Antiproliferative effects of selected marine organisms collected from Red Sea

Ahmed Abdel-lateff[1,2*], Walied M Alarif[3], Sultan S Al-Lihaibi[3] and Ashraf B Abdel-Naim[4]

[1]Department of Natural Products and Alternative Medicine, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia
[2]Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia, Egypt
[3]Department of Marine Chemistry, Faculty of Marine Sciences, King Abdulaziz University, Jeddah, Saudi Arabia
[4]Department of Pharmacology and Toxicology, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia

Abstract: Ten selected marine organisms representing different classes of marine fauna and flora were collected from Saudi Arabia territorial water. They were *Antipathes dichotoma*, *Rumphella* sp., *Dictoyota dichotoma*, *Hyrtios erectus, Petrosia* sp., *Heteroxenia fuscescens*, *Rumphella aggregata*, *Sinularia polydactyla*, *Sarcophyton glaucum*, *Sarcophyton trocheliophorum.* Samples were lyophilized and extracted. Their cytotoxic activity was assessed by determining their IC_{50}'s against HepG2, A549 and PC-3 cancer cell lines. The extracts showed variable activities against A549 with IC_{50} in the range 388.3-0.1µg/mL; HepG2 with IC_{50} in range 382.5-0.1µg/mL and PC-3 with IC_{50} in the range 428.6-0.1µg/mL. *Dictoyota dichotoma*, *Hyrtios erectus*, *Rumphella aggregata* and *Sarcophyton glaucum* exhibited the highest antiproliferative activity. Therefore, their impact on cell cycle was examined by flow cytometry technique. It was concluded that they cause G0/G1, S-phase and G2/M cell cycle arrest.

Keywords: Marine invertebrates, sponge, cytotoxicity.

INTRODUCTION

Marine ecosystems are often characterized by harsh atmosphere which led to the production of diverse bioactive metabolites (Aoki *et al.*, 2004; Thomas *et al.*, 2010). Thus, marine organisms are numerated as a wealthy source for bioactive natural compounds, which consequently, are considered a lead for new drugs (Hickford *et al.*, 2009; Arzumanyan *et al.*, 2013).

Local **c**ancer incidence as reported by the Saudi Cancer Registry in 2007 is more than 12,000 cases. The highest occurrence was associated with breast cancer (15.0%). Further, the most common cancers among Saudis included liver (4.8%), prostate (6.1% in men) and lung cancer (4.0%) (Saudi Cancer Registry, 2014). A computer survey indicated that three types of cancer in Saudi Arabia should be given the priority of national research plans; namely hepatocellular carcinoma, prostate cancer and lung cancer (Al-Abdin *et al.*, 2013). Therefore, the present study was designed to evaluate the potential cytotoxic effects of selected Saudi marine extracts against selected tumor cell lines namely; HepG2 (Human hepatocellular carcinoma), A549 (Human lung adenocarcinoma) and PC-3 (Human prostate cancer). Extracts with highest antiproliferative activities were also tested for their impact on cell cycle phases (G0/G1, G2/M and S).

MATERIALS AND METHODS

Marine samples

All samples (*Antipathes dichotoma*, *Rumphella* sp., *Dictoyota dichotoma*, *Hyrtios erectus, Petrosia* sp., *Heteroxenia fuscescens*, *Rumphella aggregata*, *Sinularia polydactyla*, *Sarcophyton glaucum* and *Sarcophyton trocheliophorum*) were collected from the Saudi territorial water (21°29′31″N 39°11′24″E) by SCUBA divers (Depth range 1-15 m, during 2013) and identified by Prof. Manfred Grasshoff (Senckenberg Museum, Frankfurt, Germany) and Dr. Mohsen el-Sherbiny (Marine Biology department, Faculty of Marine Sciences, KAU). A voucher specimen from each marine organism was deposited in the Department of Chemistry, Faculty of Marine Sciences, King Abdulaziz University, Jeddah, Saudi Arabia (fig. 1).

Extraction

Freeze-dried marine macro-organisms (40g) were extracted three times with 200mL of a mixture of CH_2Cl_2-MeOH (1:1, v/v) for 24 hours at 22°. The obtained viscous extracts were concentrated under reduced pressure using Rotavapor® R-210, dried by nitrogen and weighted (table 1).

