Chemical Composition, Antimicrobial, Cytotoxic and Antiplasmodial Activities of Three Sponges from Buton Islands, Indonesia

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Abstract

GC-MS analysis of the crude extracts of three different species of Indonesian marine sponges has been carried out for identification of bioactive compounds. The GC-MS analysis from Haliclona (Gellius) sp., Lamellodysidea herbacea, and Spheciospongia inconstans revealed the presence of 23, 21, 19 various compounds, respectively and mainly sterols and fatty acids. All the sponge species has been evaluated for antimicrobial activities, cytotoxicity using brine shrimp lethality bioassay and heme polymerization inhibitory activity assay for antiplasmodial activity. In this study, all the sponge species showed antimicrobial activities against at leastone of the test strains. Among them, the extract of sponge Lamellodysidea herbacea displayed activity against two Gram-positive bacteria (S. aureus and B. subtilis) and the Gram-negative bacteria V. cholerae, with inhibition zones of 10.3, 9.2 and 9.5 mm, respectively. The sponge Haliclona (Gellius) sp.*, showed significant activity against fungal pathogen C. albicans. The sponge Haliclona (Gellius)* sp., *displayed the ability to inhibit heme polymerization indicating an anti-Plasmodium function and also showed potent cytotoxic activity against the brine shrimp Artemia sp.*

Keywords: GC-MS analysis, antimicrobial, sponges,bioactive

Introduction

In general, natural products play a significant role in the development of drugs. The marine environment is largely unexplored and untapped in comparison with the terrestrial environment. Therefore, research efforts to the discovery of bioactive secondary metabolites have expanded from the land to the ocean. Numerous new secondary metabolites have been isolated, and many were revealed interesting pharmacological activities, most of which were described from marine invertebrates. Marine invertebrates, particular sponges, dominated these studies, due to readily available to collect by snorkeling or scuba diving.

So far, nearly 30% of all marine natural products or more than 4500 compounds have been isolated from marine sponges (Mehbub *et al.,* 2014). Sponges not only produce the largest number of all marine natural products currently known but also show the most significant chemical diversity of marine natural products including alkaloids, peptides, terpenes and polyketides as most important groups of compounds (Proksch *et al.,* 2003). Anti-cancer, anti-infective, anti-inflammatory, neuroprotective, antifouling and several other bioactivities have been disclosed for members of chemical compounds from marine sponges (Mehbub *et al.,* 2014; Blunt *et al.,* 2016).

In term of our ongoing research program aimed at the discovery of marine bioprospecting from Indonesian coast held as one of the richest biodiversity hotspots in the world. We had the opportunity to analyze three different species of sponges. These sponges were collected from Buton Islands (Southeast Sulawesi), and from the crude extract of sponges we have evaluated antimicrobial activities against four human pathogenic bacteria and one pathogenic fungus as well as cytotoxic activity using the brine shrimp lethality bioassay. We also characterized the chemical constituent in the crude extracts by using modern sensitive gas chromatography–mass spectrometry (GC–MS).

Material and Methods

Sponge materials and extraction

Three marine sponges were collected by scuba diving from three sites of the Buton Islands and immediately frozen at -20^{o}C until extracted. A voucher record of each specimen was deposited at the Research Center for Oceanography of

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ijms.undip.ac.id
DOI: 10.14710/ik.ijms.22.3.147-154
Received : 09-02-2017
Accepted : 12-04-2017

Indonesian Institute of Sciences. The sponge species, locations, and depths of the collection were listed in Table 1. Identification of the sponges was based on their morphological characteristics. Each sponge sample with 200-250 g wet weight was homogenized and extracted with 500 ml MeOH and CHCl3 (3:1) at room temperature for 24 h. The sample was filtered, and the residue was repeatedly extracted (2 x 500 mL). Then, each sponge extract was evaporated under reduced pressure to obtain crude extracts.

Antibacterial activity

The screening for antibacterial activity of sponge extracts was carried out using the agar disk diffusion technique as described by Qaralleh *et al.* (2010), with slight modification. Method Briefly, the sample was prepared with a concentration of 100 µg.mL^{-1} in MeOH. A 20µl sample was dropped on a filter paper disc with 6 mm diameter. The paper disc was then placed on a Mueller Hinton Agar (Himedia) in a petri dish that had been inoculated with test bacteria (10^{7}CFU.mL^{-1}). Four reference strains of human pathogens were used in this work, including two Gram-negative bacteria (*Eschericia coli* ATCC 25922, *Vibrio cholerae* (ATCC 14035), two Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633). Inhibition of bacterial growth activity appeared as a clear zone around the paper disc. The inhibition zone was observed after incubation at 30°C for 20-24 hours and then measured using a caliper. As positive controls, ampicillin 10 µg.mL^{-1}will be utilized and methanol as a solvent control. All the assays were performed in triplicate.

