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NEW ANTIBACTERIAL ACTIVITIES OF BROMINATED C_{18} AND C_{20} FATTY ACIDS ISOLATED FROM MARINE SPONGE *Xestospongia testudinaria* AGAINST SHRIMP PATHOGENIC BACTERIA

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ABSTRACT

The two known brominated C_{18} and C_{20} fatty acids (methyl ester) possessing acetylenic bonds (**1,2**) have been obtained from ethyl acetate extract of *Xestospongia testudinaria* collected in Badi island, Spermonde Archipelago, Makassar, Indonesia. The C_{18} and C_{20} compounds were determined using NMR and mass spectral data. Both C_{18} and C_{20} exhibited antibacterial activities against the shrimp pathogenic bacteria (*Vibrio* sp.). Compound **1** (C_{18}) showed significant inhibitory activity with the highest inhibition zone of 7.86 mm against *Vibrio harveyi* (inhibition zone of positive control (ciprofloxacin) was 24.8 mm). The two known compounds might be considered as a source of antibacterial drugs against *Vibrio* sp.

Keywords: *Xestospongia testudinaria*, Spermonde Archipelago, Brominated methyl ester, Antibacterial, *Vibrio* sp.
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INTRODUCTION

Marine sponges have the most marine natural products (MNPs) with unpredictable chemical structures since the early 1970s in different countries. Their secondary metabolites showed valuable biological activities.[1] There are approximately 2.500 types of sponges found in the Spermonde Archipelago.[2] *Xestospongia testudinaria*, known as class Demospongia, order Haplosclerida, family Petrosiidae, is quite abundant in the Spermonde islands. Badi island, one of the islands of the Spermonde, has a lot of sponge *X. testudinaria*. However, it is yet unexplored and needs further investigation regarding its secondary metabolites. Sponges genus Xestospongia have chemical diversity i.e. alkaloids, quinones, sterols, and brominated polyacetylene fatty acids.[3-9] Specifically, previous studies of brominated polyacetylene fatty acids isolated from sponge *Xestospongia* sp. have been reported from the various sea. These compounds can be seen by determining their chain length, unusual unsaturation patterns, acetylenic bonds, and bromine atom have unique chemical structures, and functions as secondary metabolites. [5,10-17]

With unique chemical structures, sponge *X. testudinaria* can be a rich source of natural chemicals for antibacterial. It has antibacterial activities with various inhibitory zones and pathogenic bacteria (Gram-positive and Gram-negative bacteria). Several studies have reported the antibacterial activities against human bacterial pathogens.[3,18-21] However, there are no studies related to antibacterial tests against shrimp pathogenic bacteria (*Vibrio* sp.) Therefore, this study has isolated and elucidated the two known compounds (brominated fatty acids) from the ethyl acetate extract of *X. testudinaria*, and determined antibacterial activities against shrimp pathogenic bacteria. The outcome of this research may support the existence of data and can provide new information related to the biological activities of compounds.

EXPERIMENTAL

Material

Sponge *X. testudinaria* was collected using hand with scuba diving on the inner reef at depths 10-18 m in Badi Island, South Sulawesi Province, Indonesia. The sample was identified at Marine Science and Fisheries Faculty, Hasanuddin University, Makassar, South Sulawesi Province, Indonesia.

Extraction and Purification

Samples (100 g) were cut into small pieces and extracted in various solvents using n-hexane, ethyl acetate, and acetone. Furthermore, fractionation and purification methods were conducted using the sequential application of Sephadex LH-20 chromatography, Waters Sep-Pak Silica 2 g, and reversed-phase HPLC to give pure samples. Reversed-phase HPLC purifications were performed on a Waters 1525 Binary HPLC pump attached to a Waters 2998 Photodiode Array Detector and equipped with Empower Software. Merck Type 5554 silica gel plates and Whatman MKC18F plates were used for analytical thin-layer chromatography (TLC). *Para*-Anisaldehyde stain with subsequent heat application was used to visualize TLC spots.[22]

Identification of Pure Compound

The chemical structure was investigated using the ^{1}H-NMR, ^{13}C-NMR, and MS data. The ^{1}H-NMR, ^{13}C-NMR spectra were recorded on a Bruker AV-600 spectrometer with a 5 mm CPTCI cryoprobe. ^{1}H and ^{13}C chemical shifts are referenced to the residual DMSO-d_6 (δ 2.49) and DMSO-d_6 solvent peak (δ 39.5), respectively. Low-resolution ESIMS were recorded on Bruker HCT Ultra PTM Discovery System, while high-resolution TOFESIMS were recorded on a Waters / Micromass LCT time-of-flight (TOF) mass spectrometer equipped with an electrospray ion source.

