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Antimicrobial characteristics of marine polychaetes collected from Alexandria beaches

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Received 3rd August 2010, Accepted 4th November 2010

Abstract

Five marine polychaete species isolated from seawater, Alexandria, Egypt, were taxonomically identified and then investigated as a source of natural products which can be used as antibacterial and antifungal of human and fish pathogens. Three of these species were from family Neriididae; genus *Nereis* (*Nereis falsa*), genus *Perinereis* (*Perinereis nuntia typica*) and genus *Pseudonereis* (*Pseudonereis anomala*); one species from family Oenonidae; genus *Halla* A. (*Halla parthenopeia*), and finally one species was from family Serpulidae; genus *Hydroides* (*Hydroides elegans*). Ethanol crude extracts of the current marine worms were tested against different bacterial and fungal pathogens. Positive significant results were detected with *Halla parthenopeia* and *Hydroides elegans.* The crude extract of *H. elegans* had abroad spectrum antimicrobial effect against different bacterial pathogens. Activity units ranged from 6.4 for *B. cereus* to 15.5 for *P. aureginosa*. Moreover, *H. elegans* extracts had the most suppression percentage against pathogenic fungi expressed in diameter. Suppression percentage ranged from 40 to 100% against *A. niger* and *R. solani*, respectively. As well as, suppression percentages of crude extracts against fungi, represented by dry weight, ranged from 15.66 to 96.9% for *H. elegans* crude extract and from zero to 90.10% for *H. parthenopeia* crude extract. The gas liquid chromatography mass spectrometer of *H. parthenopeia* and *H. elegans* extracts was determined and the main constituents detected were organic acids and their derivatives.

Keywords: Marine worms, Polychaetes; Marine bioactive substances; Dodecane; Cholesterol; Hexadecanoic acid

1. Introduction

An increasing number of disease outbreaks have been recorded in marine invertebrates and human from viral, bacterial and fungal infections, which are largely influenced by environmental conditions, such as pollution and climate warming (Mydlarz *et al*., 2006). Nowadays, research about marine natural product to control diseases appeared as an impact of pathogenic bacteria and fungi; for example, Porifera (sponges) and Chordata (including ascidians) have dominated as the major contributing phyla of novel bioactive compounds (Blunt *et al*., 2007). Sponges, in particular, are responsible for a large number of these compounds, which exhibit a wide range of activities including antitumor. For example, 13-Deoxytedanolide is a potent antitumour macrolide isolated from the marine sponge *Mycale adhaerens* (Nishimura *et al*., 2005); as an antiviral: sponge aqueous extract was tested for anti-herpetic, anti-adenovirus and anti-rotavirus activities, (Da Silva, 2006), as antibacterial: antibacterial extraction from sponges were used against certified strains of bacteria (*Staphylococcus aureus* and *Escherichia coli*) and yeast (*Candida albicans*) giving 80% positive results (Galeano and Martínez, 2007) and antifungal activity (Concepcion *et al*., 1994). Few studies were made on marine worms especially polychaetes. Marine worms dwell in sediments, indicating the requirement of antimicrobial strategy for their survival. Perinerin, Arenicins-1, 2 and Hedistin have been isolated from polychaete and echiuroid worms. Arenicin-1 and -2 were isolated from coelomoycytes of the polychaete *Arenicola marina* (Ovchinnikova *et al*., 2004). Perinerin isolated from homogenates of the polychaete *Perinereis aibuhitensis*, is a highly cationic, hydrophobic peptide (Pan *et al*., 2004). It had an antifungal and antibacterial activity against Gram-negative and Gram–positive bacteria. Organobromine compounds were produced naturally by marine creatures (sponges, corals, sea slugs, tunicates, sea fans), also brominated compounds such as brominated indoles, 2,3,4-tribromopyrrole and brominated phenols were produced naturally, by common polychaete worms (Gribble, 2000). The antimicrobial activities of dominant fatty acids were

assessed against marine pathogenic bacteria by Benkendorff *et al*. (2005).

In this study, we screened five polychaete species (*Nereis falsa, Perinereis nuntia typical, Pseudonereis anomala, Halla parthenopeia* and *Hydroides elegans*) as a source of natural products which can be used as antibacterial and antifungal agents against human and fish pathogens.

2. Material and methods

2.1. Isolation and identification of polychaete species

Five polychaete species (*Nereis falsa, Perinereis nuntia typica, Pseudonereis anomala, Halla parthenopeia* and *Hydroides elegans*) were isolated and taxonomically identified according to Fauvel (1923& 1927), and Day (1967a&b). Samples were obtained from sediment substrata from El-Ebrahimia beach (Alexandria, Egypt) and Lake Timsah by Scoba diving.