Antifungal activity

The sample was prepared with a concentration of 100 µg.mL^{-1} in MeOH. A 20µl sample was dropped on a filter paper disc with 6 mm diameter. As a test fungi, *Candida albicans* ATCC 10231 was used and inoculums containing 10^{4} CFU.mL^{-1} was spread on potato dextrose agar.The anti-fungal activity was measured as a diameter of the inhibitory zones. The inhibition zone was observed after incubation at room temperature for 24–48 h. Nystatin (30 µg.mL^{-1}) was used as positive control and methanol as a solvent control. All the assays were performed in triplicate (Qaralleh *et al.,* 2010)

Cytotoxic activity

The cytotoxic activity of sponge extracts was evaluated using the brine shrimp lethality bioassay with different concentrations (50, 100, 200, 400 mg.mL^{-1}) as described by Ullah *et al*. (2013), with some modification. Extract and controls were prepared in triplicates. The brine shrimp eggs were placed in hatching tank containing 1 L of sea water, aerated for 48 h a room temperature to hatch, under continuous light. After 48 h, 10 brine shrimps were placed in a small container filled with sea water and different concentrations of the extracts. Survivors were calculated after 24 h of incubation, and the percentage of deaths at each concentration and controls (seawater) were determined.

Heme polymerization inhibitory activity assay

A total of 100 mL solution of 1 mM hematin in 0.2 M NaOH was put into a 96-well microculture plate, and then a 50 mL assay solution with various concentrations, ranging from 0.3125 to 20 mg.mL^{-1} (Figure 1.), was added to each well. Glacial acetate acid (50 mL, pH 2.6) was added to the microculture to initiate a heme polymerization reaction. The microculture was then incubated at 37 ºC for 24 h to obtain perfect polymerization. After the period of incubation, the microculture was centrifuged and the resulting deposits were washed three times using 200 mL of dimethyl sulfoxide (DMSO). The solution of 0.1 M NaOH (200 mL) was subsequently added to the deposits in each well of microculture. Absorbance values were read at 405 nm using a microplate reader, Infinite® 200 PRO (Tecan Austria GmbH). The value of heme polymerization inhibitory activity was in IC_{50} (Basilico *et al.,* 1998). Aquadest and chloroquine were used for negative and positive control, respectively. The percentage inhibition of heme polymerization was calculated by the formula:

$$\text{Inhibition} = \frac{\beta - \text{hematin0} - \beta - \text{hematin1}}{\beta - \text{hematin0 X } 100\%}$$

Note :
β-hematin$_0$ = Concentration of negative control
β-hematin$_1$ =Concentration of fraction test

Table 1. Sponge species and collection sites

Sponges	Places of collection	Depth (m)	Voucher specimen number
Haliclona (*Gellius*) sp.	Wa Ara Village, Lakudo District (122°29'17.05"E 5°36'9.76" S)	1-5 m	1BTN2016
Lamellodysidea herbacea	Kapoa Village, Kadatua District (122°28'30.25"E 5°31'1.42"S)	1-5 m	2BTN2016
Spheciospongia inconstans	Wabula Village, Wabula District (122°52'2.75"E 5°36'55.94"S)	1-5 m	3BTN2016

Zoochemical analysis

The qualitative analysis of the possible secondary metabolites present in the sponge samples was done following a conventional standardized protocol described by Abioye *et al*. (2013).

Gas Chromatography Mass Spectroscopy (GC-MS) analysis

GC-MS analysis was was carried out at the Regional Health Laboratory (Labkesda), DKI Jakarta. The potent open-column samples were injected into Agilent Technologies 7890 GC-Mass with autosampler and 5975 Mass Selective Detector and Chemstation Data System. This instrument was set to electron impact using ionization mode with electron energy 70eV. The column used for analysis was a capillary column HP Ultra 2L, length (m) 30x0.25 (mm) I.D. X 0.25 (µm) film thicknesses

Results and Discussion

Sponges (phylum Porifera), constitute one of the most primitive of multicellular animals (Metazoa) and play important to the overall ecology of coral reefs. Natural products chemists have been isolating bioactive lead compounds from sponges and commonly described as chemical defense tools to protect against a predator (Burns *et al.,* 2003; Rohde *et al.,* 2015). Sponges have continuously been an important part of Indonesian coral reef communities. Since, the first marine compound, named Laulimalide, isolated from Indonesian sponge *Hyattella* sp., continuing until now more than 50 publications have reported on the bioactive compounds from Indonesian marine sponges (Putra and Murniasih, 2016).

