The Antibacterial Activity of some Red Sea Soft Corals species

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ABSTRACT

The present study aimed to investigate the antibacterial properties of crude extracts from some soft coral species from the Red Sea, Hurghada, Egypt. Crude extracts of *Lobophytum pauciliforum, Dendronephthya hemprichi, Sarcophyton gracile, Sarcophyton glaucum, Sinularia gardineiri, Sinularia leptoclados, Nephthea pacifica, Sarcophyton acutum, Sarcophyton spongosium* and *Xenia macrospiculata*, were tested against fish and human pathogenic bacteria. The well cut - diffusion technique was used to determine the absolute activity units (AU) and the minimal inhibitory concentrations (MICs) using disc-diffusion technique were determined against the most affected bacterial pathogens (*E. coli* and *S. aureus).* The AU of the ethanolic crude extract ranged from 1.4 to 25.0 for *Sarcophyton acutum* and *Lobophytum pauciliforum*, respectively. On the other side, ethyl acetate crude extracts showed the highest AU (16.0) for *L. pauciliforum* against *S. aeurus*, followed by (11.1) of *N. pacifica* and *X. macrospiculata* against). The MIC of *L. pauciliforum* ethyl acetate crude extract was recorded as (50 mg ml^{-1}) against both *E. coli* and *S. areaus* ATCC 6358.

Extracts from some soft corals showed the ability to inhibit the growth of some pathogenic bacteria indicating that it could be used for medical purposes.

Keywords: Antibacterial activity, Soft corals, MIC, Cytotoxicity.

INTRODUCTION

The ocean is considered to be a great source of potential drugs, and hence marine natural products have attracted the attention of biologists and chemists for the past five decades (Bhakuni and Rawat, 2005). Over 5000 novel compounds have been isolated from shallow waters to 900-m depths of the sea (Somnath and Ghosh, 2010). The recent studies on the bioactive compounds isolated from marine organisms have shown that they have anti-cancer, anti-bacterial, anti-fungal or anti-inflammatory and other pharmacological activities (Borowitzka and Borowitzka, 1992; Febles *et al.*, 1995; Mayer and Hamann, 2005; Somnath and Ghosh, 2010). Chemicals such as alkaloids, phenols, steroids, terpenoids, are secondary metabolites that have toxicological, pharmacological and ecological significance (Somnath and Ghosh, 2010). The evolutionary success of soft corals in areas of high levels of predation has been attributed to their production of significant amounts of secondary metabolites, especially terpenes (Sammarco *et Al.*,1985). Many soft corals show predator deterrence activity (Coll *et al.*, 1982; Taglialatela-Scafati *et al.*, 2002). Soft corals elaborate a large variety of sesquiterpenoids and diterpenoids. Several of these are found to be toxic. Guaiazulene from the gorgonian *Euplexaura erecta* exhibits mild

activity against *Pseudomonas aeruginosa*. Subergorgic acid, a cardiotoxin, is obtained from the Pacific gorgonian coral *Subergorgia suberosa* (Groweiss *et al*., 1985). Pseudopterolide, an unusual diterpene with a 12-member ring from the gorgonian *Pseudopterogorgia acerosa* shows unusual cytotoxic properties (Banduraga *et al*., 1982). The soft corals are very widespread throughout the tropical Indo-Pacific area including the Red Sea. The Red Sea reefs are made up of four main families; Xeniidae, Nephtheidae, Alcyoniidae and Tubiporidae containing about 250 species. All of these families and species are found scattered abundantly along the reefs of the Red Sea coast. Although they are abundant on the Red Sea reefs, a few studies on the soft corals were carried out in comparison to hard corals. Most of the studies focused on their abundance, diversity and growth. However, studies on the biological and microbial activities on the Red Sea soft corals are almost lacking. Thus, the present study was suggested to investigate compounds extracted from some soft coral species common in the Red Sea at Hurghada, Egypt and whether they are effective in curing some microbial infections. In addition, MICs and cytotoxicity were demonstrated.

MATERIALS AND METHODS

Sampling of soft corals

Soft corals were sampled off the National Institute of Oceanography and Fisheries at Hurghada on the Egyptian Red Sea coast. Ten species were collected using SCUBA diving technique and identified (Thomson & Dean, 1931; Gohar, 1940; Ezz Al-Arab, 2009). The samples were then washed by distilled water and frozen for further the purpose of extraction of bioactive substances.

