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Antimicrobial activity of the sea star (*Astropecten spinulosus*) collected from the Egyptian Mediterranean Sea, Alexandria

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ABSTRACT

A species of sea star was collected from the Mediterranean Sea, Alexandria, Egypt. It was identified based on general morphological and anatomical features as *Astropecten spinulosus*. The antibacterial and antifungal activities were investigated via the standard techniques. Data obtained revealed that the inhibition zones as a factor for antibacterial activity of *A. spinulosus* ranged between 0 and 18 mm. The highest antibacterial activity was detected against *P. aeruginosa* (18 mm) for ethanol extract, followed by *B. subtlis* (14 mm) for methanol extract, then by *P. aeruginosa* (13 mm) for both ethyl acetate and methanol extract. Different solvent extracts recorded inhibition zones as antifungal activity ranged between 8 to 10 mm. the most suppressed fungus was *P. crustosum* by acetone and ethanol extracts as 80 and 90%, respectively. Weakly, *A. terreus* was suppressed by ethanol and methanol extracts of *A. spinulosus* as 10 and 20%, respectively. The suppression was not taken place against other fungi by any solvent extract. Regarding to investigating the efficacy of some commercial antibiotics (mm), data confirmed that the Gram negative bacteria were more resistant than Gram positive bacteria. On the other side, result of GC-MS/MS of crude extract observed the presence of several bioactive constituents, most of which had antimicrobial activities.

INTRODUCTION

The phylum Echinodermata is contains almost 7000 living and 13,000 fossil species in the world's oceans and possesses 5 classes, namely Crinoidea, Asteroidea, Ophiuroidea, Echinoidea, and Holothuroidea (Pawson, 2007; Zulliger and Lessios, 2010). They are respectively more commonly known as sea stars, sea urchins, sea cucumbers, sea lilies, and feather stars. They inhabit every ocean, in all climate zones and across all ranges of the depth (Uthicke *et al*., 2009).

Sea stars have significant ecological role as essential predators in the structure and function of intertidal and subtidal benthic communities, since they promote heterogeneity

and diversity (Abd El Hafez, 2018). Sea stars have been investigated widely in much more fields such as; paleontology, evolutionary biology, reproduction, conservation, genetics, biochemistry, and biogeography. Also, they play are considered as model organisms for understanding climate change (Lawrence, 2013). The genus *Astropecten* (Gray, 1840) comprises more than 100 extant species world-wide (Mah and Hansson, 2008; Ventura, 2013).

Specifically, the genera of *Astropecten* has most species among sea stars and its members are distributed worldwide, inhabiting soft-bottom ecosystems from polar to tropical waters and from intertidal areas to the deep sea (Zulliger and Lessios, 2010). Indeed, this genus includes six species in the Mediterranean Sea: *Astropecten aranciacus* (Linnaeus 1758), *Astropecten bispinosus* (Otto 1823), *Astropecten irregularis pentacanthus* (Pennant 1777), *Astropecten platyacanthus* (Philippi 1837), *Astropecten jonstoni* (Delle Chiaje 1827), and *Astropecten spinulosus* (Philippi 1837). However, the last three are endemic to the Mediterranean. *Astropecten* species is voracious predators feeding mainly on gastropods and bivalves.

Moreover, EL-Beshbeeshy (1995) described 12 species along the Egyptian Mediterranean waters, which belong to Echinoidea, Asteroidea and Ophiuroidea, these are: *Stylocidaris affinis* (Philippi, 1845), *Psammechinus microtuberculatus* (Blainville, 1825), *Schizaster canaliferus* (Lamarck, 1816), *Brissopsis lyrifera* (Forbes, 1841), *Echinocardium cordatum* (Pennant, 1777), *Sphaerodiscus placenta* (Muller et Troschel, 1842), Astropecten aranciacus (Linne, 1758), *Astropecten bispinosus* (Otto, 1823), *Astropecten spinulosus* (Philippi, 1937), *Echinaster sepositus* (Gray, 1840), *Amphiura filiformis* (Muller, 1776), and *Ophiura texturata* (Lamarck, 1816).

On the other side, there is an increase in scientific interest on marine crustaceans, molluscs, and echinoderms, particularly on their secondary metabolites with obvious antimicrobial properties (Casas *et al*., 2011; Rahman, 2014). Clearly, echinoderms appear as promising source for novel products (Sudek *et al*., 2007). However, sea stars are benthic free living echinoderms have evolved with rich sources of bioactive metabolites such as steroidal glycosides, steroids, anthraquinones, alkaloids, glycolipids, and phospholipids (Molinski *et al*., 2009). Especially, steroidal glycosides and related compounds are predominant metabolites in sea stars and have a broad variety of biological activities such as cytotoxic, hemolytic, repellent, antineoplastic, antimicrobial (Haug *et al*., 2002; Rahman, 2014), antifungal, antiviral (Schumacher *et al*., 2011), and anti-inflammatory (Villier *et al*., 2004). For instance, Layson *et al.* (2014) obtained considerable antibacterial activity from starfishes; *Linckia laevigata* and *Oreaster nodusus*. All their extracts showed antibacterial activity against *E. coli*. Also, some polyhydroxylated steroids from starfish *Leptasterias ochotensis* exhibited antibacterial activity (Malyarenko *et al*., 2015).

Therefore, the current investigation aimed to discover antimicrobial agents from sea star; *Astropecten spinulosus* habiting in the Egyptian Mediterranean Sea, Alexandria as alternative source to antibiotics. Also, this study extended to detect the bioactive compounds in the crude extract of *A. spinulosus* using GC-MS analysis.

