FACULTY OF AGRICULTURE

Minia J. of Agric. Res. & Develop.
Vol. (44), No.1, pp.31 - 46, 2024

ANTIBACTERIAL ACTIVITIES OF SOME MARINE SPONGES AND CORAL

Sally M.A. El-Shafei [1], Moaz M. Hamed [2], Esraa M. Megahed [1], Hosni M.S. Abd El-Salam [1] , Gamal F. Abd El-Naem [1], Ahmed G. Darwish [1]

[1] Department of Agricultural Chemistry, Faculty of Agriculture, Minia University, El-Minya , Egypt.

[2] Marine Environment Division, National Institute of Oceanography and Fisheries, Cairo, Egypt.

Received : 20 Jan. 2024 **Accepted: 2 Feb. 2024**

ABSTRACT

Two species of sponge *i.e., Xenia macrspiculata* and *Clathria arbuscula*, as well as one species of coral, *i.e., Crella (Grayella) cyathophora* representing marine invertebrates were collected from the Red Sea, Hurghada, Egypt. The antibacterial activities of their crude extracts was investigated against, *Against G+ve bacteria namely, Staphylococcus aureus (NIOF-B16), enterococcus faecalis(NIOF-B21) and Bacillus cereus (NIOF-B33) as well as G-ve ones namely, E. coli(NIOF-B17), Vibrio fluvial(NIOF-B24), Pseudomonas aeroginosa(NIOF-B23), and Salmonella ttyphimurium (NIOF-B35).*The extracts were screened for the presence of compounds such as terpenoids, tannins, anthocyanins, glycosides, phenols, flavonoids and alkaloids that could be responsible for bioactivity. The extracts exhibited an antibacterial effect against the all of the examined strains, resulting in average inhibition zones ranging between 8.0 ± 0.1 and 20.0 ± 0.3 mm. The presence of steroids, terpenes, phenols, alkaloids, and tannins found in the extract fractions seemed to be the cause of the wide spectrum antibacterial activity of these sponges Most likely, this is the first report on *Xenia macrspiculata*, *Clathria arbuscula*, and *Crella (Grayella) cyathophora's* antibacterial activity.

Key words: Antibacterial activity, sponge, coral, Rad Sea

INTRODUCTION

A wide variety of marine life forms can be found, such as sponges, seaweeds, corals, soft corals, ascidians, gorgonians, sea pens, algae, fungi, and marine-associated microorganisms. These organisms are thought to be significant sources for the identification of structurally diverse and bioactive secondary metabolites, which in turn yield a wide range of novel secondary metabolites with intriguing medical potential, pharmaceutical significance, and a variety of biotechnological applications **Wali *et al.* (2019) and Thomas *et al.*,(2010)).** Marine sponges, or Porifera, are among the oldest living things on Earth. They are deep-sea creatures that have been there for about 580 million years, and they may also be found in freshwater environments. Although just around 15,000 species have been identified, there are yet more un-identified varieties in the genuine aquatic ecosystems **Hooper and vanSoest,** (**2002).** Sponge-like filter feeders, known as aquiferous systems, move vast amounts of water via a network of specialized canals. Numerous tiny intake gaps (Ostia) and one or more larger outlet gaps (Oscula) make up this system. A current forms within the sponge as a result of the water being translocated by pressure or specialized cells called choanocytes that have**.** Flagella **Hooper and vanSoest,** (**2002).** Since the nineteenth century, soft corals (Cnidaria: Anthozoa: Octocorallia) have been the focus of several research because they frequently equal or surpass the total coverage of scleractinian corals in coral reef ecosystems. They are also significant structural elements of coral reef communities, dominant space occupants, and contributors to coral reef biomass. Seapens, gorgonians, and soft corals are all members of the subclass Octocorallia. The Alcyonacea order, which includes the families Xeniidae, Nephtheidae, and Alcyoniidae, is home to the majority of soft corals. Members of the genera Sinularia, Lobophytum, and Sarcophyton, which make up the family Alcyoniidae, are among the most common benthic invertebrates on the coral reefs of Okinawa and other Pacific Ocean regions **Tursch and Tursch,** (**1982).** Biologically active secondary metabolites with unique chemical structures may be found in abundance in marine sponges. Anti-inflammatory, anticancer, immunosuppressive or neurosuppressive, antiviral, antimalarial, antibiotic, and antifouling agents are the most common categories for the bioactive chemicals derived from sponges. The chemical variety seen in sponge products is astounding. Apart from the unique nucleosides, sponges have also been reported to contain bioactive terpenes, sterols, cyclic peptides, alkaloids, fatty acids, peroxides, and derivatives of amino acids . (many of which are halogenated) **(Joseph and Sujatha 2011; Sipkema *et al., 2*005).** Therefore, the current work was suggested to extract and study the potent antibacterial agents from the Red Sea sponges and coral species.

MATERIALS AND METHODS

Marine Biological Station (MBS):

The sampling location Fig. 1; situates 5 kilometers north to Hurghada city. The coral reefs directly abut to the mainland and other three main reefs independently are enclosed by water. The specimens collected at depths ranges between 1– 7 meters. The general features and Taxonomic position of the collected samples are presented in Fig.1 and Table, 1, respectively.

