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ANTIBACTERIAL ACTIVITY AND GC-MASS ANALYSIS OF ORGANIC EXTRACT FROM PERSIAN GULF *HALICLONA SP*

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ABSTRACT

Marine organisms given the opportunity to develop more than terrestrial organisms, potentially producing new molecules in the properties of interest therefore marine organisms give us more molecules in which the result would be more information for research. As marine organisms, sponges are a variety of chemicals, including terpenes, polysaccharides and sterols have medicinal properties, antioxidant, antiviral, antibacterial, antifungal, anticancer and AIDS inhibition. Persian Gulf has many species of sponges and *Haliclona sp.* was collected from Kharg Island, then rinsed and finally extracted with ether as solvent. Five combinations of cholestanol, 2-(Acetoxymethyl)-3-(methoxycarbonyl)bi-phenylene carboxylate, silicic acid diethyl bis(trimethylsilyl) ester, 2,4-dimethyl-Benzo[H]Quinoline and 1-(3,5-dimethylpyrazolyl),2-(2-benzothiazolylthio) ethanone were analyzed using gas chromatography and mass spectrometry methods. Finally antimicrobial capacity was determined.

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Introduction

Marine animals in general and marine invertebrates in particular are promising organisms for preparation of biological active compounds.[1] Marine sponges (Porifera) are considered to be true "chemical factories" producing natural compounds which have been identified and elucidated by physical and spectroscopic properties, but their bioactivities are still unknown.[2] *Haliclona* sponges have produced many natural materials which can be used for preparation of steroids,[3] polyketides,[4] macrolides,[5]alkaloids,[6]terpenes,[7] with antioxidant, antimicrobial and anticancer activities.
In this research work we report the GC-Mass analyzing results of several compounds corresponded to organic extracts from Persian Gulf marine sponge, *Haliclona* species for possible antibacterial activity of them.

Materials and Methods

Collection of sample

Marine sponge was collected from Kharg Island of Persian Gulf at depths of 10-15 m in September 2013. Sample was transported and stored frozen prior to exhaustive extraction in organic solvent. Taxonomic was done and certified by Iranian Fisheries Science Research Institute Bandar Abbas. The sample was identified as *Haliclona sp.* as shown in figure 1.

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Figure 1. Persian Gulf marine sponge, *Haliclona species*

Preparation of the extract

50 g of sponge sample was extracted with diethyl ether (100 ml) at 25 °C overnight on a shaker. The extract was filtered by a Buchner funnel using suction and kept in the refrigerator -20 °C for antibacterial activity and further GC-Mass analysis.

Microorganisms and culture media

All microorganisms i.e., Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Basillus subtilis were obtained from the Microbiology Laboratory of Ahwaz Golestan Hospital.

Antimicrobial assay

Well agar diffusion method was used for microbial test of extract. Nutrient agar was utilized as culturing media for microorganisms. For antibacterial activity, 20ml of agar was poured in each petri plate, allowed to set at 37 °C for 24 hr. Cultures were swabbed in nutrient agar plates using sterile cotton.

The organic extracts were prepared by transferring 10, 20, 30, 40 and 50 mg of each extract into, 1ml of DMSO (Merck, Germany), swab them and incubated at desired condition. The inhibitory zone (mm) was applied for antibacterial activity. Antibiotics such as vancomycin, tetracycline, penicillin, gentamycin, ceftriaxone, amoxicillin were used as positive controls. DMSO and Et_2O were also tested as negative control to ensure that they do not interfere with the tests.

Haliclona sp. extract demonstrate considerable antibacterial activity, was selected for determination of MIC.[8]

Minimum inhibitory concentration (MIC)

Small volume microtitre plates and a stock of 50 mg/ml of extract into DMSO were used in this method.[8] To determine MIC of extract, 100µL of Mueller Hinton broth was transferred into microtitre plate. From this reason, 100 µL was placed into wells of column 1. The contents of well were mixed thoroughly, 100 µL from column 1 and added to column 2, two-fold serial dilution. Then, each well is inoculated with 50 µL of strain of microorganism pre-adjusted at 0.5 McFarland Standard.

Minimum bactericidal concentration (MBC)

For determination of MBC, samples with negative microbial growth were subjected for determination of living cells after 24 h of incubation.

