

Effects of Indonesian marine sponges ethanol extracts on the lipid profile of hyperlipidemic rats

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ABSTRACT

This study was aimed to investigate the effects of ethanol extracts of Indonesian marine sponges (*Callyspongia* sp., *Melophlus sarasinorum*, and *Xestospongia* sp.) on the lipid profile of hyperlipidemic rats. The antihyperlipidemic study of these sponges is firstly reported in this study. Experimental hyperlipidemic rats were induced by daily intake of propylthiouracil (1.8 mg/200 g b.wt and quail yolk (10 ml/kg) for the duration of 3 weeks. Hyperlipidemic rat groups were administered orally with three doses (30, 60, and 120 mg/kg) of the ethanol extracts for 1-week onward. Blood sample was then collected via intracardiac puncture and serum was biochemically analyzed. Ethanol extracts of *Callyspongia* sp., *M. sarasinorum*, and *Xestospongia* sp. at doses of 60 and 120 mg/kg exhibited a significant reduction of cholesterols, triglycerides, and low-density lipoprotein. These doses also significantly increased the high-density lipoprotein level. Levels of atherogenic indices (Atherogenic Index, Atherogenic Index Plasma, Castelli's Risk Index-I, and Castelli's Risk Index-II) were also decreased by both doses with percentages protection ranging from 70.6% to 81.6%. These results showed that ethanol extracts of *Callyspongia* sp., *M. sarasinorum*, and *Xestospongia* sp. exhibited a lipid-lowering activity in hyperlipidemic rats. Hence, these extracts could be used as sources of lead molecules in the development of natural lipid-lowering agents from marine species.

INTRODUCTION

Cardiovascular Disease (CVD) is one of the major causes of death in the global human population. In 2016, 17.9 million people died each year from CVD (WHO, 2017). The risk factors of CVD include hyperlipidemia, which is a condition with elevated levels of low-density lipoprotein (LDL), cholesterol, and triglycerides and with low levels of high-density lipoprotein (HDL) cholesterols. Medications such as statins can be used to lower the cholesterol levels in the blood (Clark *et al.*, 2012). However, in Indonesia, native people preferably apply traditional medicines as an alternative than using chemical drugs to prevent

and reduce cholesterol levels in the blood. This behavior is also supported by the rich biodiversity of this nation (Dahuri, 2003)

Indonesia's vast marine biodiversity provides an opportunity for biota to be used as a new source for new medicines. One of the marine biotas that has bioactive compounds and has the potential as a medicinal ingredient is marine sponges. Sponges such as *Callyspongia* sp., *Melophlus sarasinorum*, and *Xestospongia* sp. can be studied for their activity for their lowering cholesterol level activity in the blood (Artanti *et al.*, 2016; Dahuri, 2003). In previous studies, these sponges are reported to exhibit potential pharmacological activities, such as antibacterial, anticancer, antifungal, and many unknown benefits (Cita *et al.*, 2017; Menggelea *et al.*, 2015; Tapilatu, 2015). Compounds isolated from sponges are secondary metabolites containing steroids, alkaloids, flavonoids, terpenoids, saponins, and phenols (Hariani *et al.*, 2014; Ivanchina *et al.*, 2011; Menggelea *et al.*, 2015). In

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addition, compounds isolated from sponges-associated fungi showed a lipid-lowering activity assayed using *in vitro* and *in vivo* models (Blunt and Munro, 2008). Thus, in this study, we aimed to determine the effectiveness of *Callyspongia* sp., *M. sarasinorum*, and *Xestospongia* sp. ethanol extracts as an antihyperlipidemic based on the lipid profile in male Wistar rats.

MATERIALS AND METHODS

Sponges collection

Sponge samples of *Callyspongia* sp. (1 kg), *M. sarasinorum* (1.8 kg), and *Xestospongia* sp. (1.2 kg) (Fig. 1) were collected by hand with SCUBA diving from the reef slope area of the Lapuko beach of Moramo district, Southeast Sulawesi, Indonesia, at a depth of 10 m, in May 2015. The expert staff of the Faculty of Fisheries and Marine Science of Universitas Halu Oleo carefully handled the procedure of samples collection and specimen identification. The collected sponges were separately stored in their respective cool boxes and urgently brought back to the laboratory for analyses. Specimens of these sponges were deposited with voucher numbers of UHO-2015-01, UHO-2015-02, and UHO-2015-04, respectively.

Extraction

The dried sponge samples of *Callyspongia* sp. (0.98 kg), *M. sarasinorum* (1 kg), and *Xestospongia* sp. (1 kg) were macerated with ethanol (96%) for three times each for 24 hours and filtered using Whatman filter papers. Their filtrates were concentrated under reduced pressure and yielded dried ethanol extracts. Each extract were labeled accordingly as EC (6 g) for *Callyspongia* sp., EM (10 g) for *M. sarasinorum*, and EX (8 g) for *Xestospongia* sp. All extracts were then stored in amber containers and kept in a refrigerator at 4°C until further use.

