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Identification of Bioactive Compounds and Evaluation of the Antimicrobial and Anti-biofilm Effect of *Psammocinia sp.* and *Hyattella sp.* Sponges from the Persian Gulf

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Abstract

Biofilms are microbial communities that bind to surfaces resist adverse conditions. Increasing the survival of bacteria in biofilm structures compared to their planktonic form causes problems in drug treatment. On the other hand, drug resistance in the world is increasing and the need to discover and identify new compounds with the antimicrobial effect is felt. Marine sponges are adapted to unique marine environments and can fight pathogens of these ecosystems without having a dedicated defense system. This study aimed was to investigate the antimicrobial and anti-biofilm effects and also to identify the bioactive compounds of two samples of *Psammocinia* sp. and *Hyattella* sp. sponges. Six bacteria *Pseudomonas aeruginosa*, *Acinetobacter baumanni*, *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* were tested. The extract was first extracted using dichloromethane and methanol (DCM: MeOH) (1:1 v/v) solvents. The planktonic form was investigated using Disk diffusion and agar well diffusion methods. The minimum inhibitory concentration (MIC) was determined by the microdilution method and then the minimum bactericidal concentration (MBC). Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) were performed to identify the compounds of each extract. No zone of inhibition (ZOI) was observed on the planktonic form of *K. pneumonia* due to both extracts. MIC have about 10 to 20 mg/ml and MBC in about 20 to 80 mg/ml was determined. The results showed that the effect of both extracts on the degradation of the biofilm formed by *B. cereus* was less than other bacteria. The results of GC-MS showed the presence of phenol, butanedioic acid, propanoic acid and Benzeneacetaldehyde compounds. This study showed that marine sponges at the Persian Gulf can be a good candidate for the extraction of bioactive compounds that use as antimicrobial agents.

Keywords Antimicrobial · Anti-biofilm · Sponge · Bioactive compounds · Secondary metabolites · *Psammocinia* sp. · *Hyattella* sp. · Gas chromatography-mass spectrometry · GC-MS

Introduction

Biofilms are microbial communities attached to surfaces in an extracellular matrix (ESM) that have greater drug and antibiotic resistance than their planktonic form. Increasing drug resistance is one of the global concerns for the treatment of acute and chronic infections associated with the formation of microbial biofilms. Studies have shown that more than 90% of bacteria can form biofilms (Sadeghian et al. 2012; Li and Lee 2017; Lu et al. 2019; Masák et al. 2014). One of the new approaches that have been considered over the last two decades to prevent the formation of biofilms is the use of natural products (Lu et al. 2019; Tan and Vanitha 2004).

Each year, about 200 new molecules of marine sponges are reported, making them a vast and diverse source of natural compounds with medicinal and therapeutic properties over the past six decades. Among these features can be cited as antimicrobial properties (Ancheeva et al. 2017; Rane et al. 2014; Zhang et al. 2017).

Marine sponges from the phylum Porifera are metazoans that existed about 700 to 800 million years ago. Populations of them are found in tropical oceans, moderate waters, and freshwaters (Hentschel et al. 2002; Khoddami et al. 2018). Sponges have a high interaction with marine ecosystems and because they are attached to the solid seabed, they are not able to escape in the face of adverse conditions. For this reason, they fight predators and pathogens by producing certain

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compounds called secondary metabolites (Mahon et al. 2003; Mehbub et al. 2014; Paul et al. 2001; Mohammadi et al. 2019; Proksch 1994). Secondary metabolites are introduced as organic compounds that do not play a direct role in the growth and reproduction of living organisms (Mehbub et al. 2014; Tilviv 2004). Most of the secondary metabolites produced by sponges are very potent. So that they are still stable and active in saline and diluting ocean conditions (Abad et al. 2011; Mehbub et al. 2014).

Little research has been done on marine organisms in the Persian Gulf as a source of natural marine products (Seradj et al. 2012). The aim of this investigation was to recognize sponge with antimicrobial activity for chemical and pharmacological studies. Also, in this study, the antibiofilm activity of sponges extracts against some pathogenic bacteria was evaluated.

Material and Method

Techniques for investigating the anti-biofilm and antimicrobial effects, as well as the materials used, are listed below.

Collection and Identification of Marine Sponge

The marine sponges were collected at the Persian Gulf, Iran. These sponges were gathered from a depth of 10–12 meters in Lesser Tunb islands (26º14′ N-55º08′ E,68.8 km^2) (Khoobdel et al. 2014) in November 2015. Three repetitions were collected for each marine sponge species. They were transported alive in seawater to the laboratory and maintained at 4ºC in a refrigerator before extraction. The collected sponges were identified according to the protocol of Hooper and Boury-Esnault (Boury-Esnault and Rützler 1997; Hooper 2000). Identification results have shown that these two sponge belongs: *Psammocinia* sp. and *Hyattella* sp. genus. Figure 1 show the macroscopic image of these sponges.