2.2. Preparation of polychaete crude extracts

Forty grams of each marine worm were macerated with 100 ml of 70 % aqueous ethanol. After soaking for two weeks, they were filtered through Whatman 542 filter paper. Ethanol was evaporated using rotary evaporator to obtain soluble extracts (Ballantine, 1987).

2.3. Microbial indicator strains

The bacterial indicators were; *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 8739, *Vibrio damsela, Vibrio fulvilalis, Bacillus cereus, Bacillus* cereus 1318, *Streptococcus faecalis and Escherichia coli.* The fungal indicator strains used were *Fusarium oxysporum***,** *Rhizoctonia solani,* and *Aspergillus niger.*

2.4. Media

2.4.1. Nutrient broth

(Atlas, 1995) composed of (g\L): yeast extract, 2; beef extract, 1**;** peptone, 5; sodium chloride, 5. Agar (15-20) was added for obtaining nutrient agar.

2.4.2. Potato dextrose broth medium

Diced potatoes were boiled in 500 ml of distilled water until thoroughly cooked, filtered through cheese cloth and water was added to the filtrate up to 1L, then 20 g dextrose were added. Agar (15-20g\L) was added to obtain potato dextrose agar (El-Masry *et al*., 2002).

2.5. Bacterial and fungal cultures

All bacterial pathogenic strains were maintained on nutrient agar slants. Bacterial inocula were prepared by inoculating 100 ml of nutrient broth medium, and incubated shaken (250 rpm) at 30˚C for 24h until late logarithmic phase of growth (A_{550}=1). Fungal pathogenic strains were activated and\ or maintained on potato dextrose agar slants incubated at 28˚C.

2.6. Microalgae strain

The microalgae strain used for cytotoxicity experiment was kindly provided by National Institute of Oceanography and Fisheries (NIOF).

2.7. Antimicrobial bioassay

2.7.1. Antibacterial bioassay

The well-cut diffusion technique was used to test the ability of the crude extracts to inhibit the growth of indicator bacteria. Fifty millimeters of nutrient agar medium inoculated with indicator microorganisms were poured into plates. After solidifying, wells were punched out using 0.5 cm cork borer, and each of their bottoms was then sealed with two drops of sterile water agar. One hundred microliters of the tested crude extracts were transferred into each well after sterilizing by ultra-filtration using 0.22 µl sterilized filters. All plates were incubated at appropriate temperature for 24 - 48 h. After incubation period, the radius of clear zone around each well (Y) and the radius of the well (X) were linearly measured in mm, where dividing Y^2 over X^2 determines an absolute unit (AU) for the clear zone. The absolute unit of crude extract, which indicates a positive result, was calculated according to the following equation (Yang et *al*., 1992):

$$AU= Y^2\pi/X^2\ \pi$$

2.7.2. Antifungal bioassay

2.7.2.1. In plates

Glucose peptone agar plates were used to test the ability of crude extracts to inhibit the growth diameter of indicator fungi. These plates were amendment with different ratios of the filtered crude extracts (5, 10, 15 and 25 %). The discs of reference fungal strains (diameter of 5mm) were placed on these plates; one disc for each plate. All plates were incubated at 28ºC for a week. The diameter of fungus was measured daily, and the suppression percentages were calculated comparing to control (Hadacek and Greger, 2000).

2.7.2.2. In liquid cultures

Glucose peptone broth was prepared to test the ability of crude extracts to suppress growth of indicator fungi in form of dry weight. The conical flasks were amended with different ratios of the filtered crude extracts (5, 10, 15 and 25 %). Each flask received one disc (5mm) of fungal strain. All flasks were incubated at 28ºC for a week. The dry weight of each treatment was estimated, and the suppression percentages were

calculated comparing to control (El-Abyed and Saleh, 1971).

2.8. Cytotoxicity assay (Cordero *et al*., 2005)

This assay was applied to detect the cytotoxicity of ethanolic crude extracts against microalgae; *Tetraselims chuii* used successfully in the aquaculture feeding of shrimps larvae and other invertebrates. Several ratios (0.25, 0.5, 1.0, 2.0 and 5.0 %) of crude extracts were added into *Tetraselims chuii* culture and then count was followed daily for a week. All the counts obtained were compared to control (without any crude extracts).

2.9. Chemical Composition Analysis

Extracts produced by *Hydroides elegans* and *Halla parthenopeia* were prepared for GC-Mass analysis. Extracts prepared as mentioned before were concentrated until complete dryness and finally resuspended in appropriate volume of the solvent.