Antimicrobial activities

The antimicrobial activities from the marine sponges were evaluated against four human pathogenic bacteria and one pathogenic fungus using the agar disk diffusion assay (Table 2.). According to agar disk diffusion assay of bacteria recorded in this study, all the sponge extracts showed antimicrobial activity against at least one of the test strains.

Against the Gram positive-bacteria, the marine sponge *L. herbacea* extract displayed the highest activity with inhibition zone 10.3 and 9.2 mm against *S. aureus* and *B. subtilis*, respectively. Furthermore, in vitro growth inhibition of *V. cholerae* was observed in the extract of sponge *L. herbacea*, with inhibition zone 9.5 mm. The antibacterial activity from *L. herbacea* might be due to the presence of Polybrominated Diphenyl Ethers (PBDEs) that have been reported to have inhibitory potential against the Gram-positive bacteria and the Gram-negative bacteria (Liu *et al.,* 2016). *Haliclona* (*Gellius*) sp., showed the highest activity with inhibition zone 9.5 mm against *B. subtilis*. The crude extract of sponge *S. inconstans* showed relatively high activity against the gram negative-bacteria *E. coli* (Table 2.).

Based on the results from the antifungal assay, *Haliclona (Gellius)* sp., exhibited to be the most promising antifungal activity. In other studies, the antifungal compound from *Haliclona* sp. was discovered (Clark *et al.,* 2001; Wattanadilok *et al.,* 2007; El-Amraoui *et al.,* 2013). Antifungal compounds were isolated from *Haliclona* include a sphingosine derivatives, named haliscosamine from from the Moroccan marine sponge *Haliclona viscose* (El-Amraoui *et al.,* 2013), nortetillapyrone from *Haliclona cymaeformis*, collected from the Gulf of Thailand (Wattanadilok *et al.,*2007), and alkyl amino alcohols, halaminols A-C from sponge *Haliclona* sp., collected on the Great Barrier Reef (Clark *et al.,* 2001).

Brine shrimp assay

The results of the brine shrimp bioassay showed that all the sponge extracts showed cytotoxic activity. Mortality of *Artemia* larvae is shown after a 24 hours exposure to the various concentrations of the sponge extracts. The LC_{50} values of the crude extract of *Haliclona* (*Gellius*) sp., *L. herbacea*, and *S. inconstans* were found 92.7, 211.3, 109.4 µg.mL^{-1}, respectively (Table 3.). The crude extracts resulting in LC_{50} values less than 100 µg.mL^{-1} were categorized as having strong cytotoxic and indicated the presence of potent bioactive compounds for further investigation such as anticancer. *Artemia* nauplii have been shown to present their highest sensitivity to marine invertebrates such as sponges and soft corals (Putra and Murniasih 2016).

Heme polymerization inhibitory activity assay

The crude extract of sponge *Haliclona* (*Gellius*) sp., *Spheciospongia inconstans*, and *Lamellodysidea herbacea* were showed inhibitory activity with IC_{50} values of 11.3, 37.8 and 170.7 µg.mL^{-1}, respectively. According to Baelmans *et al.* (2000),a compound could be considered to have heme polymerization inhibitory activity if it has heme polymerization inhibitor IC_{50}values smaller than the limit of chloroquine diphosphate, (37.5 mM or 12 mg.mL^{-1}). Thus, the extract of *Haliclona* (*Gellius*) sp. displayed heme polymerization inhibitory

Table 2. Antimicrobial activities in the crude extracts of three marine sponges

Sponge species	Zone of inhibition (mm)				
	S. aureus	*B. subtilis*	*E. coli*	*V. cholerae*	*C. albicans*
Haliclona (*Gellius*) sp.	8.70	9.50	8.30	6.60	9.2
L.amellodysidea herbacea	10.30	9.20	8.00	9.50	5.2
Spheciospongia inconstans	8.10	8.30	9.45	8.90	7.8
Ampicillin	31.60	10.5	28.70	23.7	-
Nystatin	-	-	-		20.1