Preparation of Sponges Extracts

For extraction, 100 g of freeze-dried sponge samples were used. First, the samples were crushed into smaller pieces and placed in a polar and non-polar solvent of dichloromethane (DCM) and methanol (MeOH) in a ratio of 1: 1 v/v (Pech-Puch et al. 2020) in a shaker for 48 hours. The obtained extract was then filtered using Whatman No. 1 paper filter and thus large pieces of sponge were removed from the extract. To remove the solvent and concentrate the extract, after filtration, the obtained extract was incubated for 40 hours at 40 ° C. After the incubation period, the remaining extract was used to evaluate the antimicrobial and anti-biofilm effect (Hamayeli et al. 2019).

Bacteria

Six antibiotic-resistant pathogenic bacteria were used in this research includes: *Pseudomonas aeruginosa* (ATCC 27,853), *Acinetobacter baumannii* (ATCC 1611), *Bacillus cereus* (ATCC 1298), *Klebsiella pneumoniae* (ATCC 700,603), *Escherichia coli* (ATCC 35,218), *Staphylococcus aureus* (ATCC 1189).

Disk Diffusion Method

Microbial susceptibility was determined by Kirby-Bauer method (Hudzicki 2009) using paper blank disks with a diameter of 6.4 mm. Initially, a concentration of 100 mg/ml of each extract was prepared in DCM: MeOH (1:1 v/v) solvent. Blank disks were placed at this concentration for 1 h and then at room temperature for 30 min. Microbial culture of each of the studied bacteria with turbidity equivalent to 0.5 McFarland was performed on Mueller-Hinton Agar (MHA) medium and the disks were placed on the culture medium. The plates were incubated for 18 h at 37 ° C and then the diameter of the zone of inhibition (ZOI) was measured and reported in

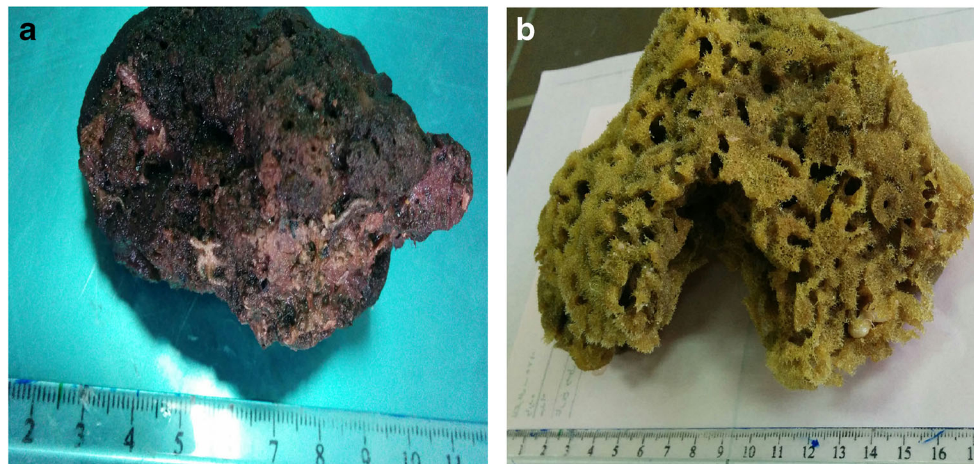


Fig. 1 The morphology of marine Sponges that studied in this research: (**a**) *Psammocinia* sp. and (**b**) *Hyattella* sp.

mm. Data were expressed as mean ± standard deviation. The blank disk containing DCM: MeOH (1:1 v/v) solvent was used as a control.

Agar Well Diffusion Method

Wells with a diameter of 6 mm were punched in plates containing MHA medium and microbial culture was performed with turbidity equivalent to 0.5 McFarland of each bacterium. Then 50 μl of the same solution prepared from the extracts was placed in the wells in the previous step. The plates were incubated at 37 ° C for 18 h and then ZOI was reported. Solvent-containing wells were considered as controls (Balouiri et al. 2016). Data were expressed as mean ± standard deviation.

Determination of the Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC)

The broth microdilution method was followed according to the Clinical and Laboratory Standards Institute (CLSI) protocol supplement M100 (2017) (CLSI 2017; Reller et al. 2009) to determine the MIC. Initially, 100 mg of each extract in 1 ml of sterile nutrient broth medium was well vortexed and from this concentration of 100 mg/ml, 7 more dilutions (50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 mg/ml) were prepared by serial dilution. In each well of the 96-well plate, 200 μl of the dilutions prepared were added, along with 50 μl of bacterial culture (0.5 McFarland) and 10 μl of sterile nutrient broth medium. Thus, the final concentration of each extract was 80, 40, 20, 10, 5, 2.5, 1.25 and 0.625 mg/ml in each well. Three control wells containing extracted nutrient broth culture medium, non-extracted broth nutrient medium, and microbial culture in broth medium (0.5 McFarland) were considered. The microplate was incubated for 18 h at 37 ° C. After this period, optical density (OD) was determined and recorded at 630 nm using an ELISA reader (Biotek ELx800). The lowest concentration of the extract in which bacterial growth was inhibited was determined as MIC. 100 μl of the well designated as MIC was applied to MHA medium to determine MBC (Mohsenipour and Hassanshahian 2016).

