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Antibacterial and Cytotoxic Activities of Four Red Sea Soft Corals from Gulf of Aqaba-Jordan

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Abstract: About 40% of worldwide identified soft corals are native to the Red Sea. They represent a source of compounds with potential pharmaceutical applications. Soft coral samples collected from Jordanian coast of Gulf of Aqaba were identified morphologically and anatomically and found to be *Sinularia polydactyla*, *S. grayi*, *S. copressa* and *Sarcophyton* sp. They were extracted with ethyl acetate and the resulting crude extracts were partially purified on lsilica gel column producing fractions with antimicrobial and/or cytotoxic activities. Gram positive *Bacillus subtilis* was the only inhibited test bacterial strain and Jurkat was the most affected cell line. Crude extract of *S. compressa* contains the most potent antibacterial compounds with MIC (8-62.5 μg mL^{-1}) and antitumor metabolites with IC_{50} (10-30 μg mL^{-1}). These activities are due to presence of compounds with different polarities.

Key words: Soft coral, antimicrobial, cytotoxicity, Red Sea, Gulf of Aqaba, Jordan

INTRODUCTION

Marine organisms are considered, as a potential source for biologically active natural products. Large numbers of these metabolites are novel that are largely unknown from terrestrial sources and have incredible diversity in chemical structures (Nofiani *et al*., 2012). It is estimated that 20 out of >14000 marine secondary metabolites are in clinical trials (Hill, 2006). The bioactivity profiles of these marine metabolites include neurotoxic, anti-inflammatory, antiviral, antitumor, antimicrobial or cytotoxic properties and are of considerable biotechnological interest (Higa *et al*., 2001; Faulkner, 2002; Kumar and Lakshmi, 2006; Tsai *et al*., 2013).

Soft corals are one of the dominant phyla that contribute in production of novel bioactive compounds (Blunt *et al*., 2014). They have as other sessile marine invertebrates, primitive immune systems and evolved mechanisms to synthesize chemicals to help defend against predation (Harvell *et al*., 1988), competitors (Coll, 1992) and infections with microbes and fouling organisms (Pawlik and Fenical, 1992; Kim, 1994; Koh *et al*., 2002; Changyun *et al*., 2008). Most of the produced compounds are structurally unique, biologically active and fall into the terpene class of compounds and in some cases, highly functionalized steroids (Groweiss and Kashman, 1978; Carmely and Kashman, 1981; Coll *et al*., 1985; Gerhart and Coll, 1993). The 40% of worldwide identified soft corals are native to the Red Sea (Hegazy *et al*., 2012). They were chemically (Ne'eman *et al*., 1974; Kashman *et al*., 1980, 1981, 1982) and biologically (El Sayed and Hamann, 1996; Radwan *et al*., 2002; Temraz *et al*., 2006) studied with emphasis on species belonging to Sarcophyton and Sinularia genera. However, recently natural products scientists are interested in isolating metabolites as potential drug leads from Red Sea soft corals.

In a screening of Red Sea soft corals isolated from Gulf of Aqaba, their extracts exhibited antimicrobial and cytotoxic activities. Preliminary purification by column chromatography yielded several bioactive fractions. Herein, the researcher report isolation, identification, bioactivity-guided fractionation and bioactivity of extracts obtained from 3 *Sinularia* species and a *Sarcophyton* sp. soft corals.

MATERIALS AND METHODS

Collection and of soft coral samples: Soft corals were collected by SCUBA diving in the Red Sea/Gulf of Aqaba during April, 2008. They were collected from reef slope in front of the Marine Science Station (MSS) (Fig. 1) at depths ranging from 5-15 m. A part from each colony was preserved in 70% ethanol for identification and a part was air dried in shadow and deep-frozen in a plastic bag at -20°C till the time of extraction.

Identification of soft corals was based on examining their anatomical, as well as morphological features. The appearance of the surface lobes and lobules, their length, arrangement of polyps on them and the distances between polyp pits were measured using a dissecting

