may be affected by the presence of impurities thus the biological activity of pure compounds would be higher and stronger than the crude extracts (Qaralleh *et al.*, 2010). In addition, due to the different locations of *Haliclona* species in Terengganu, Malaysia, and other places, the organisms might have been adapted to different ecosystems with different temperatures, light exposure, depth, and pressure. These parameters could affect the biological activities of the secondary metabolites. In the present study, the sponge methanol extracts were evaluated as potential new anticancer agents by using MTT colorimetric assay (Table 4).

As marine sponges have been continuously studied for their abundant secondary metabolites, few of the compounds that have been isolated were identified to be used in therapeutic drug development. Based on the present study, marine sponges comprised several bioactive metabolites in the methanol extract. These compounds might hold boundless potential as novel drug candidates due to their low toxicity, natural safety, and wide acceptance in the community (Malve, 2016). The current study demonstrated the cytotoxic activity as a measure of the percentage of cell viability or cell mortality calculated by MTT assay for the respective cell lines used, in a dose-dependent manner in 24 h to 72 h of extract incubation.

Based on the National Cancer Institute (NCI) guidelines, the limit of activity for crude extract was set to less than 30 μ g/mL after the exposure time of 72 h for the inhibition at 50% (IC₅₀). Conversely, if the extracts

inhibit 50% of the cell growth at concentrations lower than 20 µg/mL, they are considered exceedingly cytotoxic (Mahavorasirikul et al., 2010; Vijayarathna & Sasidharan, 2012).

The result of this report revealed that the methanol extract of *Theonella* and *Haliclona* species showed potent cytotoxic effects on all three cell lines. The values of IC₅₀ obtained were lower than the specified characterization by the NCI, USA. An IC₅₀ value below the stringent value was noted which falls within the NCI standard and thus can be assumed to be one of the promising anticancer drug potentials. The cell's morphology was prominently observed in the methanol extract showing excessive blebbing and vacuolation signifying the autophagic mechanism for the death of the cell. From the data that has been recorded, the methanol extract of *Theonella* and *Haliclona* species may have effective cytotoxic activity against the human breast carcinoma (MCF-7), liver carcinoma (HepG-2), and human cervical carcinoma (HeLa) cell lines. Further studies might introduce the compounds responsible for the high cytotoxicity that can be further used in the drug development of chemotherapeutic medicine.

Table 3. Result of Antibacterial assay of different concentrations of sponge extracts

Sponge/control	Conc (mg/mL)	Zone of inhibition (mm)									
		<i>M. luteus</i>	<i>S. uberis</i>	<i>B. cereus</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. enterica</i>	<i>V. parahaemolyticus</i>	<i>P. aeruginosa</i>		
<i>Haliclona fascigera</i>	50	11 ± 0.40	7.83 ± 0.23	8.33 ± 0.23	12 ± 0.205	9 ± 0.23	8.66 ± 0.23	8.66 ± 0.23	7.66 ± 0.23		
	25	9.5 ± 0.40	6.66 ± 0.23	7.66 ± 0.23	10 ± 0.408	7.5 ± 0.23	7.33 ± 0.23	7.16 ± 0.23	7 ± 0.40		
	12.5	8.16 ± 0.62	6.66 ± 0.23	6.66 ± 0.23	8.33 ± 0.23	6.66 ± 0.23	n. d	7 ± 0.47	n. d		
	6.25	6.83 ± 0.23	n. d	7 ± 0.40	n. d	n. d	n. d	n. d	n. d		
<i>Theonella swinhoei</i>	50	n. d	6.66 ± 0.23	7 ± 0.40	n. d	n. d	n. d	6.5 ± 0.20	n. d		
	25	n. d	6.66 ± 0.23	7 ± 0.40	n. d	n. d	n. d	6.5 ± 0.23	n. d		
	12.5	n. d	6.66 ± 0.23	7 ± 0.40	n. d	n. d	n. d	6.5 ± 0.23	n. d		
	6.25	n. d	6.66 ± 0.23	7 ± 0.40	n. d	n. d	n. d	n. d	n. d		
<i>Theonella cf cupola</i>	50	n. d	6.66 ± 0.23	7 ± 0.40	n. d	n. d	n. d	6.5 ± 0.30	n. d		
	25	n. d	6.66 ± 0.23	7 ± 0.40	n. d	n. d	n. d	6.5 ± 0.40	n. d		
	12.5	n. d	6.66 ± 0.23	7 ± 0.40	n. d	n. d	n. d	6.5 ± 0.23	n. d		
	6.25	n. d	6.83 ± 0.23	7 ± 0.40	n. d	n. d	n. d	n. d	n. d		
Streptomycin	-	30.33 ± 0.47	20.33 ± 0.47	20.33 ± 0.47	n. d	19 ± 0.48	20 ± 0.48	12 ± 0.23	12 ± 0.40		
Chloramphenicol	-	39.5 ± 0.40	29.5 ± 0.40	2.95 ± 0.40	30 ± 0.81	32.8 ± 0.62	30 ± 0.48	40 ± 0.5	9 ± 0.40		
DMSO	-	n. d	n. d	n. d	n. d	n. d	n. d	n. d	n. d		

n. d: not detected

Table 4. Results of cytotoxicity assay

Sample	IC ₅₀ (µg/mL) ± SD			
	HeLa	MCF-7	HepG-2	Vero
<i>Theonella swinhoei</i>	3.72 ± 0.38	16.21 ± 0.27	13.80 ± 0.25	60.25 ± 0.05
<i>Theonella cf cupola</i>	3.59 ± 0.08	16.2 ± 0.33	4.79 ± 0.23	60.25 ± 0.06
<i>Haliclona fascigera</i>	1.03 ± 0.01	2.51 ± 0.48	7.08 ± 0.16	9.120 ± 0.03
Tamoxifen	11.04 ± 0.02	14.53 ± 0.25	n.t	n.t

n.t: not tested

CONCLUSION

Marine sponge *H. fascigera* exerted positive results of activities in all three *in vitro* biological studies. It showed higher antioxidant and antibacterial activities compared to both *T. swinhoei* and *T. cf cupola*. *Haliclona fascigera* could be useful as one of the sources of novel antibiotics. Results of the biological activities in this study indicate promising potential biological compounds from marine sponge collected from Bidong Island, Terengganu, Malaysia thus need to be further studied to discover new possible novel compounds that can be used in pharmaceutical studies to fight against diseases.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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