World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

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Identification and fatty acids composition of *Xestospongia vansoesti* and *Aaptos suberitoides*

Ali M. Al-Talafha [1,*] and Omar Khaled Al-Mobideen [2]

[1] *Department of applied Biology, faculty of science, Tafila Technical university, PO Box 179, 66110.Tafila. Jordan.*
[2] *Department of paramedics, Prince Al-Hussein bin Abdullah II Academy of Civil Defense, Al- Balqa' Applied University, Jordan.*

World Journal of Biology Pharmacy and Health Sciences, 2024, 18(01), 399–407

Publication history: Received on 18 March 2024; revised on 24 April 2024; accepted on 27 April 2024

Article DOI: https://doi.org/10.30574/wjbphs.2024.18.1.0238

Abstract

Taxonomic identification is the most significant step in any research, and this is especially true of the sponge fauna. Two marine sponges were identified based on their morphology, skeleton, and spicules characteristics and the fatty acid composition was also studied for *Xestospongia vansoesti* and *Aaptos suberitoides*. In both species, the main saturated acids were 16:0 and 18:0. Saturated fatty acids C14-18 of iso /anteiso-structure were detected in both species with different percentages. The positional isomers of octadecenoic acid were the most common in both species with 6.78 % in *Xestospongia vansoesti* and 5.3% in *Aaptos suberitoides*. Two demospongic acids C25:2 Δ 5, 9 and C26:2 Δ 5, 9 were detected in both species and the percentage of 5,9-Hexacosadienoic acid in *Aaptos suberitoides* is high with 7.26% of the total fatty acids content. The characterization of 5,9-pentacosadienoic acid was achieved based on the MS spectra to confirm the presence of demospongic acids in both species.

Keywords: *Xestospongia vansoesti*; *Aaptos suberitoides;* Sponge; Fatty acids; Demospongic acids

1. Introduction

Sponge identification is still the most important part in modern sponge biology due to the huge diversity of these organisms. In the Southeast Asia region, more than 1500 sponge species were identified and this number is growing every year (Lim, de Voogd, & Tan, 2009). Malaysian waters are at the heart of one of the world`s most biodiverse region. The seas surrounding Malaysia support thousands of animal and plant species. The sponge fauna in Malaysia is highly diverse due to the surrounding tropical waters (Qaralleh et al., 2011). Sponge taxonomic identification is the most critical step in any investigation. However, sponges consider as one of the most difficult organisms for identification purposes (Hooper, Kennedy, & Van Soest, 2000). Sponges are filtered feeders which help in filtering large amount of water through the water flow from their bodies to the surrounding and capturing small food particles including microorganisms. Due to this kind of nutrition sponges contain a high diverse of symbionts, which contain unusual fatty acid that can be used as valuable biomarkers (Corallini & Gaino, 2001). In general, lipid classes and fatty acids composition are markedly different in their biochemical properties and chemical structures from lipids obtained from other organisms (Barnathan et al., 2003; Corallini & Gaino, 2001).

Sponge phylum has been successfully adapted to their surrounding environment and this could be due to the distinctive features in the structure of the cell membrane, particularly the fatty acid composition and sterol interactions are presumed to play an essential role in cell membrane functions (Barnathan et al., 2003; Carballeira and Alicea, 2001; Rod'kina, 2005). Thus, sponge lipids consider as one of the richest sources of fatty acids composition including very long chain fatty acids (C23 to C34, representing up to 80%), and these fatty acids are called demospongic acids. The demospongiae class is a novel source for the fatty acids structure, in particular, the unusual long chain Δ5,9 fatty acid.

* Corresponding author: Ali M. Al-Talafha

Several studies have been conducted on demospongic acids detected in marine sponges with no counterpart in the terrestrial world (Ando et al., 1998; Bergé and Barnathan, 2005; Makarieva et al., 2002).

Two sponge species were collected from two different regions; the first species is *Xestospongia vansoesti*. *X. vansoesti* is a toxic sponge, and was identified for the first time in Philippines (Bakus & Nishiyama, 2000). It is a common sponge species in the coastal water of Balok- Malaysia. The second species was collected and identified from Bidong Island - Malaysia which is *Aaptos suberitoides*. *A. suberitoides* was identified for the first time by Van Straelen, (1933). To best of our knowledge, no information is available about the fatty acids composition of both species. This study aims at extending knowledge on sponge documentation and fatty acids composition of the selected sponge species.