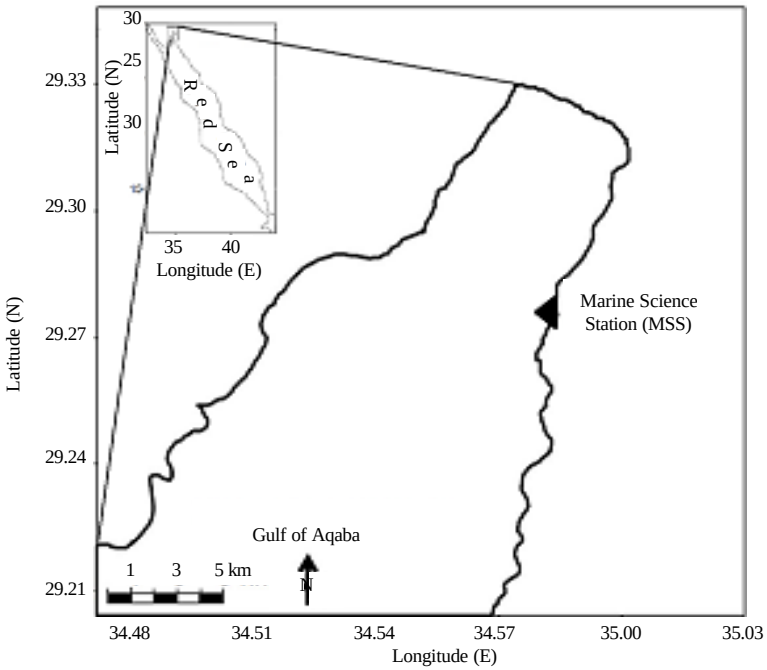

Fig. 1: Location of marine science station on the Jordanian coast of Gulf of Aqaba

light microscope (Nikon/Japan). Moreover, parts from the surface layer, as well as interior of the colony lobes and stalk had been dissolved in 5-10% sodium hypochlorite to dissimilate the organic tissues in order to obtain loose sclerites. These sclerites were then observed under a compound light microscope (Nikon/Japan) to determine their form and measure their size. All data were compared with available literature (Verseveldt, 1974, 1980, 1982; Verseveldt and Benayahu, 1978, 1983) to reach to the genus level and even if possible to the species level.

Extraction and partial purification of bioactive crude extracts from soft coral: Dried soft coral samples (200-300 g) were ground with a blender and the powder was exhaustively extracted with ethyl acetate. The solvent was dried over sodium sulfate (anhydrous) and concentrated *in vacou* at 45°C. The resulting crude extract was dissolved in methanol to a final concentration of 100 mg mL^{-1} and stored at 4°C.

Crude extracts that showed antibacterial activity against *Bacillus subtilis* ATCC 6633 were subjected to partial purification through silica gel (Merck 60, 0.063~0.2 μm), as a stationary phase and elution with increasing polarity of the mobile phase (i.e., starting from 100% cyclohexane through cyclohexane-ethyl acetate mixture, ethyl acetate-methanol mixture till 100% methanol) under atmospheric pressure.

***In vitro* bioactivity assays:** The antibacterial activity of was determined by measuring the caused inhibition zones in Agar diffusion test and deducing the Minimum Inhibitory Concentration (MIC) by serial dilution assay according to the National Committee for Clinical Laboratory Standards (NCCLS, 2004). The test microorganisms used in this study were *Staphylococcus aureus* ATCC 43300, *Escherichia coli* ATCC 25922, *Klebsilla pneumoniae* ATCC 13883 and *Bacillus subtilis* ATCC 6633, seeded on Luria-Bertani (LB) agar plates (0.5% tryptone, 0.5% yeast extract, 1% NaCl, 1.8% agar). Aliquots corresponding to 300 μg of the concentrated extract were used to evaluate the antibacterial activities in the agar diffusion assay. While Minimum Inhibitory Concentration (MIC) was determined using serial dilutions of test samples and positive control (Chloramphenicol) starting from a final concentration of 500 and 100 μg mL^{-1}, respectively.

Cytotoxic activity was assayed as described previously (Al-Zereini *et al*., 2007). Jurkat (human acute T cell leukaemia) and Colo-320 (human colorectal adenocarcinoma) cells were grown in RPMI1640 medium supplemented with 10% Fetal Calf Serum (FCS), 65 mg mL^{-1} of penicillin G and 100 mg mL^{-1} of streptomycin sulfate. The cells were incubated at 37°C in a humidified atmosphere containing 5.0% CO_2. Cytotoxicity was determined quantitatively

by staining with a tetrazolium salt-XTT (Sodium 3, 3′-(1-[(phenylamino) carbonyl]-3, 4-tetrazolium)-bis (4-methoxy-6-nitro) benzene sulphuric acid hydrate).

Statistical analysis: Means and standard deviations were deduced using Excel software.