3. Results and Discussion

Polychaete species were isolated from marine sediment and then identified according to Fauvel (1923 & 1927), and Day (1967a & b). Three of them were from family Nereididae (*Nereis falsa*, *Perinereis nuntia* typica and *Pseudonereis anomala*); one was from family Oenonidae (*Halla parthenopeia)* and the last one was from family Serpulidae (*Hydroides elegans*). However, these species were characterized as follows:

3.1. Perinereis nuntia typica

The worm was reddish in color with body length reaching to 20cm. Proboscis paragnaths were arranged as follows: MI= 2 in tandem; MII= 3 rows of 10-11 in a triangle. MIII= rows in a rectangle and two paragnaths on both sides far away; MIV= 21-22 paragnaths in two curved rows; MV= 3 large in a triangle; MVI= 8-11 in curved row on each side; MVIII= 2 anterior rows of large ones and 5 below (Figure 1).

3.2. Nereis falsa

There was proboscis provided with large conical paragnaths. MI= 2-3 in line, MII= wedge of several equal dents, MIII= about 20 dents forming oval shape, MIV= wedge of several dents. Area V does not bear paragnaths, MI= 4 dents in aquare, VII and VIII= 2-3 irregular rows of subequal dents (Figure 2).

3.3. *Pseudonereis anomala*

There were paragnaths of three types; conical, pectinate and transverse, paragnaths of area VI arranged in a single row. Paragnaths in II, III and IV flattened and sharply triangular, generally forming regular comb-like rows but sometimes irregular. Paragnathes were typically arranged as follows: I= 1 large cone; II= 17-32 in oblique rectangular group of 4-6 short transverse rows; III= = 37-82 in 3-6 transverse arcs; IV= 32-68 in rectangular group of 4-5 rows; V = 0; VI = 5-9 cones in single transverse arc, ; VII& VIII = 14-22 in 2 alternating rows, anterior with very large cones, posterior with small cones (Figure 3).

3.4. *Hydroides elegans*

This worm was characterized by operculum with two vertical: distal vertical chitinized with central spine and Ca 16 marginal spines, each with 2-3 lateral spinules and two fine medial spinules on inner margin; proximal vertical symmetric funnel with rounded, blunt radii. There were thoracic setigers with limbate setae shown (Figure 4).

3.5. *Halla parthenopeia*

The body of worm was very long up to 90 cm. The head was with three small antennae. There were two long maxillary carriers on both sides of the maxillae; there were plates which named lateral carriers. Maxillary parts: MI = 7+4, MII= 7+7, MIII = 5+5, MIV = 4 +4, MV =1 +1. Maxillae V without lateral carriers were shown. Three types of setae were present namely: limbate, hispide capillary and bidentate hooded hooks (Figure 5).

Several marine sources have been studied well, along several decades, to extract bioactive substances in the form of anti-oxidants, anti-tumour, anti-malarial, anti-inflammatory, anti-viral, anti-bacterial and anti-fungal agents (Benkendorff, 2001; Pan *et al*., 2004; Benkendorff *et al.* 2005; Nishimura *et al*., 2005; Da Silva, 2006; Blunt *et al*., 2007; Galeano and Martínez, 2007**).** The marine polychates although had not been studied enough in such manner (Ovchinnikova *et al*., 2004). Therefore, the present research was directed towards investigating the antimicrobial activity of five marine worms.

The ethanolic crude extracts of the five marine worms were prepared and then screened against different bacterial and fungal pathogens. In general, the crude extracts of all marine worms showed positive records against at least two microbial pathogens. On the other hand, *Halla parthenopeia* and *Hydroides elegans* showed the most significant records. The crude extract of *H. elegans* had abroad spectrum antimicrobial effect against all indicator microorganisms. The crude extract of *H. parthenopeia* had abroad spectrum antimicrobial effect especially against; *Vibrio fluvials, Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus Fusarium solani* and *Rhizoctonia solani*, Table (1).