Inhibition of Biofilm formation

The formation of microbial biofilm in plate microtiter and its staining with crystal violet (CV) is described by Masumipour and Hassanshahian (2016). Then, 100 μl of each microbial suspension with turbidity equivalent to 1 McFarland and 100 μl of each of the extracts prepared by serial dilution were placed in 96-well polystyrene microplate wells. The final concentration of extracts in each well was three dilutions of 12.5, 6.25, and 3.12 mg/ml was estimated. Three control wells were considered as microbial suspension wells from each bacterial strain, wells containing sterile culture medium and wells containing an equal amount of extract and culture medium. The microplates were then incubated at 37 ° C for 24 h in a stationary state. The formed biofilms were stained by CV and at the end, 160 μl of glacial acetic acid 33% (v/v) were added to the wells (Stepanović et al. 2000). OD was recorded at 630 nm by the ELISA reader (Biotek ELx800) (e Silva et al. 2017).

Disruption of an Established Biofilm

Biofilm formation was established using microbial suspension (1 McFarland) in 96-well polystyrene microplate for 24 h at 37 ° C under static conditions. At this stage, two control wells containing culture medium and microbial suspension were also considered. After this time, the wells were gently drained under aseptic conditions and washed twice to remove planktonic cells. Then 100 μl of extract and 100 μl of culture medium were added to each well. The concentrations used in the extracts were the same as in the previous step (12.5, 6.25, and 3.12 mg/ml). Since the extracts had color, the control well containing equal amounts of the extract and culture medium was placed. The microplates were incubated for 24 h at 37 ° C and then CV was stained and an OD of 630 nm was recorded (e Silva et al. 2017).

Statistical Analysis

Differences for individual parameters between control and treated groups were tested with Duncan's test by analysis of variance (ANOVA) using SPSS version 16.0 for Windows. Differences were considered significant if the P value less than 0.01, 0.05 and 0.001. All experiments were performed in triplicate and repeated three times.

Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS)

The marine sponge extracted with DCM: MeOH and investigated using GC Agilent Technologies CP 3800 GC with Split/Splitless Inlet United States (2004), CP-SIL 5CB column (30 m, 0.1 mm and ID 0.32 μm) equipped with Flame Ionization Detector (FID), as well as GC-MS Agilent Technologies, Varian Saturn 2000 United States (2004), the HP-5MS column (60 m, 0.25 mm, 0.25 μm). The specification of the detector was a GC/MS/MS and Mass range from 10 to 650 amu. equipped with Electron Impact Ionization (EI) ion source and NIST MS Library V.2.0.1. The oven was programmed a primary temperature 70ºC (hold for 2 min) to the terminal temperature 300ºC at the rate 10 C/min (hold for 10 min, 35 min in total) (Hassanshahian et al. 2019). H_2 at the rate of 30 ml/min was used as the carrier gas in constant flow mode.

Table 1 The antimicrobial effect of *Psammocinia sp.* and *Hyattella sp.* sponges against six planktonic bacteria

Bacteria	*Pseudomonas aeruginosa*		*Acinetobacter baumannii*		*Bacillus cereus*		*Klebsiella pneumoniae*		*Escherichia coli*		*Staphylococcus aureus*	
Sponge extract	*Psammocinia sp.*	*Hyattella sp.*	*Psammocinia sp.*	*Hyattella sp.*	*Psammocinia sp.*	*Hyattella sp.*	*Psammocinia sp.*	*Hyattella sp.*	*Psammocinia sp.*	*Hyattella sp.*	*Psammocinia sp.*	*Hyattella sp.*
Disk diffusion (dia: mm)	10 ± 0/9 SD	8 ± 0/8 SD	12 ± 1/0 SD	8 ± 0/5 SD	8 ± 0/9 SD	9 ± 0/4 SD	0	0	0	0	10 ± 1/4 SD	8 ± 1/7 SD
Agar well plate (dia: mm)	0	20 ± 1/6 SD	16 ± 0/8 SD	10 ± 0.9SD	25 ± 0/4 SD	11 ± 0/3 SD	0	0	30 ± 1/6 SD	14 ± 0/9 SD	21 ± 0/6 SD	0
MIC (mg/ml)	-	20	10	10	20	20	-	-	-	-	10	-
MBC (mg/ml)	-	40	20	40	40	80	-	-	-	-	40	-

Due to lack of extract, MIC and MBC were not performed for the samples that did not have ZOI in the disk diffusion and agar well diffusion testes

Results

The results obtained from the effect of extracts on the planktonic form and biofilm of bacteria as well as the analysis of GC-MS are reported below.

