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Antimicrobial activity of some Egyptian marine invertebrates, Red Sea

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ABSTRACT

Three species of sponge and one species of sea cucumber representative to marine invertebrates were collected from the Red Sea, Hurghada, Egypt. They were identified based on general morphological and anatomical features as *Cinachyrella arabica*, *Ciocalypta penicillus*, *Axinella verrucosa*, and *Holothuria atra*. Their antibacterial and antifungal activities in crude extracts were investigated and data revealed that the positive values were recorded in the range of 8 to 20 mm by the ethyl acetate crude extract obtained from *C. arabica*. *E. coli* was the most affected bacterium followed by *S. typhimurium* that was 18 mm by the ethyl acetate crude extract obtained from *A. verrucosa*. Also, the fungal suppression % was in the range of 10-100% by all kinds of sponge crude extracts. However, the ethanolic extract of *C. arabica* and ethyl acetate extract of *C. penicillus* had the highest suppression %, followed by the ethanolic extract of *C. penicillus* as of 90%. Likewise, the highest activity was detected by *H. atra* methanolic crude extract against *A. hydrophila*, followed by the same extract against *S. aureus* (14 mm). Only *P. notatum* was the fungus that affected by both ethanolic and methanolic extracts with 20% suppression. On the other side, results of GC-MS/MS of crude extract observed the presence of several bioactive constituents, most of which had antimicrobial activities.

INTRODUCTION

The multi-resistant nature of pathogens to antibiotic is the serious threat and has stimulated search for novel antimicrobial agents from various natural sources (Abubakar *et al*., 2012). The resistance to current antibiotics remains a significant challenge for pathogenic bacterial infections (Mayer *et al*., 2007). Therefore, the search for alternative antimicrobial agents from alternative sources became an essential demand. It is widely

accepted that new drugs, especially antibiotics, and that the most propitious source remains natural products (Bull and Stach, 2007; Mayer *et al*., 2007).

During the last decade, there has been an increase in research on marine crustaceans, molluscs, and echinoderms, particularly interest on their secondary metabolites with desirable antimicrobial properties (Casas *et al*., 2011). Many of these organisms produce their antibacterial factors as a first line of defense against pathogenic microbes (Haug *et al*., 2002).

On the other hand, sponges are the most well studied animals among the marine invertebrates, from which a vast array of bioactive compounds have been isolated (Hu *et al*., 2015). Actually, they are considered as the chemical factory in marine environment because of its immense production of chemically diverse compounds. Other than the chemical diversity, these compounds possess remarkable bioactivities (Kim and Dewapriya, 2012). Also, they were considered for years as a rich source of natural products and metabolites for antibiotics with strong inhibitory activity against bacteria, fungi and microbes Research showed that many bioactive compounds from various sponge species can be useful for the development of new antibiotics and antimicrobial drugs (Shen *et al.,* 2012). Specifically, Youssef *et al.* (2013) identified three new alkaloids from the Red Sea sponges which displayed antimicrobial activity against several pathogens, antioxidant activity and cancer growth inhibition activities. However, they concluded the importance of drug leads from Red Sea sponges.

Echinoderms have been, and continue to be, examined as a source of biologically active compounds with biomedical applications. Sea cucumber has been valued in Chinese medicine for hundreds of years as a cure for a wide variety of ailments (Kelly, 2005). Some more recently isolated compounds, mainly from sea cucumbers including those with antitumor, antiviral, anticoagulant, anti-angiogenic, anticancer, antihypertension, anti-inflammatory, antimicrobial, antioxidant, antithrombotic, antitumor and wound healing have been ascribed to various species of sea cucumbers. When wild stocks decline, the demand created in the market place raises to the price of the product and, consequently, culturing is more likely to become viable economically (Jawahar *et al*., 2002; Althunibat *et al*., 2009).

Therefore, the current work was suggested to extract and partially characterize, via GC/MS technique, the potent antimicrobial agents from the Red Sea sponges and sea cucumber species.

MATERIALS AND METHODS

Experimental animals and samples collection

Sponge and sea cucumber samples were collected from the sites located around Hurghada city, Egypt. The sponge specimens were collected by using SCUBA diving and snorkeling at the depth of (5-100 m). Sea cucumber specimens with a size range of 10 to 30 cm in length and 30 to 180 g weight were collected manually from the intertidal zone at the depth of 1-5 m. All fresh samples were then washed with seawater at the sampling

site to remove the adhered sediments and impurities, and then put in polyethylene bags. Quick rinsing of the samples with tap water was done in the laboratory on the same day to get rid of the remaining impurities and epiphytes.

Reference microbes and culture media

During this work, there five Gram positive bacterial pathogens (*Bacillus subtlis* ATCC 6633, *B. cerues, Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermids, Enterococcus faecalis* ATCC 29219) besides three Gram negative bacterial ones (*Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739) *Aermonas hydrophila,* and *Vibrio damsela* were used as reference strains. Also, there one yeast species (*Candida albicans*) was used as reference strains. As well as, there seven fungal pathogens (*Penicillum crustosum, Penicillum notatum, Aspergillus terreus, Aspergillus niger,* and *Fusarum solani*). Some of these strains were kindly provided from Microbiology Laboratory (National Institute of Oceanography and Fisheries, Alexandria, Egypt). Some others purchased from the Center of Fungi, Asuit University, Egypt.

On the other side, three common media were used to culture the reference strains and determine the antimicrobial activity of sea hare extract as follows: i) Nutrient broth (NB) and nutrient agar (NA) (Atlas, 1997) were used in all microbiological tests. Bacterial inocula was prepared by inoculating 100 ml of nutrient broth medium, and incubated in a shaker (250 rpm) at 30˚C for 24 h until the late logarithmic phase of growth (A_{550} = 1). ii) Sabouraud dextrose agar; (SDA) (Guinea *et al*., 2005) was used to cultivate yeast and other types of fungi. iii) Potato dextrose broth PDB and potato dextrose agar (PDA) (Atlas, 1997) were used to culture yeasts such as *C. albicans* and *Saccharomyces cerevisiae* and molds such as *A. niger*.

Extraction of antimicrobial agents

The samples of all marine invertebrates were cut into very small pieces of about 2 mm size. The extraction was carried out with four organic solvents; acetone, aqueous ethanol (70%), ethyl acetate, and methanol by soaking the material in the respective solvents (1:10, w/v) on a rotary shaker at 150 rev min^1 at ambient temperature for 96 h. The extract from consecutive soaking was pooled and filtered using filter paper (Whatman no 4). After evaporation of the solvent, the crude extract was re-suspended in 5 ml of dimethyl sulphoxide (DMSO). The antibacterial efficiency of the tested extract in DMSO was screened against different microbial pathogens.