Table 3. Effect of the extract of sponges on brine shrimp survival

Sponges	LC_{50} (µg.mL^{-1})	Regression equation	R^2
Haliclona (*Gellius*) sp.	92,7422	y = 4,5845x - 4,0189	R^2 = 0,9961
Lamellodysidea herbacea	211,2613	y = 3,3719x - 2,8371	R^2 = 0,8682
Spheciospongia inconstans	109,4088	y = 3,9699x - 3,0932	R^2 = 0,9213

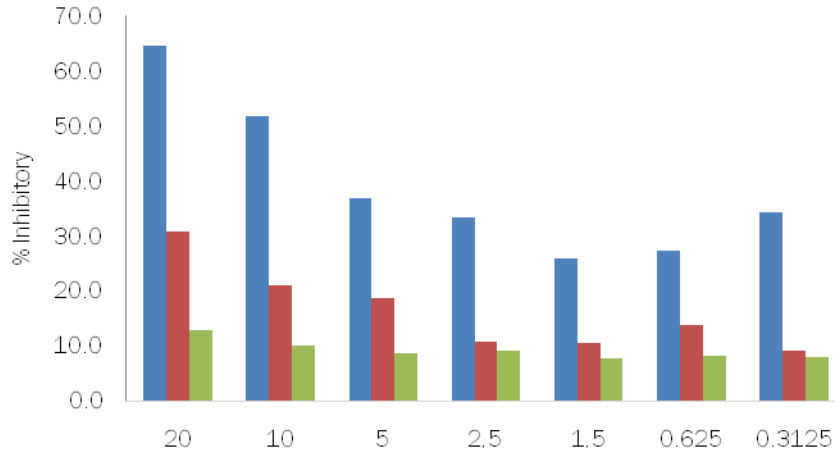


Figure 1. Heme polymerization inhibitory activity assay of sponge extracts
Note. : *Haliclona (Gellius)sp,* : *Speciospongia inconstants,* : *Lamellodysidea herbacea*

activity. Furthermore, the sponge genus of *Haliclona* are known to contain the antiplasmodial compounds named *Haliclona cyclamine* A (Mani *et al.,* 2011)

Zoochemical analysis

This zoochemical analysis of the crude extract of sponges showed that *Haliclona (Gellius)* sp., *L. herbacea*, and *S. inconstans* were positive alkaloids, saponin, and terpenoid. The extracts that tested positive for tannin was *L. herbacea*.

Over the past few years, Gas Chromatography-Mass Spectrometry (GC-MS) has become firmly established as a key technological platform for chemical compound profiling in both plant and marine organisms. Hence the present study we analyzed the crude extract of marine sponges to characterized the bioactive metabolites using GC-MS techniques. The bioactive metabolites with their retention time (RT), molecular formula, molecular weight (MW), concentration (peak area %) are presented in Tabel 5-7. The results of GC-MS from the sponge extracts contained numerous bioactive compounds belonging to various chemical classes, particularly sterols and fatty acids. Recently, more than 250 polar sterols have been isolated from marine sponges, with three types of the sterol *viz.* Δ^5-sterol, Δ^7-sterol and $\Delta^{5,7}$-sterol (Sarma *et al.,* 2005). It has been hypothesized that the sterols in cell membranes of the sponge are related to the presence of unusual fatty acids in their phospholipids.

The marine sponge genus *Haliclona* (Demospongiae) has been widely studied and well recognized as a rich source of secondary metabolites including steroids, alkaloids, cyclic peptides, terpenoids, and unsaturated fatty acids. Some of these metabolites exhibited interesting biological activities such as the cytotoxic and antimicrobial activity. Previous chemical study of the sponge, *Haliclona* sp., resulted in unusually fatty acids (Aratake *et al.,* 2009) and many sterols (Elenkov *et al.,* 1999; Cheng *et al.,* 2013). In the Black Sea *Haliclona* species, the Δ^5-sterols

accounted for 2% from the total sterol mixture. Furthermore, six unusual a-nor sterols resulted from the Sponge *Haliclona oculata* collected from Hainan Island, People's Republic of China (Yu *et al.,* 2006). *Haliclona (Gellius)* sp. metabolites obtained from the crude extract using GC-MS analysis revealed that there were 23 different compounds, mainly constituents of sterols (Table 5). *Haliclona (Gellius)* sp., from Buton Islands, has a similar sterol profile with those of other species belonging to the same genus (Elenkov *et al.,* 1999; Yu *et al.,* 2006). All these sponges contain Δ^5-sterols as a dominant constituent from *Haliclona (Gellius)* sp.