Antibacterial Activity

The isolated compounds were assayed by the agar diffusion method with slight modification for their antibacterial activities.[23,24] Bacterial species were donated by the Research Institute of Brackishwater Aquaculture and Fisheries Extension, Takalar, and Maros, South Sulawesi Province, Indonesia. The strain bacteria were *Vibrio harveyi* (M-120), *V. alginolyticus,* and *V. parahaemolyticus* (T-170) (shrimp pathogenic bacteria). The bacterial inoculum was used 10^{8} cfu/ml that was standardized against 0.5 McFarland using a spectrophotometer at 600 nm wavelength. The isolated compounds with a concentration of 4 µg (25 µl /disc) were put in sterile paper discs with 6 mm diameter. [25,26]

RESULTS AND DISCUSSION

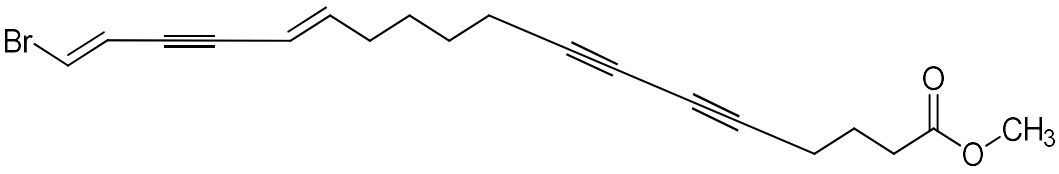
Brominated fatty acids were successfully isolated from the EtOAc extract of *X. testudinaria*. The frozen material (3.4 kg, dry weight) was extracted sequentially with n-hexane, ethyl acetate, and acetone. The EtOAc-crude extract (1.06 g) was treated with flash column chromatography to afford two known brominated polyunsaturated fatty acids. Based on ^{1}H-NMR and ^{13}C-NMR data, the presence of bromine atom was linked to olefinic bond at chemical shift 7.04 ppm; 119.3 ppm (**1**) and 7.05 ppm; 119.4 ppm (**2**). The ^{1}H-NMR data showed a methyl proton signal with singlet multiplicity at a chemical shift of 0.89 ppm (3H, s). The H atom on the carboxyl group of compound **1** dan **2** was replaced by an alkyl methyl group (CH_3) at the terminal (COO-CH_3) so that a methyl ester is formed.

The overall result appeared in ^{1}H-NMR (Table-1) and ^{13}C-NMR (Table-2) and compared to the published data.[10,27] The molecular formula of known compound **1** (C_{18}) (Fig.-1) was established as $C_{19}H_{21}O_2Br$ by HRESIMS (*m*/*z*: 360.07 M^{+}, colorless amorphous). It was concluded as Methyl 18-Bromo-(13E,17E)-octadeca-13,17-diene-5,7,15-triynoate. The methyl ester was bound to methylene at a chemical shift of 2.35 ppm at the beginning of the structure. The data of ^{13}C-NMR showed the presence of enyne conjugation system between δ 145.8 and δ 90.6; δ 90.6 and δ 117.4. Olefinic proton δ 6.18 ppm with methylene proton δ 2.11 ppm indicated allylic methylene. The first report of this compound has reported from the marine sponge *Petrosia volcano*, Hoshino, Hachijo-Jima Island.[27] Another study has isolated compound 1 from Chinese marine sponge *X. testudinaria.*[10] Badi Island, South Sulawesi, can be a new location for the discovery of compound 1 from sponge *X. testudinaria*.

Table-1: ^{1}H-NMR Data of Compound 1 (C_{18}) and 2 (C_{20}) (DMSO-d6, 600 MHz)

Position	Experimental (C_{18})	References [27]	Experimental (C_{20})	References[11]
	^{1}H-NMR	^{1}H-NMR	^{1}H-NMR	^{1}H-NMR
OMe	3.58	3.67	3.56	3.39

1				
2	2.35	2.44	2.29	2.00
3	1.69	1.83	1.49	1.39
4	2.30	2.31	1.32	1.13
5			1.44	1.19
6			2.34	1.93
7				
8				
9	2.29	2.12		
10	1.43	1.50		
11	1.43	1.50	5.66	5.28
12	2.11	2.26	6.27	5.96
13	6.18	6.14	2.21	1.61
14	5.68	5.53	2.21	1.61
15			6.14	5.87
16			5.70	5.33
17	6.52	6.28		
18	7.04	6.43		
19			6.52	6.18
20			7.05	6.38



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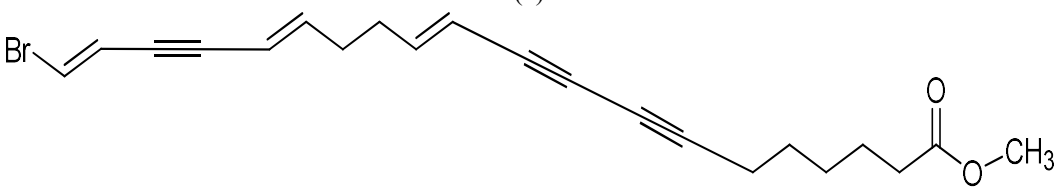

(2)

Fig.-1: Chemical Structures of C_{18} (**1**) Methyl 18-bromo-(13E,17E)-octadeca-13,17-diene-5,7,15-triynoate and C_{20} (**2**) Methyl 20-bromoeicosa-11*E*,15*E*,19*E*-triene-7,9,17-triynoate.