Gas chromatography-mass spectrum (GC-MS)

GC-MS Agilent GC 7890, Mass 5975 fitted with a fused HP-5ms (5% phenyl methylpolysiloxane) capillary column (0.25 mm × 30 m, 0.25 µm film thickness) with helium gas at 1 ml/minute are used. The sample was injected (sampling time, 5 minutes). The respective temperatures and ionization chamber were 290°C and 280°C. Temperature programs for the column oven were as follows: program, 60°C for 1 minute, elevated to 130°C at 20°C/minute, then to 210°C at 10°C/minute, then to 260°C at 10°C/minute, then to 300°C at 10°C/minute; it was finally maintained at 300°C.

Identification of compounds

Interpretation of mass spectra was done according to National Institute Standard and Technology (NIST 08s), WILEY 8 and FAME.

Results

The *Haliclona sp.* extract was tested for antimicrobial activity against 4 bacterial species namely E.coli, P. aeruginosa, S. aureus and B. subtilis by the well agar diffusion method. All extracts in table1 with 50 mg/ml concentration exhibited activity. All test microorganisms was present mainly in test extract. Moreover, the data in this table indicate that this extract was more effective as compared to other marine sponges.[8]

Table 1. Antimicrobial activity of Persian Gulf sponge

Microorganisms	Diethyl ether	Tetracycline	Penicillin	Amoxicillin	Ceftriaxone	Gentamicin	Vancomycin
E. coli	21±2.31	13±1.2	17±1.01	17.33±1.5	25.32±1.7	28.33±1.33	19.67±1.35
P. Aeruginosa	6.33±2.5	18.6±1.25	8.41±1.04	5.31±1.21	13.67±1.33	18.31±1.21	18.7±1.25
S. aureus	28.67±2.5	18.6±1.33	7.37±1.43	6.45±1	8.33±1.22	18.42±1.04	10.33±1.23
B. subtilis	26.67±1.35	25.33±1.11	19.12±1.21	21.39±1.02	35.51±1.13	23.12±1.35	16.46±1.35

Table 2. MIC and MBC of Persian Gulf sponge

Extract	**Microorganisms**							
	E.Coli		P.Aeruginosa		S.Aureus		B.Subtilis	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Diethyl ether	12.5	50	12.5	25	12.5	50	3.12	50

The extract was effective against pathogenic bacteria. The MIC of extract at 3.12 mg/ml show inhibition the growth of B. subtilis.

Finally desired extract was tested to TLC for the preliminary compound separation and then to GC-MS analysis to characterize the compound. GC-MS of the Haliclona sp. (Figure 2 and Table 3) revealed compounds namely Cholestanol, 2-(Acetoxymethyl)-3-(methoxycarbonyl)bi-phenylene carboxylate, silicic acid diethyl bis(trimethylsilyl)ester, 2,4-dimethyl-Benzo[H]Quinoline, and 1-(3,5-dimethylpyrazolyl) 2-(2-benzothiazolylthio) ethanone. Cholestanol was identified as sterol compound with high abundant (82.75%). MS spectra of five mentioned compounds are given in Figures 3,4.