The absolute activity units of the crude extract of *H. elegans* ranged from 6.4 for *B. cereus* to 15.5 for *P.*

Table 1: Screening the antimicrobial activity of marine polychaetes

Indicator microorganisms	Marine polychaete(*)				
	1	2	3	4	5
Bacteria:					
Vibrio damsela	+	-	-	+	+
Vibrio fluvials	-	++	-	-	+++
Bacillus cereus	-	+	+	-	++
Bacillus cereus 1318	-	+	-	-	+
Pseudomonas aeruginosa	-	+	-	+	++++
Staphylococcus aureus	+	+	+	-	+++
Escherichia coli	-	-	-	-	+
Streptococcus faecalis	-	-	-	-	+
Fungi:					
Fusarium solani	-	++	-	-	+++
Rhizoctonia solani	-	+	-	-	++
Aspergillus niger	-	-	-	-	-

(*)1: *Pseudomereis anomala*; 2: *Halla parthenopeia*; 3: *Perimereis nuntia typical*; 4: *Noveis fasta* and 5: *Hydroides elegans*.

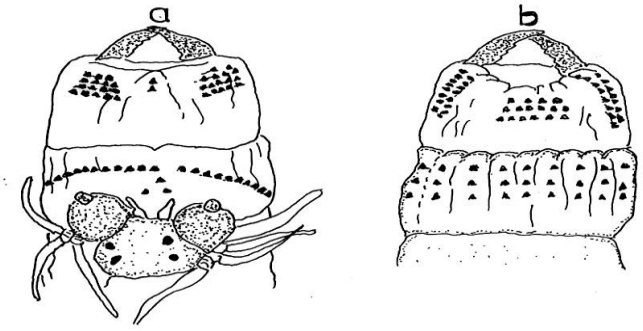

Figure 1: *Perinereis nuntia typical*; a: proboscis (dorsal) and b: proboscis (ventral)

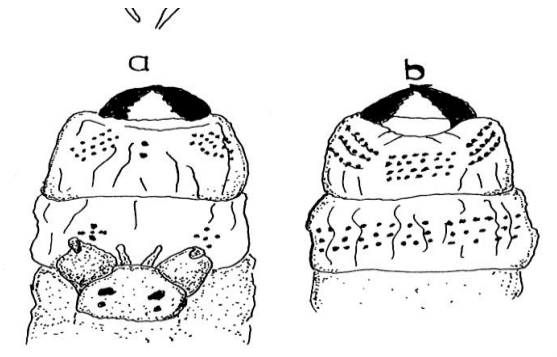

Figure 2: *Nereis falsa*; a: proboscis (dorsal) and b: proboscis (ventral)

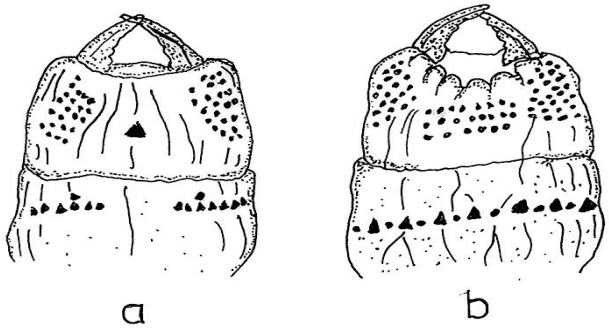

Figure 3: *Pseudonereis anomala*; a: proboscis (dorsal) and b: proboscis (ventral)

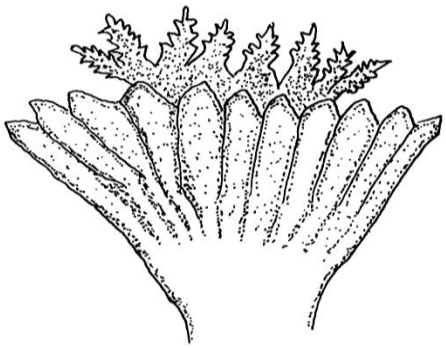

Figure 4: *Hydroides elegans*; with operculum

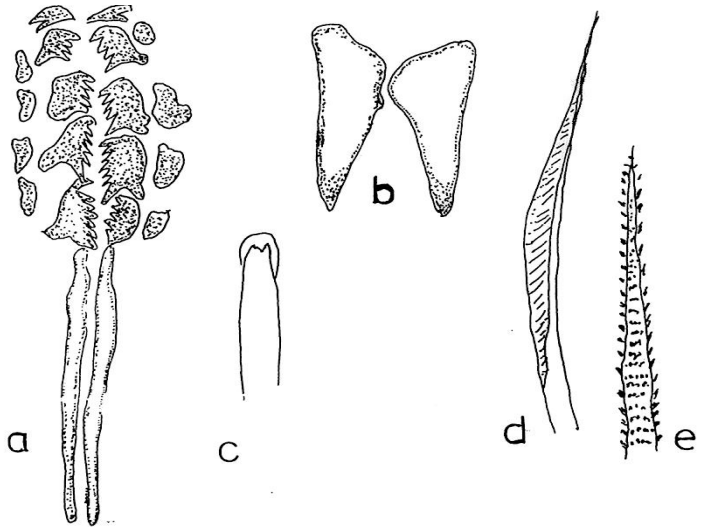

Figure 5: *Halla parthenopeia;* a: Maxillae, b: Mandible, c: Bidentate hooked seta, d: Limbate capillary seta and e: Hispid capillary seta

aureginosa, while, the crude extract of *H. parthenopeia* had absolute activity units ranged from 3.0 for *V. fluvials, P. aeruginosa* and *Staphylococcus aureus* to 6.5 for *B. cereus*, (Table 2).

