Taylor & Francis
Taylor & Francis Group

Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

A new sterol from the Vietnamese marine sponge Xestospongia testudinaria and its biological activities

Hien Minh Nguyen, Takuya Ito, Nwet Nwet Win, Hung Quoc Vo, Hoai Thi Nguyen & Hiroyuki Morita

To cite this article: Hien Minh Nguyen, Takuya Ito, Nwet Nwet Win, Hung Quoc Vo, Hoai Thi Nguyen & Hiroyuki Morita (2018): A new sterol from the Vietnamese marine sponge Xestospongia testudinaria and its biological activities, Natural Product Research, DOI: 10.1080/14786419.2018.1465057

To link to this article: https://doi.org/10.1080/14786419.2018.1465057

Published online: 23 Apr 2018.

NATURAL PRODUCT RESEARCH, 2018
https://doi.org/10.1080/14786419.2018.1465057

Taylor & Francis
Taylor & Francis Group

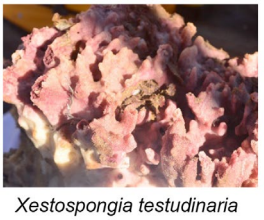
A new sterol from the Vietnamese marine sponge *Xestospongia testudinaria* and its biological activities

Hien Minh Nguyen[a], Takuya Ito[a], Nwet Nwet Win[a,b], Hung Quoc Vo[c], Hoai Thi Nguyen[c] and Hiroyuki Morita[a]

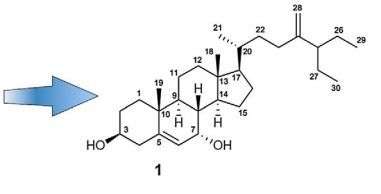
[a]Institute of Natural Medicine, University of Toyama, Toyama, Japan; [b]Department of Chemistry, University of Yangon, Yangon, Myanmar; [c]Faculty of Pharmacy, Hue University of Medicine and Pharmacy, Hue University, Hue City, Vietnam

ABSTRACT

A new sterol, langcosterol A (**1**), together with two known sterols **2** and **3**, were isolated from the marine sponge *Xestospongia testudinaria* collected in Vietnam. Their chemical structures were elucidated on the basis of extensive spectroscopic analyses and comparisons with published data. The new compound **1** and the known compound **3** exhibited moderate cytotoxic activities against three human cancer cell lines (A549, lung cancer; MCF7, breast cancer; HeLa, cervical cancer) and a human normal cell line (WI-38 fibroblast), with IC_{50} values ranging from 29.0 to 68.0 µM.



Xestospongia testudinaria



1

ARTICLE HISTORY
Received 9 February 2018
Accepted 11 April 2018

KEYWORDS
Xestospongia testudinaria; sterol; antibacterial activity; cytotoxicity; Vietnam

1. Introduction

Sponges of the genus *Xestospongia* belong to the Petrosiidae family. They are one of the most widespread genera in several regions around the world and have been proven to be a rich source of diverse secondary metabolites, such as alkaloids, quinones, and brominated polyacetylenic acids (Bergmann and Feeney 1949; Goad 1978; Kanazawa et al. 1979; Seldes et al. 1985; Ingavat et al. 2009; Zhou et al. 2010; Ramanjooloo et al. 2015; Yang et al. 2017). Polyhydroxy sterols have also been isolated from the *Xestospongia* sponges (Miyaoka et al. 1997; Cheng et al. 2016), as in the cases of other marine organisms, such as algae,

CONTACT Takuya Ito itot@inm.u-toyama.ac.jp; Hiroyuki Morita hmorita@inm.u-toyama.ac.jp
Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2018.1465057.

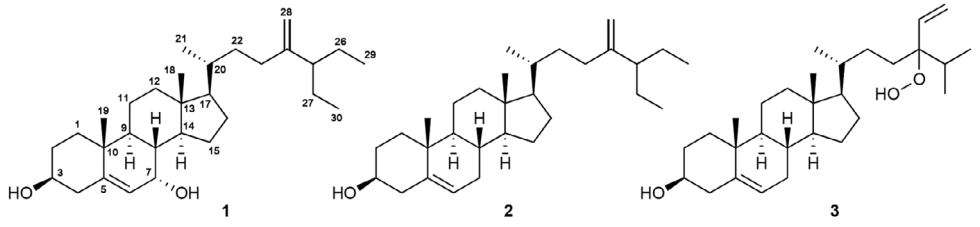


Figure 1. Structures of compounds **1**–**3** from *Xestospongia testudinaria*.