Antimicrobial Effect of Extracts on Planktonic Form

The ZOI of two sponge extracts that assayed by disc diffusion and agar-well plate methods against bacteria were illustrated in Table 1. As shown in this table the ZOI obtained by the agar-well plate was higher than ZOI obtained by the disc diffusion method. The most sensitive bacteria to the antibacterial effect of *Psammocinia sp.* and *Hyattella sp.* extract was *B. cereus* and *P. aeruginosa* respectively. The MIC and MBC results were presented in Table 1. According to this table, the values of MIC and MBC were different for each bacteria.

Anti-biofilm Effect of Extracts on Bacteria

The effect of sponge extracts on the inhibition of biofilm formation by pathogenic bacteria was studied. The results were shown in Figs. 2 and 3. As shown in this figure the maximum biofilm inhibition by two sponge extracts related to *P. aeruginosa* (90.86%). These extracts had the lowest inhibitory effect against biofilm formation of *S. aureus* (36.74%). Also, it can be concluded from this figure that with an increase in extract concentration the biofilm formation was more inhibited. For the destruction of biofilm structure, the *Psammocinia sp.* extract had the best antibiofilm activity against *K. pneumoniae* biofilm (90.32%) and *Hyattella sp.* extract had the optimum antibiofilm activity on *E. coli* biofilm (94.33%). The most resistant biofilm structure between studied bacteria was *B. cereus* (39.20%) (Fig. 4).

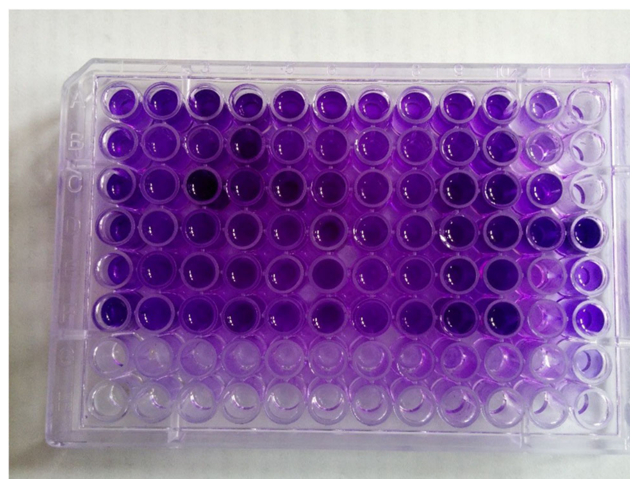


Fig. 2 96-well polystyrene microplate stained with crystal violet

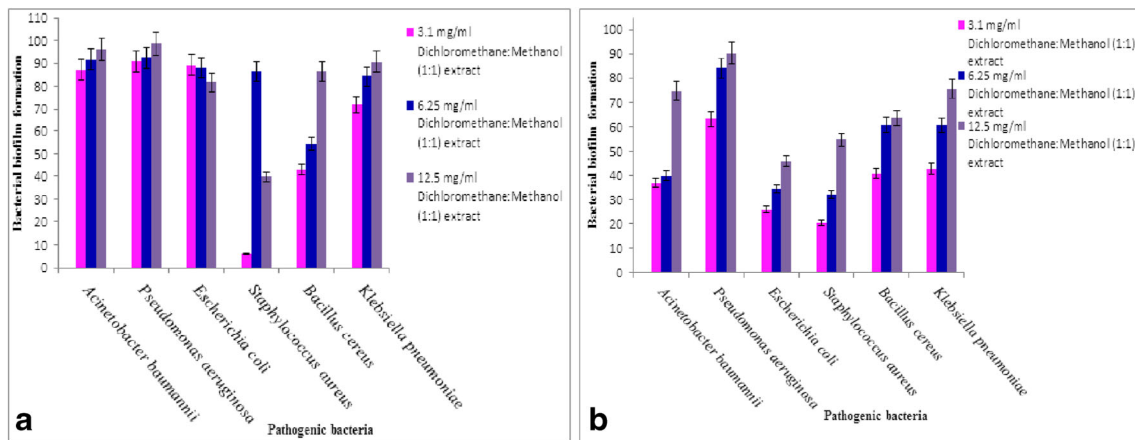


Fig. 3 Percentage reduction of biofilm formation for test bacteria treated with different concentrations of sponge extracts for 24 hours: (**a**) *Psammocinia* sp. (**b**) *Hyattella* sp.

Statistical Analysis

The effect of bacteria genus and different concentration of sponge extracts on biofilm formation and destruction were analyzed statistically by Duncan's test. The results were presented in Table 2. This table confirmed that for biofilm inhibition and destruction *Hyattella* sp. extract was significant. Also for biofilm destruction type of bacteria were significant with *Psammocinia* sp. extract ($P < 0.05$).