MATERIALS AND METHODS

Collection of sea star samples

Sea star samples were collected from the Eastern Harbor (29885 E longitude and 31205 N latitude) located at Alexandria, Egypt (Fig.1). The sample was collected manually, including the holdfast from the Scout club located in Eastern Harbor in the intertidal zone at the depth of (5-100 m). The fresh samples were washed with seawater at the sampling site to remove the adhered sediments and impurities, and then put in polyethylene bags. Quick rinsing of the sea star with tap water was done in the laboratory on the same day to neglect any remaining impurities or epiphytes.

Fig. 1. A map of northern Egypt showing sampling stations off Alexandria City

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, *Klebsilla pneumoniae*, and *Escherichia coli* ATCC 8739) were used as reference strains. Also, there five yeast species (*Candida albicans, C. krusei, C. tropicalis, C. glabrata*, and *Rhodotorula mucilaginosa*) were 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, five common media were used to culture the reference strains and determine the antimicrobial activity of sea star extracts (Atlas, 1997; Guinea *et al*., 2005). They were: nutrient broth (NB), nutrient agar (NA), Sabouraud dextrose agar (SDA) to cultivate dermatophytes and other types of fungi, potato dextrose (PDB), and potato dextrose agar (PDA) to culture on are yeasts such as *C. albicans* and *Saccharomyces cerevisiae* and molds such as *A. niger*.

Preparation of the crude extracts

The samples of sea star were cut into so small pieces of about 2 mm size. The extraction was carried out via soaking in acetone, ethanol, ethyl acetate, and methanol (1:10, w/v) on a rotary shaker at 150 rev min^{-1} at ambient temperature for 96 h. The extracts were then pooled and filtered using Whatman filter paper. After evaporation of the solvent, the crude extracts were 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 NA for bacteria and SDA 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 determined by well-cut diffusion technique, in which wells were punched out using a sterile 0.7 cm cork-porer in nutrient agar plates containing the tested strains. About 100 µL of each crude extract was transferred into each well. They were incubated at 4°C for 2 h, and then were incubated at 37°C for 24 h. The results were estimated by measuring the diameter of inhibition zone three times for each well and expressed in millimeter (Amer and Ibrahim, 2019).

Antifungal bioassays

By pouring technique

Different crude extracts of sea star were tested against the reference 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 the fungal growth. The radius-growth of each fungus was measured to calculate the suppressive eff ect (%) of crude extract (Amer and Ibrahim, 2019).

By well-cut diffusion technique

One disc of the seven fungal growths was separately put on the top of a plate containing PDA medium. About 100 µL of each sea star crude extract was transferred into each well. All plates were incubated at 28ºC until the control was completely covered with the 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 sea star, small discs of the five antibiotics were put associated with each microbial strain. All plates were incubated at 37°C for 24 h (Khan *et al*., 2019; Shaaban *et al*., 2020). The results were estimated through measuring the diameter of inhibition zone three times for each well and expressed in millimeter.

GC-MS/MS analysis of sea star extract

The most potent crude extracts of sea star obtained previously 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 $mlmin^{-1}$. 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.

RESULTS AND DISCUSSION

The characterization of the current species of sea hare was done based on general morphological and anatomical features. It was small starfish, usually 6-8 cm and maximum just under 10 cm in diameter with very short superomarginal plates, which fully covered by scales and very small spines. There three small spines were observed on the top of plate as real spines, while the other spines were too small. The color of these spines is the same of the superomarginal plates and it was brown or clear brown. As well as, the inferomarginal spines were long and pointed and they had the feature color blue-purple. It looked like a slender starfish with rounded ends of the arms. Its aboral side had dark reddish-brown color. These findings made us satisfied to identify this species as; *Astropecten spinulosu*. However, data in Fig. 2 confirmed such manner, while data in Table 1 show the modern classification position of this sea hare.

Fig. 2. General external feature of *A. spinulosus* sample collected from the Mediterranean Sea, Alexandria Egypt.

Table 1. Modern classification position of *A. spinulosus* within Kingdom Animalia.

Item	Position
Kingdom	Animalia
Phylum	Echinodermata
Subphylum	Eleutherozoa
Class	Asteroidea
Order	Paxillosida
Family	Astropectinidae
Genus	Astropecten
Species	*A. spinulosus*

Astropecten spinulosus is a species of sea star belongs to family Astropectinidae. Generally, starfishes of genus Astropecten live on sandy, muddy or gravel seabed. So, they remain largely buried under sediment during the day (Koukouras and Kitsos, 2010). This species lives only in the Mediterranean Sea and it prefers sandy seabed in areas very rich in algae from 1 to 50 m deep (Hansson, 2001). It is a carnivore and feeds on molluscs, which it catches with its arms and then takes to the mouth trapping the prey by the long, moving prickles around the mouth cavity (Koukouras and Kitsos, 2010).

Amazingly, sea stars, or starfish, secret a huge number of secondary metabolites, Therefore, our investigation was to focus on the antimicrobial properties of bioactive substances in crude extracts of sea star; *A. spinulosus* collected from the Mediterranean Sea, Alexandria, Egypt.