2. Materials and methods

2.1. Sample collection

Two sponge species were collected in this experiment. First sponge species was collected from coastal water of Balok-Pahang and the second was collected from Bidong Island - Terengganu, Malaysia, South China Sea in 2015. All the samplings were done by the mean of scuba diving. Data about the animal, area of collection, date and depth were also recorded. The collected species were immediately placed in a plastic box with seawater from the site and equipped with aeration pumps and then transported to the laboratory for further analysis.

2.2. Sponge documentation

2.2.1. Sponge Morphology

Underwater photographs were taken to identify the sponge species and to record the general morphological features figure 1 and 2.

2.2.2. Nitric Acid digestion and Spicule Preparation

The sponge samples were preserved in 70% ethanol. Nitric acid spicule preparation was performed according to Hooper, (2000) with some modifications. Briefly, several drops of concentrated nitric acid were placed on the sample tissue fragment to digest the organic matter in a screw-capped test tube. The test tube was gently shaken and stored at room temperature for one day to allow the acid to digest the tissues. Then, the mixture was centrifuged (5 min, 2000x g) and the acid was discarded. The pellet was washed with distilled water and dislodged by shaking the Eppendorf and then the mixture was again centrifuged (5 min, 2000x g). Distilled water was pipetted off and replaced by adding ethanol to speed up drying. When decanting the last fluid from the tube, a small amount was left and dislodged to facilitate the removal of the spicules. Spicules after being brought into suspension in the residual fluid were pipetted out on a slide on the warming plate. After drying (1 h), spicules morphology was observed using light microscopy (Nikon, series 901) at different magnification.

2.2.3. Section preparation

Section preparation was performed according to Ackers et al. (1985). The sponge samples were kept in 70% ethanol to appear harder. The sample tissues were prepared by using clean scalpel and slices were made with approximate size (1 to 2 mm^3). After that, the slices were soaked in absolute ethanol until they were free of water (48 h). Then, the water-free slices were soaked in clearing agent (xylol) was used to determine the structure of the mineral skeleton and to clear the section. This mixture was left for 3 weeks until the slices look translucent. Then, the translucent slices were removed from the mixture and transferred directly to the slide. Slides were left to dry for one day and were then observed using light microscope at different magnifications.

2.2.4. Sponges Final Identification

Sponge were analysed for their spicules and skeleton structure, final identification was achieve by an expert according to the descriptions of each species. After the description of the selected species, data were sent by email to Dr. Nicole de Voogd (Naturalis Biodiversity Center Leiden, South Holland, Netherlands). The two examined sponges` species were identified by Dr. Nicole de Voogd.

2.3. Fatty acids analysis

2.3.1. Total Lipid Extraction

The freeze-dried sponge were cut in small pieces and exhaustively extracted by homogenization in chloroform /methanol (2:1 v/v), the whole mixture was agitated for 24 hours in an orbital shaker at room temperature. The homogenate was filtrated to recover the liquid phase then was centrifuged at 2000 rpm. After that, the lower chloroform layer containing lipids was evaporated under vacuum in a rotary evaporator and the total lipid extract was kept for further analysis (Folch, Lees, & Sloane-Stanley, 1957).

2.3.2. Preparation of Fatty Acid Methyl Ester (FAME)

The preparation of FAME was followed method previously reported (Ichihara & Fukubayashi, 2010). Lipid sample in screw capped test tubes was dissolved by adding 0.2 ml toluene. After that, 1.5 ml of the methanol solvent and 0.3 ml of HCl reagent were added and the mixture was left for 1 hour at 95 ºC using a water bath. After this treatment, 2 ml of water and 2 ml of hexane were added and the tubes were vortexed for a while. At this point, two layers were formed and the upper layer of hexane was separated and transferred to a vial for GC-MS analysis.

2.3.3. Gas-Liquid Chromatography Mass Spectrophotometer (GCMS)

The analysis was performed by using GC-MS Model 6890 N gas chromatography system coupled with detector Model 5973 mass spectrometry selective detector (Agilent Technologies, USA, serial number. US14113031) and the FAME samples were injected automatically onto the HP-5MS column (Agilent 19091S-433, 30 m dimension, 0.25 mm i.d., 0.25 m film thickness) using automatic injector Model 7683 Series Injector. All the spectra obtained were recognized by comparing MS spectrum with standard library (Wiley Registry of Mass Spectral data).