coelenterata, bryozoa, molluska, echinodermata, arthropoda, tunicates, and chordata (Djerassi 1981; Sarma et al. 2005). Since several promising candidates for antibacterial and antitumor pharmaceuticals have been exposed from marine sponges, we have isolated antibacterial and cytotoxic secondary metabolites from Vietnamase *Spongia* sp. in our previous studies (Nguyen et al. 2016, 2017; Ito et al. 2018). In our ongoing research for the discovery of new bioactive metabolites mainly from marine organisms, the purification of the ethyl acetate extract of Vietnamese *X. testudinaria* led to the isolation of a new sterol, langcosterol A (**1**), along with two known related sterols, xestosterol (**2**) (D'Auria et al. 1993; El-Gamal Ali et al. 2016) and 24-hydroperoxy-24-vinyl cholesterol (**3**) (Kokke et al. 1979; Chen et al. 2014; Seif-Eldin et al. 2015; Cheng et al. 2016) (Figure 1). Herein, we describe the structural elucidation of the new compound **1**, and the antibacterial and cytotoxic activities of the isolated compounds **1**–**3**.

2. Results and discussion

Compound **1** was obtained as a white powder, and its molecular formula was found to be $C_{30}H_{50}O_2$ by HREIMS in conjunction with NMR spectroscopic data (Table S1 and Figures S1–S7). The IR spectrum of **1** indicated the presence of hydroxyl and terminal methylene groups at 3392 and 1473 cm^{-1}, respectively. All protons and carbons were assigned by ^1H, ^{13}C, HMQC, HMBC, COSY, and NOESY experiments (Figures S1–S6). The ^1H NMR spectrum of **1** displayed two singlets of methyl groups [δ_H 0.70 (s, H-18), 1.01 (s, H-19)], a doublet of a methyl group [δ_H 0.96 (d, J = 6.6 Hz, H-21)], two triplets of methyl groups [δ_H 0.81 (t, H-29/H-30, overlapped)], and an exomethylene [δ_H 4.77 (br, s), 4.69 (br, s), H-28]. In addition, two oxygenated methine signals at δ_H 3.50 (m, H-3) and 3.87 (m, H-7) suggested the presence of two hydroxyl groups. Analyses of the ^{13}C NMR and HMQC spectra revealed the existence of five methyl carbons [δ_C 11.6 (C-18), 12.0 (C-29/C-30), 18.3 (C-19), 18.8 (C-21)], twelve secondary carbons [δ_C 108.7 (C-28), 42.2 (C-4), 39.2 (C-12), 37.0 (C-1), 34.3 (C-22), 31.4 (C-2), 29.7 (C-23), 28.2 (C-16), 26.4 (C-26), 26.3 (C-27), 24.3 (C-15), 20.7 (C-11)], seven tertiary carbons [δ_C 123.9 (C-6), 55.7 (C-17), 50.2 (C-25), 49.4 (C-14), 42.3 (C-9), 37.5 (C-8, 35.7 (C-20)], four quaternary carbons [δ_C 152.6 (C-24), 146.2 (C-5), 42.0 (C-13), 37.4 (C-10)], and two oxygenated secondary carbons [71.4 (C-3), 65.3 (C-7)]. The above NMR data of 1 was closely similar to that of xestosterol (2), except for the presence of a hydroxyl group at C-7, suggesting that 1 is 7-hydroxyxestosterol. The HMBC correlations (Figure S8) from H-6 (δ_H 5.62)/H-8 (δ_H 1.45)/H-9 (δ_H 1.22)/H-14 (δ_H 1.45) to C-7 and H-7 to C-5/C-6/C-9/C-14, as well as the COSY correlations (Figure S8) of H-6/H-7/H-8 further confirmed the existence of 7-OH group in 1.

Table 1. Cytotoxic activities of compounds **1**–**3**.

	IC_{50} (μM)			
Compound	A549	MCF7	HeLa	WI-38
1	63.1	55.8	67.4	68.0
2	>100	>100	>100	>100
3	29.0	43.9	54.9	43.4
5-Fluorouracil[a]	4.46	4.41	6.4	9.06

A549 (human lung cancer), MCF7 (human breast cancer), HeLa (human cervix cancer), WI-38 (human fibroblast).
[a]Positive control.