The antibacterial and antifungal activity was investigated by using the standard techniques. Generally, the activities via well-cut diffusion method were calculated in terms of inhibition zone diameter (mm). The activities via pouring technique were expressed in terms of suppression percentage (%). In the present investigation, a pronounced antimicrobial activity has been observed against some bacterial and fungal strains. The crude extract of *A. spinulosus* showed a considerable activity against many of bacterial and fungal strains. However, the values of the antibacterial activity ranged between 0 and 18 mm as inhibition zones. As well as, the highest antibacterial activity was detected against *P. aeruginosa* (18 mm) for ethanol extract, followed by *B. subtlis* (14 mm) for methanol extract, then by *P. aeruginosa* (13 mm) for both ethyl acetate and methanol extract. *Escherichia coli* also affected by acetone, ethanol, and methanol extracts as 10, 11, and 12 mm inhibition zones, respectively. Clearly, the other Gram positive bacteria were not affected at all (Table 2).

By different solvent extracts of *A. spinulosus,* the antifungal activity was recorded in several cases against both molds and yeasts strains. Data presented in Table 2 conducted that the *P. crustosum, P. notatum, A. terreus,* and *C. albicans* were not affected at all by any type of solvent-extract tested. The positive records as inhibition zones ranged between 8 to 10 mm. Obviously, the most effective solvent was shown to be ethanol, followed by methanol and then acetone, while the crude extract by ethyl acetate was not effective to inhibit any fungus.

The result in Table 3 exhibited *P. crustosum* was suppressed by acetone and ethanol extracts of *A. spinulosus* at 80 and 90%, respectively (Fig. 3). Also, *A. terreus* was suppressed weakly by ethanol and methanol extracts of *A. spinulosus* at 10 and 20%, respectively. The suppression was not taken place against *P. notatum, A. niger, A. flavus, F. solani,* and *F. oxysporum* by any solvent extract.

Table 2. The antibacterial activity of *A. spinulosus* crude extract against different bacterial, fungal reference strains (molds and yeasts) via well-cut diffusion technique.

Pathogen	Zone of inhibition (mm)/extract-solvent			
	A	**E**	**EA**	**M**
Gram +ve bacteria:				
B. subtlis	11	12	12	14
B. cerues	٠	٠	٠	٠
S. aureus	٠	٠	٠	٠
S. epidermids	٠	٠	٠	٠
E. faecalis	٠	٠	٠	٠
Gram -ve bacteria:				
P. aeruginosa	12	18	13	13
K. pneumoniae	٠	٠	٠	٠
E. coli	10	11	٠	12
Fungi (molds):				
P. crustosum	٠	٠	٠	٠
P. notatum	٠	٠	٠	٠
A. terreus	٠	٠	٠	٠
A. niger	8	8	0	9
A. flavus	0	8	0	8
F. solani	٠	٠	٠	٠
F. oxysporum	9	10	0	9
Fungi (yeasts):				
C. albicans	٠	٠	٠	٠
C. tropicalis	٠	٠	٠	٠
C. krusei	8	9	0	8
C. glabrata	٠	10	٠	8
R. mucilaginosa	٠	8	٠	8

*AU refers to the antibacterial activity which was calculated according to the equation mentioned in methodology section. NI means inhibition was not happened. A= acetone, E= ethanol, EA= ethyl acetate, and M= methanol.

Fig. 3. Antifungal activity of *A. spinulosus* crude extracts via pouring method against *P. crustosum;* control (Left), treated by acetone extract (Middle), and treated by ethanol extract (Right).

Table 3. Antifungal activity of *A. spinulosus* crude extracts on the fungal strains via pouring method.

Fungal pathogens	Antifungal activity (Suppression %*)/extract			
	A	E	EA	M
P. crustosum	80±0.05	90±0.1	NS	NS
P. notatum	NS	NS	NS	NS
A. terreus	NS	10±0.7	NS	20±0.5
A. niger	NS	NS	NS	NS
A. flavus	NS	NS	NS	NS
F. solani	NS	NS	NS	NS
F. oxysporum	NS	NS	NS	NS

*Suppression % refers to the antifungal activity which was calculated according to the equation mentioned in methodology section. NS means suppression not taken place. A= acetone, E= ethanol, EA= ethyl acetate, and M= methanol.

On the other hand, the bioactive substances formed by marine echinoderms have attracted attention due to their antimicrobial, antifungal, antiprotozoal, antiviral especially anti HIV activity, antihelminthic, and anticancer activities (Zapata and Amemiya, 2000; Datta *et al.,* 2015). Indeed, there are many researches on different metabolites of unnique structure that exhibit biological activities (Alam *et al*., 2012; Kiran *et al*., 2014; Rahaman *et al*., 2014).

Although the work done on the antimicrobial activity from sea stars are little, there were some studies confirmed this property. Early, Shimizu *et al.* (1990) extracted biological active compounds from starfishes, *Asterias forbesi* and *Asterina pectinifera*, which were found to inhibit the multiplication of influenza virus in chicken embryos. Recently, Prabhu and Bragadeeswaran (2013) found a significant activity of their crude extract as antimicrobial agent from *Ophiocoma marmorata*. Also, Layson *et al.* (2014) obtained considerable antibacterial activity from brittle star; *Ophiocoma ochoenleinii*, as well as, starfishes; *Linckia laevigata* and *Oreaster nodusus*. All their extracts showed antibacterial activity against *E. coli*. In addition, some polyhydroxylated steroids were obtained from starfish *Leptasterias ochotensis* and exhibited antibacterial and cytotoxic activities (Malyarenko *et al*., 2015).