Methyl 20-bromoeicosa-11*E*,15*E*,19*E*-triene-7,9,17-triynoate (compound **2**) (C_{20}) (Fig.-1) that confirmed with the presence of *m*/*z*: 386.09 M^+. Its molecular formula was determined to be $C_{21}H_{23}O_2Br$. It has colorless amorphous. The methyl ester was bound to methylene at a chemical shift of 2.29 ppm at the beginning of the structure. Based on ^1H–^1H COSY, HSQC, HMBC data, compound **2** had an enediyne system that was linked together at carbon 15 - 20 (Fig.-1). The data of ^1H-NMR and ^{13}C-NMR of compound **2** were identical with references. This compound has been reported for the first time from *Xestospongia* sp., Papua New Guinea.[11] There are no studies of compound 2 that have been reported anymore. Therefore, this research could be additional data dan reference for compound 2.

Table-2: ^{13}C-NMR Data of Compound 1 (C_{18}) and 2 (C_{20}) (DMSO-d6, 600 MHz)

Position	Experimental C_{18}	References		Experimental (C_{20})	References [11]
	^{13}C-NMR	[27]	[10]	^{13}C-NMR	
OMe	51.3	51.6	51.6	51.1	50.9
1	172.7	173.5	173.4	173.2	173.1
2	32.1	32.7	32.7	33.0	33.7

3	23.1	23.5	23.5	23.8	24.5
4	17.7	18.7	18.6	27.6	28.3
5	78.1	75.8	76.1	27.2	28.0
6	65.7	66.1	66.1	18.5	19.4
7	65.2	65.4	65.3	84.5	84.1
8	77.1	77.4	77.5	65.0	66.5
9	18.0	19.0	18.9	73.1	74.7
10	27.0	27.6	27.6	74.1	74.2
11	27.2	27.6	27.7	108.9	110.1
12	31.8	29.7	29.8	147.6	146.1
13	145.8	145.2	144.3	31.3	32.0
14	109.3	109.6	109.1	31.4	32.0
15	90.6	90.6	90.0	144.8	143.9
16	85.0	84.6	88.5	109.8	110.5
17	117.4	117.7	117.8	90.4	90.9
18	119.3	117.7	117.7	85.2	85.5
19				117.4	118.0
20				119.4	118.1

Most of the brominated polyunsaturated fatty acids have been known that have one or more olefinic and/or acetylenic groups. Based on literature reviews, the antibacterial activities of acetylenic fatty acids were reported. Antibacterial activities of them have various inhibition zone against Gram-positive and Gram-negative bacteria.[28-30] One of the studies showed significant inhibition zones of acetylenic fatty acids and minimal-inhibitory concentrations (MIC) at 64 and 128 mg/ml against *Staphylococcus aureus* and *Escherichia coli*, respectively.[28] In this report, the antibacterial activities of C_{18} and C_{20} of *X. testudinaria* against *Vibrio* sp. are shown in Table-3.

Table-3: Antibacterial Activities of C_{18} and C_{20} (4 µg/25 µl)

Name of Bacteria	Zone of Inhibition (mm) (mean ± SD)		Control (+)
	Compound **1** (C_{18})	Compound **2** (C_{20})	(Ciprofloxacin)
Vibrio harveyi	7.86 ± 0.24	6.20 ± 0.10	24.80 ± 1.32
Vibrio parahaemolyticus	6.50 ± 0.20	6.23 ± 0.06	21.63 ± 0.47
Vibrio alginolyticus	7.56 ± 0.46	6.63 ± 0.58	23.77 ± 0.84

The two known compounds were tested for antibacterial activities against *Vibrio harveyi* (M-120), *V. alginolyticus,* and *V. parahaemolyticus* (T-170) (shrimp pathogenic bacteria). As the result, compounds **1** and **2** possess weak inhibitory activities against *Vibrio* sp.[31] However, compound **1** showed higher inhibition zones than compound **2** by the agar diffusion method. Compound **1** can inhibit *V. harveyi* with the highest inhibition zone (7.86 mm), followed *V. alginolyticus* (7.56 mm), and *V. parahaemolyticus* (6.50 mm). This research was the first study of antibacterial activities against *Vibrio* sp.

From this result, it can be known what kind of pharmaceuticals will be utilized by the biological test.[1] The importance of information from this research can be used as supporting data regarding structure elucidation of the two known compounds, and additional data for their biological activities, in particular, antibacterial activities. For more details, the effectiveness of the compounds can be assayed based on further pharmaceutical tests.

CONCLUSION

The two known brominated (C_{18} and C_{20}) polyunsaturated fatty acids (methyl ester) were gained from the EtOAc extract of *Xestospongia testudinaria*. Compound **1** (C_{18}) could inhibit shrimp pathogenic bacteria *Vibrio harveyi* (M-120), *V. alginolyticus,* and *V. parahaemolyticus* (T-170) higher than compound 2 (C_{20}).

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