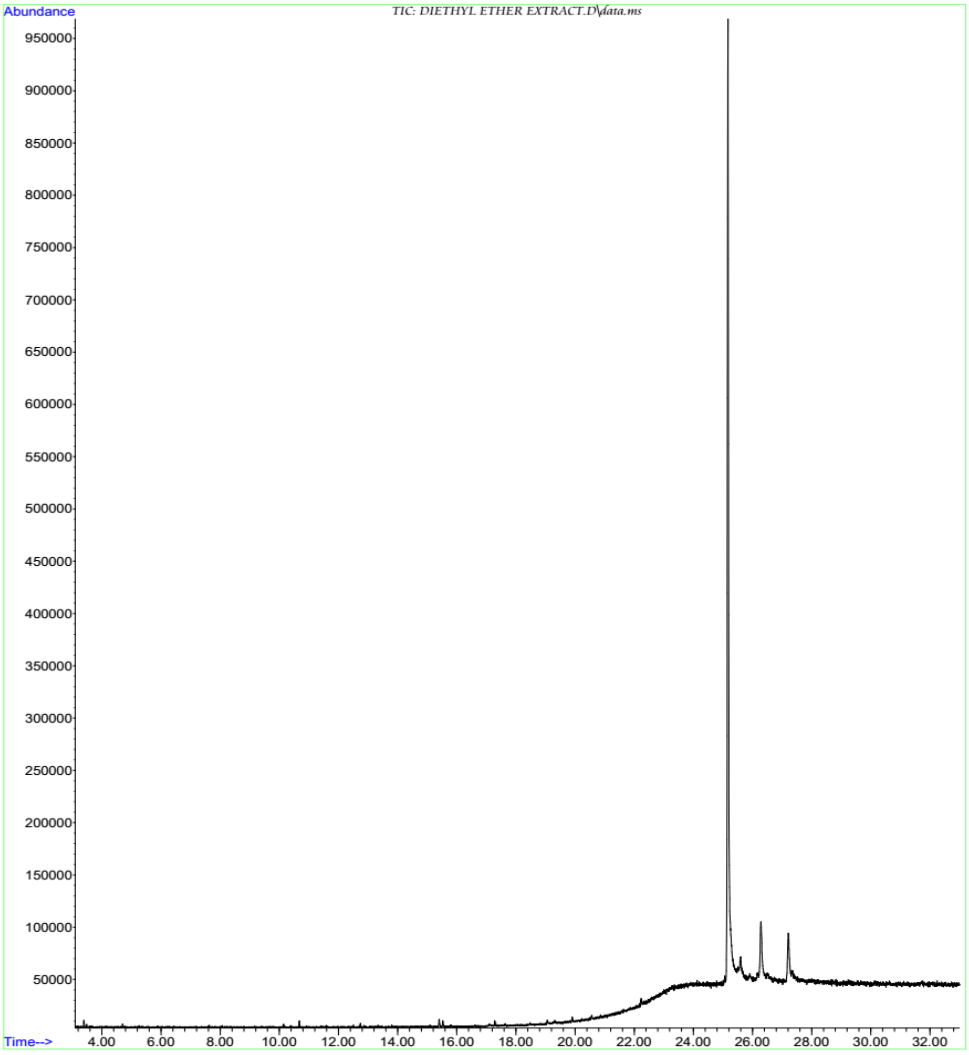

Figure 2. GC Spectrum of Haliclona sp. extract

Table 3. Bioactive of components identified in the extract

Entry	RT	Name of compound	Peak area %	Nature of compound
1	25.168	Cholestanol	82.75	Sterol
2	25.340	2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene	2.12	Aromatic compounds
3	25.603	Silicic acid, diethyl bis(trimethylsilyl) ester	1.93	Silicon ester
4	26.284	2,4-dimethyl-Benzo[H]quinoline	7.65	Aromatic compounds
5	27.217	1-(3,5-dimethylpyrazolyl), 2-(2-benzothiazolylthio) Ethanone	5.55	Aromatic compounds

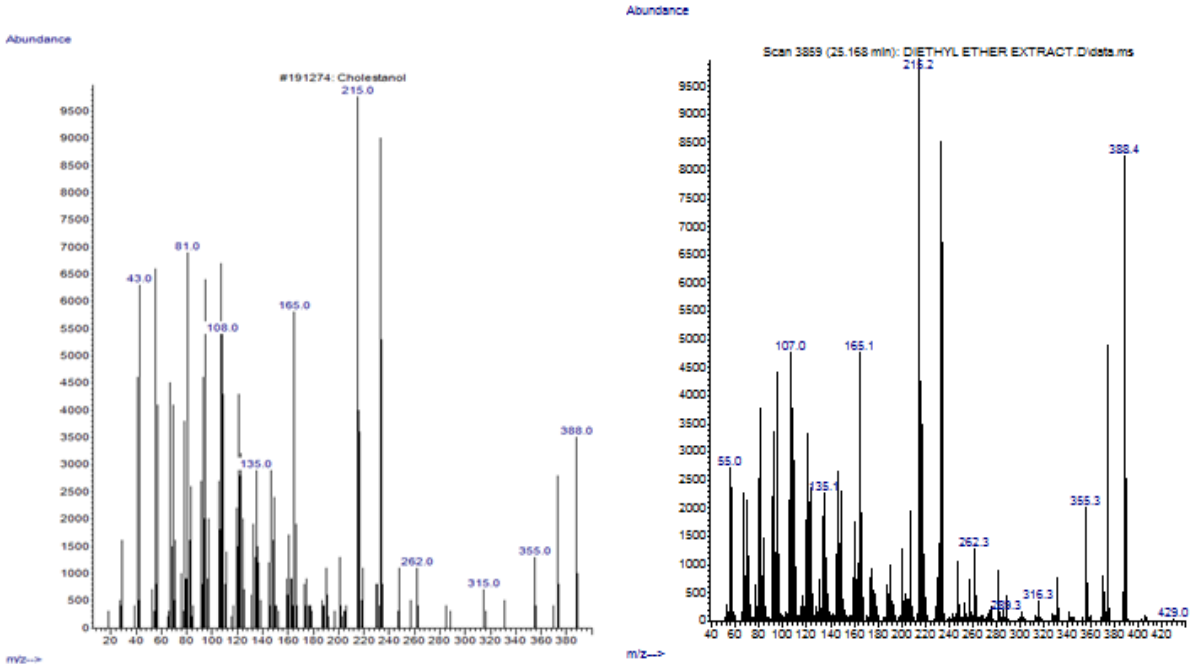

Figure 3. Mass pectrum of *Haliclona sp.* extract with RT=25.168 (right) and cholestanol reference (left)