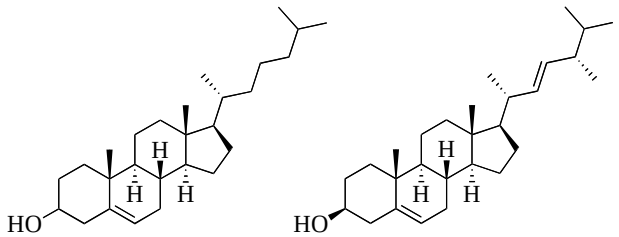
One of the most widely studied from the sponges of the family Dysideidae is the species of the genus *Lamellodysidea* (formerly known as *Dysidea*), *L. herbacea*. The majority of its secondary metabolites can be divided into three chemical classes: polychlorinated amino acid derivatives, sesquiterpenoids, and polybrominated diphenyl ethers (PBDEs) (Hanif *et al.,* 2007). These secondary metabolites have been found to exhibit avariety biological activities such as antimicrobial, anti-inflammatory and cytotoxic activity. The GC-MS analysis of *L. herbacea* extract revealed the presence of various compounds (21 different compounds), such as phenolic compounds, hexadecanoic acid, methyl ester and benzyl alcohol

Table 4. Zoochemical analysis from the crude extract of sponges

Zoochemical	*Haliclona(Gellius)* sp.	*Lamellodysidea herbacea*	*Spheciospongia inconstans*
Alkaloid	+	+	+
Tannin	-	+	-
Saponin	+	+	+
Terpenoid	+	+	+

Table 5. GC-MS spectral analysis of the crude extract of the sponge *Haliclona(Gellius)* sp.

No.	RT (min)	Name of the compound	Molecular formula	Molecular weight	Peak Area (%)
1.	27.968	1,3,5-trimethyl-3,7-diazabicyclo[3.3.1]nonan-9-ol	$C_{10}H_{20}N_2O$	184	1.81
2.	28.155	Hexadecanoid acid, methyl ester	$C_{17}H_{34}O_2$	270	1.55
3.	28.941	Piperazine, 2,5-dimethyl-3-(2-methylpropyl)	$C_{10}H_{22}N_2$	170	2.68
4.	29.368	(9E)-9-octadecenoic acid	$C_{18}H_{34}O_2$	282	1.15
5	29.389	N-(N-allylformamide)ethyleneimine	$C_6H_{10}N_2O$	126	1.60
6.	29.506	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	2.23
7.	30.009	13-methyloxacyclotetradecan-2-one	$C_{14}H_{26}O_2$	226	1.21
8.	30.037	n-Propyl decyl ether	$C_{13}H_{28}O$	200	2.31
9.	30.844	Hexanedioic acid, BIS(2-ethylhexyl) ester	$C_{22}H_{42}O_4$	370	6.97
10.	31.389	Trans-2-methyl-4-n-pentylthiane, S,S-dioxide	$C_{11}H_{22}O_2S$	218	4.39
11.	31.775	8-hexadecenal, 14-methyl-, (Z)	$C_{17}H_{32}O_2$	252	4.63
12.	32.140	Thiophene, 2,5-bis(1,1-dimethylethoxy)	$C_{12}H_{20}O_2S$	228	1.09
13.	32.402	15-hydroxypentadecanoic acid	$C_{15}H_{30}O_3$	258	5.28
14.	32.864	(S)(+)-Z-13-methyl-11-pentadecen-1-ol acetate	$C_{18}H_{34}O_2$	282	3.91
15.	33.512	Methyl 3-ethoxycarbonyl-2-octylcyclopropeneoctanoate	$C_{23}H_{40}O_4$	380	1.71
16.	33.926	Cholest-5-en-3-ol (3.beta)-, acetate	$C_{29}H_{48}O_2$	428	2.48
17.	35.340	hexadecadienoic acid, methyl ester	$C_{17}H_{30}O_2$	266	1.97
18.	35.615	3□-hydroxy-11-cholenic acid methyl ester	$C_{25}H_{40}O_3$	388	1.77
19.	35.822	(22E)-cholesta-5,22-dien-3-ol	$C_{27}H_{44}O$	384	7.24
20.	36.284	cholesterol	$C_{27}H_{46}O$	386	13.91
21.	36.850	ergosta-5,22-dien-3-ol, (3.beta.,2,2E,24S)	$C_{28}H_{46}O$	398	12.43
22.	37.712	ergosta-5,24(28)-dien-3.beta.-ol	$C_{28}H_{46}O$	398	4.83
23.	39.235	□-sitosterol	$C_{29}H_{50}O$	414	8.60



cholesterol ergosta-5,22-dien-3-ol, (3.beta.,2,2E,24S)