3. Results

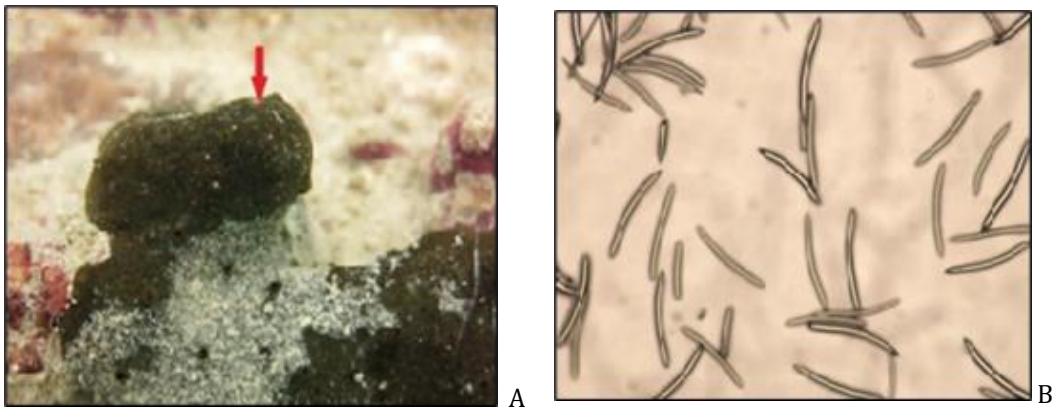

Figure 1 A Underwater photo. B: Spicules morphology (40×). Species name: *Xestospongia vansoesti*.

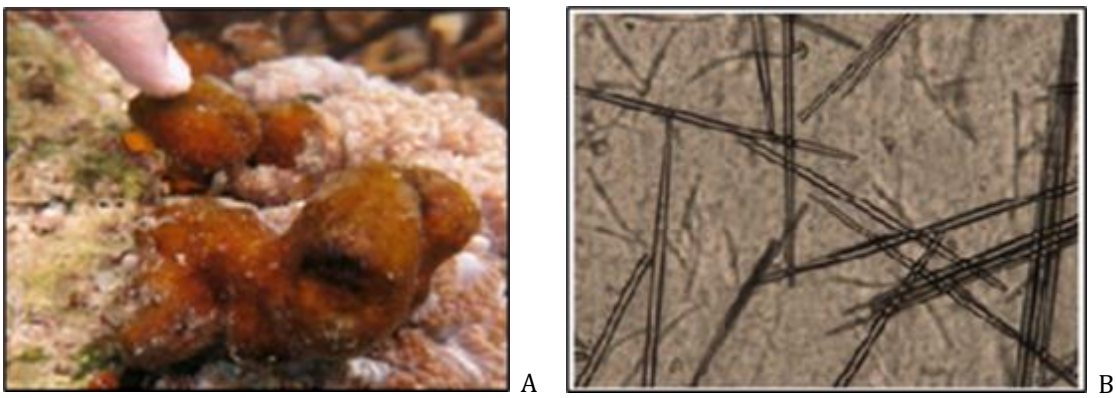

Figure 2 A underwater photo. B: Spicules morphology (40×) .Species name: *Aaptos suberitoides*.

3.1. Sponge Identification

3.1.1. Taxonomy

- Phylum Porifera Grant, 1836
- Class Demospongiae Sollas, 1885
- Order HaploscleridaTopsent, 1894
- Suborder Petrosina Boury-Esnault & Van Beveren, 1982
- Family Petrosiidae Van Soest, 1980
- Genus: *Xestospongia de Laubenfels* 1932
- *Xestospongia vansoesti* Bakus & Nishiyama, 2000

Material examined: Malaysia, Pahang state, Coastal water of Balok, collected from a depth 10 – 16 m on 20 October 2015. Preserved sponge sample is dark brown and the shape is slightly digitate. "3°56.233' N 103°22.627° E".

Description: thickly encrusted to digitate. Dark green to black and the sponge secretes a brown mucous. Dense meshwork of multispicular tracts of oxeas (Bakus & Nishiyama, 2000).