The NOESY correlations of H_3-19/H-2b (δ_H 1.52), H_3-19/H-4b (δ_H 2.29), H-3/H-2a (δ_H 1.85), and H-2a/H-4a (δ_H 2.32) suggested that the A-ring exists in the chair conformation. The chemical shift of C-3 and the NOESY correlations of H-3/H-4a and H-6/H-4a indicated the *β*-orientations of the hydroxyl group at C-3 and the methyl group at C-19 (Guyot et al. 1982; Chen et al. 2014; Edgar et al. 2014). The observed NOESY correlations of H-8/H_3-19/H_3-18, H-9/H-12b (δ_H 1.16)/H-14, and H_3-18/H-15b (δ_H 1.11) indicated that the B- and C-rings exist in the twist-chair and chair conformations, respectively. The chemical shifts were all consistent with the 7-OH group adopting an axial orientation and the four rings being trans-fused (Calderón et al. 2004; Cui et al. 2011; Tian et al. 2011) The relative configurations of the hydroxyl group at C-7 and the methyl group at C-18 were thus identified as the *α*- and *β*-forms, respectively. The NOESY correlations of H-17/H-12b and H-14/H-17 suggested *α*-orientation of H-17. According to the observed NOESY correlations and from a biogenetic perspective (Nes 2011), the relative configuration of **1** was determined as shown Figure 1. Consequently, compound **1** was elucidated to be a new sterol, and named langcosterol A.

The structures of the isolated known sterols, xestosterol (**2**) and 24-hydroperoxy-24-vinyl cholesterol (**3**) were identified by comparisons to their reported physicochemical data, including NMR and MS.

The isolated compounds **1**–**3** were assessed for their cytotoxicities against three human cancer cell lines (A549, lung cancer; MCF7, breast cancer; HeLa, cervical cancer) and a human normal cell line (WI-38, fibroblast), and antibacterial activities against two Gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*, and two Gram-negative bacteria, *Klebsiella pneumoniae* and *Escherichia coli*. Compounds **1** and **3** exhibited moderate cytotoxicities against the tested cancer cell lines and the normal cell line, with IC_{50} values ranging from 29.0 to 68.0 μM (Table 1). All three compounds did not show any antibacterial activities against the tested strains.

3. Experimental

3.1. General experimental procedures

Optical rotations were recorded on a JASCO P2100 polarimeter. Infrared spectra were recorded with KBr pellets on a Jasco FT/IR-460 Plus spectrometer. UV–vis spectroscopy was recorded on a NanoDrop™ 2000C spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). NMR spectra were recorded at 600 MHz (^{1}H NMR) and 150 MHz (^{13}C NMR) on a Varian UNITY 600 spectrometer, and at 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR) on a JEOL ECA500II spectrometer. Chemical shift values are expressed in *δ* (ppm) downfield from TMS, as an internal standard. The mass spectra, including high-resolution mass spectra, were

recorded on a JEOL MStation JMS-700 spectrometer. Open column chromatography was performed with normal phase silica gel (silica gel 60 N, spherical, neutral, 40–50 μm, Kanto Chemical Co., Inc., Tokyo, Japan) and C18-reversed phase silica gel (Cosmosil 75C18-OPN, Nacalai Tesque, Inc., Kyoto, Japan). TLC was performed on silica gel GF_{254} pre-coated (Merck) plates, with detection by visualization with a UV lamp at 254 and 365 nm, followed by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 and heating to 150 °C. A vertical type rectangular chamber (Yazawa, Tokyo, Japan) was used for normal phase preparative TLC under saturated developing solvent conditions. Bacterial culture 96-well plates were purchased from TPP (TPP Techno Plastic Products AG, Switzerland).

3.2. Sponge material

The marine sponge *X. testudinaria* was collected in the coastal waters of Son Cha, Lang Co, Thua Thien-Hue city, Vietnam, in June 2015, and identified by Thai Minh Quang, Bs, the marine sponge taxonomist at the Vietnam Academy of Science and Technology, Institute of Oceanography, in Nha Trang, Vietnam. A voucher specimen (E54558) was deposited at the Oceanography Museum, Institute of Oceanography, in Nha Trang, Vietnam.