The Chemical Composition of Sponge Extract

The bioactive compounds of each sponge extracts were revealed by GC-MS. The results were presented in Table 3. Also, the chromatographs for each sponge extract were illustrated in Figs. 5 and 6. These figures show that phenolic and benzoic aromatic compounds are predominant in the two sponge extracts.

Discussion

A drug called Hymendin is a type of marine sponge for the treatment of tuberculosis in preclinical trials (Masoumipour et al. 2018). So far, other drug compounds with anti-viral and anti-cancer properties, such as Vidarabine and Cytarabine from sea sponges have been able to obtain Food and Drug Administration (FDA) approval (Mayer et al. 2010).

In February 2017, the World Health Organization (WHO) published a document outlining a list of pathogens that are a priority for research and development of new antibiotics. Based on this, bacteria such as *A. baumannii*, *P. aeruginosa*, and *S. aureus* resistant to antibiotics are a priority (WHO 2017).

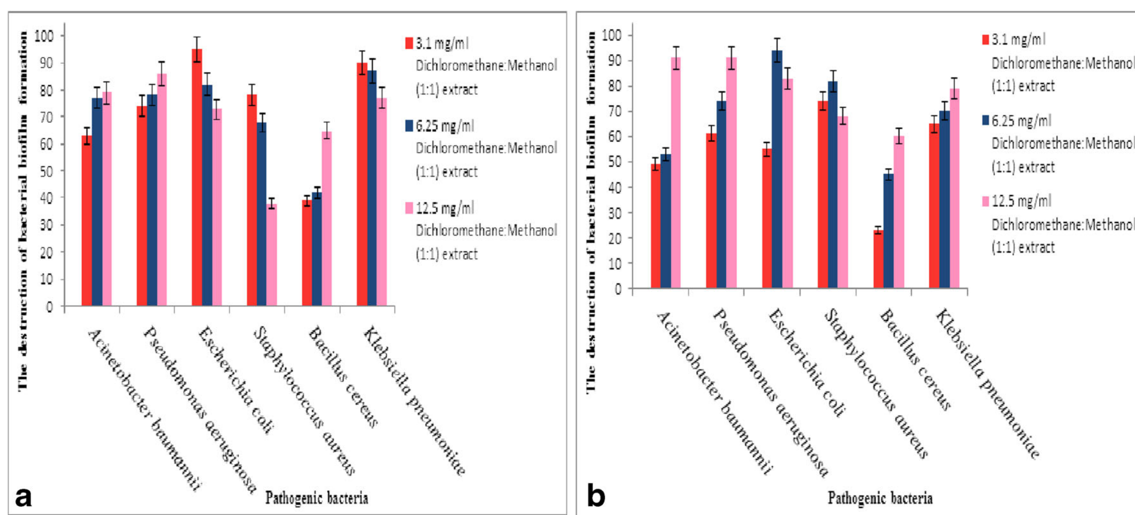


Fig. 4 Percentage disruption of biofilm for test bacteria treated with different concentrations of sponge extracts for 24 hours: (**a**) *Psammocinia* sp. (**b**) *Hyattella* sp.

Table 2 Statistical analysis of the results by Duncan's test. Ms; Mean square, Df: Degrees of freedom, Sig: Significant

		Biofilm formation			Biofilm destruction		
Sponges	Variables	Df	Ms	Sig	Df	Ms	Sig
Psammocinia sp.	Bacteria	5	0/063	-	5	0/060	*
	Concentration (mg/ml)	2	0/015	-	2	0/002	-
	total	7			7		
Hyattella sp.	Bacteria	5	0/081	***	5	0/051	*
	Concentration (mg/ml)	2	0/126	***	2	0/090	**
	total	7			7		

*p < 0/05, **p < 0/01, ***p < 0/001, -: No significant level

Govinden-Soulange et al. (2014) in investigating the antimicrobial effect of *Biemna tubulosa* and *Stylissa* spp. sponges, mention the reason for the high level of MIC as the use of crude extracts, which contain a mixture of active and inactive compounds (Govinden-Soulange et al. 2014). The use of crude DCM: MeOH extracts of *Psammocinia* sp. and *Hyattella* sp. sponges, as shown in Table 1, was estimated at 10 to 20 mg/ml. It can probably be concluded that the use of refined extracts or any component of the compound alone can reduce the MIC (De and James, 2002).