Colour: Dark green to black. Specimens out of the water turn to dark brown.

Skeleton: Ectosomal specialization is lacking, and the choanosomal mesh is near to the surface about 150 μm (Bakus & Nishiyama, 2000).

Spicules: Curved oxeas with sharp ends Figure 1 C.

- Porifera Grant, 1836
- Class Demospongiae Sollas, 1885
- Order Suberitida Topsent, 1894
- Family Suberitidae Van Soest, 1980
- Genus Aaptos Gray, 1867
- *Aaptos suberitoides* Brøndsted, 1934

Material examined: Malaysia, Terengganu state, Bidong Island, collected from a depth 4 –8 m on 15 September 2015 "5.6167°N, 103.0667°E. Preserved sponge sample is dark brown and it forms massess of globular osculiferous lobes

Skeleton: Ectosomal is rubbery and dark coloured, the consistency is fleshy tough, compact and spongy, the skeleton is radiate with tracts and contains many spicules erect with points from the centre of the lobe.

Colour: reddish black with canary yellow for interior.

Spicules: Monaxon rounded at one end and pointed at the other end with one end blunt and the other end pointed (Setiawan, Nurhayati, & Muzaki, 2009).

The description of the selected species were compared with the previous studies, and the taxonomic identification of these two species were revealed to be consistent with *Xestospongia vansoesti* and *Aaptos suberitoides* (Bakus & Nishiyama, 2000; Van Straelen, 1933).

3.1.2. Remarks

Xestospongia vansoesti was identified for the first time by Bakus and Nishiyama (2000) as a new species. This sponge produces copious brown mucus containing chemicals poisonous to hard corals. The first described species in this study belong to *Petrosiidae* family. The second studied species is *Aaptos suberitoides* which belong to Suberitidae family and this species has masses of globular osculiferous lobes, with a smooth and sometimes elevated surface. The colour is reddish black with vivid yellow for the interior. The consistency is compact and spongy. The skeleton consists of many spicules erect with points toward the ectosome (Setiawan et al., 2009).

3.2. Fatty acid analysis

Based on the GC-MS analysis, fatty acids from the total lipids of *Xestospongia vansoesti* and *Aaptos suberitoides* contained more than 45 components as shown in Table 1. Saturated fatty acids made up 45.14% of the total fatty acids in

Xestospongia vansoesti and 41.62% in *Aaptos suberitoides*. The major saturated fatty acids in both species were C16:0 and C18:0. Saturated fatty acids C14-18 of iso /anteiso-structure were detected in both species with different percentages. All the branched fatty acids were iso or anteiso, with methyl branching on the penultimate and antepenultimate carbon atom.

Total monounsaturated fatty acids exceeded 11% in *Xestospongia vansoesti* and 7.97% of the total fatty acids in *Aaptos suberitoides*. The percentage of polyunsaturated fatty acids (PUFA) in *Xestospongia vansoesti* was 8.09% while in *Aaptos suberitoides* was 12.25% of the total fatty acids content. Two demospongic acids C25:2 Δ 5, 9 and C26:2 Δ 5, 9 were identified in the lipids of both species. Of these, the main one was 5,9-Hexacosadienoic acid in *Aaptos suberitoides* with 7.26% of the total fatty acids content. Dienoic acids C18:2 Δ 6, 9, C18:2 Δ 8, 11 and C18:2 Δ 9, 12 were also detected in both species with different percentages. The following isomeric octadecenoic acids were detected in *Xestospongia vansoesti*: 7-octadecenoic acid (2.59%), 8- octadecenoic acid (2.09%), 9- octadecenoic acid (0.4%), 10- octadecenoic acid (0.56%), and 11- octadecenoic acid (1.14%), also same octadecenoic isomers were found in *Aaptos suberitoides* with different percentages.