Preparation of soft coral crude extracts

Fifty grams of each soft coral sample were macerated with 100 ml of 70 % aqueous ethanol and ethyl acetate solvents. After soaking for two weeks with shaking twice daily, they were filtered through Whatman 542 filter paper. Ethanol and ethyl acetate solvents were evaporated using rotary evaporator to obtain crude extracts (Ballantine, 1987).

Antibacterial activity against indicator strains

1- Well-cut diffusion technique:

Fifty millimeters of nutrient agar medium inoculated with indicator microorganisms (0.5%) 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 crude extract. All plates were incubated at appropriate temperature for 24-48 hrs. 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 each crude extract, which indicates a positive result in the antimicrobial action, was calculated according to the following equation (El-Masry et *al*., 2002):

$$\mathbf{AU = \pi Y^2 / \pi X^2}$$

2- Pouring technique

Nutrient agar was prepared and then 24 hrs old - indicator strain was added in 0.1%. The crude extracts were added to yield 1, 2, 3, 4, and 5%. Control was prepared without any crude. After incubation period, the count of indicator pathogen used was determined. Comparing the treated with crude extracts to control, then the suppression percentages were calculated according to the following equation (Al-Ajlani and Hasnain, 2006):

$$\text{Suppression \%} = \frac{\text{C control} - \text{C treatment}}{\text{C control}} \text{ x } 100$$

Where: (C) refers to bacterial count as cfu ml^{-1}.

Minimal inhibitory concentrations (Parveez *et al.*, 2007)

The MICs were detected by applying eight concentrations in mg per one ml for each crude extract obtained either by ethanol 70% or ethyl acetate. All crude extracts were prepared as stock solutions of 50 mg/ml. All selection agents were filter sterilized and stored at 4°C. Diagnostic sensitivity test (D.S.T.) agar medium was autoclaved and cooled to 50°C in a water bath prior to the addition of the indicator bacteria (*Escherichia coli*, and *Staphylococcus aureus* ATCC 6538). All crude extracts were added to the concentrations of 25, 50, 75, 100, 200, 300, and 500, 700 and 900 mg l^{-1}. Three replicates (plates) were used for each treatment as well as the control. The means of replicates were used in the final calculations.

Cytotoxicity assay (Dvorak *et al.*, 1999)

This assay was applied to detect the cytotoxicity of ethanolic crude extracts against brine shrimp; *Artemia salina* used successfully in the aquaculture feeding of fish and shrimps larvae and other invertebrates. Several ratios (1, 3, 5, 10, 20, and 50 %) of crude extracts were added into *Artemia salina* culture and then count was followed daily for four days. All the counts obtained were compared to control (without any crude extracts). Using the values on died individuals in given concentrations determines the percent of mortality according the following formula:

$$\mathbf{Mm_{ct} = N_{Mm} \cdot 100/N_0}$$

Where:

$\boldsymbol{Mm_{ct}}$ is mortality of individuals in time t (%), N_{Mm} is average number of dead individuals, $\boldsymbol{N_0}$ is initial number of living individuals put into every concentration at the test start. The EC_{50} values were assessed using non-linear regression, where mortality is related to decimal logarithm of concentration.

Individual EC values were determined for each replicate separated and average value should be determined afterwards. EC_{50} values cannot differ more than 30 %.

RESULTS

Screening of antibacterial activity of Red Sea soft coral

Ten species of soft corals were tested for the antimicrobial activities. These species were *Lopophytom pauciliforum, Dendronephthya hemprichi, Sarcophyton gracile, Sarcophyton galucum, Sarcophyton spongosium, nephthyea pacifica, Sarcophyton acutum, Sinularia gardinieri, Xenia macrospiculata. Sinularia leptoclados.*

In general, the crude extracted from the ten soft coral species showed positive records against at least two bacterial pathogens. The results in (Table 1) confirmed that the absolute activity units (AU) of the ethanolic crude extract ranged from 1.4 to 25.0 for *Sarcophyton acutum* and *Lopophytum pauciliforum*, respectively.