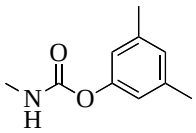
Figure 2. Major sterols compounds from of the crude extract the sponge *Haliclona(Gellius)* sp.

(Table 6.). Some of the GC-MS peaks remained unidentified, due to of lack of authentic samples and library data of corresponding compounds, particularly for polychlorinated amino acid derivatives, and polybrominated diphenyl ethers (PBDEs).

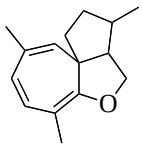
The GC-MS chromatogram of the crude extract of sponges *S. inconstans* exhibited 19 different compounds (Table 7). Δ^5-sterols and fatty acids were identified as a dominant constituent from *S. inconstans*. Stigmasta-5,22-dien-3β-ol, acetate, and 26-Nor-5- cholesten-3β−ol-25-one were shown

Table 6. GC-MS spectral analysis of the crude extract of the sponge *Lamellodysidea herbacea*

No.	RT (min)	Name of the compound	Molecular formula	Molecular weight	Peak Area (%)
1.	20.963	1H-cyclohepta[B]cyclopenta[C]furan,2,3,3A,4-tetrahydro-3,6,9-trimethyl	$C_{15}H_{20}O$	216	9.68
2.	21.721	Phenol,3,5-dimethyl-methylcarbamate	$C_{10}H_{13}NO_2$	179	10.17
3.	25.086	Heptadecane	$C_{17}H_{36}$	240	1.55
4.	26.045	1-methyl-4-(1-methylethylidene)-1-cyclohexene	$C_{10}H_{16}$	136	1.00
5.	27.314	Neophytadiene	$C_{20}H_{38}$	278	2.46
6.	27.769	6-octen-1-ol, 3,7-dimethyl-, acetate	$C_{12}H_{22}O_2$	198	1.03
7.	27.907	Benzenemethanol, .alpha.,4-dimethyl	$C_9H_{12}O$	136	9.79
8.	28.196	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	6.94
9.	28.238	2-cyclononen-1-one, 7-acetyl-3-methyl-9-(1-methylethylidene)-, (Z)-(.+-.)	$C_{15}H_{22}O_2$	234	3.39
10.	28.424	Bicyclo[3.3.0]octan-2-one, 7-ethylidene	$C_{10}H_{14}O$	150	1.10
11.	29.044	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.31
12.	29.437	Cyclohexanone, 5-methyl-2-(1-methylethyl)-,cis	$C_{10}H_{18}O$	154	1.24
13.	30.023	(9z)-9,17-octadecadienal	$C_{18}H_{32}O$	264	2.54
14.	30.092	Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.39
15.	30.327	1,3-dichloro-2-hydroxycarbazole	$C_{12}H_7Cl_2NO$	252	4.45
16.	30.809	1,4,8-cyclododecatriene	$C_{12}H_{18}$	162	1.16
17.	30.885	Bicyclo[4.3.1]dec-1(9)-ene	$C_{10}H_{16}$	136	2.18
18.	31.196	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	2.69
19.	31.451	Palmitoyl chloride	$C_{16}H_{31}ClO$	274	5.28
20.	32.361	4-methyl-thiazol-5-acetaldehyde	C_6H_7NOS	141	3.28
21.	33.092	Squalene	$C_{30}H_{50}$	410	1.35