Moreover, Abd El-Hafez (2018) extracted three new steroids (**1**, **2,** and **3**) from a sea star; *Acanthaster planci* collected from the Egyptian Red Sea. These compounds were named as 5α-cholesta-4(27), 24-dien-3β, 23β-diol; 5α-cholesta-24-en-3β, 20β-diol-23-one; and 5α-cholesta-9(11)-en-3β, 20β-diol; respectively. All compounds showed activity against *P. aeruginosa* and *S. faecalis*, and compound **2** had the highest activity (21 mm) against *P. aeruginosa*, while there was no antifungal activity observed. In the same work, the author extracted two compounds (**1** and **2**) named as; 7, 11-epoxy-9(15)-himachaladiene-4-ol (O7-ophiocomane) and 8, 11-epoxy-9(15)-himachaladiene-4-ol (O8-ophiocomane from brittle star; *Ophiocoma dentate*, collected from Hurghada, Egyptian Red Sea. However, the maximum antibacterial activity of crude extract was recorded against *S. aureus* (16 mm) followed by (14 mm) against *V. damsel*, while, both pure compounds showed antibacterial activity against *P. aeruginosa* as 9 and 10 mm, respectively. The anti-*C. albicans* activity was no detected against at all. More recently, Senthikumari and Revathi (2018) performed the antibacterial activity of *Ophiocoma erinaceus* and *Ophiomastrix annulosa* extracts against some bacterial pathogens. The result of *O. erinaceusc* extract showed maximum zone of inhibition against *P. aeruginosa* (4 mm), followed by *Proteus* sp. at (2.2 mm). Also, the extract of *O. annulosa* exhibited

maximum zone of inhibition of 3 mm against *Bacillus s*p. followed by 2 mm for both *E. coli* and *Proteus* sp.

On comparison level, the activity of several commercial antibiotics (mm) was examined and then compared to the results of *D. setosum* 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, E. faecalis* and *K. pneumoniae* 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**.** However, data confirmed that the Gram negative were more sensitive than Gram positive.

Furthermore, our study confirmed that the Gram negative bacteria were more resistant than Gram positive ones. This may be due to Gram negative bacteria have a largely impermeable thick cell wall (Exner *et al*., 2017). Also, the inhibition of the most potent crude extract *A. spinulosus* was lower than all commercial antibiotics may due to the bioactive substance in such crude is exposed to the dilution effect. So, it did not show high clearance zone around the tested bacteria.

On the other hand, the results of GC-MS/MS of acetone-extract revealed the presence of several bioactive constituents, with 5 major compounds (Fig. 4 & Table 5). The chemical profiles of them are mainly: 2-(N,N-Bis(2-chloroethyl)amino)methyl-2,3-dihydro-6-hydroxy-3-oxopyridazine (19.10%), Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-decamethyl (20.53%), Carbonotrithioic acid, dimethyl ester (78.17%), Cyclohexasiloxane, dodecamethyl (86.45%), and Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl (72.54%).

Table 4. Effect of the different commercial antibiotics on the bacterial reference strains in comparing to *A. spinulosus* crude extract.

Reference bacterium	**Inhibition zone (mm)* of *A. spinulosus* 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	0	6	6	6	6	0
K. pneumoniae	0	0	0	7	0	9
P. aeruginosa	18	0	0	0	12	0
E. coli	12	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.

Susceptible/sensitive is considered when clearance inhibition zone detected around well or disc.

Also, the results of GC-MS/MS of ethanol-extract revealed the presence of several bioactive constituents, with 17 major compounds (Fig. 4 & Table 5). The chemical profiles of them are mainly: Dimethyl sulfone (33.99%), 8,11-Octadecadiynoic acid, methyl ester (12.18%), Methyl N-(N-benzyloxycarbonyl-beta-l-aspartyl)-beta-d-glucosaminide (8.89%), 2-Cyclopentene-1-carboxylic acid, 1-methyl (2.11%), [4-(1,3-Benzodioxol-5-yl)-6,8,9-trimethyl-3-oxabicyclo[3.3.1]non-6-en-1-yl]methyl acetate (12.73), N,N'-Ethylenebis(2-[2-hydroxyphenyl]glycine) (6.06%), 12-Oxatricyclo [4.4.3.0(1,6)]tridecane-3,11-dione (10.29%), 4,25-Secoobscurinervan-4-one, O-acetyl-22-ethyl-15,16-dimethoxy-, (22à) (10.41%), 2-Amino-3-(4-hydroxyphenyl)-propanoic acid (11.90%), Cyclohexasiloxane, dodecamethyl (88.41%), Pyridine-4-carbohydrazide, N2-(3,4-methylenedioxy-6-nitrobenzylideno), (6.57%), Undefined (8.85%), 4,25-Secoobscurinervan-4-one, O-acetyl-22-ethyl-15,16-dimethoxy-, (22à) (24.08%), Z,Z,Z-1, 4,6,9-Nonadecatetraene (6.92%), 7,10,13-Hexadecatrienoic acid, methyl ester (5.46%), Ergosta-5,22-dien-3-ol, acetate, (3á,22E) (10.96%), and Bis[bicyclo[3.2.0]hept-2-en-4-yl]ether (8.55%).

In addition, the results of GC-MS/MS of ethyl acetate-extract revealed the presence of several bioactive constituents, with 19 major compounds (Fig. 4 & Table 5). The chemical profiles of them are mainly: Pentanoic acid, 2-propyl-, 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester, endo (15.64%), Pyridinium, 1-amino-, chloride (41.18%), 1,3,2-Dioxathiolane, 2-oxide (15.00%), S-Methyl methanethiosulphonate (42.33%), Dihydroxanthin (6.18%), Carbonotrithioic acid, dimethyl ester (40.87%), Cyclohexasiloxane, dodecamethyl (6.90%), psi.,.psi.-Carotene, 3,4-didehydro-1,2-dihydro-1-methoxy (12.91%), 2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine (9.74%), Cycloheptasiloxane, tetradecamethyl (89.32%), Spiro-6-(bicyclo[3.2.1]octane)-2'-(oxirane), 7,8-di(hydroxymethyl)-5-methyl-2-isopropyl (13.03%), Ergosta-5,22-dien-3-ol, acetate, (3á,22E) (5.86%), (5á)Pregnane-3,20á-diol, 14à,18à-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate (0.54%), Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl) (2.73%), phthalic acid, di(2-propylpentyl) ester (26.90%), Ergosta-5,22-dien-3-ol, acetate, (3á,22E) (6.33%), Glycine, N-[(3à,5á)-24-oxo-3-[(trimethylsilyl)oxy]cholan-24-yl]-, methyl ester (12.80%), and Cholesteryl formate (15.15%).