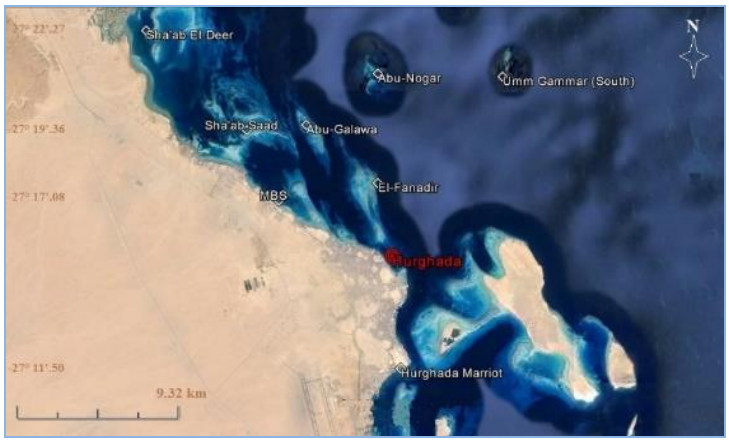

Fig 1: Location in Red Sea, Hurghada

Table 1: Classification position of sponges and coral

Sample/scientific classification			
Position	**Sponge**	**Sponge**	**coral**
Kingdom	**Animalia**	**Animalia**	**Animalia**
Phylum	Porifera	Porifera	Cnidaria
Class	Demospongiae	Demospongiae	Anthozoa
Order	*Poecilosclerida*	*Poecilosclerida*	*Alcyonacea*
Family	*Microcionidae*	*Crellidae*	*Xeniidae*
Genus	*Clathria*	*Crella*	*Xenia*
Subgenus	*Clathria (Clathria)*	*Crella(Grayella)*	------
Species	*Clathria (Clathria) arbuscula* ***Row,*** **(1911)**	*Crella(Grayella) cyathophpora* ***Carter,*** **(1869)**	*Xenia macrspiculata* ***Gohar,*** **(1940)**

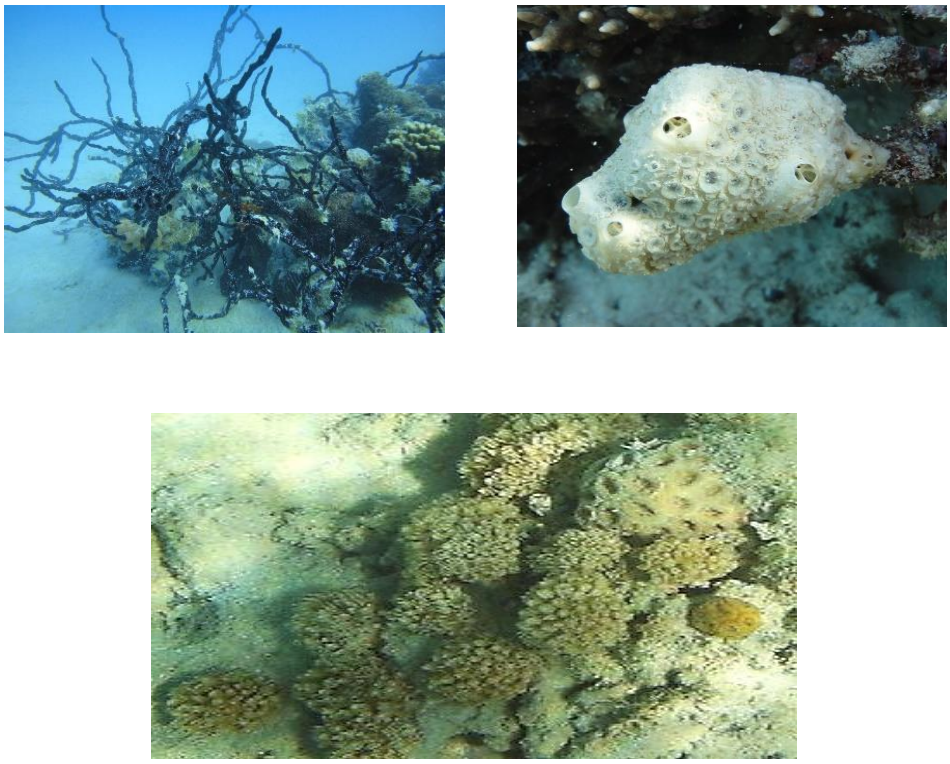

Fig. 2: General features of collected sponges and coral.

Marine Sponge materials

The marine sponge *Clathria arbuscula*, *Crella cyathophora*, and *Xenia macrspiculata* **Figure (2)** were collected from the Red Sea- Hurghada by diving in the Red Sea at depth 1-7 m, GPS: 27°17'12.4"N, 33°46'38.8"E in May 2021 and identified by Professor Mohammed Abdel Latif Ezz El-Arab, National institute of oceanography and fisheries, Hurghada, Egypt. Sample were transferred to the laboratory in plastic containers in seawater, cut into small pieces, and left to dry.

Bacterial strains

The pathogenic bacterial strains used in this investigation included *Staphylococcus aureus* (NIOF-B16), *Escherichia coli* (NIOF-B17), *Enterococcus faecalis* (NIOF-B21), *Pseudomonas aeruginosa* (NIOF-B23), *Vibrio fluvial is* (NIOF-B24),

Vibrio damsela (NIOF-B29), *Bacillus cereus* (NIOF-B33) and *Salmonella typhimurium* (NIOF-B35) **Abdel-Hameed *et al.* (2023).** The NIOF Microbiological Lab (National Institute of Oceanography and Fisheries, Red Sea branch, Egypt) provided these particular marine pathogenic strains. The pathogenic strains were kept on nutrient agar slants, which were folded with 25% glycerol and kept for extended preservation at -4 °C.