Very long chain fatty acids C22:0, C23:0, C24:0, C25:0, C26:0 and C27:0 were also found in both species. This fatty acid composition was of interest because it presented several very long chain fatty and the characterization of these fatty acids was possible by mass spectral data of the methyl ester Fig 5. A gas chromatogram of FAME from the sponge *Xestospongia vansoesti* and *Aaptos suberitoides* is shown in Fig 3 and 4. The chromatogram displays the presence of most dominant saturated fatty acids and very long chain fatty acids were detected in this experiment. The results of the gas chromatography-mass spectrometry showed 5,9 Pentacosadienoic acid, methyl ester with matching higher than 95% comparing in the standard library. 25:2Δ5,9 was identified based on the MS. The molecular ions at m/z 392 corresponded to the molecular weight of FAME of 25:2. Its MS of FAME had a diagnostic ion at m/z 140 and 250 that is typical when the cleavage occurred between C7 and C8 followed by proton transfer. At m/z 141 and 250 are the fragment from the cleavage of C7 and C8 followed by proton loss. Hence, this proved that 5,9–25:2 was present Fig 5.

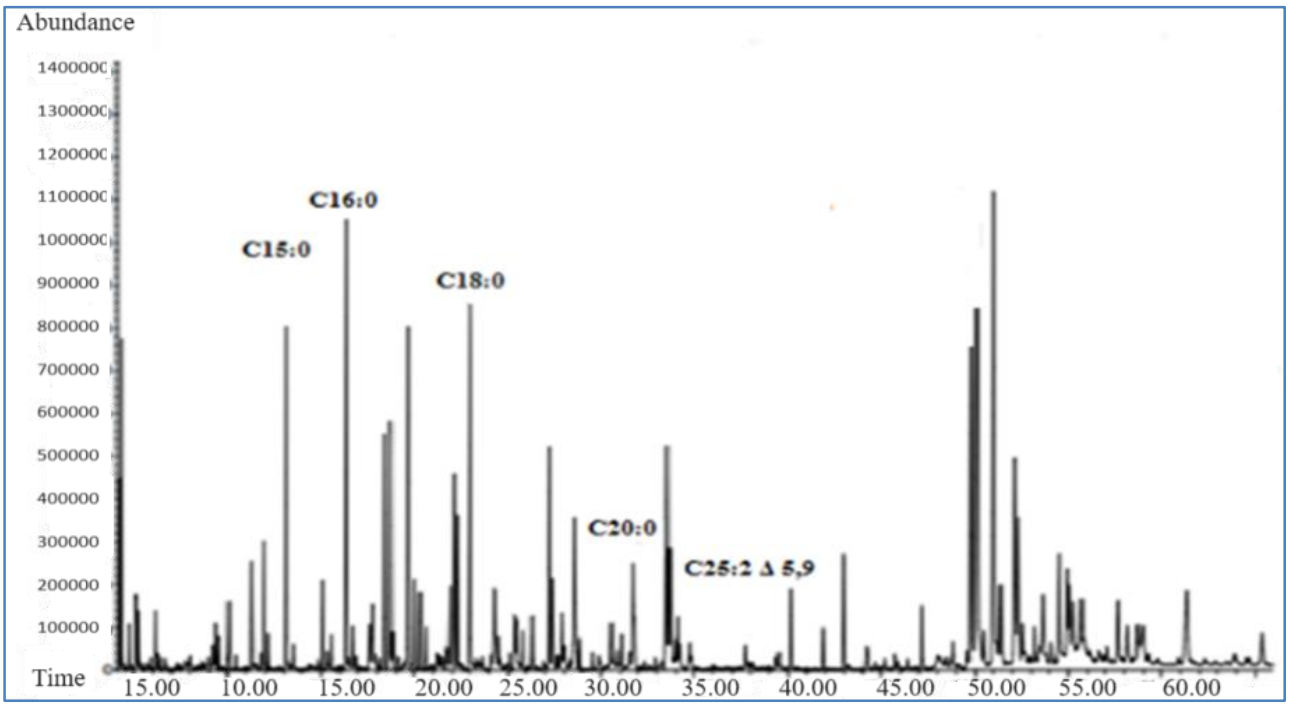

Figure 3 Gas chromatogram of the FAME from the total lipids of the sponge *Xestospongia vansoesti*. The chromatogram was obtained from HP-5MS column (Agilent 19091S-433, 30 m dimension, 0.25 mm i.d., 0.25 m film thickness)