Moreover, the results of GC-MS/MS of methanol-extract revealed the presence of several bioactive constituents, with 14 major compounds (Fig. 4 & Table 5). The chemical profiles of them are mainly: Pyridinium, 1-amino-, chloride (38.71%), Pyridinium, 1-amino-, hydroxide, inner salt (18.58%), S-Methyl methanethiosulphonate (68.44%), 4,25-Secoobscurinervan-4-one, O-acetyl-22-ethyl-15,16-dimethoxy-, (22à) (3.76%), 6-Chloro-3-(2-nitro-1-phenylethyl)-3,4-dihydro-1H-naphthalen-2-one (3.51%), Cyclohexasiloxane, dodecamethyl (94.82%), (5á)Pregnane-3,20á-diol, 14à,18à-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate (1.66%), pirost-8-en-11-one, 3-hydroxy-, (3á,5à,14á,20á,22á,25R) (0.93), Cycloheptasiloxane, tetradecamethyl (45.34%), Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl (58.28%), Undefined (12.40%), Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl) (7.85%), Spirost-8-en-11-one, 3-hydroxy-, (3á,5à,14á,20á,22á,25R) (5.22%), and Astaxanthin (17.34%).

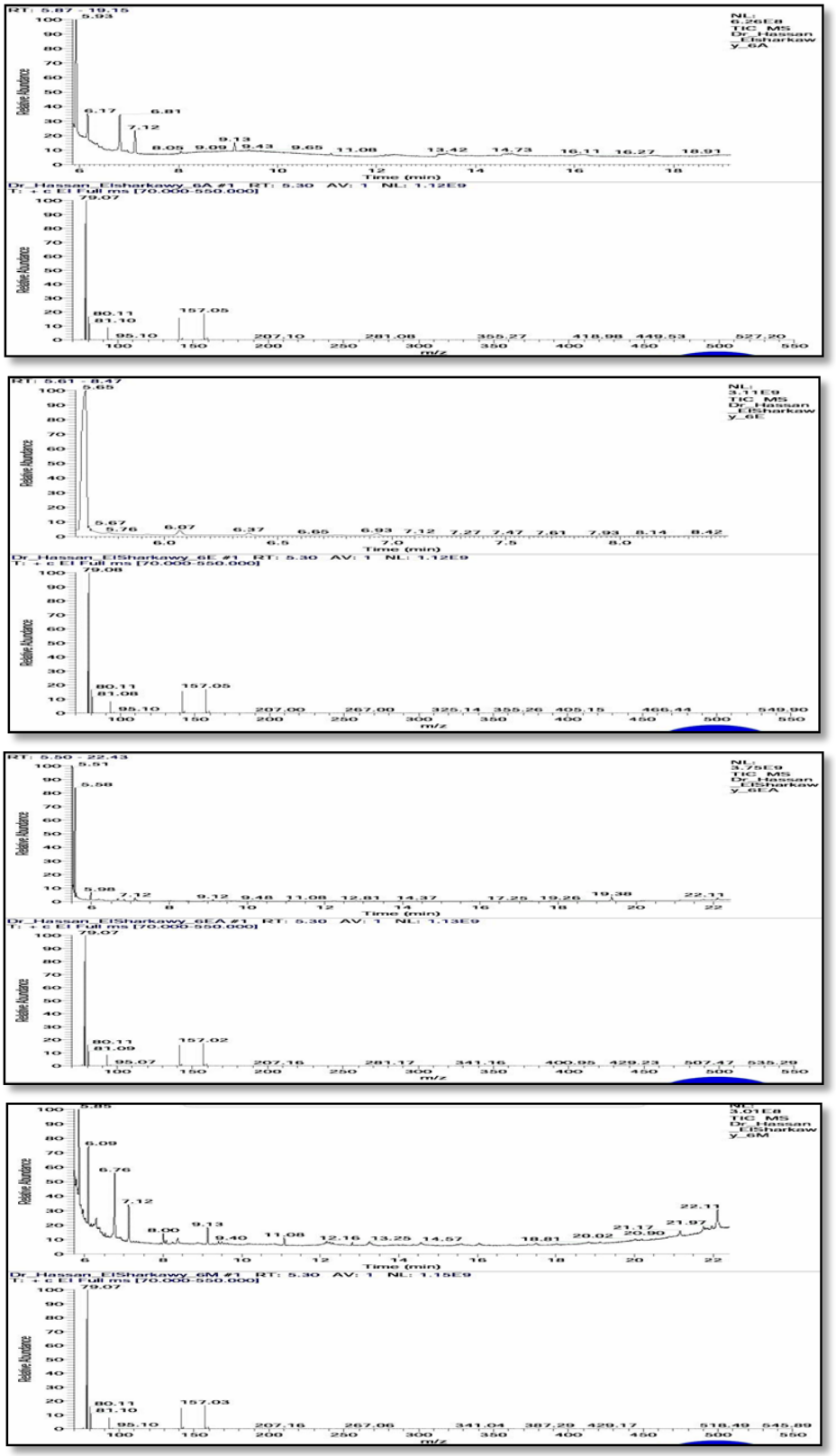

Fig. 4. GC-MS/MS chromatogram of *A. spinulosus* acetone (A), ethanol (B), ethyl acetate (C), and methanol (D) extracts showing the retention time and molecular weights of the identified compounds.