Marine sponge methanolic extracts:

Samples of *Xenia macrspiculata, Clathria arbuscula,* and *Crella cyathophora* were cut into small pieces and used for preparing the methanol extract.where 17.5, 16.44, and 29.65 g extracts were obtained from the extraction of 500 g fresh weight using 950 , 350 and 450 mL 99.8% methanol. Each extract was shaken for 3 hours at 150 rpm and 25°C than leaved in the dark place over night. Whatman sheets were then used for repeated extract filtration, and the residue was further extracted using the solvent for 3 days. The leftover methanol from each extract was evaporated at 40°C using a rotary evaporator according to the methods described by **Erdogan orhan *et al.* (2012)** with some modification.

Qualitative chemical Analysis:

The qualitative analysis of the sponge and coral extracts was carried out following the methodology outlined by **Harborne (1973)** as follows:

Detection of Steroids:

For detection of steroids; an equivalent amount of concentrated H2SO4 was added to a test tube's sidewalls after one milliliter of the extract of the extract and ten milliliters of chloroform were combined. The development of H2SO4 layer fluoresces yellowish green, while the top layer becomes red was considered as an indication of the presence of steroids . **(Gibbs, 1974).**

Detection of Terpenoids:

Concentrated H2SO4 and two milliliters each of acetic anhydride and extract were thoroughly mixed. The formation of blue-green ring , indicated presence of terpenoids . **Ayoola *et al.*, (2008).**

Detection of Tannins:

When two milliliters of the examined extract were mixed with few drops of 1% lead acetateand a reddish precipitate was formed, this indicated the presence of tannins **Treare and Evans, (1985).**

Detection of Saponins:

Five milliliters of the examined extract were agitated in twenty milliliters of distilled water in a graded cylinder for 15 mins . Foam production indicates the presence of saponins **Kumar *et al.*, (2009).**

Detection of Anthocyanins:

Detection of anthocyanins tn the extracts was performed by adding 2 milliliters of the examined extract to 2 ml of 2N HCl and 2 ml of 33% ammonia The change of the pink-red color into blue-violet, means the presence of anthocyanins **Paris and Moyse, (1969).**

Detection of Glycosides:

According to **Khandewal ,(2008),** 5 mL of the examined extract were mixed with two milliliters of glacial acetic acid and one drop of 5% FeCl3. Concentrated H2SO4 was then put into the test tube, The formation of a brown ring indicates the presence of glycosides.

Detection of Emodins:

Emodins were detected in the examined by adding 3 ml of benzene and

2 ml of 33% NH4OH to the extract and the presence of emodins is indicated by the appearance of red color **Rizk, (1982).**

Detection of Alkaloids:

For detection of alkaloids; a little amount of Mayer's regent (Techno Pharmchem Co.) was applied to the extracts and the presence of alkaloids was indicated by the formation of a cream-colored precipitate **Gibbs, (1974).**

Detection of Phenol:

The presence of phenols in the sponge and coral extracts was examined by mixing 2 mL of the extract with 0.5 mL of FeCl3 (5%) solution; the creation of a strong hue indicated the presence of phenols. **Gibbs, (1974).**

Detection of Flavonoids:

The presence of flavonoids in the examined extracts was shown by adding few drops of a 20% sodium hydroxide solution to 2 or 3 mL of the examined extract in a test tube and the formation of a bright yellow color. If the mixture became colorless after addition of a few drops of diluted HCl ,, this indicates the presence of flavonoids **Khandewal, (2008).**

Test Microorganism

The pathogenic bacterial strains used in this investigation included *Staphylococcus aureus (NIOF-B16), enterococcus faecalis(NIOF-B21) and Bacillus cereus (NIOF-B33) as well as G-ve ones namely, E. coli(NIOF-B17), Vibrio fluvial(NIOF-B24), Pseudomonas aeroginosa(NIOF-B23), and Salmonella ttyphimurium (NIOF-B35).***Abdel-Hameed, *et al.*, (2023)**. The NIOF Microbiological Lab (National Institute of Oceanography and Fisheries, Red Sea branch, Egypt) provided these particular marine pathogenic strains. The pathogenic strains were kept on nutrient agar slants, which were folded with 25% glycerol and kept for extended preservation at -4 °C.

Preliminary Tests for Antibacterial activities of sponge and coral extracts

The antibacterial activity of the sponge and coral extracts was preliminary examined against the G+ve strains Staphylococcus aureus (NIOF-B16), enterococcus faecalis(NIOF-B21) and Bacillus cereus (NIOF-B33) and G-ve ones *E. coli* (NIOF-B17), *Vibrio fluvial* (NIOF-B24), *Pseudomonas aeruginosa* (NIOF-B23), *and Salmonella ttyphimurium* (NIOF-B35) adopting the agar-well diffusion technique as described by **Gaswi *et al.*, (2022)** in surface-inoculated Muller Hinton agar plates. Eight mm diameter wells in each plate were filled with , 100 µL (25 mg/mL) of the examined extract.