Animals and ethics

Male Wistar rats (100–200 g) used in this study were obtained from the veterinary clinic Drh. Rachmad Priyadi, Surabaya, Indonesia. The animals were adapted to the new environment for 1 week under standard environmental conditions, including ambient temperature of 25°C–27°C for 12/12 hours light and dark cycle. They were fed with standard commercially rats diet. Either food or water was provided *ad libitum* throughout the period of the study. The body weights of rats were daily recorded and no significant change was observed when compared with

those records from the beginning of the study. All experiments involving animals in this study were conducted in accordance with the Animal Ethics Committee of Halu Oleo University (approval number 11676/UN29.20/PPM/2017).

Experimental design

After 1 week of acclimatization, four animals were continuously maintained with standard rat diet and designated as the normal control group (Group I). Hyperlipidemia in 44 rats was induced by propylthiouracil (PTU) (1.8 mg/200 g of body weight) and quail yolk (Qy) (10 ml/kg of body weight). The induction was made two times a day for 3 weeks. In the morning, rats were given orally with quail yolk each 5 ml, while the combination of PTU (1.8/200 g b.wt.) and quail yolk (5 ml) was administered in the evening. At day 21 (3 weeks), hyperlipidemic rats were divided into designated groups (Groups II to XI) and treated accordingly for another 1 week.

Group I:	Normal control (standard diet, no treatment)
Group II:	High cholesterol control (PTU-quail yolk diet, no treatment)
Group III:	Positive control (PTU-quail yolk diet, 0.18 mg/200 g b.wt. simvastatin)
Group IV:	PTU-Qy diet + 30 mg/kg of <i>Callyspongia</i> sp. extract (EC30)
Group V:	PTU-Qy diet + 60 mg/kg of <i>Callyspongia</i> sp. extract (EC60)
Group VI:	PTU-Qy diet + 120 mg/kg of <i>Callyspongia</i> sp. extract (EC120)
Group VII:	PTU-Qy diet + 30 mg/kg of <i>M. sarasinorum</i> extract (EM30)
Group VIII:	PTU-Qy diet + 60 mg/kg of <i>M. sarasinorum</i> extract (EM60)
Group IX:	PTU-Qy diet + 120 mg/kg of <i>M. sarasinorum</i> extract (EM120)
Group X:	PTU-Qy diet + 30 mg/kg of <i>Xestospongia</i> sp. extract (EX30)
Group XI:	PTU-QY diet + 60 mg/kg of <i>Xestospongia</i> sp. extract (EX60)
Group XII:	PTU-QY diet + 120 mg/kg of <i>Xestospongia</i> sp. extract (EX120)

The designated groups of hyperlipidemic animals were treated orally with sponge extracts and simvastatin. Extracts and simvastatin (0.18 mg/ml) were diluted homogeneously in 0.5% Na-



Figure 1. (A) *Callyspongia* sp.; (B) *M. sarasinorum*; (C) *Xestospongia* sp.

CMC as the vehicle. After 1 week of treatment, blood was collected intracardially and transferred into Ethylenediaminetetraacetic acid (EDTA) tubes. Blood samples were let to stand for 1 hour at room temperature. Samples of blood-EDTA were then centrifuged at 3000 rpm for 10 minutes and serum was separated for further biochemical analyses.

Measurement of serum lipid profile

Level of total cholesterol (TC), total triglycerides (TG), and high-density lipoproteins (HDL) in serum were spectrophotometrically measured using assay kits (Rajawali Nusindo, Catalog No. 1096778, Jakarta, Indonesia). The very-low-density lipoproteins (VLDL) were calculated as $VLDL = [TG/5]$. The serum level of low-density lipoproteins (LDL) was calculated using Friedewald's formula as $LDL = TC - (TG/5 + HDL)$ (Friedewald *et al.*, 1972).

Atherogenic risk index, percentage protection, and atherogenic indices

Atherogenic Index (AI) was calculated as $AI = [(TC - HDL)/HDL]$. Meanwhile, percentage protection of marine sponges extracts treated hyperlipidemic rats toward atherogenicity was calculated as $\% \text{ protection} = [(AI_{PTU-QY} - AI_{treated}) / AI_{PTU-QY}] \times 100$. Atherogenic indices that are Atherogenic Index Plasma (AIP) was calculated as $AIP = \log [TG/HDL - C]$, Castelli's Risk Index-I (CRI-I) was calculated as $CRI-I = [TC/HDL - C]$, and Castelli's Risk Index-II (CRI-II) was calculated as $CRI-II = [LDL/HDL]$ (Chikezie *et al.*, 2018; Dianita *et al.*, 2016; Dobiášová *et al.*, 2001).