Cytotoxicity and cell cycle assays

Three cell lines HepG2 (Human hepatocellular carcinoma cells), A549 (Human lung adenocarcinoma cells) and PC-3 (Human prostate cancer cells) were obtained from the Holding Company for Biological Products & Vaccines (VACSERA), Giza, Egypt. They were cultured and maintained according to the supplier instructions. Cytotoxicity and cell cycle analysis were performed in our laboratory as previously described (Alarif *et al.* 2013).

**Corresponding author:* e-mail: ahmedabdellateff@gmail.com, aabdellateff@kau.edu.sa

Table 1: Description of the marine samples and the yield of the organic extracts

No	Marine organism	Classes	Family	Weight (mg)	Description
1	*Antipathes dichotoma*	Black coral	Antipathidae	100	It has black skeleton. Distinct features vary greatly within this genus, containing symmetrically aligned as well as irregularly shaped corals, a range of different colors and colonies that can be either sparsely branched or closely packed.
2	*Rumphella sp.*	Soft Coral	Gorgoniidae	80	Colonies grey, light yellow or light brown. Surface layer of coenenchyme with wart-clubs. Interior with spindles. Zooxanthellae present.
3	*Dictoyota dichotoma*	Algae	Dictyotaceae	70	Flat thallus and regular dichotomous branches with parallel sides to 30cm long, the tips usually bifid. Outer layer of small cells enclosing a single layer of large cells no more than one cell thick even near the base. Branches 3 to 12mm wide, membranous without a mid-rib.
4	*Hyrtios erectus*	Sponge	Thorectidae	100	It forms pinkish-gray tubes. Surface of the sponge is densely conulose. The skeleton is dense, anisotropic, and consisting of sand-filled primary and secondary fibers, near the surface forming fascicles. The surface aspect and skeletal characters conform to the genus Hyrtios.
5	*Petrosia sp.*	Sponge	Petrosiidae	60	Large, compact structure without defined shape and visible oscules
6	*Heteroxenia fuscescens*	Soft coral	Xeniidae	70	Feathery pinnate tentacles forming a clump up to 60 cm (24 in) across. Its polyp stalks are 2.0 in long, pulsating rhythmically around 40times/ minute.
7	*Rumphella aggregate*	Soft coral	Plexauridae	50	Colony of dirty white to grey in colour when alive and brown when dried. Size of the colony is up to one meter and bushy in structure. Colonies are forming compact large shrubs with whip - like branches. The branches are smooth, thick and have blend tips. The polyps are yellowish brown in colour, monomorphic and retractile in the smooth
8	*Sinularia polydactyla*	Soft coral	*Alcyoniidae*	110	The colonies resemble a set of fingers, which held upright. This minute identification process will often involve a close look at sclerites, which are minuscule calcareous particles that live embedded on corals' tissue. This species may be found in the lower areas of a reef, especially where turbidity is higher.
9	*Sarcophyton trocheliophorum*	Soft coral	Alcyoniidae	70	It is called elephant ear coral or Green toadstool coral. It is one of the other leather coral in the Sarcophyton genus. It has a thick smooth, single stalk with a flared, smooth mushroom-shaped top that can be folded or funnel-shaped. The flesh is firm and soft, yet can be easily torn. The polyps can retract all the way, giving them a smooth look.
10	*Sarcophyton glaucum*	Soft coral	Alcyoniidae	110	It has a thick smooth, single stalk with a flared, smooth mushroom-shaped top that can be folded or funnel-shaped. The flesh is firm and soft, yet can be easily torn. The "top" is called a capitulum and within that area are found long autozooid polyps for feeding and many also have siphonozooid polyps for water movement.

Table 2: Cytotoxicity of the extracts of marine macro-organisms against three cancer cell lines

Extract			IC_{50} µg/mL*		
			HepG2	A549	PC-3
1	B	*Antipathes dichotoma*	382.5±19.00	142.2±10.03	66.6±4.01
2	G	*Rumphella sp.*	32.5±1.50	388.3±22.02	18±1.012
3	ID	*Dictoyota dichotoma*	5.9±0.18	35.6±2.05	19.2±1.01
4	IE	*Hyrtios erectus*	0.1±0.012	0.1±0.030	0.1±0.012
5	IP	*Petrosia sp.*	19.8±1.01	18.1±1.04	18.5±1.01
6	IH	*Heteroxenia fuscescens*	17.6±1.085	100.1±7.07	10.2±1.01
7	IR	*Rumphella aggregate*	5.5±0.35	28.3±1.09	13.2±0.812
8	ISS	*Sinularia polydactyla*	1.5±0.045	7.1±0.062	5.8±0.014
9	ST	*Sarcophyton trocheliophorum*	108.2±8.01	168.8±9.07	428.6±21.03
10	SE	*Sarcophyton glaucum*	1.7±0.010	7.453±0.052	6.7±0.02
DOX†		Doxorubicin	0.5±0.018	0.2±0.032	0.5±0.012