Minimum Inhibitory Concentration (MIC) of sponge and coral extracts

As described by **Mahmud *et al.*, (2012)**; tetrazolium microplate assay was adopted to assess the minimum inhibitory concentrations (MICs) of the examined extracts on the test strains in Muller–Hinton broth (Becton Dickinson, Sparks, MD, USA). The examined extracts were prepared in DMSO to be ranging from 15 to 0.25 mg/mLwhere 200 µL of each concentration were added in triplicate to the wells, and the plates were incubated for 18–24 hours at 37 °C ± 0.5. Fifty µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) with a concentration of 0.2 mg/mL were added to each well, and incubated at 37 °C for 30 minutes. The bacterial suspension without the extract

served as the positive control, while the corresponding solvent blank (DMSO) was used as the negative control. To determine the percentage reduction of the dye (indicating the inhibition of bacterial growth), we measured the absorbance at 570 nm relative to a reference wavelength of 650 nm, which was achieved by introducing DMSO to the spectrophotometer **Pourali *et al.* (2017).**

Comparative evaluation of the antibacterial activities of the sponge and coral extracts

The antimicrobial activity of the sponge and coral extracts was evaluated in comparison with 4 commercial antibiotics, following the agar disc diffusion method as described by **Bauer *et al.* (1966)**. Approximately 25 mg/mL of each extract was added. For the comparative study, four standard commercial antibiotic discs were utilized: 30 mg/disc of Amikacin, 30 mg/disc of Tetracycline, 5 mg/disc of Flucloxacillin and 2 mg/disc of Clindamycin (Oxoid Ltd, England). These discs were placed on the surfaces of inoculated plates with the test bacterial strain Plates were incubated at 37 °C overnight and the inhibition zone diameters each disc was measured (mm) . The assays were conducted in duplicate **Abd-Elnaby *et al.* (2016).**

Statistical Analysis

Data were analyzed using One Way ANOVA test. The results were expressed as mean ± standard division and values of $P_{< 0.05}$.by prism 7.

Ethics Committee Approval

All procedures carried out in the current study were approved by the ethical committee for the care and use of animals, microorganisms, and living cell cultures in education and scientific research, Faculty of Agriculture, Minia university, El-Minya, Egypt and assigned the following approval number:. 0170523.

RESULTS AND DISCUSSION

Qualitative Screening of zoo-chemicals of marien sponge :

The results presented in **Table. (2**) show the phytochemical screening of methanolic extract from marine sponge *Clathria (Clathria) arbuscula , Crella (Grayella) cyathophora* ،and the coral *Xenia macrspiculata*. All these extracts displayed different types of terpenoids, tannins, anthocyanins, glycosides ,phenols, flavonoids and alkaloids compounds .

Table (2): chemical screening of marine sponges methanol 99.8% , *Clathria (Clathria) arbuscula*،*Crella (Grayella) cyathophora* and *Xenia macrspiculata* .

	***Clathria (Clathria) arbuscula* (RS)**	***Crella (Grayella) cyathophora* (C)**	***Xenia macrspiculata* (CS)**
Terpenoids	+	++	+++
Tannins	++	+++	++
Saponins	+++++++	+	_
Anthocyanins	+++	+	_
Glycosides	+++	++	++
Phenols	++	+++	+
Favonoids	+	+++	+
Alkaloids	+	++	+
Cumarine	-	++	+++

(++++++),(+++),(++),(+) and (-) indicate to very high, high, moderat and low absent concentration respectively.

According to earlier research, sea sponges were shown to contain secondary metabolites such alkaloids, flavonoids, and steroids. These findings are supported by chemical testing(**Hanani *et al.*, 2005 and . Rumagit , 2015).**According **Mayefis *et al.*, (2021),**the sea sponge Porifera contained a variety of compounds, such as tannins, steroids, alkaloids, and flavonoids Analysis of the different bioactivities of a methanol extract of the sea sponges, Demospongiae,revealed the presence of triterpenoids, steroids, phenols, and alkaloids **Athira and Keerthi, (2016). Govinden-Soulange *et al.*, (2014**) revealed that the secondary metabolites found in both marine sponges seem to be in a methanolic extract of the sponge, alkaloids, steroids, saponins, and tannins (both hydrolysable and condensed tannins).

Anti-microbial activity

In this study, eight distinct strains of marine pathogenic bacteria, *Against G+ve bacteria namely, Staphylococcus aureus, enterococcus faecalis* and *Bacillus cereus* as well as *G-ve ones namely, E. coli, Vibrio fluvial,Pseudomonas aeroginosaand Salmonella ttyphimurium* were examined. The extracts exhibited an antibacterial effect against all of tested strains, resulting in average inhibition zones ranging between 8.0 ± 0.1 and 20.0 ± 0.3 mm **(Table 3 and Figure 3**). The extract from RS had a very positive impact on the examined bacteria, showing the highest effect on both *Staphylococcus aureus* (NIOF-B16), *Escherichia coli* (NIOF-B17), *Enterococcus faecalis* (NIOF-B21) and *Vibrio fluvialis* (NIOF-B24) giving an inhibitory zone diameter of 18mm (18 mm). However, this extract had a moderate effect on both *Pseudomonas*

aeruginosa (NIOF-B23), *Vibrio damsel* (NIOF-B29) and *Salmonella typhimurium* (NIOF-B35) with inhibitory zones of 16.0 ± 0.2 , 14.0 ± 0.1 and 14.0 ± 0.2 mmin diameter, respectively. finally, there was an acceptable impact on *Bacillus cereus* (NIOF-B33) with inhibition zones measuring (12.0 ± 0.4 mm). The CS extract exerted a potent influence on *Staphylococcus aureus* (NIOF-B16), as indicated by inhibition zones measuring 20.0 ± 0.3 mm. Furthermore, the extract demonstrated an impact on the other pathogenic bacteria in an effective manner, with inhibition zones ranging from 16.0 ± 0.1 mm to 10.0 ± 0.1 mm. The antibacterial effect of the coral C extract was magnificent and acceptable on all the bacteria as it appears in **Table 3.** In the case of the negative control (DMSO), there was no observable zone of inhibition.