Chemical screening

The extracts of *Callyspongia* sp., *M. sarasinorum*, and *Xestospongia* sp. were chemically screened to detect the occurrence of alkaloids, flavonoids, tannins, triterpenoids and steroids, and saponins. These types of compounds were screened by using the Dragendorf reagent, magnesium-chloric acid reagent, ferric chloride reagent, Liebermann-Burchard reagent, and foam test, respectively (Harborne, 1973).

Statistical analysis

Results were statistically analyzed using SPSS Statistics 17.0 (IBM Inc., USA). The comparison of data among groups was carried out using the One-way ANOVA, followed by Tukey's Test. The values of $p < 0.05$ were considered statistically significant. Pearson's correlation was analyzed using the GraphPad Prism 5 (Graphpad Software, Inc., La Jolla, USA). Data were presented as mean \pm standard deviation (SD).

RESULTS AND DISCUSSION

Cholesterol-rich dietary intake has been closely linked with the development of hypercholesterolemia in humans and animals. The imbalance of circulating cholesterol influx and efflux through the liver and cholesterol esterification has been a feature of lipid metabolism disturbances. Excessive cholesterol and fats in the plasma will accumulate the formation of macrophage foam cells which then deposited in the arterial walls, leading to an early stage of vascular atherosclerotic lesions (Douglas and Channon, 2014; Yu *et al.*, 2013). The role of hypercholesterolemia in triggering atherosclerosis, along with other risk factors, such as

diabetes, hypertension, smoking, male gender, and inflammatory conditions has been recognized and received a particular concern among clinicians and health care professionals. The accumulation of atherogenic conditions is associated with evidence of cardiovascular diseases and chronic kidney diseases (Falk, 2006).

Indications of hypercholesterolemia are characterized by increased levels of cholesterol and triglycerides in plasma as well as a high ratio of LDL to HDL. In this study, hypercholesteremic condition made in rats as an animal model was developed by feeding the rats with PTU and quail yolk. The use of PTU in lowering hyperthyroidism has been associated with increased cholesterol levels (Santillo *et al.*, 1999). Hence, PTU induction in rats resulted in a hypothyroid hypercholesterolemia status. In addition, feeding rats with quail yolk has been reported to increase the cholesterol level due to its lipid nutritional compositions (Tungsaringkarn *et al.*, 2013). Our study showed that induction of hyperlipidemic condition in rats for 3 weeks with PTU and quail yolk was successfully developed, indicated by significant elevation of cholesterol (56.8%), triglycerides (77.9%), and LDL (86.3%) productions as compared to the normal control group (Fig. 2). On the contrary, the HDL level in these rats was significantly reduced about 62.9%, which suggested as a result of exogenous cholesterol and LDL up-take as well as the inhibition of cholesterol synthesis related to HDL origins (Deprès *et al.*, 2000). The comparison of lipid profile in rats serum between the normal control group and hyperlipidemic groups after induction with PTU-quail yolk diet was significantly different ($p < 0.05$), indicating an event of atherogenic condition. The development of atherogenicity may be due to the reduction of HDL level (Dianita *et al.*, 2016). The diet of PTU-quail yolk increased the risk of atherogenicity in rats, showing by significant elevations of AI, AIP, CRI-I, and CRI-II values by 94.4%, 144.2%, 85.1%, and 95.3%, respectively (Table 1).

In this study, treatment of hyperlipidemic rats with ethanol extracts of *Callyspongia* sp., *M. sarasinorum*, and *Xestospongia* sp. found to reduce levels of TC and TG. Significant reduction of cholesterol level was showed by *Callyspongia* sp. extract at 120 mg/kg (EC120 group), decreasing by 40.2%. This was followed by EM60, simvastatin, EM120, EX60, and EX120 groups with percentage decrements of 28.8%, 28.3%, 25.1%, 22.6%, and 20.2%, respectively. The reduction of cholesterol in the EC120 group found to be lower than simvastatin group ($p > 0.05$), indicating a potent TC-lowering activity. Moreover, a significant reduction of triglycerides level was exhibited by EM60 and EM120 groups with percentage decrements of 42.5% and 36.9%, respectively, and surprisingly lower than simvastatin (21.5%). In addition, EC120 and EX60 groups did reduce the triglycerides level by 28.4% and 27.9%, respectively. Treatment of hyperlipidemic rats with the extracts of marine sponges also reduced their LDLs level. Significant decreased LDLs level was shown by hyperlipidemic rats given with *Callyspongia* sp. at 120 mg/kg (EC120 group), having a lipoprotein reduction by 153.5%. This LDL-lowering activity was comparable to the simvastatin group (147.8% reduction). On the other hand, EM60 and EX120 groups exhibited a similar reduction in serum LDL with percentages reduction of 79% and 60%, respectively. Furthermore, significant increase of serum HDL level in hyperlipidemic rats with extracts of marine sponges was observed in groups treated with *Xestospongia* sp. extract at 60 mg/kg (EX60 group) and *Callyspongia* sp. extract at 120 mg/kg (EC120 group) in which the HDL levels increased