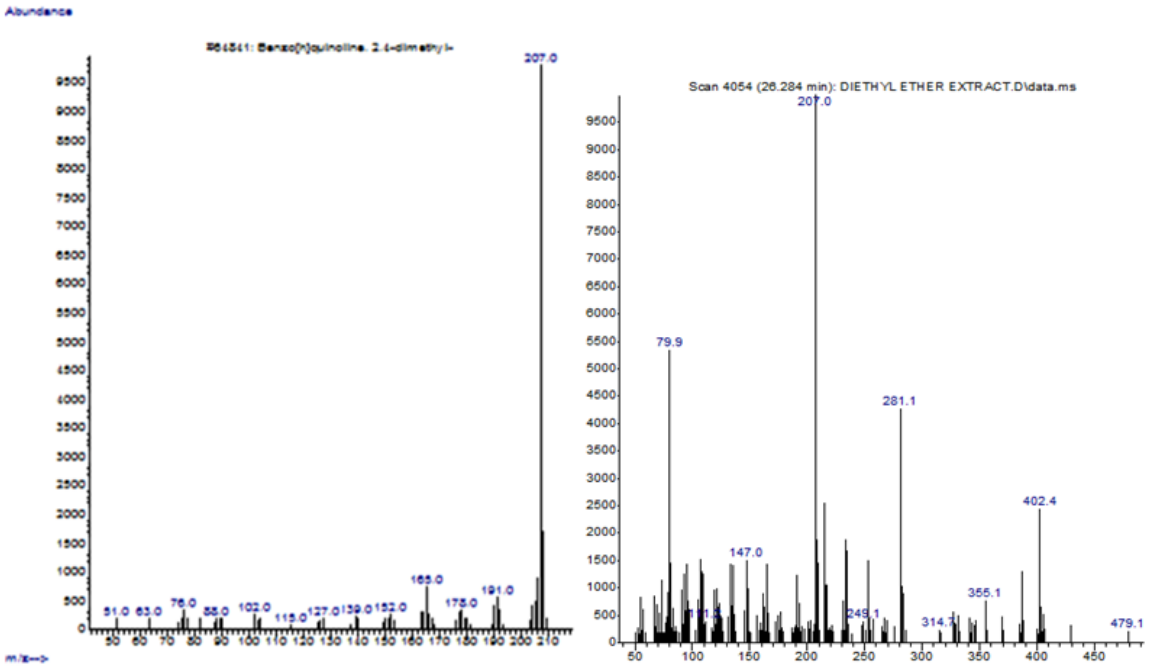

Figure 4. Mass pectrum of extract with RT=26.284 (right) and 2,4-dimethyl-Benzo[H]quinoline reference (left)

Discussion

Antibacterial activity has previously been described in marine sponge species.[9]A variety of antimicrobial compounds, including terpenes[10-12], sterols[13] and alkaloids[14] have been isolated from them. The antibacterial activity of extract in Table 1 against gram-negative bacteria proved more effective than gram-positive. Also this extract was effective against S. aureus compared with penicillin, amoxicillin, tetracycline, vancomycin, gentamycin and ceftriaxone.

Marine supplies are a potent source for unexplored chemical moieties that may have vital biological and economic properties. Marine diaspora has proven its importance as several of compounds of economic significance are isolated and mass produced across the globe. Very little of these living organisms was studied and therefore provides ample opportunities to hunt for vital substances.