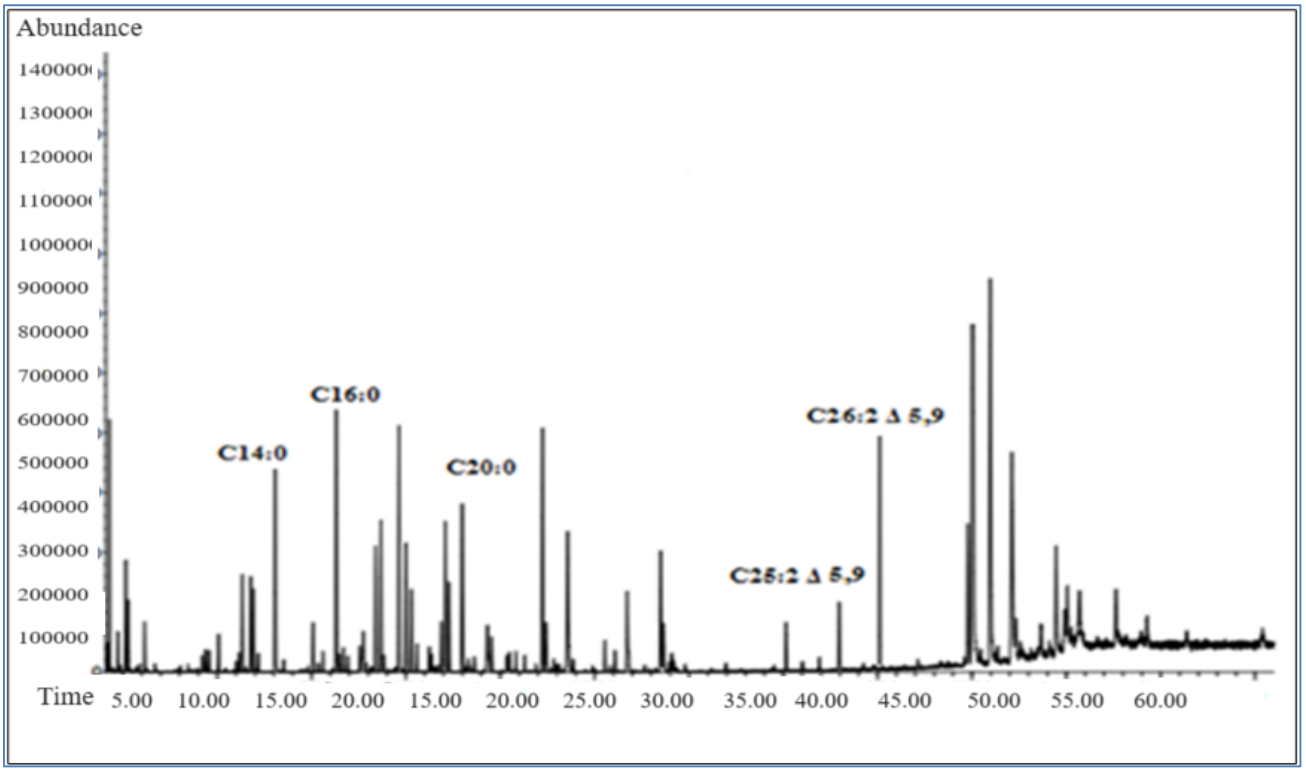

Figure 4 Gas chromatogram of the FAME from the total lipids of the sponge *Aaptos suberitoides*. The chromatogram was obtained from HP-5MS column (Agilent 19091S-433, 30 m dimension, 0.25 mm i.d., 0.25 m film thickness)