In order to assess the susceptibility of the tested strains to the all extracts, the minimum inhibitory concentration (MIC) were evaluated and presented in **Table 3**. The RS extract exhibited the lowest MIC for *Escherichia coli* (NIOF-B17) (8.0 ± 0.5 mg/mL). The MIC values for *Staphylococcus aureus* (NIOF-B16), *Enterococcus faecalis* (NIOF-B21), *Pseudomonas aeruginosa* (NIOF-B23), *Vibrio fluvialis* (NIOF-B24), *Vibrio damsel* (NIOF-B29), *Bacillus cereus* (NIOF-B33) and *Salmonella typhimurium* (NIOF-B35) were 8.5 ± 0.4, 10.0 ± 0.5, 9.5 ± 0.1, 10.0 ± 0.2, 12.5 ± 0.1, 15.0 ± 0.2 and 11.5 ± 0.2 mg/mL, respectively. On the other hand, The CS extract exhibited the lowest MIC represented against *Staphylococcus aureus* (NIOF-B16) and *Escherichia coli* (NIOF-B17) were 4.5 ± 0.5, and 7.5 ± 0.1 mg/mL, respectively, while the highest MIC was represented against *Bacillus cereus* (NIOF-B33) (15.0 ± 0.03 mg/mL). In addition, the values of MIC concentrations of compound S ranged from 14.0 ± 0.1 to 5.5 ± 0.4 mg/mL.

Table (3): Bacterial growth inhibition zone diameters (mm) by the sponge and coral extracts

Pathogens	Inhibition Zone diameter (mm)			
	RS ***Clathria***	**CS** ***Xenia***	**C** ***Crella***	**DMSO (Negative (Control)**
Staphylococcus aureus (NIOF-B16)	18.0 ± 0.1	20.0 ± 0.3	16.0 ± 0.2	0.0 ± 0.0
Escherichia coli (NIOF-B17)	18.0 ± 0.3	16.0 ± 0.1	16.0 ± 0.4	0.0 ± 0.0
Enterococcus faecalis (NIOF-B21)	18.0 ± 0.1	14.0 ± 0.2	14.0 ± 0.4	0.0 ± 0.0
Pseudomonas aeruginosa (NIOF-B23)	16.0 ± 0.2	12.0 ± 0.1	10.0 ± 0.2	0.0 ± 0.0
Vibrio fluvialis (NIOF-B24)	18.0 ± 0.2	14.0 ± 0.1	14.0 ± 0.1	0.0 ± 0.0
Vibrio damsel (NIOF-B29)	14.0 ± 0.1	14.0 ± 0.2	16.0 ± 0.2	0.0 ± 0.0
Bacillus cereus (NIOF-B33)	12.0 ± 0.4	10.0 ± 0.1	8.0 ± 0.1	0.0 ± 0.0
Salmonella typhimurium (NIOF-B35)	14.0 ± 0.2	12.0 ± 0.2	14.0 ± 0.1	0.0 ± 0.0

The data are represented as mean ± SD in mm of inhibition zone demonstrated, contrasted utilizing ANOVA, with a significance level (p-value) ≤ 0.05.

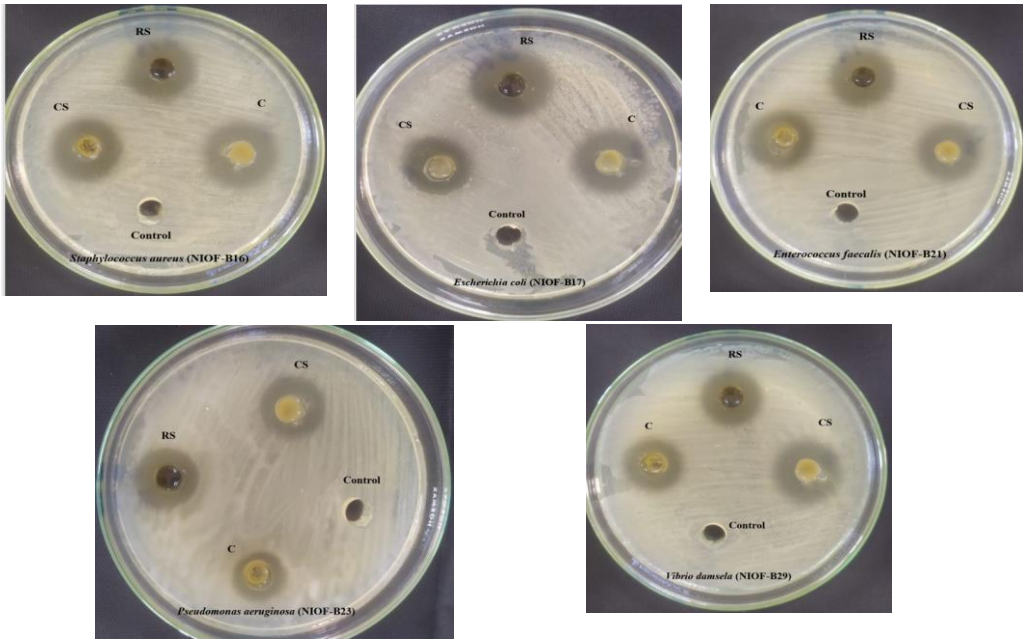

Figure 3: An image showing the effect of extracts some selected microbial pathogens