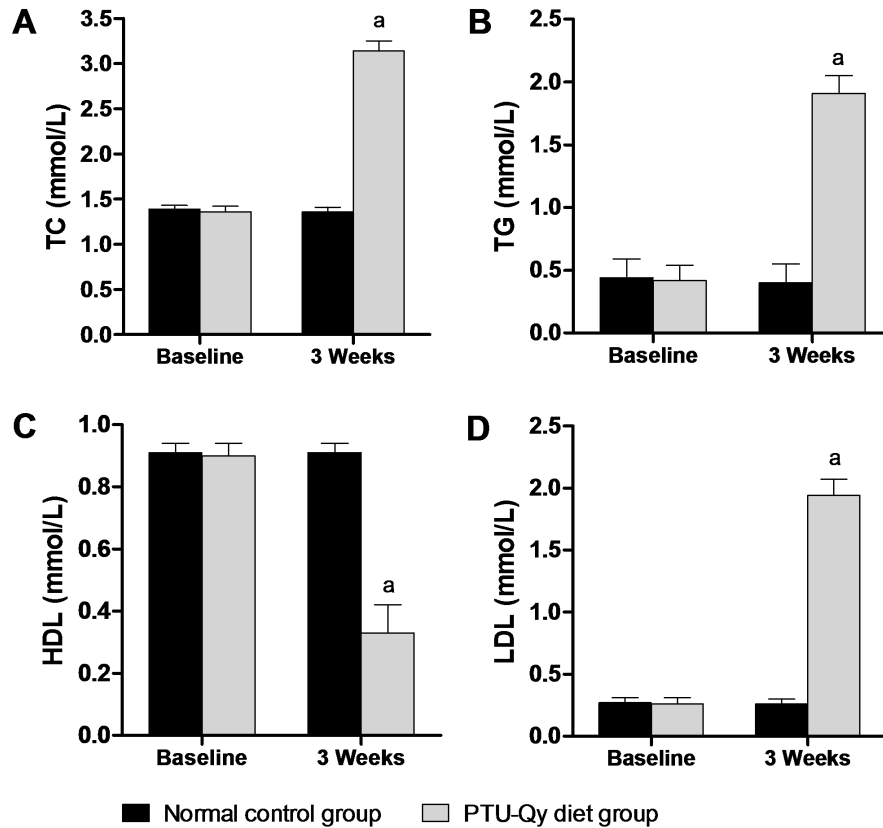


Figure 2. The increased levels of cholesterol (A), triglycerides (B), and LDL (D) and the decrement of HDL level (C) in the hypercholesterolemic rats group induced by PTU-Qy diet. Data are presented in mean \pm SD ($n = 4$ for the normal control group and $n = 44$ for PTU-Qy diet group). ^aSignificantly different versus normal control group ($p < 0.05$).

Table 1. Elevation of atherogenicity risk of PTU-Qy diet-induced rats.

Group	Duration	AI	AIP	CRI-I	CRI-II
Normal	Baseline	0.52 \pm 0.08	-0.33 \pm 0.15	1.52 \pm 0.08	0.29 \pm 0.05
	3 weeks	0.50 \pm 0.09	-0.38 \pm 0.15	1.50 \pm 0.09	0.29 \pm 0.05
PTU-Qy	Baseline	0.51 \pm 0.09	-0.34 \pm 0.12	1.51 \pm 0.09	0.30 \pm 0.05
	3 weeks	9.18 \pm 2.89 ^a	0.77 \pm 0.13 ^a	10.18 \pm 2.89 ^a	6.33 \pm 2.04 ^a

Data are presented in mean \pm SD ($n = 4$ for the normal control group and $n = 44$ for PTU-Qy diet group). ^a Significantly different versus normal control group ($p < 0.05$).

by 62.5% and 58.0%, respectively, comparable to simvastatin (61.6%). The rising of HDL level also observed EM60 and EX120 groups, having their serum HDL levels increased by 54.1% and 54.8%, respectively. Surprisingly, the HDL levels in EM60, EC120, and EX60 groups were restored significantly, comparable to the normal control and simvastatin groups ($p > 0.05$). These results are listed in Table 2 and illustrated in Figure 3.