Antibacterial and anti-yeast bioassay

All reference strains of bacteria and yeasts were examined as pathogens. A volume of 15 ml of the sterilized nutrient agar for bacteria and Sabouraud dextrose agar for yeast were poured into sterile caped test tubes and were allowed to cool to 50°C in a water bath. A half of ml of inocula (10^8 CFU for bacteria and yeast) were added. The

tubes were mixed using a vortex for 15-30 s. Thereafter, each test tube contents were poured onto a sterile 100 mm diameter Petri dish for solidification (Khan *et al*., 2019). The activity was evaluated using well-cut diffusion technique. Wells were punched out using a sterile 07 cm cork-porer in nutrient agar plates containing the tested microorganisms. About 100 µl of each crude extract were transferred into each well. They were subjected to 4°C incubation for 2 h, and then were later incubated at 37°C for 24 h. The results were obtained by measuring the diameter of inhibition zone three times for each well and expressed in millimeter (Amer and Ibrahim, 2019).

Antifungal bioassay

By pouring technique

The crude extracts were tested against the indicator fungi by adding aliquots of it to PDA medium at a concentration of 10% (v/v). One disc of the seven fungal growths was separately placed on the center of a plate containing crude extract-PDA medium. All plates were incubated at 28ºC until the control was completely covered with fungal growth. The radius-growth of each indicator fungus was measured to estimate the suppressive eff ect (%) of crude extract against the indicator fungi (Amer and Ibrahim, 2019).

By well-cut diffusion technique

One disc of the five fungal growths was separately put on the top of a plate containing PDA medium. About 100 µl of each crude extract were transferred into each well. All plates were incubated at 28ºC until the control was completely covered with fungal growth. The results were obtained by measuring the inhibition zone diameter three times for each well and expressed in millimeter (Amer and Ibrahim, 2019).

Antibiotic susceptibility test

Five commercial antibiotics: Cephalexin (CL, 30 µg), Rifampicin (RF, 30 µg) Piperacillin (TZP, 10 µg) Metronidazole (MTZ, 20 µg), and Amikacin (AMK, 30 µg) were selected to test their inhibition capacity against the bacterial strains besides the yeast strain *C. albicans*. The microbial strains were inoculated in the sterilized prepared medium. Instead of the crude extract of marine invertebrates, small discs of the five antibiotics were put associated with each microbial strain. All plates were subjected to 4°C incubation for 2 h, and then later incubated at 37°C for 24 h (Khan *et al*., 2019; Shaaban *et al*., 2020). The results were estimated by measuring the diameter of inhibition zone three times for each well and expressed in millimeter.

GC-MS/MS analysis of sea star extract

The different crude extracts were prepared with by soaking the fresh animal material in pure ethanol (1:10, w/v) and the filtrate was subjected to gas chromatography-mass spectrometry (GC-MS) analysis (Perkin Elmer, Waltham, MA, USA) according to

(Muller *et al*., 2002). The analyses were performed in Agilent 7693 series GC system equipped with an OV-5 capillary column (length 30 m 9 diameter 025 mm 9 film thickness 025 lm; Ohio Valley Specialty Chemical, Inc., Marietta, OH, USA) and an Agilent 5975C network selective mass detector, with initial temperature 90°C for 1 min, reaching to 300°C for 30 min, the splitless mode with injection volume 1 µl (total run time 6187 min). The mass spectrometer was operated in the electron impact (El) mode at 70 eV in the scan range 60-600 m/z. The helium was used as the carrier gas pressurized to 2223 psi, whereas the gas flow was 122 ml/min. The chemical constituents of the extract were identified by comparing the GC-MS peaks with retention times of standards, and the mass spectra obtained were compared with those available in the Mass Spectral Library NIST 2015. The percentage of each component was estimated as the ratio of the peak area to the total chromatographic area.

Statistics

The statistical software SPSS 17 was used for statistical analysis. One-way analysis of variance (ANOVA) test was performed to determine the differences between various groups. $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Marine invertebrates are extremely diverse, widely distributed and are always exposed to huge microbial challenges from the oceanic environment which is continuously altering (Otero-González *et al*., 2010). On the other hand, they are considered a promising field for the discovery of novel drugs. Marine sponges have efficiently provided potent drugs against several critical diseases as cancer, malaria, microbial, viral and many inflammatory diseases (Perdicaris, 2013). Therefore, the current study was suggested to screen three sponge species and one sea cucumber, collected from the Egyptian Red Sea at Hurghada, as a source of bioactive substances, which can be used as antibacterial agents against several human and fish pathogens. The four species of sponges and sea cucumber, collected for the current study, were identified as; Cinachyrella *arabica* (Carter, 1869), *Ciocalypta penicillus* (Bowerbank, 1862), *Axinella verrucosa* (Esper, 1794), and *Holothuria atra.* All these species have been habited commonly in the Egyptian Red Sea, Hurghada. However, its classification positions and general features of them within Kingdom Animalia are presented in Table 1 and Fig. 1, respectively**.**

In general, marine sponges (Hegde *et al*., 2002) and sea cucumbers (Chen, 2003) have capability of providing several antimicrobial agents. Indeed, the extracts of numerous marine invertebrates have shown broad spectra of antibacterial activity. Some of them were obviously active against pathogenic bacteria and yeasts (Marmouzia *et al.*, 2018). However, the antimicrobial effects of the crude extracts obtained from the

identified four marine invertebrates, including the antibacterial and antifungal activities, were estimated and then expressed in inhibition zone (mm).

Table 1: Classification position of sponges and sea cucumber within Kingdom Animalia.

Position	**Sample**/scientific classification			
	Brown sponge	**Yellow sponge**	**Long finger sponge**	**Sea cucumber**
Kingdom	Animalia	Animalia	Animalia	Animalia
Phylum	Porifera	Porifera	Porifera	Echinodermata
Class	Demospongiae	Demospongiae	Demospongiae	Holothuroidea
Order	Spirophorida	Halichondrida	Halichondrida	Holothuriidae
Family	Tetillidae	Halichondriidae	Axinellidae	Holothuriida
Genus	Cinachyrella	*Ciocalypta*	*Axinella*	*Holothuria*
Species	*C. arabica* (Carter, 1869)	*C. penicillus* (Bowerbank, 1862)	*A. verrucosa* (Esper, 1794)	*H. atra*

Cinachyrella arabica *Ciocalypta penicillus* *Axinella verrucosa*

Hololhuria atra

Fig. 1: General features of collected sponges and sea cucumber.