Table 1: Screening the antibacterial activity of ethanolic crude extracts of soft corals species using well cut - diffusion technique

Soft coral species	Antibacterial activity (AU*)						
	S.a.	*E.c.*	*S.f.*	*P.a.*	*B.c.*	*V.d.*	*V.f.*
L. pauciliforum	5.9	25.0	1.7	4.0	3.8	-	1.6
Dendronephthea hemprichi	-	-	-	-	-	-	-
S. gracile	-	-	-	-	-	-	-
S. glaucum	1.7	18.4	4.0	8.2	1.5	1.8	2.7
S. gardineiri	-	-	-	-	-	-	-
N. pacifica	-	-	-	-	-	-	-
S. acutum	-	7.6	-	-	-	-	-
S. leptoclados	-	-	-	-	-	-	-
S. spongosium	-	-	7.1	2.3	-	1.4	-
X. macrospiculata	2.3	6.1	2.8	1.7	1.7	1.9	2.2

S.a.= *Staphelococcus auerus* **ATCC 6538, E.c.=** *Escherichia coli*,**E.f.=** *Enterococcus faecalis,* **P.a.=** *Pseudomonas aeruginosa* **ATCC 8739,, B.c.=** *Bacillus cereus* **1318, V.d.=** *Vibrio damse l* **and V.f**.*= Vibrio fulvilali*s.

The highest activity (25.0 and 18.4) were recorded against *E. coli*. This was occured by *L. pauciliforum* and *S. glaucum.* These values followed by 8.2 and 5.9 for *S. glaucum* against *P. aueroginosa* and for *L. pauciliforum* against *S. auerus*, respectively. On the other side, ethyl acetate crude extracts showed the highest AU (16.0) for *L. pauciliforum* against *S. aeurus,* followed by (11.1) of *N. pacifica* and *X. macrospiculata* against *S. aeurus* (Table 2).

Table 2: Screening the antibacterial activity of ethyl acetate crude extracts of soft corals species using well cut - diffusion technique

Soft coral species	Antibacterial activity (AU*)						
	S.a.	*E.c.*	*E.f.*	*P.a.*	*B.c.*	*V.d.*	*V.f.*
L. pauciliforum	16.0	9.9	9.9	8.2	2.6	1.4	8.2
Dendronephthea	6.3	6.6	2.2	6.6	-	-	5.9
S. gracile	-	1.5	-	1.3	-	-	-
S. glaucum	1.7	1.9	1.5	1.6	1.8	1.2	1.4
S. gardineiri	-	4.0	-	3.4	-	-	-
N. pacifica	2.9	5.2	1.9	11.1	1.7	1.2	1.8
S. acutum	-	6.1	-	2.3	-	-	-
S. leptoclados	2.1	-	-	-	-	-	2.3
S. spongosium	-	2.2	-	7.1	-	-	-
X. macrospiculata	4.0	7.1	3.3	11.1	2.3	1.6	4.0

S.a.= S. auerus ATCC 6538, *E.c.= Escherichia coli, S.f.= Enterococcus faecalis, P.a.= **Pseudomonas aeruginosa*** ATCC 8739,, *B.c.= Bacillus cereus* ATCC 1318, *V.d.= Vibrio damsela* and *V.f.= Vibrio fulvilalis*.

However, the extract of *L. pauciliforum* was the most effective in the inhibition of all tested bacterial pathogens. On contrary, the extracts of *S. gracile, S. acutum* and *S. spongosium* were the lowest effective against these indicators. From these results, it was noted that the crude extracts of *L. pauciliforum* had a broad spectrum antimicrobial effect against all indicator microorganisms. In addition, the crude extracts of *S. glaucum*, *N. pacifica* and *X. macrospiculata* had considerable effect in the inhibition of indicator bacteria tested here. Moreover, the highest results of antibacterial activities are shown as inhibition zones in several macrogarphs (Fig.1). To confirm the efficacy of the most effective crude extracts of soft corals (*L. pauciliforum, S. glaucum, N. pacifica* and *X. macrospiculata*), the suppression percentages in bacterial count (cfu/ml) for the most affected bacterial pathogens were demonstrated using different concentrations (1-5%). The most suppression

percentages against *E. coli* were recorded for *L. pauciliforum* as of 74% and 70% for ethanol crude and ethyl acetate crude extract. Generally, the suppression percentages ranged from 31-74% for all ratios examined. The highest suppression percentages against *P. aeruginosa* were recorded as 52% for *N. pacifica* and as 48% for *X. macrospiculata* for ethyl acetate crude extract, respectively. Generally, the suppression percentages ranged from 31- 52% for all ratios examined. The most suppression percentages against *P. aeruginosa* were recorded as of 38% for *N. pacifica* and as of 34% for *X. macrospiculata* for ethanolic crude extract. Generally, the suppression percentages ranged from 0 - 38% for all ratios examined. However, all of these pathogens were strongly affected in the ascending direction, i.e. the more the concentration of crude extract, the higher the suppression percentage of the bacterial pathogen. These results revealed the efficacy of the soft coral extracts.