In this study, GC-MS as a good method can be analyze the unknown organic materials from Persian Gulf sponge *Haliclona sp.*. We obtained mixtures of sterols with other compounds which investigated by chromatography on silica gel columns. GC investigation of isolated mixture showed at least 5 different compounds. So this mixture was subjected to GC-MS. For example

the reference mass spectrum and fragmentation pattern corresponded to cholestanol were displayed in figure 5 and figure 6.[15]

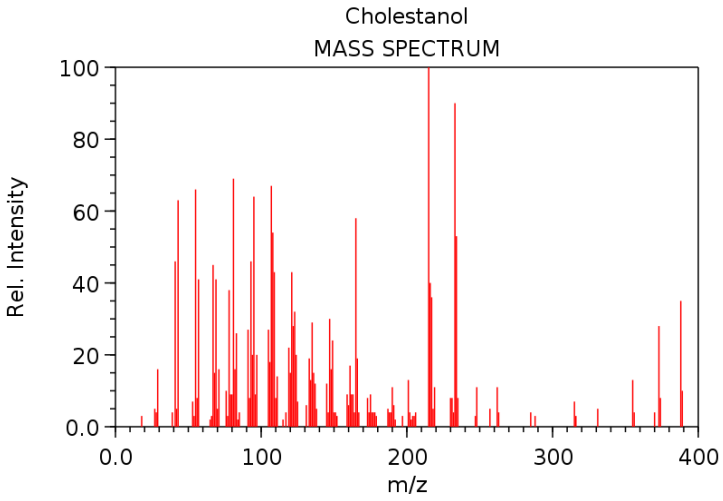

Figure 5. Mass pectrum the reference of cholestanol

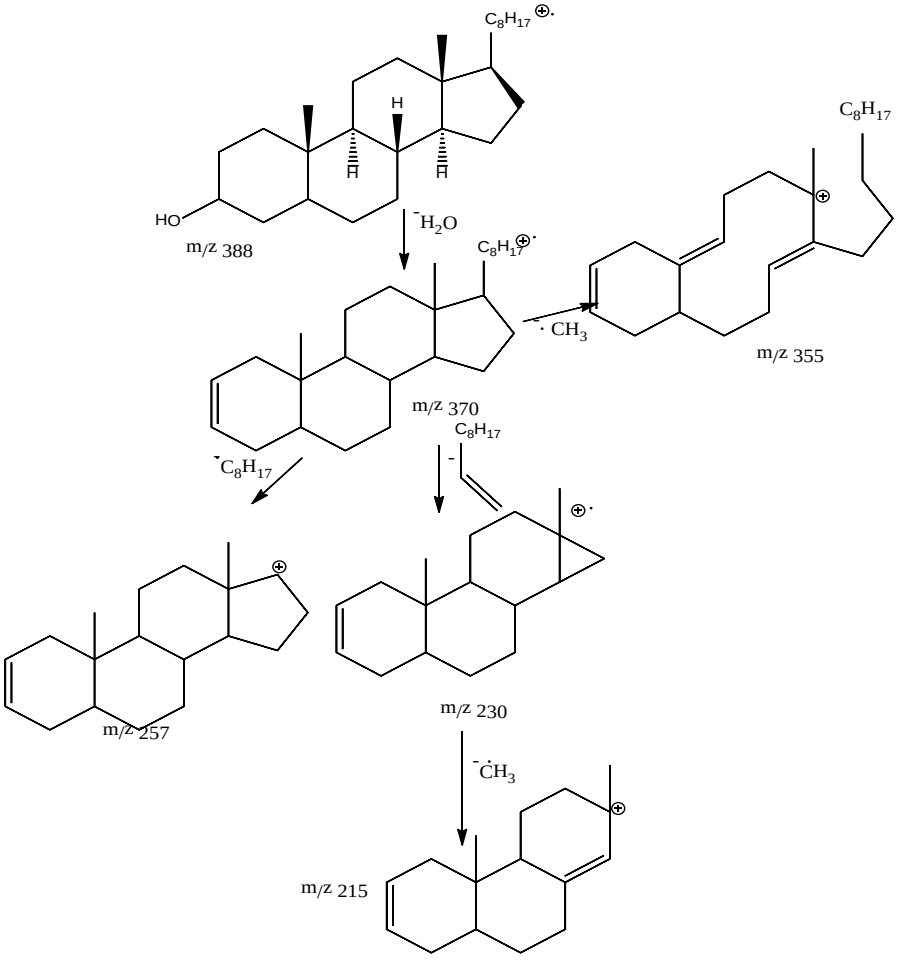

Figure6. Fragmentation pattern of cholestanol

Conclusion

Persian Gulf sponge *Haliclona sp.* produces numerous unique biomaterial of potential medicinal value. This species has several components. From it, five different compounds were identified by GC-MS. Most of these compounds are widely can used in pharmaceutical, medicinal, cosmetic and other fields. The results clearly showed that high antimicrobial activity of extract against various gram-positive and negative bacteria. The MIC of extract inhibited the growth of B. subtilis at 3.12 mg/ml.

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