RESULTS AND DISCUSSION

The Jordanian Red Sea coast (Gulf of Aqaba) is dominated with alcyonacean soft corals. Therefore, current study was initiated to screen the potential use of crude extracts isolated from 9 soft coral samples as source of biologically active compounds. These collected samples were found to belong, based on anatomical and morphological characteristics to 2 *Alcyonacean* genera; namely, *Sinularia polydactyla* (Ehrenberg in 1834), *Sinularia grayi* (Tixier-Durivault in 1945) *Sinularia compressa* (Tixier-Durivault in 1945) and *Sarcophyton* sp. The extract yields were 2.1, 2.2, 1.3 and 15% for *S. polydactyla*, *S. grayi*, *S. compressa* and *Sarcophyton* sp., respectively. The bioactivity of their crude extracts against tested microorganisms in agar diffusion test is summarized in Table 1 and their cytotoxicity against tumor cell lines is listed in Table 2.

The crude extracts obtained from tested soft coral showed activity just against the gram positive *Bacillius subtilis*. The inhibition zones caused by 300 μg disc^{-1} ranged between 10-11 mm for *S. polydactyla* and *S. grayi* and 18 mm for *S. compressa* while extract from *Sarcophyton* sp. caused 14 mm inhibition zone. Therefore, the order of antibacterial potency for soft coral extract is *S. polydactyla*≤*S. grayi*<*Sarcophyton* sp.<*S. compressa*.

Table 1: Bioactivity of soft coral crude extracts against tested microorganisms in Agar diffusion test

	Test microorganism; inhibition zone (mm±SD) 300 μg disc^{-1}				
Isolates	*N. coryli*	*K. pneumoniae*	*E. coli*	*S. aureus*	*B. subtilis*
Sinularia polydactyla	-	-	-	-	9.7±0.58
Sinularia grayi	-	-	-	-	11±1.00
Sinularia compressa	-	-	-	-	18.3±1.5
Sarcophyton sp.	-	-	-	-	14.3±0.58

Table 2: Cytotoxic activities of the soft coral crude extracts

	Jurkat (μg mL^{-1})		Colo-320 (μg mL^{-1})	
Soft coral	IC_{50}^{a}	IC_{90}^{b}	IC_{50}	IC_{90}
Sinularia polydactyla	>100	>100	50-100	>100
Sinularia grayi	30-50	50	100	>100
Sinularia compressa	10-30	30	30-50	100
Sarcophyton sp.	30-50	50	50-100	>100

[a, b]Inhibition in proliferation of 50 and 90% of cells

In addition, soft corals possessed antitumor compounds with Jurkat being most affected cell line. Crude extract of *S. compressa* was more effective in inhibiting the proliferation of both Jurkat and Colo-320 cells with IC_{90} (inhibition in growth of 90% of tumor cells) of 30 and 100 μg mL^{-1}, respectively. While *S. poldactyla*, *S. grayi* and *Sarcophyton* sp., showed moderate to weak cytotoxic effect against both cell lines.

Bioactivity guided fractionation of soft corals crude extracts resulted in obtaining fractions that have either antimicrobial or antitumor activities (Fig. 2). These bioactivities are summarized in Table 3-5.

The 4 bioactive fractions were obtained from crude extract of *Sinularia polydactyla*, fractions A and B contains semi-polar antibacterial compounds, fraction C with moderately polar antibacterial and cytotoxic compounds and D fraction exhibited antitumor activity. Crude extract of *Sinularia grayi* produced 5 bioactive fractions; fractions A and B have cytotoxic nonpolar compounds, fraction C with semipolar antibacterial and cytotoxic substances and fractions D and E with moderately polar to polar compounds with antibacterial and antitumor properties. While *Sinularia compressa* offered 7 fractions; fraction A has nonpolar antitumor compounds and semi-polar to polar fractions (B-G) with antibacterial and antitumor substances.

Moreover, extract of *Sarcophyton* sp., give rise to 2 nonpolar antitumor fractions (A and B), 3 semipolar to moderately antibacterial and antitumot fractions (C-E) and 2 moderately polar fractions (F and G) exhibiting antibacterial and cytotoxic activities.

During this study, extracts of collected soft corals exhibited antibacterial and/or cytotoxic activities. As detected in antibacterial test, nonpolar to moderately polar fractions were more potent inhibitors against growth of the gram positive bacterium *B. subtilis*. Among the Sinularia soft corals, *Sinularia grayi* fractions exhibited weak activities with MIC value between 125-250 μg mL^{-1}, nonpolar fractions of *Sinularia polydactyla* were moderately active with MIC 16-250 μg mL^{-1} and *Sinularia compressa* has the most potent active fractions with MIC 8-62.5 μg mL^{-1}. While active fractions of *Sarcophyton* sp., have MIC of 125-250 μg mL^{-1}.