Atherogenic indices are one of the important parameters that can be used to indicate the occurrence of atherosclerosis event (Chikezie *et al.*, 2018; Dobišová *et al.*, 2001). Our results showed that treatments with extracts of marine sponges decreased the atherogenic index (AI) and atherogenic indices levels (AIP, CRI-I, and CRI-II) in hyperlipidemic rats (Table 3). Simvastatin, EC120, EM60, EM120, EX60, and EX120 groups lowered the AI, CRI-I,

and CRI-II levels comparable to the normal control group ($p > 0.05$), and their levels were significantly different as compared to PTU-Qy diet group ($p < 0.05$). The lowering AIP levels of simvastatin and all sponges extracts were significantly different from normal control and PTU-Qy diet groups ($p < 0.05$). Indeed, AIP levels of EC120, EM60, EM120, EX60, and EX120 were comparable to the simvastatin group. It was consistent with AI, AIP, CRI-I, and CR-II levels that extracts of *Callyspongia* sp. (120 mg/kg), *M. sarasinorum* (60 and 120 mg/kg), and *Xestospongia* sp. (60 and 120 mg/kg) exhibited protection against atherogenicity in hyperlipidemic rats with percentages protection in the range of 70.6%–81.6% (Table 4). Pearson's correlation analysis revealed a significant positive correlation of AIP versus LDL level of all groups ($r = 0.9225$). Meanwhile, linear regression analysis of AIP

Table 2. Level of cholesterol, triglycerides, HDL, and LDL of the normal control group and hypercholesterolemic rats before and after treatment with *Callyspongia* sp., *M. sarasinorum*, and *Xestospongia* sp. extracts.

Group	Cholesterol level		Triglycerides level		HDL level		LDL level	
	3 weeks	4 weeks	3 weeks	4 weeks	3 weeks	4 weeks	3 weeks	4 weeks
Normal	1.36 ± 0.05 ^{ac}	1.36 ± 0.05 ^{ac}	0.40 ± 0.15 ^{ac}	0.44 ± 0.12 ^{ac}	0.91 ± 0.03 ^{ac}	0.85 ± 0.07 ^a	0.26 ± 0.04 ^{ac}	0.31 ± 0.06 ^{ac}
PTU-Qy	3.15 ± 0.12 ^b	3.21 ± 0.13 ^{bc}	1.92 ± 0.16 ^b	1.93 ± 0.14 ^{bc}	0.31 ± 0.07 ^b	0.31 ± 0.09 ^{bc}	1.97 ± 0.10 ^b	2.02 ± 0.11 ^{bc}
Simvastatin	3.17 ± 0.10 ^a	2.47 ± 0.17 ^{ab}	1.93 ± 0.15 ^b	1.58 ± 0.13 ^{ab}	0.37 ± 0.15 ^b	0.97 ± 0.22 ^a	1.91 ± 0.11 ^b	0.77 ± 0.17 ^{ab}
EC30	3.05 ± 0.07 ^a	2.90 ± 0.11 ^{ab,bc}	1.91 ± 0.08 ^b	1.82 ± 0.10 ^{bc}	0.34 ± 0.06 ^b	0.46 ± 0.07 ^{bc}	1.84 ± 0.04 ^b	1.60 ± 0.07 ^{ab,bc}
EC60	3.11 ± 0.04 ^a	2.77 ± 0.10 ^{ab,bc}	1.92 ± 0.16 ^b	1.73 ± 0.13 ^b	0.35 ± 0.06 ^b	0.61 ± 0.09 ^{ab,bc}	1.87 ± 0.05 ^b	1.38 ± 0.18 ^{ab,bc}
EC120	3.09 ± 0.12 ^a	2.20 ± 0.03 ^{ab,bc}	1.96 ± 0.08 ^b	1.53 ± 0.05 ^{ab}	0.32 ± 0.07 ^b	0.77 ± 0.08 ^a	1.87 ± 0.12 ^b	0.74 ± 0.05 ^{ab}
EM30	3.03 ± 0.14 ^a	2.72 ± 0.21 ^{ab,bc}	1.89 ± 0.13 ^b	1.76 ± 0.14 ^b	0.33 ± 0.06 ^b	0.51 ± 0.13 ^{bc}	1.83 ± 0.14 ^b	1.41 ± 0.13 ^{ab,bc}
EM60	3.17 ± 0.07 ^a	2.46 ± 0.08 ^{ab}	1.94 ± 0.12 ^b	1.36 ± 0.10 ^{ab}	0.35 ± 0.06 ^b	0.76 ± 0.06 ^a	1.94 ± 0.09 ^b	1.08 ± 0.06 ^{ab,bc}
EM120	3.19 ± 0.10 ^a	2.55 ± 0.03 ^{ab}	1.94 ± 0.07 ^b	1.42 ± 0.05 ^{ab}	0.34 ± 0.07 ^b	0.69 ± 0.03 ^{ac}	1.96 ± 0.11 ^b	1.21 ± 0.04 ^{ab,bc}
EX30	3.19 ± 0.08 ^a	3.07 ± 0.09 ^{bc}	1.66 ± 0.17 ^b	1.55 ± 0.11 ^{ab}	0.31 ± 0.10 ^b	0.43 ± 0.11 ^{bc}	2.12 ± 0.17 ^b	1.93 ± 0.17 ^{bc}
EX60	3.12 ± 0.20 ^a	2.55 ± 0.13 ^{ab}	1.88 ± 0.07 ^b	1.47 ± 0.05 ^{ab}	0.29 ± 0.12 ^b	0.78 ± 0.06 ^a	1.97 ± 0.13 ^b	1.10 ± 0.07 ^{ab,bc}
EX120	3.19 ± 0.07 ^a	2.66 ± 0.10 ^{ab}	1.94 ± 0.07 ^b	1.67 ± 0.05 ^{ab}	0.31 ± 0.10 ^b	0.68 ± 0.12 ^{ac}	2.00 ± 0.16 ^b	1.22 ± 0.15 ^{ab,bc}