Growth of fungal strains was affected by the polychaetes extracted as indicated by growth diameter on agar plates. *Hydroides elegans* extract had the most suppression percentage against the pathogenic fungi fungus *F. solani*. Growth suppression of *F. solani* was remarkably affected by the two tested extracts and was dependent on concentration used when 25% extract was used; fungal growth was inhibited by 90 and 100% by extract of *H. elegans* and *H. parthenopeia*, respectively. On the contrary, *R. solani* was slightly affected with 25% suppression in case of *H. elegans* extract and 20 % suppression in case of *H. parthenopeia* extract. While *A. niger* was not affected at all, (Table 3).

In the liquid culture experiment, the yield of *F. solani*, *R. solani* and *A. niger* (mg) in glucose peptone broth under the effect of four different concentrations (5, 10, 15 and 25%) of crude extracts was investigated. However, the suppression percentages of crude extracts against fungi were ranged from 15.66 to 96.9% for *H. elegans* crude extract and from zero to 90.10% for *H. parthenopeia* crude extract, (Table 4).

In accordance to our data, Benkendorff (2001) screened the antibacterial activity of 39 mollusks and 4 polychaetes against 3 human pathogenic bacteria; *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Activity was detected in the egg masses from 34 species, including 2 polychaetes and mollusks from two classes and 18 families. Antibacterial activity in molluskan egg masses was found to extend across the marine, estuarine, freshwater, and terrestrial environments. Both gelatinous egg masses and tough egg capsules were found to inhibit microbial growth, suggesting that physical protection alone may not be sufficient to protect the eggs. Antimicrobial activity was observed in the fresh egg masses but not in the well-developed egg masses of a subset of species. However, the results of this study indicated that a wide range of invertebrates use chemical defense to protect their early stage embryos against bacterial infection.

The work was extended to determine the GC-MS of *Hydroides elegans* and *Halla parthenopeia* extracts. Data in Table (5) reveal that the main constituents of *H. elegans* extract were dodecane, heptadecane, pentadecane, 1,2-benzendicarboxylic acid, cholesterol and aspidofractinine-3-methanol (Figure 6). On the other hand, Table (6) shows the GC-MS of *H. parthenopeia* extract which contained hexadecanoic acid, cholest-5-en-3-ol, Heptadecene-8-carbonic acid-1, 2-methylpentanal-5-d3, 1-tetradecene and 9-octadecenal (Figure 6).

However, fatty acids can act as anionic surfactants and have antibacterial and antifungal properties at low pH (Hayes and Berkovitz, 1979), in addition to being selective against Gram positive organisms (Kabara *et al.*, 1972) by targeting the structure and function of bacterial cell walls and membranes.

Barnathan (2009) reviewed marine organisms, in particular invertebrates, which proved to be a major source of unique fatty acid (FA) structures originating from unusual biosynthetic pathways. Among them, non-methylene-interrupted (NMI) FA occur in various mollusks in the wide ranges of concentrations (upto20%). Such NMIFA have also been reported from algae, echinoderms, sponges, tropical rays, and many other invertebrates.

The ability of fatty acids to interfere with bacterial growth and survival has been known for several decades (Kabara *et al.*, 1977; Ababouch *et al.*, 1992), although their effectiveness against marine pathogens has never been tested. Structure- function relationship studies on free fatty acids against human pathogenic bacteria indicate that antimicrobial activity can depend on both the chain length and the degree of unsaturation (Kabara *et al.*, 1977). It has also been demonstrated that compounds, such as cholesterol, can antagonize the antimicrobial properties of fatty acids (Galbraith *et al.*, 1971).