3.3. Extraction and isolation

The dried *X. testudinaria* (1.0 kg) was chopped into small pieces, which were macerated with 95% EtOH (3 × 3 L) for 48 h each at room temperature. After the suspension was filtered, the resulting solution was evaporated under reduced pressure to give the EtOH-soluble portion (30.0 g), and then the concentrated total extract was partitioned into an EtOAc (3 × 1 L)-water (1 L) mixture to give the EtOAc-soluble extract (20.2 g). The EtOAc-soluble extract was subjected to normal phase open column chromatography (CC), eluted stepwise with *n*-hexane–EtOAc (8:2) and EtOAc–MeOH (9:1), to afford six fractions (Fr. A–F). Fr. B (8.2 g) was subjected to normal phase open CC, eluted with *n*-hexane–EtOAc (9:1), to yield **2** (12.0 mg). Fr. C (6.2 g) was subjected to normal phase open CC, eluted with *n*-hexane–EtOAc (7:3), to give sub-fractions (Fr. C-1-3). Fr. C-2 (1.8 g) was purified by normal phase preparative TLC, developed by *n*-hexane–EtOAc (7:3), to obtain **1** (1.1 mg) and **3** (3.0 mg).

3.3.1. Langcosterol A (1)

White amorphous powder; $[\alpha]_D^{23}$ -23.0 (*c* 0.1 $CHCl_3$); IR (KBr) υ_{max}: 3392, 1473, and 1375 cm^{-1}; ^1H NMR (500 MHz, $CHCl_3$): δ_H 1.87 (m, H-1), 1.85 and 1.52 (m, H-2a and H-2b), 3.50 (m, H-3), 2.32 (m, H-4a), 2.29 (m, H-4b), 5.62 (dd, J = 5.4, 1.8 Hz, H-6), 3.87 (m, H-7), 1.45 (m, H-8), 1.22 (m, H-9), 1.51 (m, H-11), 2.02 (m, H-12a), 1.16 (m, H-12b), 1.45 (m, H-14), 1.72 (m, H-15a), 1.11 (m, H-15b), 1.92 (m, H-16), 1.20 (m, H-17), 0.70 (s, H-18), 1.01 (s, H-19), 1.57 (m, H-20), 0.96 (d, 6.6, H-21), 1.86 (m, H-22a), 1.23 (H-22b), 1.77 (m, H-23), 1.79 (m, H-25), 1.38 (m, H-26/H27), 4.77 (brs, H-28), 4.69 (brs, H-28), 0.81 (t, H-29/H-30); ^{13}C NMR (150 MHz, $CHCl_3$): δ_C 37.0 (C-1), 31.4 (C-2), 71.4 (C-3), 42.2 (C-4), 146.2 (C-5), 123.9 (C-6), 65.3 (C-7), 37.5 (C-8), 42.3 (C-9), 37.4 (C-10), 20.7 (C-11), 39.2 (C-12), 42.0 (C-13), 49.4 (C-14), 24.3 (C-15), 28.2 (C-16), 55.7 (C-17), 11.6 (C-18), 18.3 (C-19), 35.7 (C-20), 18.8 (C-21), 34.3 (C-22), 29.7 (C-23), 152.6 (C-24), 50.2 (C-25), 26.4 (C-26), 26.3 (C-27), 108.7 (C-28), 12.0 (C-29/30); HREIMS *m/z* 442.3803 $[M]^+$ (Calcd. for $C_{30}H_{50}O_2$, 442.3811).

3.4. Cytotoxicity assay

α-Minimum essential medium with L-glutamine and phenol red (α-MEM, Wako) was used for the HeLa, A549, MCF7, and WI-38 cell cultures. All media were supplemented with 10% fetal bovine serum (FBS, Sigma) and 1% antibiotic antimycotic solution (Sigma). For MCF7 cells, the growth medium was supplemented with 1% 0.1 mM non-essential amino acids (NEAA, Gibco) and 1% 1 mM sodium pyruvate (Gibco). The assay was performed as previously reported (Nguyen et al. 2017; Ito et al. 2018). Briefly, each cell line was seeded in 96-well plates (2×10^3 per well) and incubated in the respective medium at 37 °C, under a 5% CO_2 and 95% air atmosphere, for 24 h. After the cells were washed with PBS (Nissui Pharmaceuticals), serial dilutions of the tested samples were added. After incubation for 72 h, the cells were washed with PBS, and 100 µL of medium containing 10% WST-8 cell counting kit solution (Dojindo; Kumamoto, Japan) was added to the wells. After incubation for 2 h, the absorbance at 450 nm was measured. The concentrations of the serial dilutions of the tested samples were 100–1.0 µM for the isolated compounds and the positive control, respectively. Cell viability was calculated from the mean values of data from three wells by using the following equation, and cytotoxicity was expressed as the IC_{50} (50% inhibitory concentration) value. As a positive control, 5-fluorouracil was used (Nguyen et al. 2017; Ito et al. 2018).