Treatment of hypercholesterolemic rats was given 1 week onwards after induction for 3 weeks. Data are presented in mean (unit in mmol/L) ± SD ($n = 4$). ^aSignificantly different versus PTU-Qy control group ($p < 0.05$); ^bsignificantly different vs. normal control group ($p < 0.05$); ^csignificantly different versus simvastatin group ($p < 0.05$).

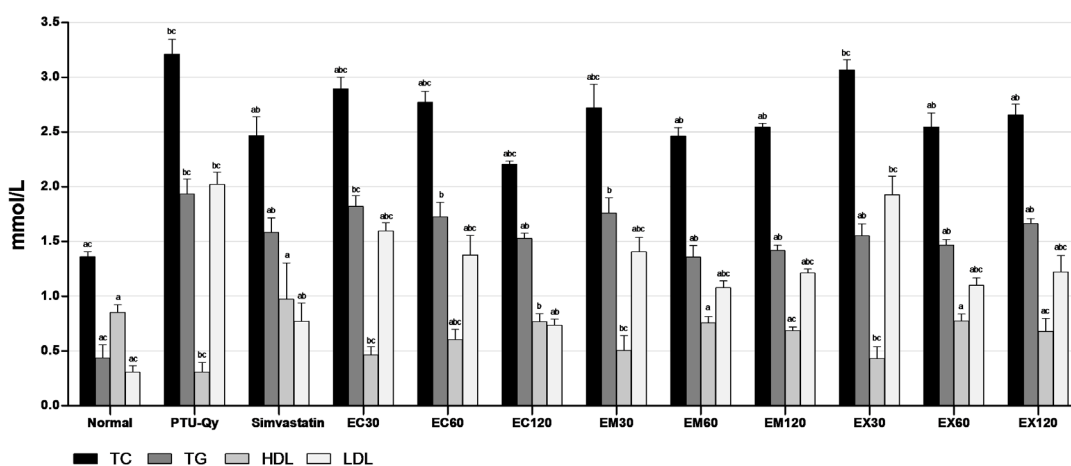


Figure 3. Effects of ethanol extracts of *Callyspongia* sp., *M. sarasinorum*, and *Xestospongia* sp. on serum lipid profile of normal and hypercholesterolemic rats. Data is presented as mean ± SD ($n = 4$). ^aSignificantly different vs. PTU-Qy control group ($p < 0.05$); ^bsignificantly different versus normal control group ($p < 0.05$); ^csignificantly different versus simvastatin group ($p < 0.05$).

versus LDL level showed a relatively fitted curve ($r^2 = 0.8510$) (Fig. 4). The reduction of these atherogenic indices by ethanol extracts of marine sponges indicated an alleviating activity of atherosclerosis. High doses of extracts also showed protections against the atherogenic condition in hyperlipidemic rats.

Biological activities of marine species on hypercholesterolemia and hyperlipidemia have been reported in some studies. Extracts and polysaccharides extracted from marine algae (seaweeds) have proven to exhibit antihyperlipidemic activity and a restoring activity on liver damage (Ren *et al.*, 1994; Wang *et al.*, 2014). In addition, compounds isolated from sponges-associated fungi showed lipid-lowering activity assayed using *in vitro* and *in vivo* models (Blunt and Munro, 2008; Li *et al.*, 2014). Our findings showed that extracts of *Callyspongia* sp. (EC), *M. sarasinorum* (EM), and *Xestospongia* sp. (EX) have a potent antihyperlipidemic activity. The role of simvastatin in reducing cholesterol level has been proven due to the inhibition of

3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR). HMGCR is a rate-limiting enzyme that produces mevalonate as a precursor for cholesterol biosynthesis in the liver. Studies also showed that this drug has properties on anti-atherosclerosis, antioxidant, anti-inflammatory, vasodilatation, and antioxidant activities (Sparrow *et al.*, 2001; Stancu and Sima, 2001; Zhou and Liao, 2009). Our study suggests that compounds present in ethanol extracts of *Callyspongia* sp., *M. sarasinorum*, and *Xestospongia* sp. may exhibit antihyperlipidemic property similar to simvastatin and possibly other statin drugs. Statins therapy for hypercholesterolemia and hyperlipidemia may be effective when LDL and TG levels reduced and modest HDL level increased (Trentman *et al.*, 2016). However, the roles of compounds in extracts for treating hyperlipidemic condition may also vary; at least there are several types of marketed drugs used for this purpose, including inhibitors of HMGCR in the liver (endogenous production), inhibitors of intestinal cholesterol

Table 3. Atherogenic indices of normal and hypercholesterolemic rats after treatment with *Callispongia* sp., *M. sarasinorum*, and *Xestospongia* sp. extracts.