The result in the Table 2 showed that there were rather than fluctuations in the antimicrobial activities values from sponges extracts. Generally, there four pathogenic bacteria were not affected at all, they were; *S. aureus*, *S. epidermids*, *A. hydrophila*, and *V. fluvialis.* The other pathogens were clearly influenced in moderate activities (~10-14 mm) such as, high activities (~15-19 mm) or very high activity (~20 and more). Particularly, the positive values were recorded in the range of 8 to 20 mm. However, *E. coli* was the most affected bacterium by the ethyl acetate crude extract obtained from *C.*

arabica, followed by *S. typhimurium* that was 18 mm by the ethyl acetate crude extract obtained from *A. verrucosa*, etc. Amazingly, different crude extracts of *C. arabica* were the most potent recording 8 positive activities, while 5 and 4 positive records were detected only for different crude extracts of *A. verrucosa* and *C. penicillus*, respectively.

Similarly, Ibrahim *et al.* (2018) obtained very high bioactivity values finding that the acetone extract of *Spongia* sp. was the most effective against *A. hydrophila* (39.7 mm) followed by ethanol extract (35.3 mm), while the crude extracts of *C. penicillus* and *A. verrucosa* showed antibacterial activities against *E. coli* were 29.1, 29.1, and 28.0 mm for ethanol, acetone, and methanol extracts, respectively. Also, Abou-Elela *et al.* (2009) tested crude extracts of marine sponge against different bacterial pathogens and found that extracts of *Spongia officinalis* exhibited the highest inhibiting activity. The inhibition zones ranged from 13.0 mm against *S. aureus*, *S. faecalis* and *P. aeruginosa* to 15.4 mm against *E. coli*. Besides that, it has been observed that chloroform crude extracts were more effective than those of ethanol crude. Moreover, sponge's chloroform crude extracts acted on inhibiting the growth of all pathogenic bacteria. Extracts from the sponge species *Cinachyrella* sp., *Haliclona* sp. and *Petromica citrina* showed antibacterial activity against 61% of the coagulase-negative staphylococci (CNS) strains (responsible for causing bovine mastitis), including strains resistant to conventional antibiotics. Extracts from *P. citrina* showed the largest spectrum of inhibitory activity (Laport *et al.*, 2012).

Table 2: Antibacterial activity of different sponges extracts against some reference bacteria.

Bacterial pathogen	**Inhibition zone (mm)/species crude extract**											
	Long tree sponge				**Brown sponge**				**Yellow sponge**			
	E	**EA**	**M**	**A**	**E**	**EA**	**M**	**A**	**E**	**EA**	**M**	**A**
B. subtlis	0.0	0.0	0.0	0.0	10 ±0.2	14 ±1.0	0.0	0.0	11 ±0.1	0.0	0.0	0.0
S. aureus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. epidermids	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E. faecalis	0.0	0.0	0.0	0.0	0.0	14 ±0.3	0.0	0.0	0.0	0.0	0.0	0.0
P. aeruginosa	8 ±0.7	18 ±1.2	11 ±0.5	10 ±0. 3	0.0	16 ±1.5	16 ±0.7	11 ±1.4	0.0	0.0	0.0	14 ±0. 6
E. coli	0.0	0.0	0.0	0.0	0.0	20 ±0.7	10 ±1.3	0.0	0.0	14 ±0.5	0.0	0.0
A. hydrophila	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
V. damsela	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

A= acetone, E= ethanol, EA= ethyl acetate, and M= methanol.0; no activity (Resistant), ~10 mm; moderate activity, ~ 15 mm; high activity, and ~20 mm very high activity.

The results in the Table 3 exhibited that there were rather than low values in the antifungal activities from sponges extracts except against *P. crustosum* and *A. terreus*.

Specifically, the suppression was in the range of 10-100% by all kinds of crude extracts. The ethanolic extract of *C. arabica* and ethyl acetate extract of *C. penicillus* had the highest suppression %, followed by the ethanolic extract of *C. penicillus* as of 90%. The lowest value of the suppression % was for the ethanolic extract of *C. penicillus* (10%). The other fungi were not suppressed at all, besides *C. albicans*.

Table 3: Antifungal activity of different sponge extracts against some fungal reference strains.

Fungal pathogen	Suppression %*/species crude extract											
	Long tree sponge				Brown sponge				Yellow sponge			
	E	EA	M	A	E	EA	M	A	E	EA	M	A
P. crustosum	40 ±1.3	80 ±0.5	70 ±0.7	50 ±0.6	100 ±0.0	70 ±0.9	50 ±1.0	60 ±0.1	90 ±0.0	100 ±0.0	60 ±1.1	50 ±0.4
P. notatum	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
A. niger	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
F. solani	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
A. terreus	50 ±0.7	NS	50 ±1.0	NS	NS	50 ±0.5	20 ±0.7	NS	20 ±1.5	10 ±0.0	NS	NS
*C. albicans***	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*Suppression % refers to the antifungal activity which was calculated according to the equation mentioned in methodology section. **Suppression % of *C. albicans* was calculated in mm like done for the antibacterial activity. NS means no suppression was detected. A= acetone, E= ethanol, EA= ethyl acetate, and M= methanol.

Furthermore, data in the Table 4 summarized the antimicrobial activities values from sea cucumber; *H. atra* extracts. The positive records of antibacterial activities ranged between 10 and 16 mm. The highest activity was detected by methanolic crude extract against *A. hydrophila*, followed by the same extract against *S. aureus* (14 mm). Only *P. notatum* was the fungus that affected by both ethanolic and methanolic extracts with 20% suppression, while the other fungi were not influenced at all.