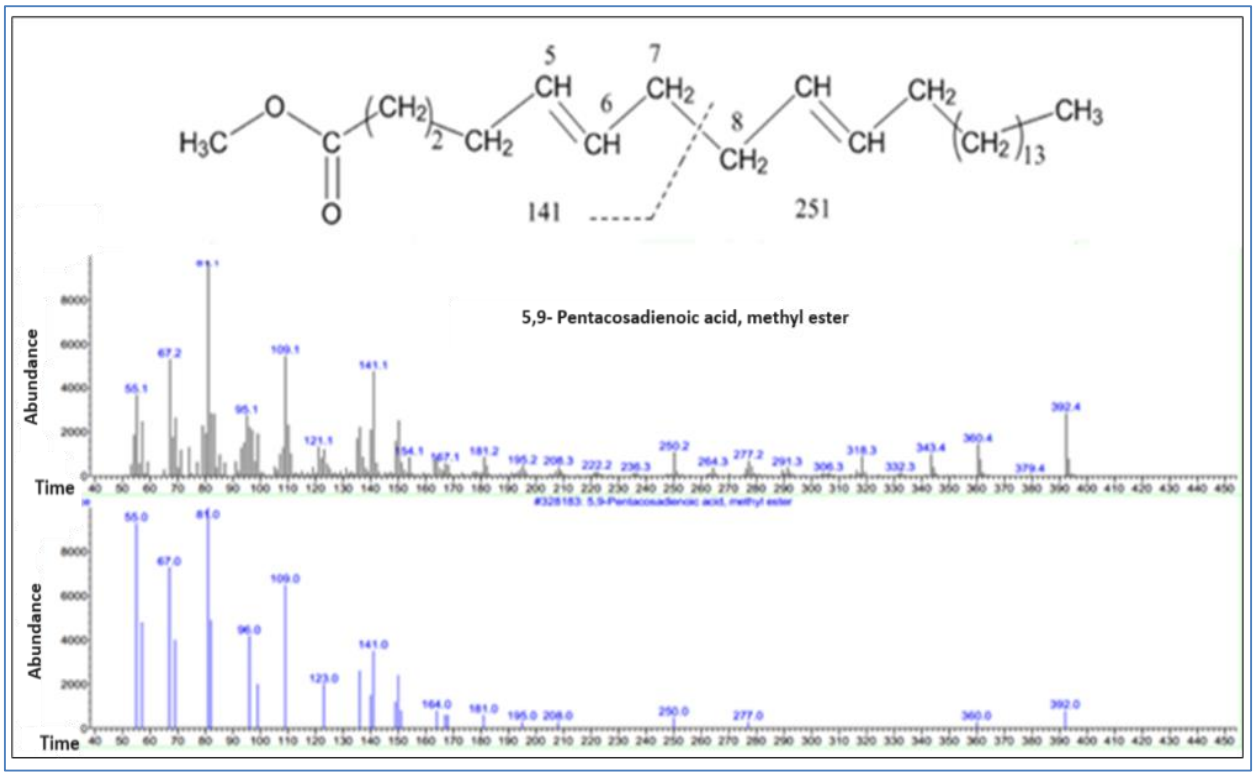

Figure 5 MS spectra of *m/z* 392 of FAME

Table 1 Fatty acids composition of marine sponge *Xestospongia vansoesti* and *Aaptos suberitoides*. % of total fatty acids

	X. vansoesti	***A. suberitoides***		***X. vansoesti***	***A. suberitoides***
Fatty acids	(Mean* ± S.D.)	(Mean* ± S.D.)	Fatty acids	(Mean* ± S.D.)	(Mean* ± S.D.)
C12:0	1.59±0.35	0.75±0.2	C18:1 (n-7)	1.14±0.07	1.07±0.23
C13:0	0.93±0.31	0.88±0.24	C18:2 Δ 6,9	1.11±0.22	1.22±0.37
C14:0	1.3±0.41	4.29±0.91	C18:2 Δ 8,11	2.34±0.38	0.45±0.23
nteiso-C15:0	1.56±0.29	2.02±0.42	C18:2 Δ 9,12	0.55±0.16	0.33±0.08
C15:0	4.19±0.37	4.05±0.96	C19:0	1.3±0.19	3.38±0.7
nteiso-C16:0	0.81±0.51	0.57±0.23	C19:1(n-9)	0.83±0.54	2.05±0.42
Iso-C16:0	4.69±0.75	2.04±0.54	C20:0	3.08±0.34	0.57±0.12
C16:0	8.12±1.8	7.41±0.75	C20:1 (n−9)	1.05±0.41	0.62±0.23
Anteiso-C17:0	2.83±0.72	3.16±0.76	C21:0	3.47±0.43	0.84±0.21
Iso-C16:0	1.08±0.14	0.41±0.23	C22:0	3.24±0.54	3.55±0.77
C16:1 (n−7)	2.78±0.51	0.55±0.16	2-hydroxy C23:0	0.88±0.23	0.3±0.1
C16:1 (n−9)	0.48±0.25	0.59±0.18	C23:0	0.93±0.38	1.18±0.22
C16:0	4.97±0.46	3.5±0.69	2-hydroxy C23:0	2.35±0.24	1.54±0.28
Anteiso-C18:0	3.73±0.18	1.08±0.21	C24:0	0.85±0.39	1.07±0.24
C18:0	4.38±0.35	7.16±1.57	C25:0	3.18±0.29	1.41±0.26
10-methyl-C18:0	5.26±0.22	3.5±0.67	C25:2 Δ 5,9	1.88±0.38	0.93±0.3
11-methyl-C18:0	2.48±0.25	3.3±0.69	C26:0	2.89±0.5	0.67±0.17
Iso-C19:0	4.25±0.28	3.33±0.72	C26:2 Δ 5,9	1.74±0.16	7.26±1.4
Sdf	3.04±0.4	0.79±0.3	C27:3 (n−16)	0.51±0.06	2.06±0.39
C18:1(n-11)	2.59±0.14	0.37±0.17	C27:0	0.41±0.21	0.91±0.41
C18:1(n-10)	2.09±0.45	0.41±0.09	-	1.05±0.06	1.67±0.44
C18:1 (n-9)	0.4±0.18	2.29±0.44	-	0.89±0.5	0.94±0.26
C18:1 (n-8)	0.56±0.14	1.16±0.22	-	-	-