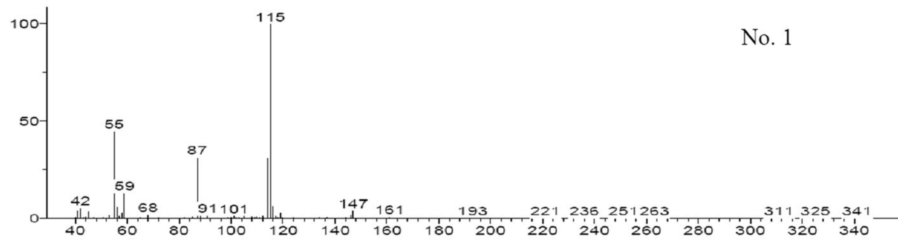
Carneiro et al. (2019) identified a type of lectin from *Aplysina fulva* sponge that, although not effective in inhibiting the planktonic growth of bacteria, significantly reduced the formation of *E. coli* and *S. aureus* biofilms (Carneiro et al. 2019). A similar result was observed in the present study of *K. pneumoniae*. As in the disk diffusion and agar well plate tests, no ZOI was observed for both *Hyattella* sp. and *Psammocinia* sp. extracts, but the extracts were able to inhibit and destroy the biofilm of this bacterium. In another study of *Callyspongia* sp. sponge with solvent, the extraction method was similar to the observed ZOI for this bacterium of 8 mm (Hamayeli et al. 2018). From all the results of this research, it can be concluded that the types of marine sponges exhibit a diverse range of antimicrobial and anti-biofilm activities.

Hamayeli et al. (2017) reported three species of *Dysidea* sp. sponges from the Persian Gulf that each extract had a different antimicrobial effect on human pathogens, whose findings could be due to the production of bioactive compounds or symbiotic microorganisms. According to their report, at a concentration of 12.5 mg/ml of *Dysidea* sp. extract, the inhibition of *B. cereus* biofilm was at a maximum. Also, the ZOI

Table 3 Chemical composition of sponge extracts obtained by GC-MS

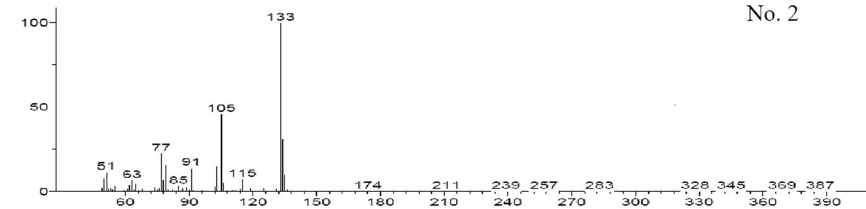
Sponge	No.	Compounds	Formula	Retention Time	Area	% of Total
Hyattella sp.	1	Butanedioic acid, dimethyl ester	$C_6H_{10}O_4$	7.512	3.733e + 4	0.285
	2	Benzaldehyde, 2,4-dimethyl-	$C_9H_{10}O$	10.552	4.178e + 4	0.319
	3	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	14.451	2.064e + 5	1.575
	4	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	$C_{18}H_{28}O_3$	19.271	4.750e + 4	0.362
	5	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	24.576	5.076e + 4	0.387
Psammocinia sp.	6	Propanoic acid, 2-hydroxy-2-methyl-, ethyl ester	$C_6H_{12}O_3$	2.498	6.189e + 6	4.055
	7	Propane, 2,2-dimethoxy-	$C_5H_{12}O_2$	2.995	1.255e + 6	0.822
	8	Benzeneacetaldehyde, 2-methoxy-α,5-dimethyl-	$C_{11}H_{14}O_2$	10.556	3.755e + 6	2.460
	9	1,4-Benzenedimethanol, α,α'-dimethyl-	$C_{10}H_{14}O_2$	11.312	1.234e + 6	0.809
	10	Benzenemethanol, 4-(1-methylethyl)-	$C_{10}H_{14}O$	11.387	1.301e + 6	0.852
	11	Phenol, 2-methyl-5-(1-methylethyl)-	$C_{10}H_{14}O$	11.509	1.706e + 6	1.118
	12	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	14.468	2.223e + 5	0.146
	13	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)-	$C_{11}H_{12}O_3$	14.461	2.223e + 5	0.146
	14	Apiol	$C_{12}H_{14}O_4$	15.894	1.287e + 5	0.084
	15	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	18.989	1.794e + 5	0.118
	16	Valtrate	$C_{22}H_{30}O_8$	23.670	2.398e + 5	0.157
	17	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	24.599	5.412e + 5	0.355

Unknown: 7.511 min, Scan: 465
Compound in Library Factor = -265



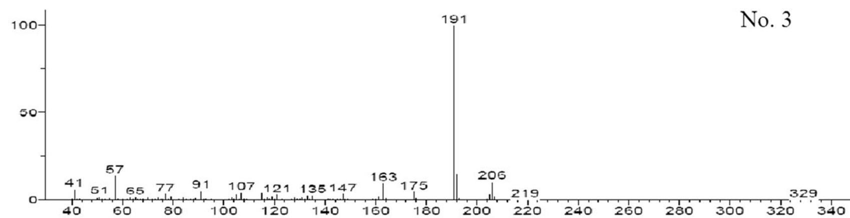
Hit 1 : Butanedioic acid, dimethyl ester
C6H10O4; MF: 779; RMF: 832; Prob 71.7%; CAS: 106-65-0; Lib: mainlib; ID: 77584.