Minimal inhibitory concentrations

The minimum inhibitory concentrations (MICs) are represented in Table (3) and Macrographs in Fig. (2). The MIC of *L. pauciliforum* ethanolic crude extract was recorded as 300 mg ml^{-1} against both *E. coli* and *S. areaus* ATCC 6358. Whereas, the MIC of *L. pauciliforum* ethyl acetate crude extract was recorded as 50 mg ml^{-1} against both *E. coli* and *S. areaus* ATCC 6358. The MIC of *S. galucum* ethanolic crude extract was recorded as 500 mg ml^{-1} against *E. coli* and (300 mg ml^{-1}) *S. areaus* ATCC 6358. Whereas, the MIC of *S. galucum* ethyl acetat crude extract was recorded as (300 mg ml^{-1}) against both *E. coli* and *S. areaus* ATCC 6358. The MIC of *N. pacifica* ethanolic crude extract was recorded as (700 mg ml^{-1}) against *E. coli* and (300 mg ml^{-1}) *S. areaus* ATCC 6358. Whereas, the MIC of *N. pacifica* ethyl acetat crude extract was recorded as (50 mg ml^{-1}) against both *E. coli* and *S. areaus* ATCC 6358. The MIC of *X. macrospiculata* ethanolic crude extract was recorded as (300 mg ml^{-1}) against both *E. coli* and *S. areaus* ATCC 6358. Whereas, the MIC of *X. macrospiculata* ethyl acetat crude extract was recorded as (500 mg ml^{-1}) against *E. coli* and (100 mg ml^{-1}) *S. areaus* ATCC 6358.

Table 3: Minimal inhibitory concentrations (MICs) of ethanolic and ethyl acetate crude extacts from selected soft coral against *E. coli* and *S. aureus*

Species	Concentration of crude extract (mg ml^{-1})								
	25	50	75	100	300	500	700	900	1000
L. pauciliforum[a]	nil^{E} nil^{S}	nil^{E} nil^{S}	nil^{E} nil^{S}	nil^{E} nil^{S}	$\underline{7}^{E}$ $\underline{7}^{S}$	12^{E} 11^{S}	15^{E} 14^{S}	18^{E} 18^{S}	26^{E} 24^{S}
L. pauciliforum[b]	nil^{E} nil^{S}	$\underline{7}^{E}$ $\underline{7}^{S}$	7^{E} 7^{S}	10^{E} 8^{S}	12^{E} 10^{S}	16^{E} 14^{S}	20^{E} 18^{S}	25^{E} 23^{S}	28^{E} 26^{S}
S. glaucum[a]	nil^{E} nil^{S}	nil^{E} nil^{S}	nil^{E} nil^{S}	nil^{E} nil^{S}	nil^{E} $\underline{7}^{S}$	$\underline{7}^{E}$ 10^{S}	11^{E} 17^{S}	16^{E} 20^{S}	18^{E} 21^{S}
S. glaucum[b]	nil^{E} nil^{S}	nil^{E} nil^{S}	nil^{E} nil^{S}	nil^{E} nil^{S}	$\underline{7}^{E}$ $\underline{7}^{S}$	12^{E} 9^{S}	15^{E} 14^{S}	18^{E} 18^{S}	20^{E} 22^{S}
N. pacifica[a]	nil^{E} nil^{S}	nil^{E} nil^{S}	nil^{E} nil^{S}	nil^{E} nil^{S}	nil^{E} $\underline{7}^{S}$	nil^{E} 11^{S}	$\underline{7}^{E}$ 16^{S}	13^{E} 20^{S}	16^{E} 24^{S}
N. pacifica[b]	nil^{E} nil^{S}	$\underline{7}^{E}$ $\underline{7}^{S}$	8^{E} 9^{S}	10^{E} 9^{S}	12^{E} 13^{S}	15^{E} 15^{S}	18^{E} 18^{S}	20^{E} 22^{S}	23^{E} 27^{S}
X. macrospiculata[a]	nil^{E} nil^{S}	nil^{E} nil^{S}	nil^{E} nil^{S}	nil^{E} nil^{S}	$\underline{7}^{E}$ $\underline{7}^{S}$	10^{E} 9^{S}	12^{E} 13^{S}	14^{E} 15^{S}	15^{E} 15^{S}
X. macrospiculata[b]	nil^{E} nil^{S}	nil^{E} nil^{S}	nil^{E} nil^{S}	nil^{E} $\underline{7}^{S}$	nil^{E} 11^{S}	$\underline{7}^{E}$ 14^{S}	10^{E} 16^{S}	12^{E} 20^{S}	14^{E} 25^{S}