On the other hand, many of the biogenic organobromine compounds have been suggested to exhibit antimicrobial or other biologic activities. In addition, many marine mollusks and polychaetes have evolved a reproductive strategy that involves the deposition of fertilized embryos in benthic egg masses (Benkendorff *et al.*, 2001). Bromoindoles and their derivates have been shown to act as antifungal (Liu and Gribble, 2002), and antimicrobial (Cuntignano *et al*., 2000). The brominated compounds are produced naturally, e.g., by common polychaete worms and algae. Brominated phenols and indoles assumed to be of biogenic origin have been detected in water and sediment extracts from the German Bight. These substances as well as some of their isomers have been tested with the zebra fish embryo test and were found to cause lethal as well as non-lethal malformations (Kammann *et al.*, 2006).

Matsushima *et al.* (2002) isolated myoactive peptides (GGNG peptides) from the marine polychaete (*Perinereis vancaurica*). The peptide was a pentadeca peptide whose amino acid sequence was similar to that of the earth worm excitatory peptides (EEP) and the leech excitatory peptide (LEP), and showed myoactivity on isolated esophagus of *P. vancaurica* with at threshold concentration of 10−10 M. The peptide was designated as polychaete excitatory peptide (PEP).

From the cytotoxicity experiment, the ethanolic crude extracts of both *Hydroides elegans and Halla parthenopeia* were clearly safe to be applied in the aquaculture alternative to synthetic antibiotics for inhibiting the microbial fish and invertebrate pathogens, (Table 7).

Table 2: Absolute activity units (±SD) of *H. elegans* and *H. parthenopeia* against pathogenic bacteria

Indicator bacteria	Marine polychaete	
	Hydroides elegans	***Halla parthenopeia***
Vibrio fluvials	6.6 ± 0.003	3.0± 0.005
Bacillus cereus	6.4± 0.005	6.5± 0.001
Pseudomonas aeruginosa	15.5± 0.001	3.0± 0.002
Staphylococcus aureus	10.0± 0.002	3.0± 0.03

Table 3: Fungal diameter and suppression % of *Fusarium solani* and *Rhizoctonia solani* growth on plates after 7 days incubation as affected by *H. elegans* and *H. parthenopeia* crude extracts

Ratio of crude extracts (%)		Fungal diameter (cm±SD)		Suppression %	
		F. solani	***R. solani***	***F. solani***	***R. solani***
Control		10± 0.001	10± 0.001	-	-
5	**a**	6± 0.01	10± 0.005	40	-
	b	4± 0.003	10± 0.005	60	-
10	**a**	5± 0.003	10± 0.003	50	-
	b	2.5± 0.002	10± 0.004	75	-
15	**a**	3.5± 0.001	8± 0.003	65	20
	b	2± 0.003	8.2± 0.003	80	18
25	**a**	1± 0.001	7.5± 0.002	90	25
	b	-	8± 0.001	100	20

[a] Crude extracts of *Hydroides elegans*
[b] Crude extracts of *Halla parthenopeia*

Table 4: Dry weights (mg) of *F. solani* and *R. solani* in glucose peptone broth under the effect of four different concentrations (5, 10, 15 and 25%) of *H. elegans* and *H. parthenopeia* crude extracts

Ratio of crude extracts (%)		Dry wt. (mg±SD)		Suppression %	
		F. solani	***R. solani***	***F. solani***	***R. solani***
Control		355± 0.001	310± 0.001	-	-
5	**a**	287± 0.002	261± 0.001	19.20	15.66
	b	299± 0.003	310± 0.002	15.77	-
10	**a**	264± 0.005	163± 0.004	25.50	47.50
	b	214± 0.003	242± 0.001	39.66	21.90
15	**a**	118± 0.003	62± 0.002	66.70	80.00
	b	71± 0.005	140± 0.004	80.00	55.00
25	**a**	11± 0.004	24± 0.002	96.90	92.33
	b	35± 0.003	101± 0.005	90.10	67.43

[a] Crude extracts of *Hydroides elegans*
[b] Crude extracts of *Halla parthenopeia*

Table 5: GC-MS of major components in *Hydroid elegans* extract

No.	Compound	Molecular formula	Molecular wt. (g/mol)	Retention time (min)	Area (%)
1	Dodecane	$C_{12}H_{26}$	170.3360	8.01	13.54
2	Heptadecane	$C_{17}H_{36}$	240.4694	8.01	13.54
3	Pentadecane	$C_{15}H_{32}$	212.4160	8.01	13.54
4	1,2 benzendicarboxylic acid	$C_8H_6O_4$	166.1316	12.31	42.06
5	Cholesterol	$C_{27}H_{46}O$	386.6561	15.54	17.18
6	Aspidofractinine-3-methanol	$C_{20}H_{26}N_2O$	310.4351	12.31	42.06