Unknown: 10.553 min, Scan: 655
Compound in Library Factor = -334



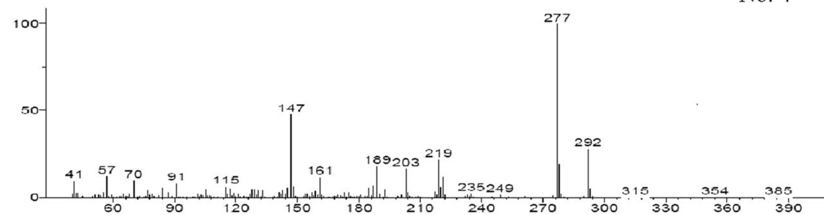
Hit 1 : Benzaldehyde, 2,4-dimethyl-
C9H10O; MF: 822; RMF: 854; Prob 18.8%; CAS: 15764-16-6; Lib: replib; ID: 18102.

Unknown: 14.439 min, Scan: 895
Compound in Library Factor = -118



Hit 1 : Phenol, 2,4-bis(1,1-dimethylethyl)-
C14H22O; MF: 884; RMF: 902; Prob 53.7%; CAS: 96-76-4; Lib: replib; ID: 23675.

Unknown: 19.271 min, Scan: 1194
Compound in Library Factor = 130



Hit 1 : Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester
C18H28O3; MF: 816; RMF: 831; Prob 95.2%; CAS: 6386-38-5; Lib: mainlib; ID: 173117.

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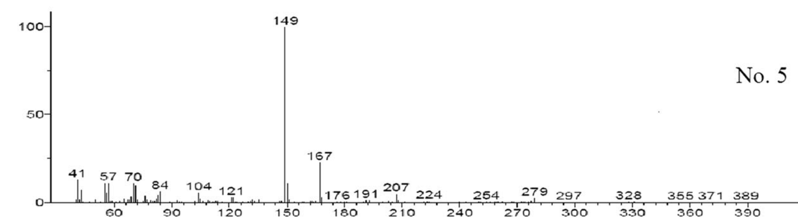

Hit 1 : 1,2-Benzenedicarboxylic acid, diisooctyl ester
C24H38O4; MF: 751; RMF: 902; Prob 25.1%; CAS: 27554-26-3; Lib: replib; ID: 20061.