To sea cucumber, Mokhlesi *et al.* (2011) evaluated the antibacterial and antifungal activities of the ethyl acetate, methanol, and water-methanol extracts of the cuvierian organ, coelomic fluid, and body wall of the sea cucumber, *Bohadschia marmorata*, collected from Persian Gulf, against *A. niger, C. albicans, S. aureus, P. aeruginosa*, and *E. coli*. Their results showed that methanol extract of body wall and water–methanolic extract of cuvierian organ (against *A. niger*) and methanol extract of body wall and water–methanolic extract of cuvierian organ (against *C. albicans*) showed significant antifungal activities but no inhibitory effect of the extracts against bacteria was observed. Ibrahim (2012) assayed the antibacterial activities of flesh and coelomic fluids of three

species of sea cucumber; *Holothuria scabra, Holothuria leucospilota,* and *Holothuria atra*, were detected against number of human and fish pathogens (*S. aureus* ATCC 6538, *P. aeruginosa* ATCC 8739, *V. damsela*, *S. faecalis,* and *E. coli*). However, considerable antibacterial activities were observed in the methanolic extracts of coelomic fluid and flesh of all *Holothuria* species against *S. aureus* ATCC 6538. Their AU ranged from 2.3 to 2.8. Noticeably, crude extracts of *H. scabra* exhibited abroad spectrum effect against all tested pathogens. Muthulakshmi *et al.* (2012) reported the biological activity due to the identified components in the methanolic extracts of *H. scabra*. They revealed that the phenolic nature of phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-compound, the steroid nature of cholesta8, 24-dien-3-ol, 4-methyl-, (3a´,4a`)- compound, and also the alcoholic nature of 2-isopropyl-5methyl-1-heptanol compound showed potent antimicrobial activity. In recent findings, Dhinakaran and Lipton, (2014) observed that the methanol extracts of *H. atra* showed antimicrobial activity against *S. aureus* MTCC737, *E. coli* MTCC443, *K. pneumonia* MTCC109, *Listeria monocytogenes* MTCC1143, and *Serratia liquefaciens* MTCC3039.

Table 4: Antimicrobial activity of different extracts sea cucumber; *H. atra* on reference strains.

Pathogen	**Inhibition zone (mm)/solvent**			
	E	**EA**	**M**	**A**
Bacteria:				
E. coli	0.0	0.0	12±0.0	0.0
E. faecalis	0.0	0.0	0.0	0.0
S. aureus	10±0.3	0.0	14±1.0	0.0
P. aeruginosa	0.0	0.0	0.0	0.0
B. subitilis	0.0	0.0	0.0	0.0
A. hydrophila	13±1.2	0.0	16±0.9	0.0
V. damsela	0.0	0.0	0.0	0.0
S. epidermids	0.0	0.0	0.0	0.0
Fungi:	**Suppression %/solvent**			
P. crustosum	NS	NS	NS	NS
P. notatum	20±0.7	NS	20±0.5	NS
A. terreus	NS	NS	NS	NS
A. niger	NS	NS	NS	NS
F. solani	NS	NS	NS	NS
C. albicans[*]	0.0	0.0	0.0	0.0

[*]Inhibition of *C. albicans* was calculated in mm like done for the antibacterial activity. NS means no suppression was detected 0; no activity (Resistant), ~10 mm; moderate activity, ~ 15 mm; high activity, and ~20 mm very high activity. A= acetone, E= ethanol, EA= ethyl acetate, and M= methanol.

On comparison level, the activity of several commercial antibiotics (mm) was examined and then compared to the results of *A. fasciata* crude extract (mm) (Table 5).

Basically, Gram positive showed obvious susceptibility towards most of the tested antibiotics. In fact, *B. subtlis* was sensitive towards Cephalexin, Rifampicin**,** and Piperacillin**,** while it was resistant towards both Metronidazole and Amikacin. Also, *B. cerues,* was sensitive towards Cephalexin, Rifampicin**,** and Amikacin**,** while it was resistant towards both Piperacillin and Metronidazole. As well as, *S. aureus* was sensitive toward both Cephalexin and Metronidazole, while it was resistant towards Rifampicin, Piperacillin, and Amikacin. On contrary, *S. epidermids* and *E. faecalis* exhibited clear resistance towards all tested antibiotics. Also, *P. aeruginosa* behaved like them except for Cephalexin and Metronidazole, where they showed intermediate sensitivity. In addition, *E. coli* was only susceptible against Cephalexin**.** By comparing the current crude extracts of sponges and sea cucumber to commercial antibiotics, it was observed that the good efficacy of these extracts. Even, they would need a strong purification process to be used in very low concentrations as commercial antibiotics.

Table 5: Effect of different commercial antibiotics on reference strains in comparing to *C. arabica* crude extract.

Reference bacteria	**Inhibition zone (mm)* of *C. arabica* crude extract**	**Inhibition zone (mm)/Antibiotic (disc/µg)**				
		Cephalexin (CL, 30 µg)	**Rifampicin (RF, 30 µg)**	**Piperacillin (TZP, 10 µg)**	**Metronidazole (MTZ, 20 µg)**	**Amikacin (AMK, 30 µg)**
B. subtlis	14	23	21	13	7	0
B. cerues	0	25	14	6	0	22
S. aureus	0	30	9	9	29	8
S. epidermids	0	10	0	0	9	0
E. faecalis	14	6	6	6	6	0
P. aeruginosa	16	0	0	0	12	0
E. coli	20	23	0	0	0	0

*These values taken were representative as the highest average from Table 2. 0; no activity (Resistant), ~10 mm; moderate activity, ~ 15 mm; high activity, and ~20 mm very high activity.

On the other hand, the results of GC-MS/MS of ethanol-extract from *C. arabica* revealed the presence of several bioactive constituents, with 11 major compounds (Fig. 2 & Table 6). The chemical profiles of them are mainly: Dimethyl sulfone (41.86%), Pyridine, 2-nitro (16.30%), 1,3,2-Dioxathiolane, 2-oxide (20.90%), S-Methyl methanethiosulphonate (88.59%), 1,2,4-Trithiolane (46.63%), 4,25-Secoobscurinervan-4-one, O-acetyl-22-ethyl-15,16-dimethoxy-, (22à) (24.07%), Methyl N-(N-benzyloxycarbonyl-beta-l-aspartyl)-beta-d-glucosaminide (6.70%), 2-Amino-3-(4-hydroxyphenyl)- propanoic acid (9.29%), Cyclohexasiloxane, dodecamethyl (86.47%), 2,7-Diphenyl-1,6-Dioxopyridazino [4,5:2',3'] Pyrrolo [4',5'-d]pyridazine (17.24%), and Cycloheptasiloxane, tetradecamethyl (77.56%). Also, the GC-MS/MS results of *C. arabica* ethyl acetate-extract revealed the presence of several bioactive constituents, with 12 major compounds (Fig. 2 & Table 6). The chemical profiles of them are mainly: undefined (59.72%), Pyridinium, 1-amino-, hydroxide, inner salt (62.20%), Methanol, (1,4-dihydrophenyl) (11.36%), Bicyclo[3.3.1]non-6-ene-3-carboxylic acid (8.16%), S-

Methyl methanethiosulphonate (64.00%), 4,25-Secoobscurinervan-4-one, O-acetyl-22-ethyl-15,16-dimethoxy-, (22à) (10.18%), Pyridine, 2-nitro (9.18%), 1,2,4-Trithiolane (76.56%), 3-Nitropyridine 1-oxide (7.42%), Carbonotrithioic acid, dimethyl ester (42.05%), 2,5-Anhydro-1-deoxy-1-(2-pyridylamino)-d-mannitol (15.53%), and Cyclohexasiloxane, dodecamethy (93.10%).