$$(\%)\text{Cell viability} = 100 \times [\text{Abs (test samples)} - \text{Abs (blank)}/\text{Abs (control)} - \text{Abs (blank)}]$$

3.5. Antibacterial assay

The antibacterial activity was assessed using the standard MTT assay, according to the previously described procedure (Nguyen et al. 2017; Ito et al. 2018). *B. subtilis* NBRC 13719, *S. aureus* NBRC 100910, *K. pneumoniae* NBRC 14940, and *E. coli* NBRC 102203 were used for this assay. These strains were tested in microdilution assays, and the MIC values were determined. Bacterial strains were inoculated on YP agar plates [1% polypeptone (Nihon Pharmaceutical Co., Ltd., Tokyo, Japan), 0.2% yeast extract (Difco, USA), 0.1% $MgSO_4 \cdot 7H_2O$, and 2% agar (Nacalai Tesque, Inc., Kyoto, Japan)] and incubated at 37 °C for 12 h. Stock solutions of samples were prepared at 10 mM in DMSO, and further diluted to various concentrations in 96-well plates containing the microbial strains incubated in YP medium. The plates were further incubated at 37 °C for 12 h. Ampicillin (Nacalai Tesque) and kanamycin (Nacalai Tesque) were used as reference reagents for the Gram-positive and Gram-negative bacterial strains, respectively. Finally, 10 µL of MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide) (Sigma Aldrich, USA) (0.5 mg mL^{-1} in isopropanol-HCl) was then added to each well and further incubated at 37 °C for 1 h.

4. Conclusion

A new sterol, langcosterol A (**1**), and two known compounds (**2** and **3**) were isolated from the marine sponge, *X. testudinaria*. All compounds lacked potency against *B. subtilis, S. aureus, K. pneumoniae,* and *E. coli*. However, **1** and **3** exhibited moderate cytotoxicities against the human lung A549, breast MCF7, and cervical HeLa cancer cell lines and the human fibroblast WI-38 normal cell line.

Supplementary data

Supplementary material related to this paper are available online, along with Table S1 and Figures S1–S9.

Acknowledgement

We thank Dr Yasuko Okamoto (Tokushima Bunri University, Tokushima, Japan) for her technical assistance with the MS measurements.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan [grant number JSPS KAKENHI Grants JP17K08333, JP15H05138, JP17H02203, and JP17H05435], the JSPS Core-to-Core Program, B. Asia-Africa Science Platforms, and a grant from the Kobayashi International Scholarship Foundation (T.I. and H.M.).

References

Bergmann W, Feeney RJ. 1949. Contribution to the study of marine products XXIII. Sterol from sponges of the family Haliclonidae. J Org Chem. 14:1078–1084.

Calderón GJ, Castellanos L, Duque C, Echigo S, Hara N, Fujimoto Y. 2004. Ophirasterol, a new C31 sterol from the marine sponge *Topsentia ophiraphidites*. Steroids. 69:93–100.

Chen Z, Liu J, Fu Z, Ye C, Zhang R, Song Y, Zhang Y, Li H, Ying H, Liu H. 2014. 24(S) Saringosterol from edible marine seaweed *Sargassum fusiforme* is a novel selective LXR β agonist. J Agric Food Chem. 62:6130–6137.

Cheng Z, Liu D, de Voogd NJ, Proksch P, Lin W. 2016. New sterol derivatives from the marine sponge *Xestospongia* sp. Helv Chim Acta. 99:588–596.

Cui EJ, Park JH, Park HJ, Chung IS, Kim JY, Yeon SW, Baek NI. 2011. Isolation of sterols from cowpea (*Vigna sinensis*) seeds and their promotion activity on HO-1. J Korean Soc Appl Biol Chem. 54:362–366.

D'Auria MV, Minale L, Riccio R. 1993. Polyoxygenated steroids of marine origin. Chem Rev. 93:1839–1895.

Djerassi C. 1981. Recent studies in the marine sterol field. Pure Appl Chem. 53:873–890.

Edgar CF, Rosa MP, Yolanda FP, Daniel R. 2014. Cytotoxic and antiproliferative constituents from *Dictyota ciliolate*, *Padina sanctae-crucis* and *Turbinaria tricostata*. Pharm Biol. 52:1244–1248.