Table (4): MIC values of the sponge and coral extracts against some pathogens

Pathogens	MIC (mg/mL)		
	RS ***Clathria***	**CS** ***Xenia***	**C** ***Crella***
Staphylococcus aureus (NIOF-B16)	8.5 ± 0.4	4.5 ± 0.5	10.5 ± 0.2
Escherichia coli (NIOF-B17)	8.0 ± 0.5	7.5 ± 0.1	9.5 ± 0.1
Enterococcus faecalis (NIOF-B21)	10.0 ± 0.5	9.0 ± 0.3	9.5 ± 0.02
Pseudomonas aeruginosa (NIOF-B23)	9.5 ± 0.1	12.5 ± 0.01	14.0 ± 0.1
Vibrio fluvialis (NIOF-B24)	10.0 ± 0.2	11.5 ± 0.2	9.5 ± 0.03
Vibrio damsela (NIOF-B29)	12.5 ± 0.1	10.0 ± 0.06	5.5 ± 0.4
Bacillus cereus (NIOF-B33)	15.0 ± 0.2	15.0 ± 0.03	12.5 ± 0.1
Salmonella typhimurium (NIOF-B35)	11.5 ± 0.2	8.5. ± 0.2	11.5 ± 0.4

The data are represented as mean ± SD in mm of inhibition zone demonstrated, contrasted utilizing ANOVA, with a significance level (p-value) ≤ 0.05.

Table (5) : Bacterial growth inhibition zoned diameters (mm) by the sponge and coral extracts in comparison with some antibiotics.

Pathogens	Inhibition Zone (mm)						
	RS *Clathria*	**CS *Xenia***	**C *Crella***	**Amikacin 30 mg/disc**	**Tetracycline 30 mg/disc**	**Flucloxacillin 5 mg/disc**	**Clindamycin 2 mg/disc**
Staphylococcus aureus (NIOF-B16)	18.0 ± 0.4	20.0 ± 0.1	16.0 ± 0.1	20.0 ± 0.5	10.0 ± 0.3	0.0	0.0
Escherichia coli (NIOF-B17)	16.0 ± 0.1	16.0 ± 0.1	16.0 ± 0.4	18.0 ± 0.4	10.0 ± 0.1	0.0	0.0
Enterococcus faecalis (NIOF-B21)	18.0 ± 0.02	14.0 ± 0.3	14.0 ± 0.05	25.0 ± 0.03	12.0 ± 0.1	6.0 ± 0.2	0.0
Pseudomonas aeruginosa (NIOF-B23)	18.0 ± 0.4	12.0 ± 0.1	11.0 ± 0.1	14.0 ± 0.1	8.0 ± 0.05	0.0	10 0 ±. 0.3
Vibrio fluvialis (NIOF-B24)	18.0 ± 0.3	18.0 ± 0.4	14.0 ± 0.2	14.0 ± 0.05	8.0 ± 0.05	0.0	0.0
Vibrio damsela (NIOF-B29)	14.0 ± 0.3	16.0 ± 0.2	16.0 ± 0.1	14.0 ± 0.2	10.0 ± 0.2	0.0	0.0
Bacillus cereus (NIOF-B33)	12.0 ± 0.4	10.0 ± 0.1	8.0 ± 0.1	10.0 ± 0.04	8.0 ± 0.05	8.0 ± 0.1	0.0
Salmonella typhimurium (NIOF-B35)	14.0 ± 0.2	12.0 ± 0.2	14.0 ± 0.1	14.0 ± 0.3	0.0	0.0	0.0

The data are represented as mean ± SD in mm of inhibition zone demonstrated, contrasted utilizing ANOVA, with a significance level (p-value) ≤ 0.05.

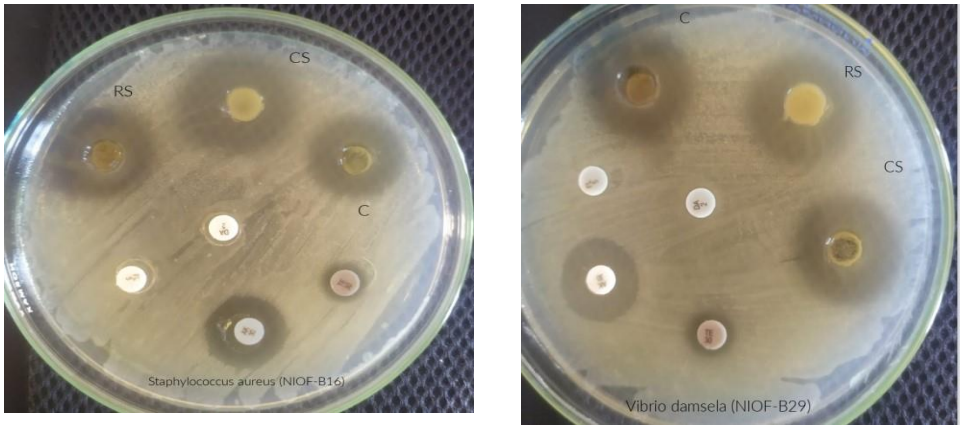

Figure 4: Effect of extracts against some pathogens in comparison with some standard commercial antibiotic discs.

According to **Webster *et al.* (2007),** the minimum inhibitory concentration (MIC) for the crude extracts of both sponges were the highest and ranged between 2.55–5.09 mg/ml. This is likely because crude extracts often include a combination of active and inactive chemicals, thus higher MICs are predicted **Govinden-Soulange *et al.*, (2014**) The findings show that the extracts from the two sea sponges had differing levels of activity against the test bacterial species. Because the extracts' actions did not rely on the Gram response, it seemed that they had a wide range of effects. Additionally, fractions were often shown to have stronger antibacterial activity **KONUKLUGİL and Gözcelioğlu (2015).** For a few numbers of crude extracts at a concentration of 250 µg/mL, sponge strong species shown antibacterial activity were detected against Gram positive (Staph aureus (MRSA), Enterococcus (VRE), and Candida albicans) bacteria.