In addition, the results of GC-MS/MS of ethanol-extract from *C. arabica* revealed the presence of several bioactive constituents, with 4 major compounds (Fig. 2 & Table 6). The chemical profiles of them are mainly: Pyridinium, 1-amino-, hydroxide, inner salt (40.23%), Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-decamethyl (14.68%), Carbonotrithioic acid, dimethyl ester (73.11%), and Cyclohexasiloxane, dodecamethyl (93.79%). Moreover, the results of GC-MS/MS of methanol-extract from *C. arabica* revealed the presence of several bioactive constituents, with 8 major compounds (Fig. 2 & Table 6). The chemical profiles of them are mainly: S-Methyl methanethiosulphonate (78.37%), 1,2,4-Trithiolane (40.93%), 2,5-Anhydro-1-deoxy-1-(2-pyridylamino)-d-mannitol (14.22%), Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid (8.90%), 2,5-Anhydro-1-deoxy-1-(2-pyridylamino)-d-mannitol (12.59%), Cycloheptasiloxane, tetradecamethyl (76.95%), Benzofuran, 7-(2,4-dinitrophenoxy)-2,3-dihydro-2,2-dimethyl (9.98%), and Cyclooctasiloxane, hexadecamethyl (36.65%).

Likewise, the results of GC-MS/MS of ethanol-extract from *H. atra* revealed the presence of several bioactive constituents, with 18 major compounds (Fig. 3 & Table 7). The chemical profiles of them are mainly: Pyridinium, 1-amino-, hydroxide, inner salt (58.68%), Ethanol, 2-mercapto (57.99%), 1,3,2-Dioxathiolane, 2-oxide (50.15%), S-Methyl methanethiosulphonate (79.92%), 3,7,7-Trimethyl-8-(2-methyl-propenyl)-bicyclo[4.2.0]oct-2-ene (21.13), 1,2,4-Trithiolane (90.07%), 3-Nitropyridine 1-oxide (19.61%), Spirost-8-en-11-one, 3-hydroxy-, (3á,5à,14á,20á,22á,25R) (6.38%), Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid (8.33%), Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid (9.32%), 4,25-Secoobscurinervan-4-one, O-acetyl-22-ethyl-15,16-dimethoxy-, (22à) (19.62%), Cyclohexasiloxane, dodecamethyl (94.87), Malonodinitrile, 2-[1-cyclopropyl-3-(4-nitrophenyl)prop-2-enylideno] (9.20), 2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine (13.71), Ethyl iso-allocholate (24.82), Cycloheptasiloxane, tetradecamethyl (81.32), 2-Cyclopropene-1-carboxylic acid, 2-(1,1-dimethyl-5-oxohexyl)-, methyl ester (10.09), and Cyclooctasiloxane, hexadecamethyl (64.99). Additionally, the results of GC-MS/MS of methanol-extract from *H. atra* revealed the presence of several bioactive constituents, with 15 major compounds (Fig. 3 & Table 7). The chemical profiles of them are mainly: Methanesulfonylacetic acid (34.70%), 2,3-Dihydro-6-hydroxy-3-oxo-2-(piperidinomethyl)pyridazine (39.23%), Acetic acid butyl-methyl-phosphinoylmethyl ester (14.13%), S-Methyl methanethiosulphonate (85.34%),1,2,4-Trithiolane (62.85%), 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde (5.83%), 4,25-Secoobscurinervan-4-one, O-acetyl-22-ethyl-15,16-dimethoxy-, (22à) (29.54%),

N,N'-Bis(Carbobenzyloxy)-lysine methyl(ester) (23.68%), 2-Propen-1-ol, 2-methyl-3-(2,6,6-trimethyl-2-cyclohexen-1-yl)-, (E) (8.49%), 2-Amino-3-(4-hydroxyphenyl)-propanoic acid (7.64%), Cyclohexasiloxane, dodecamethyl (91.46%), Z,Z,Z-4,6,9-Nonadecatriene (6.81%), Cycloheptasiloxane, tetradecamethyl (72.91%), Cyclooctasiloxane, hexadecamethyl (43.78%), and Ergosta-5,22-dien-3-ol, acetate, (3á,22E) (6.81%).