Table 5. Chemical constituents detected in different extracts of *A. spinulosus* by GC-MS/MS.

Peak No.	Compound name	RT (min)	Molecular formula	MW (m/z)	Hit	SI	RSI	Prob. (%)
***A. spinulosus* acetone extract:**								
1	2-(N,N-Bis(2-chloroethyl)amino)methyl-2,3-dihydro-6-hydroxy-3-oxopyridazine	5.92	$C_9H_{13}Cl_2N_3O_2$	265	1	582	669	19.10
2	Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-decamethyl	6.16	$C_{10}H_{32}O_4Si_5$	356	1	555	676	20.53
3	Carbonotrithioic acid, dimethyl ester	6.81	$C_3H_6S_3$	138	1	663	848	78.17
4	Cyclohexasiloxane, dodecamethyl	7.12	$C_{12}H_{36}O_6Si_6$	444	1	798	825	86.45
5	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl		$C_{16}H_{50}O_7Si_8$	578	1	774	820	72.54
***A. spinulosus* ethanol extract:**								
1	Dimethyl sulfone	5.56	$C_2H_6O_2S$	94	1	841	858	33.99
2	8,11-Octadecadiynoic acid, methyl ester	5.75	$C_{19}H_{30}O_2$	290	1	578	616	12.18
3	Methyl N-(N-benzyloxycarbonyl-beta-l-aspartyl)-beta-d-glucosaminide	6.93	$C_{19}H_{26}N_2O_{10}$	442	3	567	587	8.89
4	2-Cyclopentene-1-carboxylic acid, 1-methyl	6.07	$C_7H_{10}O_2$	126	3	592	691	2.11
5	[4-(1,3-Benzodioxol-5-yl)-6,8,9-trimethyl-3-oxabicyclo[3.3.1]non-6-en-1-yl]methyl acetate	6.32	$C_{21}H_{26}O_5$	358	1	576	613	12.73
6	N,N'-Ethylenebis(2-[2-hydroxyphenyl]glycine)	6.37	$C_{18}H_{20}N_2O_6$	360	1	598	605	6.06
7	12-Oxatricyclo[4.4.3.0(1,6)]tridecane-3,11-dione	6.65	$C_{12}H_{16}O_3$	208	2	576	620	10.29
8	4,25-Secoobscurinervan-4-one, O-acetyl-22-ethyl-15,16-dimethoxy-, (22à)	6.77	$C_{27}H_{36}N_2O_6$	484	1	573	579	10.41
9	2-Amino-3-(4-hydroxyphenyl)-propanoic acid	6.93	$C_9H_{11}NO_3$	181	1	669	714	11.90
10	Cyclohexasiloxane, dodecamethyl	7.12	$C_{12}H_{36}O_6Si_6$	444	1	745	818	88.41
11	Pyridine-4-carbohydrazide, N2-(3,4-methylenedioxy-6-nitrobenzylideno),	7.27	$C_{14}H_{10}N_4O_5$	314	2	556	678	6.57
12	Undefined	7.34	$C_{28}H_{38}Cl_2O_8$	572	1	573	585	8.85
13	4,25-Secoobscurinervan-4-one, O-acetyl-22-ethyl-15,16-dimethoxy-, (22à)	7.47	$C_{27}H_{36}N_2O_6$	484	1	591	604	24.08
14	Z,Z,Z-1,4,6,9-Nonadecatetraene	8.06	$C_{19}H_{32}$	260	1	566	652	6.92
15	7,10,13-Hexadecatrienoic acid, methyl ester	8.14	$C_{17}H_{28}O_2$	264	1	546	624	5.46
16	Ergosta-5,22-dien-3-ol, acetate, (3á,22E)	8.29	$C_{30}H_{48}O_2$	440	2	529	557	10.96
17	Bis[bicyclo[3.2.0]hept-2-en-4-yl]ether	8.42	$C_{14}H_{18}O$	202	2	590	744	8.55
***A. spinulosus* ethyl acetate extract:**								
1	Pentanoic acid, 2-propyl-, 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester, endo	5.18	$C_{16}H_{29}NO_2$	267	1	518	598	15.64
2	Pyridinium, 1-amino-, chloride	5.57	$C_5H_7ClN_2$	130	1	826	888	41.18
3	1,3,2-Dioxathiolane, 2-oxide	5.84	$C_2H_4O_3S$	108	1	543	875	15.00
4	S-Methyl methanethiosulphonate	5.97	$C_2H_6O_2S_2$	126	1	593	775	42.33
5	Dihydroxanthin	6.27	$C_{17}H_{24}O_5$	308	2	548	588	6.18
6	Carbonotrithioic acid, dimethyl ester	6.68	C3H6S3	138	1	634	792	40.87
7	2-Amino-3-(4-hydroxyphenyl)-propanoic acid	6.83	$C_9H_{11}NO_3$	181	1	582	705	6.90
8	Cyclohexasiloxane, dodecamethyl	7.12	$C_{12}H_{36}O_6Si_6$	444	1	823	852	96.28
9	psi.,.psi.-Carotene, 3,4-didehydro-1,2-dihydro-1-methoxy	7.15	$C_{41}H_{58}O$	566	1	543	551	12.91
10	2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine	8.33	$C_{20}H_{13}N_5O_2$	355	3	556	593	9.74
11	Cycloheptasiloxane, tetradecamethyl	9.12	$C_{14}H_{42}O_7Si_7$	518	1	791	876	89.32
12	Spiro-6-(bicyclo[3.2.1]octane)-2'-(oxirane), 7,8-di(hydroxymethyl)-5-methyl-2-isopropyl	9.48	$C_{15}H_{26}O_3$	254	1	573	626	13.03
13	Ergosta-5,22-dien-3-ol, acetate, (3á,22E)	12.22	$C_{30}H_{48}O_2$	440	2	564	654	5.86
14	(5á)Pregnane-3,20á-diol, 14à,18à-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate	12.81	$C_{28}H_{43}NO_6$	489	9	563	576	0.54
15	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)	14.36	$C_{27}H_{42}O_4$	430	7	583	615	2.73
16	Phthalic acid, di(2-propylpentyl) ester	19.38	$C_24H_{38}O_4$	390	1	832	927	26.90
17	Ergosta-5,22-dien-3-ol, acetate, (3á,22E)	21.16	$C_{30}H_{48}O_2$	440	5	606	689	6.33
18	Glycine, N-[(3à,5á)-24-oxo-3-[(trimethylsilyl)oxy]cholan-24-yl]-, methyl ester	21.74	$C_{30}H_{53}NO_4Si$	519	3	615	703	12.80
19	Cholesteryl formate	22.11	$C_{28}H_{46}O_2$	414	1	730	747	15.15
***A. spinulosus* methanol extract:**								
1	Pyridinium, 1-amino-, chloride	5.78	$C_5H_7ClN_2$	130	1	604	869	38.71
2	Pyridinium, 1-amino-, hydroxide, inner salt	5.85	$C_5H_6N_2$	94	1	531	890	18.58
3	S-Methyl methanethiosulphonate	6.10	$C_2H_6O_2S_2$	126	1	648	811	68.44
4	4,25-Secoobscurinervan-4-one, O-acetyl-22-ethyl-15,16-dimethoxy-, (22à)	6.30	$C_{27}H_{36}N_2O_6$	484	5	526	536	3.76
5	6-Chloro-3-(2-nitro-1-phenylethyl)-3,4-dihydro-1H-naphthalen-2-one	6.78	$C_{18}H_{16}ClNO_3$	329	6	532	586	3.51
6	Cyclohexasiloxane, dodecamethyl	7.11	$C_{12}H_{36}O_6Si_6$	444	1	803	837	94.82
7	(5á)Pregnane-3,20á-diol, 14à,18à-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate	8.11	$C_{28}H_{43}NO_6$	489	5	538	539	1.66
8	Spirost-8-en-11-one, 3-hydroxy-, (3á,5à,14á,20á,22á,25R)	8.37	$C_{27}H_{40}O_4$	428	9	537	563	0.93
9	Cycloheptasiloxane, tetradecamethyl	9.12	$C_{14}H_{42}O_7Si_7$	518	1	764	837	45.34
10	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	11.08	$C_{16}H_{50}O_7Si_8$	578	1	738	796	58.28
11	Undefined	13.24	$C_{24}H_{32}O_9$	464	2	583	604	12.40
12	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)	14.56	$C_{27}H_{42}O_4$	430	3	568	599	7.85
13	Spirost-8-en-11-one, 3-hydroxy-, (3á,5à,14á,20á,22á,25R)	21.97	$C_{27}H_{40}O_4$	428	7	603	667	5.22
14	Astaxanthin	22.11	$C_{40}H_{52}O_4$	596	2	657	661	17.34