CONCLUSION

The findings of this research are highly intriguing. It highlights the discovery of broad-spectrum antibacterial activity in certain sponge species, specifically *Clathria (Clathria) arbuscula*, *Crella (Grayella) cyathophora*, and *Xenia macrspiculata*. The study attributes this antibacterial property to the presence of natural chemical compounds such as terpenes, alkaloids, and tannins within these sponges.

REFERENCE

Abd-Elnaby, H., G. Abo-Elala, U. Abdel-Raouf, A. Abd-elwahab and M. Hamed (2016). "Antibacterial and anticancer activity of marine Streptomyces parvus: optimization and application." Biotechnology & Biotechnological Equipment 30(1): 180-191.

Abdel-Hameed, S. M., N. A. Abd Allah, M. M. Hamed and O. I. Soltan (2023). "Papaya fruit by-products as novel food ingredients in cupcakes." Annals of Agricultural Sciences 68(1): 60-74.

Abdelaleem, E. R., Samy, M. N., Desoukey, S. Y., Liu, M., Quinn, R. J., & Abdelmohsen, U. R. (2020). "Marine natural products from sponges (Porifera) of the order Dictyoceratida (2013 to 2019); a promising source for drug discovery." RSC advances, 10(57), 34959-34976.

Athira Krishnan, K. A., and Keerthi, T. R. 2016. Analyses of Methanol Extracts of Two Marine Sponges, Spongia officinalis var. ceylonensis and Sigmadocia carnosa from Southwest Coast of India for their Bioactivities. Int.J.Curr.Microbiol.App.Sci.5(2): 722-734

Ayoola, G. A.; Coker, B. A. H.; Adesegun, A. S.; Adepoju-Bello A. A.; Obaweya K.; Ezennia, C. E. and Atangbayila, O. T. (2008): "Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in south western nigeria." Tropical J. Pharm Res., 7(3): 1019-1024.

Bauer, A., W. Kirby, J. C. Sherris and M. Turck (1966). "Antibiotic susceptibility testing by a standardized single disk method." American journal of clinical pathology 45(4_ts): 493-496.

Carter, H.J. (1869). "On Grayella cyathophora, a new Genus and Species of Sponges." Annals and Magazine of Natural History. (4) 4 (21): 189-197, pl. VII.

Ebrahimzadeh, M. A.; Pourmorad, F. and Bekhradina, A. R. (2008): "Iron chelating activity, phenol and flavonoid content of some medicinal Effect of Using Aqueous Extract of Salvia officinalis L. Leaves on Some Antioxidants Status in Irradiated Rats." Egypt. J. Rad. Sci. Applic., 26, (1-2): 27-39.

Erdoğan Orhan, İ. L. K. A. Y., Özçelik, B., Konuklugil, B., Putz, A., Kaban, U., and Proksch, P. (2012). "Bioactivity screening of the selected Mediterranean sponges and three compounds from Agelas oroides." J.Records of Natural Products, 6(2).

Gazwi, H. S., N. A. Shoeib, M. E. Mahmoud, O. I. Soltan, M. M. Hamed and A. E. Ragab (2022). "Phytochemical profile of the ethanol extract of Malvaviscus arboreus red flower and investigation of the antioxidant, antimicrobial, and cytotoxic activities." Antibiotics 11(11): 1652.

Gibbs, R. D. (1974): "Chemotaxonomy of Flowering Plants: Four Volumes." McGill-Queen's Press-MQUP.

Gohar, H. A. F. (1940): "Studies on the Xeniidae of the Red Sea." Publ.

Mar. Biol. Stn. Ghardaqa, Red Sea, Egypt 2: 25-118.

Harborne, J. B. (1973): "Phytochemical nutrional and antioxidant activity evaluation of seeds of jackfruit (Artocarpusheterophyllus)." Inter. J. Pharma. & Bio. Sci., 2: 336.

Hanani E, Munim A, Sekarini R. Identification of antioxidant compounds in the Callyspongia sponges of the thousand islands. Pharm Sci Mag 2005;2 .

Hooper JNA, Van Soest RWM. Systema porifera: a guide to the classification of sponges. Kluwer Academic/Plenum Publishers. 2002.

Govinden-Soulange, J., Marie, D., Kauroo, S., Beesoo, R., & Ramanjooloo, A. (2014). Antibacterial properties of marine sponges from Mauritius waters. *Tropical Journal of Pharmaceutical Research*, *13*(2), 249-254

Joseph B, Sujatha S. (2011) "Pharmacologically Important Natural products from Marine Sponges." Journal of Natural Products, 4, 5-12.

Khandelwal, K. (2008): "Practical pharmacognosy." Pragati Books Pvt. Ltd.

Kumar, A.; Ilavarasan R.; Jayachandran T.; Decaraman M.; Aravindhan P.; Padmanaban N. and Krishnan M. R. V. (2009): "Phytochemical investigation on a tropical plant." Pakistan J. Nutr. 8(1): 83-85.

konuklugil, B., & Gözcelioğlu, B. (2015). Antimicrobial activity of marine samples collected from the different coasts of Turkey. *Turk J Pharm Sci*, *12*(3), 337-344

Maurya, S. and Singh, D. (2010): "Quantitative analysis of total phenolic content in Adhatodavasicanees." Int. J. Pharm Tech Res., 2(4): 2403-2406.