*Means are the averages of 3 replicates. The values are shown as mean ± standard deviation (S.D).

4. Disscusion

A collection of marine organisms is much more difficult compared to the terrestrial due to the difficulty of accessibility to the marine natural habitat. In addition, some problems associated with the taxonomy of these creatures and the lack of adequate biological material (Houssen & Jaspars, 2005). Any collection from marine habitat there is some information must be recorded like a place of collection, date of collection, depth, and underwater photos for the specimens (Evans & Kitting, 2010). The descriptions of the identified species were compared with the literature of the same species and the taxonomic rank for both species was shown. To extend the knowledge about the fatty acid composition in the sponge, the two identified species have not been investigated for their fatty acid composition although these species are common in the study area.

Both studied species showed a high diversity of fatty acid composition as shown in table 1, and it displayed acids of all chain lengths from C12 to C27 that were found in both species with different percentages. The most prevalent fatty acids within the chain length C12 – C22 were C16 and C18 and this is common in the sponge and other animals, plants and in bacteria (El-Beltagi, Salama, & El-Hariri, 2007; French et al., 2000; Or-Rashid, Odongo, & McBride, 2007; Sata, Kaneniwa,

Masuda, Ando, & Iida, 2002). The occurrence of demospongic acids in both species was revealed in two acids 25:2Δ5,9 and 26:2Δ5,9 with the high percentage in *Aaptos suberitoides* and this is in general consistence with the previous studies on the sponge (Barnathan et al., 2003; Kornprobst & Barnathan, 2010).

As observed in the current study, both sponges contain a various number of iso and anteiso fatty acids typical for the occurrence in bacteria such as 13-methylpentadecanoic acid, 14-methylpentadecanoic, 14-methylhexadecanoic, 15-methylhexdecanoic, 10-methyloctadecanoic, 11-methyloctadecanoic and 17-methyloctadecanoic likely originating from bacteria (Barnathan et al., 2003; Carballeira, Pagán, & Rodríguez, 1998). The branched chain fatty acids observed in this study have been reported in previous investigations like 10-methyloctadecanoic and 11-methyloctadecanoic were revealed also by Barnathan et al, (2003). Cyclopropaneoctanoic acid, 2-octyl and Cyclopropaneoctanoic acid, 2-hexyl were also found in both sponges and the occurrence of cyclopropane-containing fatty acids has been documented before in the Canary Islands sponge (Nechev et al., 2004).

5. Conclusion

Sponge phylum has the greatest fatty acid diversity among the animal kingdom and the most common feature in is high content of long chain fatty acids exceeding 22 carbon atoms. Investigations on fatty acids composition and other lipid classes in sponge phylum could be a useful approach in explaining several phenomena associated with the metabolism of sponges and in exploring the geographical diversity from shallow to deep habitats, and over wide ranges of temperature and salinity. To summarize, both sponge species are of particular interest since they identified and for their fatty acids composition revealed for the first time.

Compliance with ethical standards

Acknowledgment

The authors are grateful to the staff of INOCEM (Institute of Oceanography and Maritime Studies, IIUM) for providing the diving equipment. We extend our honest thanks to the staff of the faculty of Pharmacy (Mr. Mohd Najib Omar) for his technical assistance.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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