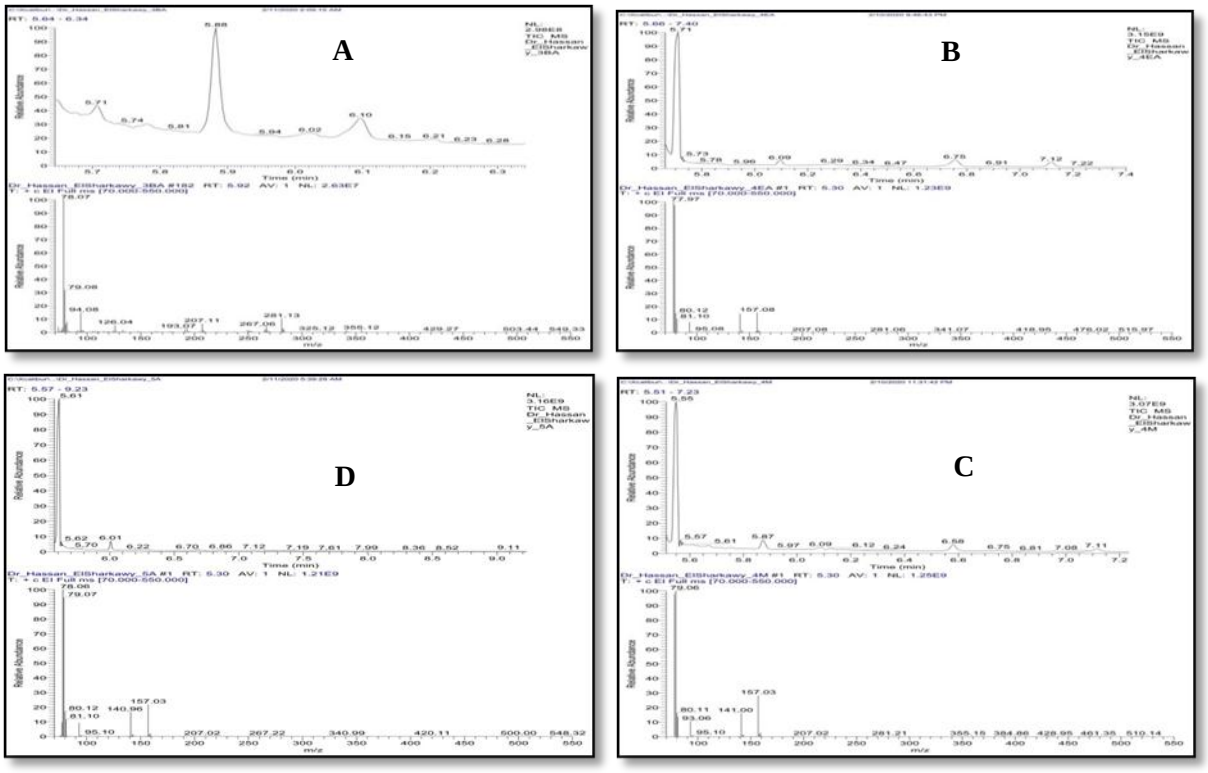

Fig. 2: GC-MS/MS chromatogram of different *C. arabica* crude extracts; ethanolic (A), methanolic (C), and acetone (D) showing the retention time and molecular weights of the identified compounds.

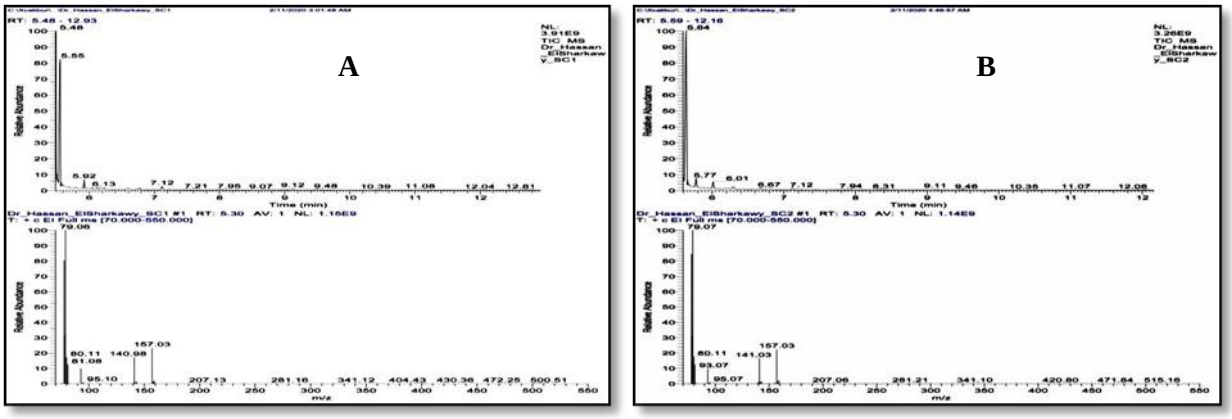

Fig. 3: GC-MS/MS chromatogram of *H. atra* ethanolic extract (A) and methanolic extract (B) showing the retention time and molecular weights of the identified compounds.

On the other side, the potential compounds with bioactivity detected by GC-MS in the our crude extracts were organic and fatty acids and their derivatives, besides much other of organic alcohols, steroids, terpenoids, amino acids, esters and benzene derivatives. However, the most of these constituents have been proven as antibacterial and antifungal agents (Ibrahim, 2012; Moustafa *et al*., 2013; Hussein *et al*., 2016; Ibrahim *et al*., 2018).

Indeed, several workers have discussed the effectiveness of the constituents that were obtained in the present crude extracts. For instance, Matsunaga *et al.* (1989) isolated an antifungal and cytotoxic cyclic tetrapeptide from a *Theonella* sponge and halicylindramides from the marine sponge *Halichondria cylindrata*. Ryu *et al.* (1994) concluded that the methanolic extract of marine sponge exhibited significant antifungal activity and antibacterial activity to both Gram-positive and Gram-negative bacteria. Also, Clark *et al.* (1998) isolated a cyclicdipsipeptide, cyclolithistide from a marine sponge; *Theonella swinhoei* (Gray, 1868), and this molecule exhibited significant antifungal activity against *C. albicans* but not against *E. coli* or *B. subtilis*. As well as, Otero-González *et al.* (2010) observed that a promising novel use of antimicrobial peptides from the marine sponge *Discodermia kiiensis*, could be a potent drug in both human and veterinary medicine based upon their potential characteristics.

Recently, Ibrahim *et al.* (2018) detected the principal components in the sponge *C. penicillus* extracts by GC-MS as fatty acids and their esters (hexadecanoic acid and octadecanoic acid), as well as, steroids and terpenoids, which have antimicrobial effects. Fatty acids are able to act as anionic surfactants; they also possess antifungal and antibacterial characteristics at low pH (AbouElela *et al*., 2009; Ibrahim and Abd El-Naby, 2010). More specifically, diterpene isonitriles isolated from the tropical marine sponge *Cymbastela hooperi*, and the sesquiterpene axisonitrile-3, isolated from the tropical marine sponge *Acanthella kletra*, were evaluated as anti-fouling, anti-algal, anti-photosynthetic, antibacterial, antifungal, and anti-tubercular agents (Wright *et al.,* 2011). Also, Halogenated alkaloids named purpuroines A-J were isolated from the marine sponge *Lotrochota purpurea*. Tests of these compounds showed inhibitory activity against diseases related to fungi and bacteria (Shen *et al.,* 2012). Shen *et al.* (2012) isolated novel cyclic bis-1,3-dialkylpyridiniums and cyclostellettamines from the sponge *Haliclona* sp. in Korea. As well as, Yang *et al.* (2012) isolated new alkaloids, (-)-8'-oxo-agelasine D, ageloxime B, (+)-2-oxo-agela-sidine C, 4-bromo-N-(butoxymethyl)1H-pyrrole-2-carboxamide and (-)-ageloxime D from the marine sponge *Agelas mauritiana*. Some of these metabolites showed antifungal activity against *Cryptococcus neoformans*, antileishmanial activity in vitro and antibacterial activity against Staphylococcus aureus and methicillin-resistant *S. aureus*.