Group	AI	AIP	CRI-I	CRI-II
Normal	0.61 ± 0.14 ^a	-0.30 ± 0.14 ^{a,c}	1.61 ± 0.14 ^a	0.36 ± 0.10 ^a
PTU-Qy	10.31 ± 3.17 ^{b,c}	0.82 ± 0.12 ^{b,c}	11.31 ± 3.17 ^{b,c}	7.20 ± 2.38 ^{b,c}
Simvastatin	1.63 ± 0.49 ^a	0.22 ± 0.11 ^{a,b}	2.63 ± 0.49 ^a	0.85 ± 0.33 ^a
EC30	5.34 ± 0.77 ^{a,b,c}	0.60 ± 0.06 ^{a,b,c}	6.34 ± 0.88 ^{a,b,c}	3.52 ± 0.65 ^{a,b,c}
EC60	3.67 ± 0.67 ^{a,b}	0.46 ± 0.08 ^{a,b,c}	4.67 ± 0.77 ^{a,b}	2.34 ± 0.58 ^a
EC120	1.90 ± 0.23 ^a	0.30 ± 0.05 ^{a,b}	2.90 ± 0.27 ^a	0.97 ± 0.16 ^a
EM30	4.59 ± 0.95 ^{a,b}	0.55 ± 0.15 ^{a,b,c}	5.59 ± 1.09 ^{a,b}	2.88 ± 0.53 ^{a,b}
EM60	2.28 ± 0.27 ^a	0.26 ± 0.06 ^{a,b}	3.28 ± 0.31 ^a	1.44 ± 0.20 ^a
EM120	2.72 ± 0.13 ^a	0.32 ± 0.01 ^{a,b}	3.72 ± 0.15 ^a	1.77 ± 0.13 ^a
EX30	6.47 ± 1.83 ^{a,b,c}	0.56 ± 0.12 ^{a,b,c}	7.47 ± 2.12 ^{a,b,c}	4.75 ± 1.68 ^{a,b,c}
EX60	2.29 ± 0.11 ^a	0.28 ± 0.04 ^{a,b}	3.29 ± 0.13 ^a	1.42 ± 0.06 ^a
EX120	3.03 ± 0.74 ^a	0.40 ± 0.10 ^{a,b}	4.03 ± 0.85 ^a	1.88 ± 0.58 ^a

Data is presented as mean ± SD ($n = 4$). ^aSignificantly different vs. PTU-Qy control group ($p < 0.05$); ^bsignificantly different versus normal control group ($p < 0.05$); ^csignificantly different versus simvastatin group ($p < 0.05$).

Table 4. Percentage protection of *Callispongia* sp., *M. sarasinorum*, and *Xestospongia* sp. extracts and simvastatin in hypercholesterolemic rats.

Group	% Protection
Simvastatin	84.2
EC30	48.2
EC60	64.4
EC120	81.6
EM30	55.5
EM60	77.9
EM120	73.6
EX30	46.5
EX60	77.8
EX120	70.6

absorptions, and inhibitors of bile acids re-uptake to the liver (Mahamuni *et al.*, 2012). Chemical screening showed the occurrence of alkaloids, flavonoids, steroids, triterpenoids, and saponins in all sponge extracts (Table 5). Moreover, tannins were only detected in the extract of *M. sarasinorum*. The prospective of these natural products in cholesterol-lowering activity is still being investigated and some studies have shown positive results. Alkaloids have been reported to exhibit cholesterol-lowering effects by improving stability of LDL receptor mRNA, increasing uptake, and conversion of cholesterol into bile acids, down-regulating cholesterol biosynthesis at mRNA level of HMGCR, and improving cholesterol transport. The combination of alkaloids is suggested to provide better cholesterol synthesis inhibition and to promote cholesterol catabolism and excretion (Kou *et al.*, 2016; Pirillo and Capatano, 2015; Zhang *et al.*, 2018b). Studies have shown that flavonoids inhibited the HMGCR activity. In addition, the antioxidant activities by flavonoids prevent lipid peroxidation and scavenge free radicals. Accumulation of excessive LDL has led to the formation of modified oxidized-LDL (oxLDL), which plays a key role during