Table 6: GC-MS of major components in *Halla parthenopeia* extract

No.	Compound	Molecular formula	Molecular wt. (g/mol)	Retention time (min)	Area (%)
1	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4256	9.71	16.98
2	Cholest-5-en-3-ol	$C_{27}H_{46}O$	386.6561	15.47	21.08
3	Heptadecane-8-carbonic acid-1	$C_{17}H_{36}$	240.4677	10.56	9.02
4	2-Methylpentanal-5-d3	$C_6H_{12}OS_3$	196.3560	4.30	1.78
5	1-Tetradecene	$C_{14}H_{28}$	196.3734	10.56	9.02
6	9-Octadecenal	$C_{18}H_{34}O_2$	282.4631	10.56	9.02

Table 7: Cytotoxicity of the crude extracts of both *Hydroid elegans* and *Halla parthenopeia* to microalga; *Tetraselims suecica*; expressed in mg ± SD

Ratio of crude extracts (%)	Incubation (days)				
	1	2	3	4	5
Control (*)	160± 0.001	192± 0.001	272± 0.002	304± 0.002	396± 0.001
0.25					
a	168± 0.002	208± 0.001	320± 0.001	344± 0.003	392± 0.001
b	164± 0.001	222± 0.002	288± 0.002	292± 0.005	320± 0.003
0.50					
a	160± 0.001	208± 0.004	336± 0.002	363± 0.001	382± 0.002
b	156± 0.001	158± 0.003	192± 0.003	256± 0.001	330± 0.002
1					
a	142± 0.001	200± 0.002	352± 0.006	376± 0.002	377± 0.005
b	151± 0.001	158± 0.005	176± 0.003	220± 0.001	308± 0.001
2					
a	128± 0.002	192± 0.001	322± 0.001	350± 0.005	367± 0.002
b	145± 0.001	174± 0.002	198± 0.003	256± 0.004	272± 0.003
5					
a	96± 0.001	170± 0.003	230± 0.001	290± 0.003	316± 0.001
b	83± 0.003	107± 0.005	160± 0.002	200± 0.006	240± 0.002