Table 6: Chemical constituents detected in different extracts of *C. arabica* by GC-MS/MS.

Peak No.	Compound name	RT (min)	Molecular formula	MW (m/z)	Hit	SI	RSI	Prob. (%)
***C. arabica* acetone extract:**								
1	Dimethyl sulfone	5.60	$C_2H_6O_2S$	94	1	874	863	41.86
2	Pyridine, 2-nitro	5.75	$C_5H_4N_2O_2$	124	1	577	735	16.30
3	1,3,2-Dioxathiolane, 2-oxide	5.87	$C_2H_4O_3S$	108	1	578	861	20.90
4	S-Methyl methanethiosulphonate	6.10	$C_2H_6O_2S_2$	126	1	743	838	88.59
5	1,2,4-Trithiolane	6.22	$C_2H_4S_3$	124	1	590	875	46.63
6	4,25-Secoobscurinervan-4-one, O-acetyl-22-ethyl-15,16-dimethoxy-, (22à)	6.30	$C_{27}H_{36}N_2O_6$	484	1	580	583	24.07
7	Methyl N-(N-benzyloxycarbonyl-beta-l-aspartyl)-beta-d-glucosaminide	6.70	$C_{19}H_{26}N_2O_{10}$	442	1	532	574	6.70
8	2-Amino-3-(4-hydroxyphenyl)-propanoic acid	6.86	$C_9H_{11}NO_3$	181	1	616	714	9.29
9	Cyclohexasiloxane, dodecamethyl	7.12	$C_{12}H_{36}O_6Si_6$	444	1	745	811	86.47
10	2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine	7.90	$C_{20}H_{13}N_5O_2$	355	1	568	599	17.24
11	Cycloheptasiloxane, tetradecamethyl	9.11	$C_{14}H_{42}O_7Si_7$	518	1	732	834	77.56
***C. arabica* methanol extract:**								
1	Undefined $\{(CH_3)_2NCl\}$	5.56	C_2H_6ClN	79	1	617	859	59.72
2	Pyridinium, 1-amino-, hydroxide, inner salt	5.57	$C_5H_6N_2$	94	1	854	918	62.20
3	Methanol, (1,4-dihydrophenyl)	5.61	$C_7H_{10}O$	110	1	535	671	11.36
4	Bicyclo[3.3.1]non-6-ene-3-carboxylic acid	5.75	$C_{10}H_{14}O_2$	166	2	521	641	8.16
5	S-Methyl methanethiosulphonate	5.87	$C_2H_6O_2S_2$	126	1	604	777	64.00
6	4,25-Secoobscurinervan-4-one, O-acetyl-22-ethyl-15,16-dimethoxy-, (22à)	5.97	$C_{27}H_{36}N_2O_6$	484	1	519	522	10.18
7	Pyridine, 2-nitro	6.05	$C_5H_4N_2O_2$	124	1	519	742	9.18
8	1,2,4-Trithiolane	6.09	$C_2H_4S_3$	124	1	638	861	76.56
9	3-Nitropyridine 1-oxide	6.12	$C_5H_4N_2O_3$	140	1	517	639	7.42
10	Carbonotrithioic acid, dimethyl ester	6.58	$C_3H_6S_3$	138	1	634	765	42.05
11	2,5-Anhydro-1-deoxy-1-(2-pyridylamino)-d-mannitol	6.75	$C_{11}H_{16}N_2O_4$	240	1	565	764	15.53
12	Cyclohexasiloxane, dodecamethy	7.11	$C_{12}H_{36}O_6Si_6$	444	1	768	840	93.10
***C. arabica* ethyl acetate extract:**								
1	Pyridinium, 1-amino-, hydroxide, inner salt	5.71	$C_5H_6N_2$	94	3	839	906	40.23
2	Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-decamethyl	6.09	$C_{10}H_{32}O_4Si_5$	356	1	532	647	14.68
3	Carbonotrithioic acid, dimethyl ester	6.75	$C_3H_6S_3$	138	1	679	843	73.11
4	Cyclohexasiloxane, dodecamethyl	7.12	$C_{12}H_{36}O_6Si_6$	444	1	823	852	93.79
***C. arabica* ethanol extract:**								
1	S-Methyl methanethiosulphonate	5.88	$C_2H_6O_2S_2$	126	1	637	818	78.37
2	1,2,4-Trithiolane	6.10	$C_2H_4S_3$	124	1	572	861	40.93
3	2,5-Anhydro-1-deoxy-1-(2-pyridylamino)-d-mannitol	6.48	$C_{11}H_{16}N_2O_4$	240	1	579	747	14.22
4	Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid	6.60	$C_8H_{10}O_2$	138	1	532	720	8.90
5	2,5-Anhydro-1-deoxy-1-(2-pyridylamino)-d-mannitol	6.77	$C_{11}H_{16}N_2O_4$	240	1	548	746	12.59
6	Cycloheptasiloxane, tetradecamethyl	9.12	$C_{14}H_{42}O_7Si_7$	518	1	749	853	76.95
7	Benzofuran, 7-(2,4-dinitrophenoxy)-2,3-dihydro-2,2-dimethyl	9.48	$C_{16}H_14N_2O_6$	330	1	531	582	9.98
8	Cyclooctasiloxane, hexadecamethyl	11.80	$C_{16}H_{48}O_8Si_8$	592	1	663	811	36.65

Table 7: Chemical constituents detected in different extracts of *H. atra* by GC-MS/MS.