symbole: a= ethanol crude extract (70%), b= ethyl acetate crude extract, E= *E. coli and S= S. aureus.* All numbers expressed in millimeters (mm) for inhibition zone around each disc. Each disc was saturated with 25µl. Numbers underlined expressed for MIC for each crude extract.

Cytotoxicity assay

In order to determine the cytotoxicty effect of bioactive compounds present in selected soft corals to be recommended in application, the EC50 value of ethanolic crude extracts was investigated (Table 4). Both concentrations 1 and 3% of ethanolic crude extracts did not show any mortality at EC50 for four days; the incubation period of toxicity experiment. Whereas, the concentration 5% showed mortality (EC50) after the fourth day ranged between 50-55% for extracts of *L. pauciliforum*, *S. glaucum*, *N. pacifica* and *X. macrospiculata*, respectively. The concentration 10% showed mortality (EC50) after the second day for extract of *X. macrospiculata* only, and after the third day ranged between 50-65% for extracts of *L. pauciliforum*, *S. glaucum*, *N. pacifica* and *X. macrospiculata*, respectively. Moreover, such concentration showed mortality at EC50 after the fourth day ranged between 65-75% for extracts of *L. pauciliforum*, *S. glaucum*, *N. pacifica* and *X. macrospiculata*, respectively. The concentration 20% showed mortality (EC50) after the second day ranged between 50-58% for extracts of *L. pauciliforum*, *S. glaucum*, *N. pacifica* and *X. macrospiculata*, respectively, and ranged between 63-75% for the same extracts after the third day. Moreover, such concentration showed mortality at EC50 after the fourth day ranged between 78-88% for the same extracts. The concentration 50% showed mortality (EC50) after the first day with 65% for extracts of *L. pauciliforum*, *S. glaucum*, *N. pacifica* and with 75% for extract of *X. macrospiculat*. Moreover, such concentration showed mortality (EC50) increased continueously after the second day until it reached into 95% for extracts of *L. pauciliforum* and *S. glaucum*, and reached into 100% for extracts of *N. pacifica* and *X. macrospiculata*. However, our data summarized that the more the crude extract ratio, the more the mortality of *Artimia salina* larvae.

Table 4: Cytotoxicity (EC_{50}) of the athanolic crude extraxts of *L. pauciliforum, S. glaucum, N. pacifica* and *X. macrospiculata* to *Artemia salina* (Mm_{ct} is mortality of individuals in time t (%) and N_{Mm} is average number of died individuals).

conc. (%)	Species	Incubation period (day)							
		1		2		3		4	
		N_{Mm}	Mm_{ct}	N_{Mm}	Mm_{ct}	N_{Mm}	Mm_{ct}	N_{Mm}	Mm_{ct}
1	*L. pauciliforum*	0	0	1	3	4	10	10	25
	S. glaucum	0	0	3	8	8	20	11	28
	N. pacifica	0	0	1	3	6	15	11	28
	X. macrospiculata	1	3	4	10	8	20	13	33
3	*L. pauciliforum*	2	5	5	13	10	25	13	33
	S. glaucum	2	5	4	10	7	18	14	35
	N. Pacifica	3	8	5	13	9	23	13	33
	X. macrospiculata	4	10	7	18	10	25	15	38
5	*L. pauciliforum*	5	13	10	25	15	38	20	50
	S. glaucum	6	15	10	25	14	35	19	48
	N. pacifica	6	15	12	30	16	40	21	53
	X. macrospiculata	7	18	13	33	18	45	22	55
10	*L. pauciliforum*	9	23	14	35	20	50	26	65
	S. glaucum	12	30	18	45	22	55	29	73
	N. pacifica	11	28	18	45	21	53	29	73
	X. macrospiculata	14	35	20	50	26	65	30	75
20	*L. pauciliforum*	15	38	19	48	25	63	31	78
	S. glaucum	15	38	20	50	27	68	32	80
	N. pacifica	16	40	22	55	29	73	33	83
	X. macrospiculata	18	45	23	58	30	75	35	88
50	*L. pauciliforum*	26	65	31	78	35	88	37	93
	S. glaucum	26	65	33	83	35	88	38	95
	N. pacifica	25	63	32	80	36	90	40	100
	X. macrospiculata	28	70	33	83	37	93	40	100
control		0	0	0	0	2	5	5	12.5