(*) Cell count = Number x 10^4

[a] *Hydroid elegans*

[b] *Halla parthenopeia*

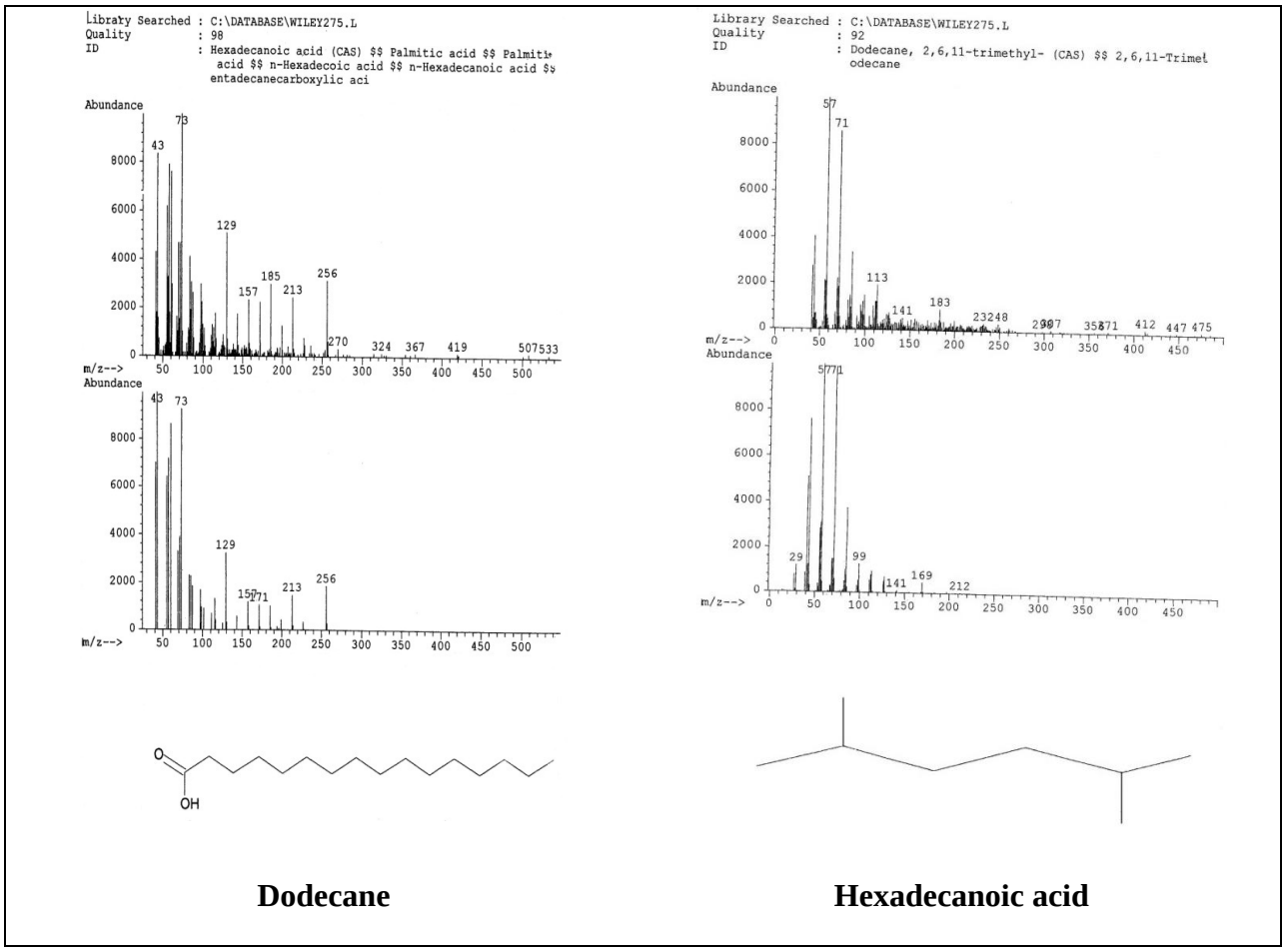

Figure 6: Mass spectrum of dodecane (Left) in the ethanolic crude extracts of *H. elegans* and Hexadecanoic acid (Right) in the ethanolic crude extracts of *H. parthenopeia*

Finally, we are satisfied to conclude that marine organisms are in great demand due to their extensive biological properties and providing source for the discovery of many new bioactive compounds. Moreover, marine polychaetes are good sources that encourage researchers in such manner.

Acknowledgement

The authors would like to thank Dr. Hanan Khairey, Hydrobiology Lab., National Institute of Oceanography & Fisheries, for the achievement of cytotoxicity experiment in this investigation.

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الخصائص المضادة للميكروبات من الديدان البحرية عديدة الأهلاب المجموعة من ماء البحر الإسكندرية

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تم تعريف خمسة أنواع من الديدان عديدة الأهلاب المجموعة من ماء البحر، الإسكندرية، مصر. ثم تم دراستها كمصدر للمنتجات الطبيعية؛ تلك التي يمكن استخدامها كمضادات بكتيرية وفطرية للممرضات الإنسان والأسماك. وقد صنفت هذه الديدان، فكانت ثلاثة من هذه الأنواع من عائلة (Neridae)، هي؛ (*Nereis falsa*)، (*Perinereis nuntia typical*) ، و(*Pseudonereis anomala*). ونوع من عائلة (Oenonidae) وهو؛ (*Halla parthenopeia*)، ونوع من عائلة (Serpulidae)، هو (*Hydroides elegans*). وقد اختبرت مستخلصات الإيثانول الخام ضد مختلف الممرضات البكتيرية والفطرية. وقد ظهرت نتائج ايجابية لكل من هلا بارثينوبيا، وهيدرويد اليجانس. وكان لمستخلص الايثانول الخام للهيدرويد اليجانس مدى واسع كمضاد بكتيري ضد الممرضات البكتيرية. وقد تراوحت وحدات النشاطية من 6.4 ضد بكتريا باسيليس سيريس إلى 15.5 ضد بكتريا سيدومنوناس اريجينوزا. بالإضافة إلى أن مستخلصات هيدرويد اليجانس أظهرت أعلى نسبة تثبيط ضد الفطريات الممرضة، وقد مُثل ذلك بالنقص في القطر الفطري. وقد تراوحت نسبة التثبيط من 40 إلى 100% ضد فطر اسبريجللس نيجر، وريزوكتونيا سولاني على التوالي. كذلك فقد مُثلت النسب التثبيطية للمستخلصات الخام بالنقص في الوزن الخام، وقد تراوحت من 15.66% إلى 96.9% لمستخلص هيدرويد اليجانس، ومن صفر إلى 90.1% لمستخلص هلا بارثينوبيا الخام. وقد حُللت مستخلصات هلا بارثينوبيا، وهيدرويد اليجانس بمطياف الكتلة اللوني للغاز السائل، وقد كانت المكونات الرئيسية لها عبارة عن أحماض عضوية ومشتقاتها.