In general, the bioactive compounds detected in the crude extract of our *A. spinulosus* were organic acids and their derivatives, besides much other of organic alcohols, steroids and terpenoids. However, the antimicrobial activities of the most of these constituents have been identified and established (Ibrahim, 2012; Hussein *et al*., 2016; Ibrahim *et al*., 2018).

For instance, Faulkner (2000) reviewed that there were three ceramides, AC-1-6, AC-1-10 and AC1-11, were obtained from Japanese *Acanthaster planci.* The cerebrosides acanthacerebroside A and astrocerebroside A from the starfish *Acanthaster planci* and *Astropecten latespinosus*, respectively, were synthesized via a chiral epoxide derived from L-quebrachitol. Three additional sulfated polyhydroxylated sterols, (20 R)-cholesta-5,24-diene-2 b,3 a,21-triol 2,21-disulfate, (20 R)- 5 a-cholest-24-ene-2 b,3 a,21-triol 3,21-disulfate and (20 R)- cholesta-5,24-diene-2 a,3 a,4 b,21-tetraol 3,21-disulfate, were isolated from the Antarctic ophiuroid *Astrotoma agassizii*. The starfish *Pteraster tesselatus* contained three similar sterol disulfates, (20 R, 25 R)-24-methyl-5 a-cholesta24(28)-ene-2b,3a,21,26-tetraol 3,21-disulfate, (20R, 25R,S)-cholest-5-ene-2 b,3 a,21,26-tetraol 2,21-disulfate and (20 R,25 R)-5 a-cholestane-2 b,3 a,21,26-tetraol 3,21-disulfate, an observation that has interesting chemotaxonomic implications.The sea star *Luidiaster dawsoni* from the Sea of Okhotsk contained (24 S, 25 R)-24-methylcholestane3b,5a,6b,15a,16b,26-hexaol. (25R)-5a-cholestane-3b,6b,15a,16b,26-pentaol, which was isolated as a cytotoxic constituent of an Antarctic starfish, has been synthesized from diosgenin in good overall yield. Datta *et al.* (2015) reported that Asterosaponins and many cerebrosides, pyrimidine nucleosides, thymine deoxyriboside and uracil deoxyribose have been isolated from the starfish; *Acanthester planei*.