The potency of nonpolar to moderately polar fractions to inhibit gram positive bacteria over gram negative bacteria indicates that the outer membrane could not be considered, as a barrier for the diffusion of such compounds but these substances may have other targets to inhibit and the degree of interference caused by these fractions with their targets determine their bioactivity. Therefore, more studies are needed and more chromatographic techniques are required to be applied in

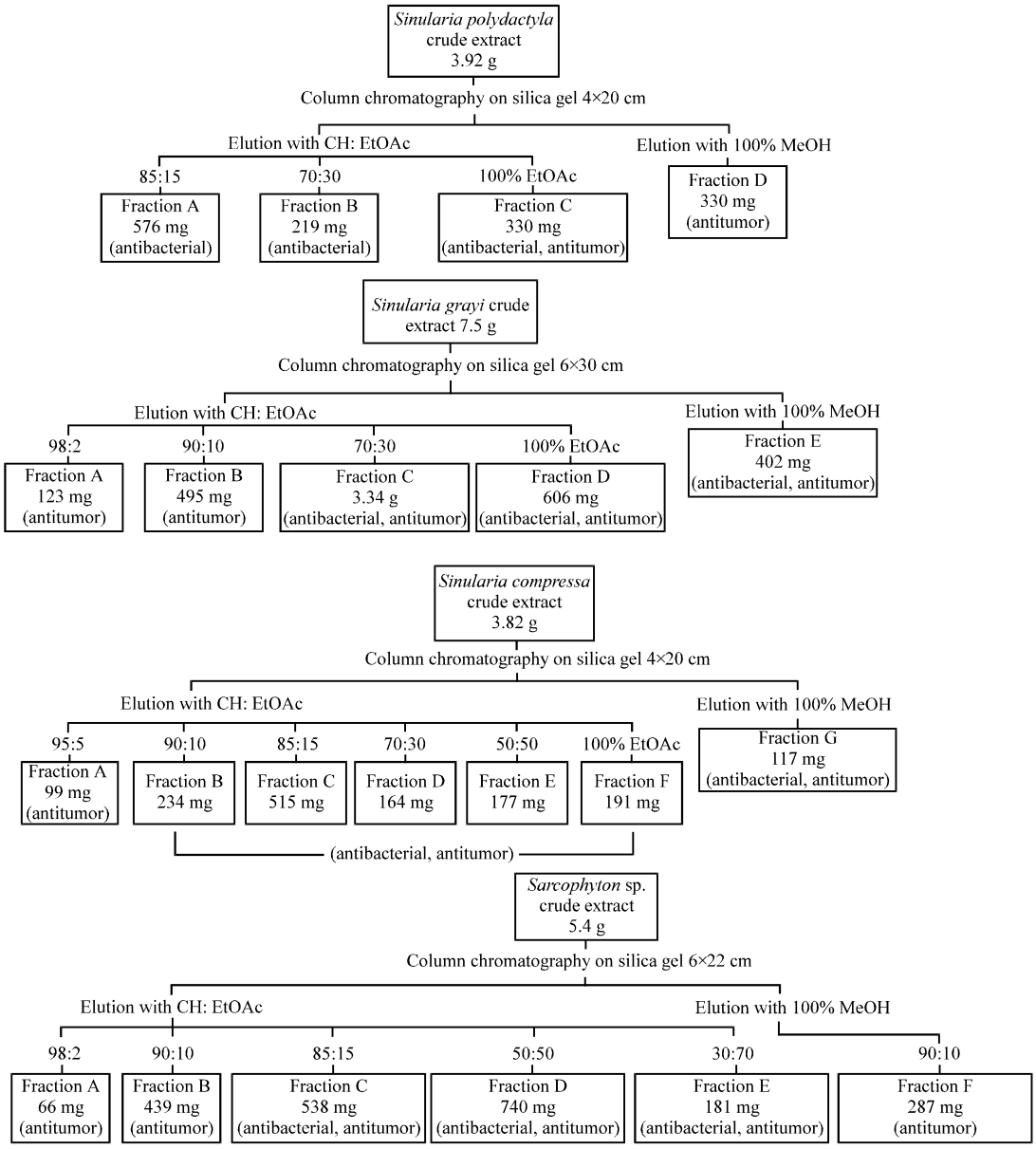

Fig. 2: Scheme of partial purification of crude extract of the collected soft corals in silica gel chromatography. Scheme shows only elution of bioactive fractions

Table 3: Bioactivity of partially purified soft coral crude extracts against *B. subtilis* in agar diffusion test

	Soft coral; inhibition zone (mm) 200 μg disc^{-1}			
Fractions	*Sinularia polydactyla*	*Sinularia grayi*	*Sinularia compressa*	*Sarcophyton* sp.
A	20	-	-	-
B	18	-	28	-
C	10	11	24	10
D	-	11	22	15
E		10	15	10
F			15	-
G			13	

a future research to obtain pure compounds and elucidate their structures. Thus, structural-activity relationship could be achieved.