DISCUSSION

The overall objective of the current study was to screen the ability of organic extracts of the soft corals collected from the Egyptian Red Sea coast to inhibit the growth of certain reference bacteria. Nowadays, research about marine natural products to control diseases appeared as an impact of pathogenic bacteria. Porifera (sponges) and Chordata (including ascidians) have dominated as the major contributing phyla of novel bioactive compounds (Blunt *et al*., 2007). The secretions from soft corals exhibit a defense mechanism (Shnit-Orland and Kushmaro, 2008). Bowden *et al*. (1984) discovered that Gorgonian soft coral; *Lobophytum crasscopiculatum* tend to yield compound; identified as cembranolides which have antimicrobial activities.

Badria *et al*. (1997) investigated bioactivity-guided fractionation of an alcohol extract of the soft coral *Sarcophyton* sp. collected from coral reefs near Hurghada, Red Sea, Egypt afforded a new lactone cembrane diterpene, sarcophytolide 1. The structure was deduced based on its spectroscopic data and from comparison with the spectral data of known closely related cembrane-type compounds. This compound exhibits good antimicrobial activity towards *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Saccharomyces cerevisiae* activity. The bioactivity-guided fractionation of an alcohol extract of the soft coral *Sarcophyton glaucum* near Hurghada, Red Sea, Egypt resulted in the isolation of a new lactone cembrane diterpene, sarcophytolide (Badria *et al.,* 1998). In antimicrobial assays, the isolated compound exhibited a good activity towards *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Saccharomyces cerevisiae*. Sarcophytolide was found to display a strong cytoprotective effect against glutamate-induced neurotoxicity in primary cortical cells from rat embryos. Preincubation of the neurons with 1 or 10 μg/ml of sarcophytolide resulted in a significant increase of the percentage of viable cells from 33±4% (treatment of the cells with glutamate only) to 44±4 and 92±6%, respectively. Administration of sarcophytolide during the post-incubation period following glutamate treatment did not prevent neuronal cell death. Pretreatment of the cells with sarcophytolide for 30 min significantly suppressed the glutamate-caused increase in the intracellular Ca^{2+} level ($[Ca^{2+}]_i$). Evidence is presented that the neuroprotective effect of sarcophytolide against glutamate may be partially due to an increased expression of the proto-oncogene *bcl*-2.

The coral secondary metabolite, sarcophytolide, might be of interest as a potential drug for treatment of neurodegenerative disorders.

Kelman *et al*. (2006) examined the antibacterial activity of five soft corals and found that only *Dendronephthya hemprichi* was active against the test bacteria. However, *Dendronephthya hemprichi* differs from the other five soft corals by the lack of symbiotic relationship with the *dinoflagellate zooxanthellae*. It therefore will be interesting to investigate the role of symbiotic *zooxanthellae*, as well as associated bacteria, in the production of natural products, especially metabolites that target co-occurring and potentially harmful microorganisms. Moreover, Kelman *et al*. (2006) explained that extracts with a low natural (whole-tissue) concentration exhibit high activity in laboratory assays indicates the presence of a highly potent compound. The corals that were used varied in their extract concentration and were not consistent with their antimicrobial activity. For example, *Sarcophyton glaucum* had a high mean extract concentration of 212.2 mg cm^{-3} but showed a much lower activity than *X. macrospiculata* that had a lower mean extract concentration of 41.9 mg cm^{-3}. These results indicated that the extracts of these corals differ in their chemical composition

as well as the potency of the active metabolites. Ali and Soliman (2009) studied the antifouling activity of crude extracts of 5 common Red Sea soft corals. The extracts were mixed with a marine paint, applied to PVC panels immersed in the seawater of Suez Bay (Red Sea). Extracts of *Sinularia heterospiculata* and *Sinularia variabilis* showed the highest and potent wide spectrum antifouling activity, particularly in the first 17 days of fouling formation. Extracts of *Sinularia polydactyla* exhibited significant selective inhibition against settlement of barnacle, while the extracts of *Lithophyton arboreum* showed significant antifouling activity against the latter successional stages of tube worms.