Mayefis, D. E. L. L. A. D. A. R. I., & Widiastuti, S. I. S. K. A. (2021). Antioxidant activity of methanol extracts natuna marine sponge (porifera) with dpphmethode. *Asian J. Pharm. Clin. Res*, *80*, 109-112.

Paris, R.and Moyse, H. (1969): "Precis de matièremedicinale." Ed Masson Paris.

Pourali, P., S. H. Badiee, S. Manafi, T. Noorani, A. Rezaei and B. Yahyaei (2017). "Biosynthesis of gold nanoparticles by two bacterial and fungal strains, Bacillus cereus and Fusarium oxysporum, and assessment and comparison of their nanotoxicity in vitro by direct and indirect assays." Electronic Journal of Biotechnology 29: 86-93.

Rizk, A. M. (1982): "Constituents of plants growing in Qatar. I. A chemical Survey of Sixty plants." Fitoterapia.

Row, R.W.H. (1911). "Reports on the Marine Biology of the Sudanese Red Sea, from Collections made by Cyril Crossland, M.A., B.Sc., F.Z.S. XIX. Report on the Sponges collected by Mr. Cyril Crossland in 1904-5. Part II. Non-Calcarea." Journal of the Linnean Society. Zoology. 31 (208): 287-400, pls 35-41. page(s): 347-349

Rumagit HM. Phytochemical test and antioxidant activity test of ethanol extract of Lamellodysidea herbacea sponge. Pharmacon 2015;4:183-92

Sipkema D, Franssen Maurice C.R, Osinga R, Tramper J. (2005) "Marine Sponges as Pharmacy." Marine Biotechnology, 7, 142–162. doi: 10.1007/s10126-004-0405-5

Thomas, T.; Rusch, D.; DeMaere, M.Z.; Yung, P.Y.; Lewis, M.; Halpern, A.; Heidelberg, K.B.; Egan, S.; Steinberg, P.D.; Kjelleberg, S. (2010) "Functional genomic signatures of sponge bacteria reveal unique and shared features of symbiosis." ISME J. 4, 1557–1567.

Treare, G. E. and Evans W. C. (1985): "Pharmacognosy 17thed." BahiveTinal, London. 149.

Tursch B, Tursch A (1982) "The soft coral community on a sheltered reef quadrat at Laing Island (Papua New Gunea)." Mar Biol 68: 321–332.

Van Soest R., N. Boury-Esnault, J. Hooper, K. d. Rutzler, N. De Voogd, B. Alvarez de Glasby, E. Hajdu, A. Pisera, R. Manconi and C. Schoenberg (2015), "World Porifera Database," available at http://www.marinespecies.org/porifera

Wali F., S. Majid, S. Rasool, S. B. Shehada, S. K. Abdulkareem, A. Firdous, S. Beigh, S. Shakeel, S. Mushtaq, I. Akbar, H. Madhkali and M. U. Rehman (2019), "Natural products against cancer: review on phytochemicals from marine sources in preventing cancer." Saudi Pharm. J., 2019, 27, 767–777.

Webster D, Taschereau P, Belland RJ, Sand C, Rennie RP. Antifungal activity of medicinal plant extracts; preliminary screening studies. J Ethnopharmacol 2007; 115: 140-146.

الملخص العربي

الأنشطة المضادة للبكتريا لبعض الأسفنج البحرى والمرجان

سالى محمد عبد العزيز الشافعى[1] – معاذ محمد حامد محمد[2] – اسراء محمد مجاهد[1] – حسنى محمد عبد السلام[1] – جمال فخرى عبد النعيم[1] – احمد جمعة درويش[1]

[1] قسم الكيمياء الزراعية – كلية الزراعة – جامعة المنيا - المنيا [2] قسم البيئة البحرية – المعهد القومى لعلوم المحيطات والمصايد - القاهرة

تم جمع نوعين من الإسفنج ونوع واحد من المرجان يمثل اللافقاريات البحرية من البحر الأحمر، الغردقة، مصر. تم دراسة نشاطها المضاد للبكتيريا في المستخلصات الخام ضد بعض الكائنات البكتيرية، وهي المكورات العنقودية الذهبية(NIOF-B16) ، الإشريكية القولونية(NIOF-B17) ، المكورات المعوية البرازية (NIOF-B21) ، الزائفة الزنجارية(NIOF-B23) ، الضمة النهرية(NIOF-B24) ، الضمة دامسيلا (NIOF-B29) ، العصوية الشمعية (NIOF-B33) والسالمونيلا تيفيموريوم.(NIOF-B35) تم فحص المستخلصات للتأكد من وجود مركبات مثل التربينويدات والعفص والانثوسيانين والجليكوسيدات والفينولات والفلافونويدات والقلويدات التي يمكن أن تكون مسؤولة عن النشاط الحيوي. أظهرت المستخلصات تأثير مضاد للجراثيم ضد جميع السلالات المختبرة، مما أدى إلى مناطق تثبيط متوسطة. يتراوح بين 8.0 ± 0.1 و 20.0 ± 0.3 ملم. يبدو أن النشاط المضاد للبكتيريا واسع النطاق لهذه الإسفنجات يرجع إلى وجود استيرويدات وتربينات وقلويدات وتنينات تم اكتشافها في أجزاء المستخلص. ربما يكون هذا هو التقرير الأول عن النشاط المضاد للميكروبات لنباتات *Clathria (Clathria) arbuscula* و *Crella (Grayella) cyathophora* و *Xenia macrspiculata*.