In addition, regardless of fraction polarity, soft corals fractions were more potent cytotoxic to tumor cell lines than antibacterial. They caused inhibition in the growth of tested cell lines at concentrations ≤50 μg mL^{-1}. Suspension cell line Jurkat was more affected than monolayer Colo-320 cell line with IC_{50} of 10-30 μg mL^{-1} for most fractions compared to IC_{50} 50-100 μg mL^{-1}, respectively.

Table 4: Minimum Inhibitory Concentration (MIC) of the active fractions from soft coral crude extracts against *B. subtilis* in serial dilution assay

	MIC (μg mL^{-1})			
Fractions	*Sinularia polydactyla*	*Sinularia grayi*	*Sinularia compressa*	*Sarcophyton* sp.
A	16s[a]	>500	>500	>500
B	16s	>500	8s	>500
C	250s	125s	16c[b]	250s
D	>500	125s	16s	125s
E		250s	62.5s	250s
F			62.5c	>500
G			62.5s	

[a]s = Biostatic; [b]c = Biocidal

Table 5: Cytotoxic activities of the partially purified fractions from soft coral crude extracts

		Jurkat (μg mL^{-1})		Colo-320 (μg mL^{-1})	
Soft coral	Fractions	IC^{a}_{50}	IC^{b}_{90}	IC_{50}	IC_{90}
Sinularia polydactyla	A	>100	>100	>100	>100
	B	>100	>100	>100	>100
	C	>100	>100	10-30	50
	D	>100	>100	50-100	100
Sinularia grayi	A	50-100	>100	>100	>100
	B	<10	10-30	>100	>100
	C	10	10-30	50	>100
	D	10	10-30	100	>100
	E	30-50	50	100	>100
Sinularia compressa	A	50	>100	>100	>100
	B	10-30	50	30-50	100
	C	10	30	50-100	>100
	D	10-30	50	100	>100
	E	10	30	30-50	100
	F	10	30	10	30
	G	10-30	50	30	50
Sarcophyton sp.	A	50	100	>100	>100
	B	100	>100	>100	>100
	C	10-30	50	100	>100
	D	10-30	50	100	>100
	E	10	30	30	>100
	F	10	30	30	>100

[a, b]Inhibition in proliferation of 50 and 90% of cells

Soft corals are known to be producers of vast range of different terpenoid compounds and their derivatives. Nearly 50% of the soft coral species in the world are known to produce bioactive metabolites (Coll *et al*., 1982; Sammarco *et al*., 1987). Alcyonacean soft corals have been reported to synthesize secondary metabolites with antimicrobial activity, as a mechanism of chemical defense to combat microbial attack (Slattery *et al*., 1995; Kelman *et al*., 1998). Nevertheless, antimicrobial chemical defense does not mean presence of antimicrobial activity in laboratory assays.

It was found that the crude extracts of the tropical soft corals *Sinularia polydactyla* and *Sinularia* sp., exhibited antifeeding activities on fish predators (Van Alstyne *et al*., 1994). Carbocyclic cembranoids from an unidentified soft coral *Sarcophyton* species had strong ichthyotoxic activity against the killifish (mosquito fish) *Oryzia latipes* (Changyun *et al*., 2008) and polyoxygenated steroids from *Sarcophyton* sp., exhibited different levels of antimicrobial activity against bacterial species *E. coli* and *Bacillus megaterium* and fungal species *Microbotryum violaceum* and *Septoria tritici* (Wang *et al*., 2013).

Soft corals are not only producer of antimicrobial metabolites but also they produce compounds with anti-inflammatory and antituomor activities. Diterpenoids-based derivatives were reported to displayed anti-inflammatory activity by inhibiting the expression of the iNOS protein and reducing the expression of the COX-2 protein in the macrophages (Su and Wen, 2011). Moreover, they exhibited cytotoxicity against cancer cell lines (Tai *et al*., 2011; Tsai *et al*., 2013).

CONCLUSION

The results agreed with the literature on activities of soft coral terpenes and terpene-derivatives against several human pathogens and tumor cells. In a study on soft corals from Red Sea, 83% of collected samples exhibited antimicrobial activity due to the production of extracts with a range of compounds of different polarities (Kelman *et al*., 2009). Sinularia and Sarcophyton species are among a large number of soft corals collected from the Red Sea coast of hurghada with extracts of interesting bioactivities not only as antibacterial or cytotoxic but also as anti-inflammatory and antipyretic agents (Badria *et al*., 1997, 1998; Hegazy *et al*., 2012).

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