El-Gamal Ali A, Al-Massarani SI, Shaala LA. 2016. Cytotoxic compounds from the Saudi Red Sea sponge *Xestospongia testudinaria*. Mar Drugs. 14:84–90.

Goad LJ. 1978. The sterols of marine invertebrates: composition, biosynthesis and metabolites. Mar Nat Prod. 2:75–172.

Guyot M, Davoust D, Belaud C. 1982. Hydroperoxy-24 vinyl-24 cholesterol, nouvel hydroperoxyde naturel isole de deux tuniciers: *phallusia mamillata* et *ciona intestinalis*. Tetrahedron Lett. 23:1905–1906.

Ingavat N, Dobereiner J, Wiyakrutta S, Mahidol C, Ruchirawat S, Kittakoop P. 2009. Aspergillusol A, an α-glucosidase inhibitor from the marine-derived fungus Aspergillus aculeatus. J Nat Prod. 72:2409–2052.

Ito T, Nguyen HM, Win NN, Kodama T, Hung VQ, Hoai NT, Morita H. 2018. Three new sesquiterpene aminoquinones from a Vietnamese *Spongia* sp. and their biological activities. J Nat Med. 72:298–303.

Kanazawa A, Teshima S, Hyodo S. 1979. Sterol from the sponges (Porifera class Demospongiae). Comp Biochem and Physiol. 62B:521–525.

Kokke WCMC, Tarchini C, Stierle DB, Djerassi C. 1979. Isolation, structure elucidation and partial synthesis of xestosterol, biosynthetically significant sterol from the sponge *Xestospongia muta*. J Org Chem. 44:3385–3388.

Miyaoka H, Shinohara M, Shimomura M, Mitome H, Yano A, Iguchi K, Yamada Y. 1997. Aragusterols E-H, new 26,27-cyclosterols from the Okinawan marine sponge of the genus Xestospongia and absolute configurations of xestokerols A and B. Tetrahedron. 53:5403–5412.

Nes WD. 2011. Biosynthesis of cholesterol and other sterols. Chem Rev. 111:6423–6451.

Nguyen HM, Ito T, Win NN, Kodama T, Hung VQ, Nguyen HT, Morita H. 2016. New antibacterial sesquiterpene aminoquinones from a Vietnamese marine sponge of *Spongia* sp. Phytochemistry lett. 17:288–292.

Nguyen HM, Ito T, Kurimoto S, Ogawa M, Win NN, Kodama T, Hung VQ, Hoai NT, Kubota T, Kobayashi J, Morita H. 2017. New merosesquiterpenes from a Vietnamese marine sponge of *Spongia* sp. Bioorg Med Chem Lett. 27:3043–3047.

Ramanjooloo A, Cresteil T, Lebrasse C, Beedessee G, Oogarah P, van Soest RWM, Marie DEP. 2015. α- Glucosidase inhibitory activity of marine sponges collected in Mauritius waters. Nat Pro Res. 29:383–387.

Sarma NS, Krishna MSR, Rao SR. 2005. Sterol ring system oxidation pattern in marine sponges. Mar Drugs. 3:84–111.

Seif-Eldin NA, Dina FK, Walied MA, Tariq RS, Magda MA, Lamiaa AS, Mohamed AG. 2015. Two new polyacetylene derivatives from the Red Sea sponge *Xestospongia* sp. Z Naturforsch C. 70:297–303.

Seldes AM, Rovirosa J, Martin AS, Gros EG. 1985. Sterols from the sponge *Haliclona chilensis*. Comp Biochem and Physiol. 81B:119–122.

Tian XR, Tang HF, Li YS, Lin HW, Chen XL, Ma N, Yao MN, Zhang PH. 2011. New cytotoxic oxygenated sterols from the marine bryozoan *Cryptosula pallasiana*. Mar Drugs. 9:162–183.

Yang M, Liang L, Wang T, Wang H, Liu H, Guo Y. 2017. Further brominated polyacetylenes with pancreatic lipase inhibitory activity from Chinese marine sponge *Xestospongia testudinaria*. J Asian Nat Pro Res. 19:732–737.

Zhou X, Xu T, Yang XW, Huang R, Yang B, Tang L, Liu Y. 2010. Chemical and biological aspects of marine sponges of the genus *Xestospongia*. Chem Biodiversity. 7:2201–2227.