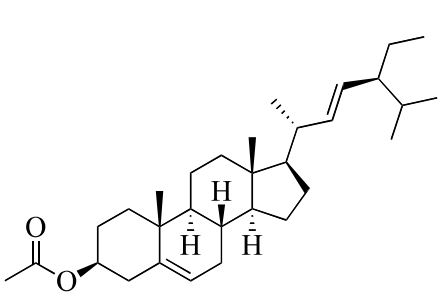


Phenol, 3,5-dimethyl-, methylcarbamate

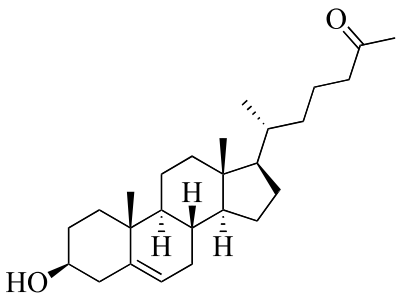


1H-cyclohepta[B]cyclopenta[C]furan,2,3,3A,4-tetrahydro-3,6,9-trimethyl

Figure 3. Major secondary metabolites from the crude extract of the sponge *Lamellodysidea herbacea*



Stigmasta-5,22-dien-3-ol, acetate,(3.beta)



26-Nor-5-cholesten-3.beta.-ol-25-one

Figure 4. Major sterol compounds from of the crude extract the sponge *Spheciospongia inconstans*

Table 7. GC-MS spectral analysis of the crude extract of the sponge *Spheciospongia inconstans*

No.	RT (min)	Name of the compound	Molecular formula	Molecular weight	Peak Area (%)
1.	27.898	1-Hydroxysulfonyl-3,4,4-trimethyl-2-azetidinone	$C_6H_{11}NO_4S$	193	1.23
2.	29.989	oleic acid	$C_{18}H_{34}O_2$	282	5.11
3.	30.237	6-methyl-2-tridecanone	$C_{14}H_{28}O$	212	3.08
4.	30.306	piperazine, 2,5-dimethyl-, cis	$C_6H_{14}N_2$	114	1.08
5.	30.726	methyl(1Z)-N-[(Z)-1-methylethyl]-3-phenylpropanimidoate	$C_{13}H_{19}NO$	205	2.47
6.	30.837	1-(hexadecyloxy)ethylene	$C_{18}H_{36}O$	268	3.46
7.	31.775	Batilol	$C_{21}H_{44}O_3$	344	3.51
8.	33.505	Spiro[4.5]decane, 6-methylene	$C_{11}H_{18}$	150	4.96
9.	33.636	(22E)-ergost-22-en-3-ol	$C_{28}H_{48}O$	400	1.86
10.	33.781	Cholesta-4, 6-dien-3-ol, benzoate, (3.beta.)	$C_{34}H_{48}O_2$	488	4.91
11.	33.926	Stigmastan-3.5-diene	$C_{29}H_{48}$	396	4.91
12.	34.291	Cholest-5-en-3-yl palmitate	$C_{43}H_{76}O_2$	625	3.42
13.	35.567	Stigmastan-6,22-dien,3,5-dedihydro	$C_{29}H_{46}$	394	7.99
14.	35.801	Stigmasta-5,22-dien-3-ol, acetate,(3.beta)	$C_{31}H_{50}O_2$	454	10.45
15.	36.270	26-Nor-5-cholesten-3.beta.-ol-25-one	$C_{26}H_{42}O_2$	386	27.65
16.	36.822	5, 6-dihydro-3a-ergosterol	$C_{28}H_{46}O$	398	5.86
17.	37.532	Cholesta-4, 6-dien-3-one	$C_{27}H_{42}O$	382	4.87
18.	39.159	beta.-sitosterol	$C_{29}H_{50}O$	414	1.60
19.	39.201	Stigmasta-5-en-3-ol	$C_{29}H_{50}O$	414	1.35

to be the main sterols in the sponge extract. The result is a previous investigation from the Caribbean sponge *Spechiospongia vesparia* found a phospholipid named (6Z)-2-methoxy-6-hexadecenoic acid and three group of sterols, Δ^5, Δ^0 and $\Delta^{5,7}$(Roberto *et al.,* 1998).

Conclusion

Marine sponges collected from Buton Islands have shown to possess various bioactive compounds and also have potential biological activities such as antimicrobial, cytotoxic and antiplasmodial activities. In our studies, the most interesting species are *L. herbacea* and *Haliclona (Gellius)* sp. These organisms will be subjected to detailed research for the isolation of biologically active compounds along with the search for new compounds.

Acknowledgements

This work was supported by a research grant from the Coral Reef Rehabilitation and Management Program-Coral Triangle Initiative (COREMAP CTI-LIPI 2016 No. 10876401/ADB LOAN No. 3094-INO), Indonesian Institute of Sciences awarded to Tri Aryono Hadi and Masteria Yunovilsa Putra

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