Specifically, Kim *et al.* (2006) conducted that the acyclic thiosulfinates (1,2-Dithiolane) possess antimicrobial, antiparasitic, antitumor and cysteine protease inhibitory activity while the natural 1,2-dithiolane-1-oxides are growth inhibitors. Also, Benkeblia *et al*. (2007) confirmed the effectiveness of the natural biologically active S-Methyl methane thiosulphonate in the development of potent antifungal agents.

The results of Lazarević *et al.* (2011) showed that the derivative of 1,2,4-trithiolane had antimicrobial properties. Among the microbes tested, the most susceptible strains were *P. aeruginosa* (minimal inhibitory/bactericidal concentration = 0.08/2.5 mg/ml and *A. niger* (minimal inhibitory/fungicidal concentration = 0.31/0.63 mg/ml. Akerina *et al.* (2015) detected bioactive compounds from the three different solvents of gonads extracts were steroid, triterpenoid and saponin. Abd El-Karim (2016) detected the suppression effect of 2-Methyl-3,5-dinitrobenzyl alcohol and tert-butyldimethylsilyl ether. Hassan (2016) detected antibacterial activity of Cycloheptasiloxane, tetradecamethyl- and cyclooctasiloxane hexadecamethyl- in its hexane extract, against several pathogens.

Several fatty acids were estimated as antimicrobial agents. Many authors (Wu *et al.*, 2006; Nielsen *et al.*, 2010; El Semary, 2012) reported that some fatty acids have cytotoxic effects on other organisms. However, Wu *et al.* (2006) and Nielsen *et al.* (2010) attributed the ability of fatty acids to increase the membrane permeability leading to membrane damage. In addition, terpenoids such as triterpenes, sesquiterpenes and diterpenes have been referred to as antibiotics, insecticidal, anthelmintic, and antiseptic agents (Parveen *et al.*, 2010). Observably, the terpenoid fraction had weak antimicrobial activity against *P. aeruginosa* and *E. coli* (Mastelic *et al*., 2005) but highly reduce the growth of *S. aureus* and *C. albicans* (Mastelic *et al*., 2005). Moreover, there is a large

number of secondary metabolites produced by sea stars in the form of lipids, including steroidal derivatives of cholesterol, and fatty acid amides of sphingosine. The steroids are mostly saponins, known as asterosaponins, and their sulphated derivatives (Ruppert *et al*., 2004).

CONCLUSION

According to the obtained results during the current work, the sea star; *Astropecten spinulosus* habiting in the Egyptian Mediterranean Sea can be useful source for novel antibiotics, since its extracts showed significant bioactive capacities with good spectrum of antimicrobial activity against the tested microbes. Further studies have been recommended to determine the structure and nature of the effective natural product. Finally, the two unidentified compounds (with $C_{28}H_{38}Cl_2O_8$ and $C_{24}H_{32}O_9$, their molecular weights equal 572 and 464, respectively), detected by the precise tool like GC-MS/MS, may be promising if it takes a chance in future study.

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Arabic summary

النشاط المضاد للميكروبات لنجم البحر (*Astropecten spinulosus*) من شاطىء البحر المتوسط، الاسكندرية، مصر.

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تم جمع أنواع من نجم البحر من البحر المتوسط، الإسكندرية، مصر. تم تعريفه بناءً على السمات المورفولوجية والتشريحية العامة على أنه *Astropecten spinulosus*. تم فحص الأنشطة المضادة للبكتيريا والفطريات من خلال التقنيات القياسية. فأظهرت البيانات التي تم الحصول عليها أن مناطق التثبيط كعامل للنشاط المضاد للبكتيريا لمستخلصات *A. spinulosus* قد تراوحت بين ٠ و ١٨ ملم. تم الكشف عن أن أعلى نشاط مضاد للبكتيريا كان ضد *P. aeruginosa* (١٨ ملم) لمستخلص الإيثانول، تلاه *B. sublis* (١٤ ملم) لمستخلص الميثانول، ثم بواسطة *P. aeruginosa* (١٣ ملم) لكل من أسيتات الإيثيل ومستخلص الميثانول. كما وقد سجلت مستخلصات المذيبات المختلفة مناطق تثبيط حول الفطريات، حيث تراوحت الفعالية المضادة للفطريات بين ٨ إلى ١٠ ملم. وكان أكثر أنواع الفطريات قمعاً هو *P. crustosum* بواسطة مستخلصات الأسيتون والإيثانول بنسبة ٨٠ و ٩٠٪ على التوالي. ، تم قمع ضعيف ضد *A. terreus* بواسطة مستخلصات الإيثانول والميثانول من *A. spinulosus* بنسبة ١٠ و ٢٠٪ على التوالي. لم يتم قمع الفطريات الأخرى بواسطة أي مستخلص-مذيب. فيما يخص دراسة فعالية بعض المضادات الحيوية التجارية (ملم)، أكدت البيانات أن البكتيريا السالبة لصبغة جرام كانت أكثر مقاومة من البكتيريا الموجبة لصبغة جرام. على الجانب الآخر، لوحظ أن من خلال نتيجة GC-MS/MS للمستخلص الخام وجود العديد من المكونات النشطة بيولوجيًا، وكان لمعظمها أنشطة مضادة للميكروبات.