Fig. 5 The GC-MS Chromatogram of *Hyattella sp.* extract

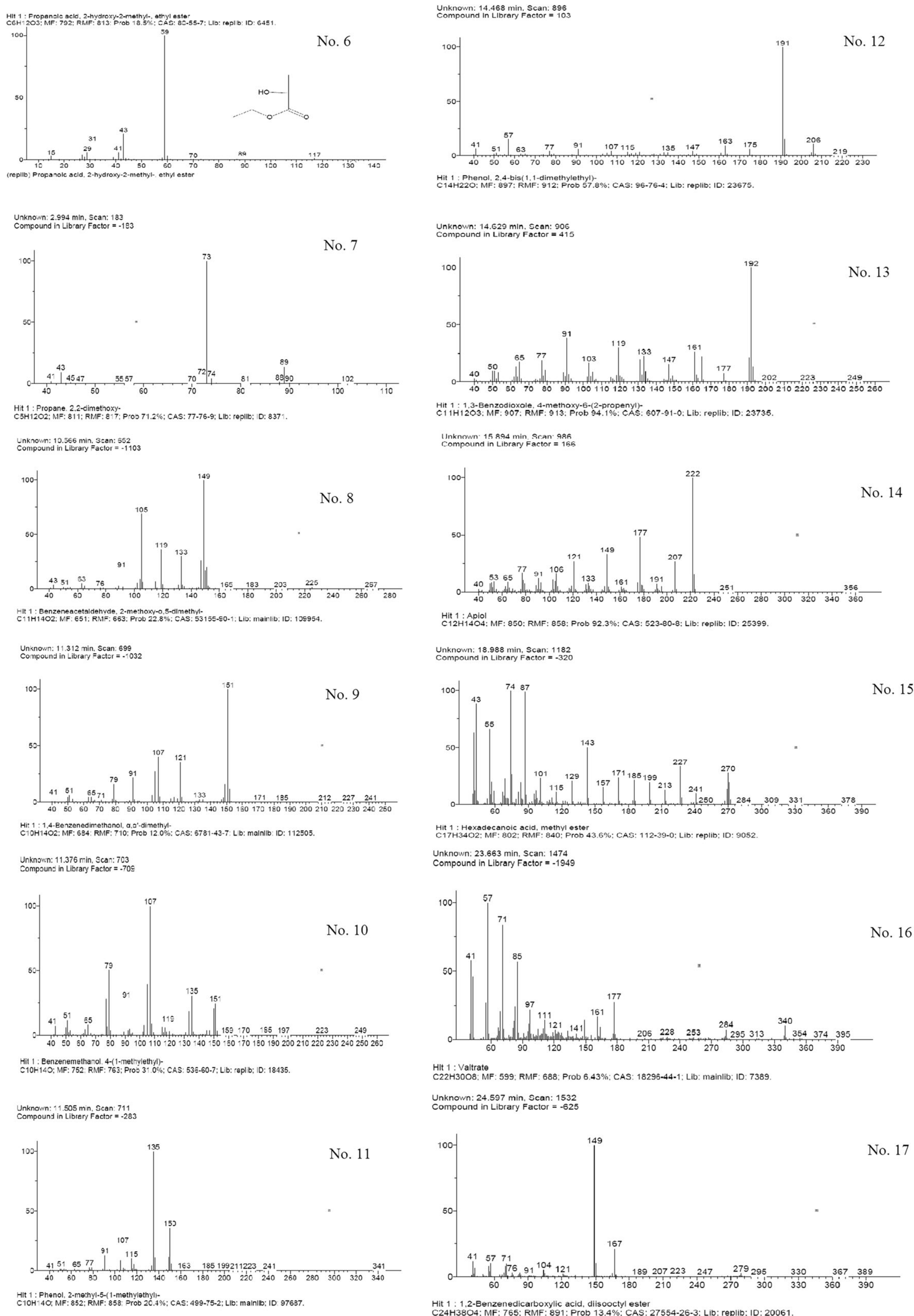

Fig. 6 The GC-MS Chromatogram of *Psammocinia sp.* extract

observed in the disk-diffusion assay for *A. baumannii* was about 9 to 12 mm (Hamayeli et al. 2017).

Kaplan (2011) states that concentrations subMIC in some antibiotics can induce the agonistic effect of biofilm formation in vitro (Kaplan 2011). Therefore, concentrations lower than MIC (according to Table 1) specified for the biofilm test (3.12, 6.25 and 12.5 mg/ml) were used in this study. Also, Cepas et al. (2019) by examining the relationship between drug resistance of three gram-negative bacteria and biofilm formation, states that antibiotic-resistant acquisition in some gram-negative bacteria can encourage or inhibit the formation of microbial biofilm. However, multidrug-resistant bacteria do not tend to produce more biofilms than non-resistant bacteria (Cepas et al. 2019). Therefore, it is thought that the presence or absence of multiple drug resistance (MDR) of the bacteria in this study does not have a significant effect on the analysis of their biofilm formation. However, the relationship between the two is still unclear.

Carefully in Fig. 3, it appears that with increasing the concentration of *Psammocinia* sp. extract, the inhibition of *E. coli* biofilm formation has decreased. This is also seen in Fig. 4 for the degradation of the biofilm formed by the *Hyattella* sp. extract on the biofilm of *E. coli*, *S. aureus* and *K. pneumonia*. Perhaps the reduction of the anti-biofilm effect of the extracts on the mentioned bacteria with the Eagle effect can be explained. In the Eagle phenomenon, the antimicrobial effect of the compound decreases with increasing concentration (Prasetyoputri et al. 2019).

Phenol the highest compound detected in both extracts was detected by GC-MS analysis. Other compounds with the highest percentage of *Psammocinia* sp. extract included propanoic acid and benzene acetaldehyde. Indraningrat et al. (2016) in their article, refer to the production of phenolic compounds by marine sponges and their microorganisms (Indraningrat et al. 2016). Shaala et al. (2020) also reported the antimicrobial effect of chlorinated propanoic acid derived a actinomycete strain from the *Callyspongia siphonella* sponge against *E. coli* and *S. aureus* bacteria (Shaala et al. 2020). Rajasabapathy et al. (2020) also reported an actinomycete strain isolated from *Orina sagittaria* sponge that had the ability to inhibit methicillin resistant *S. aureus*. The results of GC-MS showed 10 volatile organic compounds of ethyl acetate and hexane extracts of this strain (Rajasabapathy et al. 2020). In the present study, GC-MS analysis identified hexadecanoic acid, methyl ester in *Psammocinia* sp. extract and the results of agar well plate on *E. coli* and *S. aureus* bacteria with ZOI were 30 and 21 mm, respectively.

Marine animals are a good candidate for the extraction of new antimicrobial agents. The biological properties of marine extracts from Persian Gulf sponges have been less studied and identified. Overall, the results of the present study confirmed that both sponge extracts cause sufficient antibacterial effects against planktonic and biofilms typically forms. Both extracts had low destruction effects on *B. cereus* biofilm. Further research in this area can lead to the discovery of new antimicrobial drugs in the future.

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