The results of the current study propose that these soft corals may contain bioactive compounds with antifouling activity. They stated that these bioactive molecules can be isolated, purified, identified and chemically synthesized for commercial uses in the development of nontoxic and environmentally acceptable antifouling coatings. Cheng *et al*. (2009) have extracted three metabolites from the Formosan soft coral *Nephthea erecta* and one metabolite from *Nephthea chabroli*. *In vitro*, they examined their antimicrobial activities. Metabolite (1) exhibited antibacterial activity against *Enterobacter aerogenes* (ATCC13048), *Serratia marcescens* (ATCC25419), *Salmonella enteritidis* (ATCC13076), *Yersinia enterocolitica* (ATCC23715), and *Shigella sonnei* (ATCC11060). Analysis of *H. fuscescens* has led to the isolation of 6-hydroxy -α- muurolene (1), gorgosten-5(*E*)-3 β-ol (2), 1-nonadecyloxy-2,3-propanediol (3) and (2*S*,3*R*,4*E*,8*E*)-*N*-hexadecanoyl-2-amino 4,8-octadecadiene-1,3-diol (4) and sarcoaldosterol A (5) (Mohammed *et al.*, 2011). The isolated compounds were reported from several marine organisms and are identified for the first time from the soft coral *H. fuscescens* collected from the Red Sea.

The minimal inhibitory concentrations (MICs) using disc - diffusion technique were determined against the most affected bacterial pathogens (*E. coli* and *S. aureus*), applying seven concentrations (50, 100, 200, 300, 500, 700 and 1000 mg ml^{-1}) for each crude extract obtained either by ethanol 70% or ethyl acetate. The level of activity that is measured in the well-cut diffusion assay depends on both the rate of diffusion of the extract into the agar and the potency of the extract. Extracts that contain highly active compounds (i.e., more potent), but have physical properties that generate a lower diffusion rate, may appear to have low activity in the assay. This problem can be overcome by performing MIC assays in liquid media, as was shown for the cat (Kelman *et al.*, 2001). The concentration of a potent compound in the crude extract is also a major factor in the activity score that is observed in laboratory assays. The usage of antibiotic disc susceptibility tests or disc-diffusion assays has the ability to rapidly identify active metabolites and therefore is particularly useful in the initial screening for antimicrobial activity and as the means for following activity during chemical purification (Jenkins *et al*., 1998). Kelman *et al.*, (2001) found that the MIC of the coral crude extract against the *Vibrio* sp. strain *P- l* was 1.25 mg ml^{-1}, while the mean natural extract concentration of *Parerythropodium fulvum fulvum* was 28 mg ml^{-1}. This result explains the high antimicrobial activity observed in the disc-diffusion method. Both concentrations 1 and 3% of ethanolic crude extracts of the present study did not show mortality at EC50 during the incubation period of toxicity experiment. Whereas, the concentration 5% showed mortality (EC50) after the fourth day for extracts of *L. pauciliforum*, *S. glaucum*, *N. pacifica* and *X. macrospiculata*, respectively. However, our toxicity data confirmed that the more the crude extract ratio, the more the mortality of A*rtemia salina* larvae. Parallel to the results of Said, (2005) who detected cytotoxicity of cembranolide extracted from soft corals;

Lobophytum crassum and *L. rotundum* against brine shrimp *Artemia salina* larvae. Duh *et al.*, (2004) discovered eight cytotoxic steroids and sesquiterpenoids (coded from 1-8). Compounds 1, 4, and 5 exhibited cytotoxicity against P-388 cells with ED50 values of 9.45, 8.93, and 16.3 μM, respectively. Compounds 4 and 5 exhibited cytotoxicity against HT-29 cells with ED50 values of 9.03 and 10.5 μM, respectively.

CONCLUSIONS

This work introduces some important data that can be summarized in the following points:

1. The Soft coral species obtained in this study proved evidence that soft corals are valuable as a source of new bioactive molecules.
2. The compound(s) produced by soft corals possess an antimicrobial activity against a number of Gram-positive and Gram-negative bacteria.
3. The inhibitory effect of these compound(s) against human pathogens such as *S. aureus* ATTC 6538 and *P. aeruginosa* ATTC 8739 suggests promising applications in the clinical field.
4. The inhibitory effect of these compound(s) against fish pathogens such as *V. damsela* and *V. fulvialis* suggests promising applications in the aquaculture field.