Peak No.	Compound name	RT (min)	Molecular formula	MW (m/z)	Hit	SI	RSI	Prob. (%)
***H. atra* ethanol extract:**								
1	Pyridinium, 1-amino-, hydroxide, inner salt	5.55	$C_5H_6N_2$	94	1	847	902	58.68
2	Ethanol, 2-mercapto	5.69	C_2H_6OS	78	1	611	894	57.99
3	1,3,2-Dioxathiolane, 2-oxide	5.79	$C_2H_4O_3S$	108	1	604	888	50.15
4	S-Methyl methanethiosulphonate	5.92	$C_2H_6O_2S_2$	126	1	649	802	79.92
5	3,7,7-Trimethyl-8-(2-methyl-propenyl)-bicyclo[4.2.0]oct-2-ene	6.04	$C_{15}H_{24}$	204	1	564	666	21.13
6	1,2,4-Trithiolane	6.13	$C_2H_4S_3$	124	1	741	923	90.07
7	3-Nitropyridine 1-oxide	6.23	$C_5H_4N_2O_3$	140	1	556	671	19.61
8	Spirost-8-en-11-one, 3-hydroxy-, (3á,5à,14á,20á,22á,25R)	6.49	$C_{27}H_{40}O_4$	428	2	521	528	6.38
9	Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid	6.60	$C_8H_{10}O_2$	138	4	544	725	8.33
10	Benzamide, N-(2-aminophenyl)-2,4,6-trinitro	6.74	$C_{13}H_9N_5O_7$	347	1	578	656	9.32
11	4,25-Secoobscurinervan-4-one, O-acetyl-22-ethyl-15,16-dimethoxy-, (22à)	6.98	$C_{27}H_{36}N_2O_6$	484	1	575	587	19.62
12	Cyclohexasiloxane, dodecamethyl	7.12	$C_{12}H_{36}O_6Si_6$	444	1	772	847	94.87
13	Malonodinitrile, 2-[1-cyclopropyl-3-(4-nitrophenyl)prop-2-enylideno]	7.21	$C_{15}H_{11}N_3O_2$	265	1	534	634	9.20
14	2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine	7.95	$C_{20}H_{13}N_5O_2$	355	1	539	571	13.71
15	Ethyl iso-allocholate	8.04	$C_{26}H_{44}O_5$	436	1	582	588	24.82
16	Cycloheptasiloxane, tetradecamethyl	8.12	$C_{14}H_{42}O_7Si_7$	518	1	757	858	81.32
17	2-Cyclopropene-1-carboxylic acid, 2-(1,1-dimethyl-5-oxohexyl)-, methyl ester	9.48	$C_{13}H_{20}O_3$	224	1	547	635	10.09
18	Cyclooctasiloxane, hexadecamethyl	11.08	$C_{16}H_{48}O_8Si_8$	592	1	700	806	64.99
***H. atra* methanol extract:**								
1	Methanesulfonylacetic acid	5.62	$C_3H_6O_4S$	138	1	857	869	34.70
2	2,3-Dihydro-6-hydroxy-3-oxo-2-(piperidinomethyl)pyridazine	5.77	$C_{10}H_{15}N_3O_2$	209	1	622	646	39.23
3	Acetic acid butyl-methyl-phosphinoylmethyl ester	5.88	$C_8H_{17}O_3P$	192	1	583	722	14.13
4	S-Methyl methanethiosulphonate	6.01	$C_2H_6O_2S_2$	126	1	705	811	85.34
5	1,2,4-Trithiolane	6.21	$C_2H_4S_3$	124	1	648	910	62.85
6	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	6.29	$C_{23}H_{32}O$	324	1	586	591	5.83
7	4,25-Secoobscurinervan-4-one, O-acetyl-22-ethyl-15,16-dimethoxy-, (22à)	6.43	$C_{27}H_{36}N_2O_6$	484	1	587	602	29.54
8	N,N'-Bis(Carbobenzyloxy)-lysine methyl(ester)	6.58	$C_{23}H_{28}N_2O_6$	428	1	581	621	23.68
9	2-Propen-1-ol, 2-methyl-3-(2,6,6-trimethyl-2-cyclohexen-1-yl)-, (E)	6.67	$C_{13}H_{22}O$	194	1	558	617	8.49
10	2-Amino-3-(4-hydroxyphenyl)-propanoic acid	6.82	$C_9H_{11}NO_3$	181	1	587	701	7.64
11	Cyclohexasiloxane, dodecamethyl	7.12	$C_{12}H_{36}O_6Si_6$	444	1	761	829	91.46
12	Z,Z,Z-4,6,9-Nonadecatriene	7.93	$C_{19}H_{34}$	262	1	566	639	6.81
13	Cycloheptasiloxane, tetradecamethyl	9.11	$C_{14}H_{42}O_7Si_7$	518	1	709	830	72.91
14	Cyclooctasiloxane, hexadecamethyl	11.07	$C_{16}H_{48}O_8Si_8$	592	1	659	794	43.78
15	Ergosta-5,22-dien-3-ol, acetate, (3á,22E)	12.08	$C_{30}H_{48}O_2$	440	1	548	633	6.81

Likewise, It has been established that there is a variety of antimicrobial compounds have been isolated from enchinoderms including steroidal glycosides (Jawahar *et al*., 2002), polyhydroxylated sterols (Iorizzi *et al*., 2001), peptides antibiotics (Beauregard *et al*., 2001), and complement-like substances (Leonard *et al*., 1990). Zou *et al.* (2005) isolated Intercedenside D–I as cytotoxic triterpene glycoside from the sea cucumber; *Mensamaria intercedens*, and Wu *et al.* (2006) extracted Hillaside C a triterpene derived from sea cucumber *Holothuria hilla*. In addition, Fuscocineroside C compound as a triterpene glycoside was obtained from sea cucumber *Holothuria fuscocinerea* that showed cytotoxic nature against human cancer cells (Zhang *et al*., 2006). Also, Ibrahim (2012) characterized the antibacterial agent from both methanolic flash and coelomic fluid crude extracts of sea cucumber; *H. scabra* using GC/MS as carotenoids and then via preparative high performance liquid chromatography (HPLC) detected them as; xanthophyll, β-crptoxanthin, and β-carotene in of *H. scabra*. Observably, the antimicrobial properties of sea cucumbers have been linked to the presence of a wide array of bioactive substances especially triterpene glycosides (saponins), chondroitin sulfates, glycosaminoglycan (GAGs), sulfated polysaccharides, sterols (glycosides and sulfates), phenolics, cerberosides, lectins, peptides, glycoprotein, glycosphingolipids, terpenoids and essential fatty acids (Bordbar *et al.,* 2011).

The tetraterpenoid nature of palmitate and the fatty nature of oleic acid showed antimicrobial activity against both Gram-positive and G-negative organisms (Plaza *et al*., 2010). Finally, several of these compounds exhibited moderate cytotoxic and antibacterial activities against Gram-positive strains (Lee *et al.,* 2012).

CONCLUSION

Data obtained from the present study demonstrated that the marine sponges and sea cucumber are a promising source of new bioactive substances. Asa well as, the inhibition efficiency of different crude extracts against several human pathogens such; *S. aureus*, *E. faecalis*, *P. aeruginosa*, and *E. coli* suggests promising applications in the clinical field. Furthermore, the inhibitory effect of the sea cucumber extracts against fish pathogens such as *A. hydrophila* suggests promising applications in the aquaculture field.

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