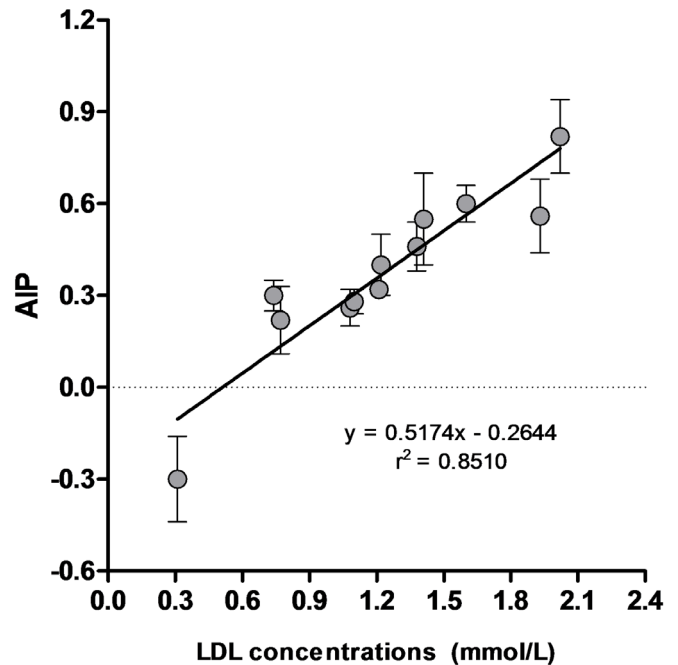


Figure 4. Linear regression curve of AIP versus LDL concentrations of all experimental groups

atherosclerosis. Flavonoids also block the oxLDL uptake by macrophages, preventing the formation of foam cells (Kumar and Pandey, 2013; Salvamani *et al.*, 2014). The lipid-lowering activity and anti-atherosclerosis of triterpenoids have reported on inhibition of LDL-induced lipid deposition aggregation in macrophages. The activity is due to down regulated of LDL receptor-related protein 1 (Zheng *et al.*, 2015). Triterpenoids have also shown inhibitory activity against *in vitro* HMGCR enzyme (Zhang *et al.*, 2018b). Steroids from plants and marine species have shown their lipid-lowering effect by binding activity to micelles, resulted in competition with cholesterol during intestinal absorption and transport. They also more hydrolyzed compared to cholesterol. Hence, their presence in intestine reduced the cholesterol solubility to form micelles, resulting in less cholesterol absorption and more cholesterol fecal excretion (Gupta *et al.*, 2011; Kritchevsky *et al.*, 1999; Trautwein *et al.*, 2003). Steroids and triterpenoids have also been reported as liver X receptors agonists which play a significant role in cholesterol homeostatis (Jayasuriya *et al.*, 2005). Saponins (triterpenoidal- and steroidal glycosides) have also been studied for their roles in cholesterol metabolism. Interaction between saponins and bile acids formed large micelles, thus interfere with the enterohepatic circulation of bile acids for intestinal absorption. As a result, the interaction then increased the fecal biliary excretion. The lipid-lowering activity of saponins is also due to their activity to modify LDL cholesterol levels (Francis *et al.*, 2002; Malinow, 1984). Polymeric tannins reduced plasma LDL levels and increased the fecal excretion of lipids and cholesterol in rats. The binding interaction between tannins-rich fiber and bile acids is suggested for cholesterol-lowering activity in humans. The lipid-lowering activity of tannins has also been associated with their antioxidant activity which prevents lipid peroxidation and

Table 5. Chemical screening of *Callyspongia* sp., *M. sarasinorum*, and *Xestospongia* sp. extracts.

Sponge sample	Code	Alkaloids	Flavonoids	Tannins	Triterpenoids/steroids	Saponins
<i>Callyspongia</i> sp.	EC	+	+	-	+	+
<i>M. sarasinorum</i>	EM	+	+	+	+	+
<i>Xestospongia</i> sp.	EX	+	+	-	+	+

scavenges free radicals (Gato *et al.*, 2013; Smeriglio *et al.*, 2017; Tebib *et al.*, 1994). Our study suggested that the combination of these types of compounds in extracts of *Callyspongia* sp., *M. sarasinorum*, and *Xestospongia* sp. may work synergistically for exhibiting lipids-lowering activity in the treated hyperlipidemic rats. The antihyperlipidemic activity of these sponges is firstly reported from this study.

CONCLUSION

Ethanol extracts of marine sponges *Callyspongia* sp., *M. sarasinorum*, and *Xestospongia* sp. exhibited a lipid-lowering activity in hyperlipidemic rats. The extracts also showed protection against atherosclerosis. The activities might be resulted in the roles of alkaloids, flavonoids, steroids, triterpenoids, saponins, and tannins. Hence, ethanol extracts of these sponges could be used as sources of lead molecules in the development of natural lipid-lowering agents from marine species.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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