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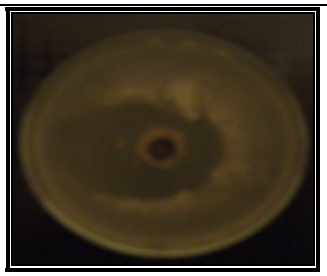 **Anti - *E. coli* of *L. pauciliforum* ethanolic crude extract**	 **Anti - *E. coli* of *S. glaucum* ethanolic crude extract**
 **Anti - *E. coli* of *Nephthea pacifica* ethyl acetate crude extract**	 **Anti - *E. coli* of *L. pauciliforum* ethyl acetate crude extract**
 **Anti - *S. fecalis* of *L. pauciliforum* ethyl acetate crude extract**	 **Anti - *V. fluvialis* of *L. pauciliforum* ethyl acetate crude extract**
 **Anti - *S. auerus* ATCC 6538 of *L. pauciliforum* ethyl acetate crude extract**	 **Anti – *P. aureginosa* of *X. macrospiculata* ethyl acetate crude extract**

Fig. 1: Macrographs showing the most records of antibacterial activities of tested crude extracts.

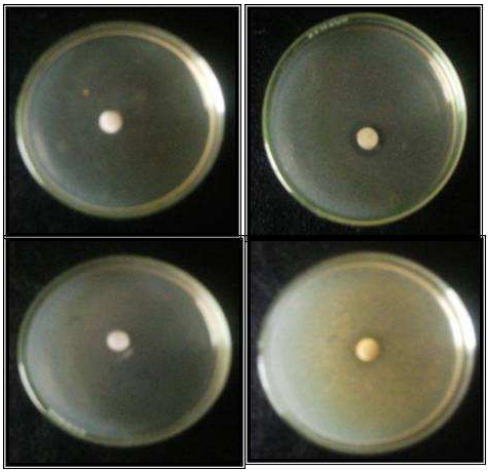

Fig. 2: Macrographs showing the inhibition zone MIC of crude extracts against *E. coli* (upper) and its control and against *S. aureaus* ATCC 6358 (lower) and its control

النشاط الضد بكتيرى لبعض أنواع المرجانيات اللينة بالبحر الأحمر

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كان الهدف الرئيسى من هذه الدراسة هو دراسة مدى قدرة المواد العضوية المستخلصة من المرجانيات اللينة على تثبيط نمو بعض أنواع البكتريا الممرضة. تم جمع المرجانيات اللينة من المنطقة المواجهة للمعهد القومى لعلوم البحار والمصايد بالغردقة و تعريفها.

ثم تم استخلاص بعض المواد من 10 أنواع من المرجانيات اللينة هى:

Lopophytum pauciliforum ،Sarcophyton Dendronephthea sp. ،Sarcophyton glaucum، gardineiri Sinularia ،Nephthea pacifica ،Sarcophyton acutum ،Lopophytum acutum Xenia ٌ Sarcophyton macrospiculata

ثم تم اختبار فعالية المواد المستخلصة من المرجانيات اللينة ضد بعض البكتيريا التى تصيب كل من الأسماك والانسان وهى:-

Staphylococcus aureus ATCC 6538, *Pseudomonas aeruginosa* ATCC 8739, *Vibrio damsela, Vibrio fulvilalis, Bacillus cereus, Bacillus* cereus 1318, *Enterococcus faecalis, Escherichia coli Fusarium oxysporum.*

أظهرت النتائج أن وحدة النشاط المطلقة Absolute activity unit (AU) للمستخلص الناتج من الكحول الإيثيلى تراوحت بين 1.4 و 25 لكل من *Sarcophyton acutum* و *Lopophytum pauciliforum* على التوالى ضد بكتريا *S.aureus* ثم *N. pacifica* and *X. macrospiculata* ضد *S.aureus*. بصفة عامة أظهرت كل من المواد المستخلصة باستخدام الكحول الايثيلى والايثيل اسيتات قدرة ضعيفة على تثبيط الفطريات.

أظهرت المواد المتسخلصة من بعض أنواع المرجانيات اللينة قدرة واضحة على تثبيط بعض البكاتريا الممرضة مما يدل على امكانية استخدامها فى الأغراض الطبية والعلاجية المختلفة.