HepG2 (Human hepatocellular carcinoma cells), A549 (Human lung adenocarcinoma cells), PC-3 (Human prostate cancer cells). Data are presented as mean ± SEM, N=3. *IC_{50}, (µg/mL): 1-10 (very potent); 11-25 (potent); 26-50 (moderate); 51-100 (weak); more than 100 (weak and considered non-cytotoxic). †Doxorubicin was used as positive control.

Table 3: FACSCAN results of the marine samples

Extract		Cell cycle phase			
		Go/G1 *	S	G2/M	Pre-G
Control	C	52.55±1.30	33.40±1.90	11.35±1.10	2.50±0.20
Dictoyota dichotoma	ID	55. 20±0.091	9.10*±0.081	5.60*±0.031	28.40*±0.111
Hyrtios erectus	IE	68.0*±0.081	7.9*±0.091	8.50*±0.061	13.80*±0.091
Rumphella aggregate	IR	70.9*±0.041	6.80*±0.141	3.90*±0.131	16.50*±0.051
Sarcophyton glaucum	SE	51.60 ±0.031	12.5*±0.081	7.10*±0.081	24.40*±0.061
Doxorubicin	DOX	62.20*±0.011	7.10*±0.121	25.90*±0.021	4.70*±0.031

Data are presented as mean ± SEM, N = 3* Significantly different from corresponding control value at $p < 0.05$

RESULTS

Ten different marine extracts, representing different classes, were assessed for their anti-proliferative effects against three solid tumor cell lines, A549, HepG2 and PC-3, with reference to a standard cytotoxic drug (doxorubicin; DOX) by employing SRB colorimetric assay. The data in table 2 indicate that the tested extracts showed considerable anti-proliferative activity against the three cell lines. Extracts of black coral (*Antipathes dichotoma*), Gorgonian (*Rumphella aggregata*), brown alga (*Dictoyota dichotoma*), sponges *Hyrtios erectus* and *Petrosia* sp., Octocorallia corals *Heteroxenia fuscescens*, *Rumphella aggregata, Sinularia polydactyla, Sarcophyton glaucum, Sarcophyton trocheliophorum* and doxorubicin (DOX) showed significant activities against HepG2 (IC_{50} 382.5; 32.5;5.9; 0.1; 19.8 ; 17.60; 5.5; 1.5; 108.2; 1.7; 0.5µg/mL, respectively), A549 (IC_{50}142.2; 388.3; 35.6;0.1;18.1; 100.1; 28.3; 7.1; 7.5; 168.8; 0.2µg/mL, respectively) and PC-3 (IC_{50} 66.7, 18,1; 19.2; 0.1; 18.5;10.3; 13.2; 5.8; 428.6; 6.7; 0.5 µg/mL, respectively). Based on these data, it was obvious that *Dictoyota dichotoma* (ID), *Hyrtios erectus* (IE), *Rumphella aggregata* (IR) and *Sarcophyton glaucum* (SE) exhibited the highest potency against HepG2 cells. Therefore, their cytotoxic activities were further substantiated by assessing their effects on cell cycle phases using DNA flow cytometric technique with reference to DOX.

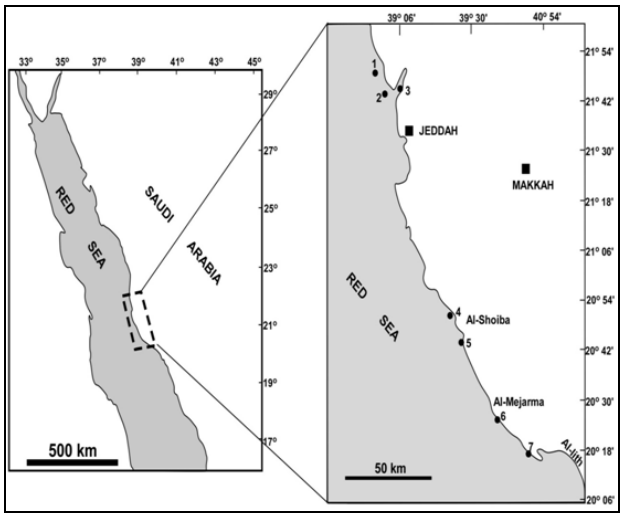
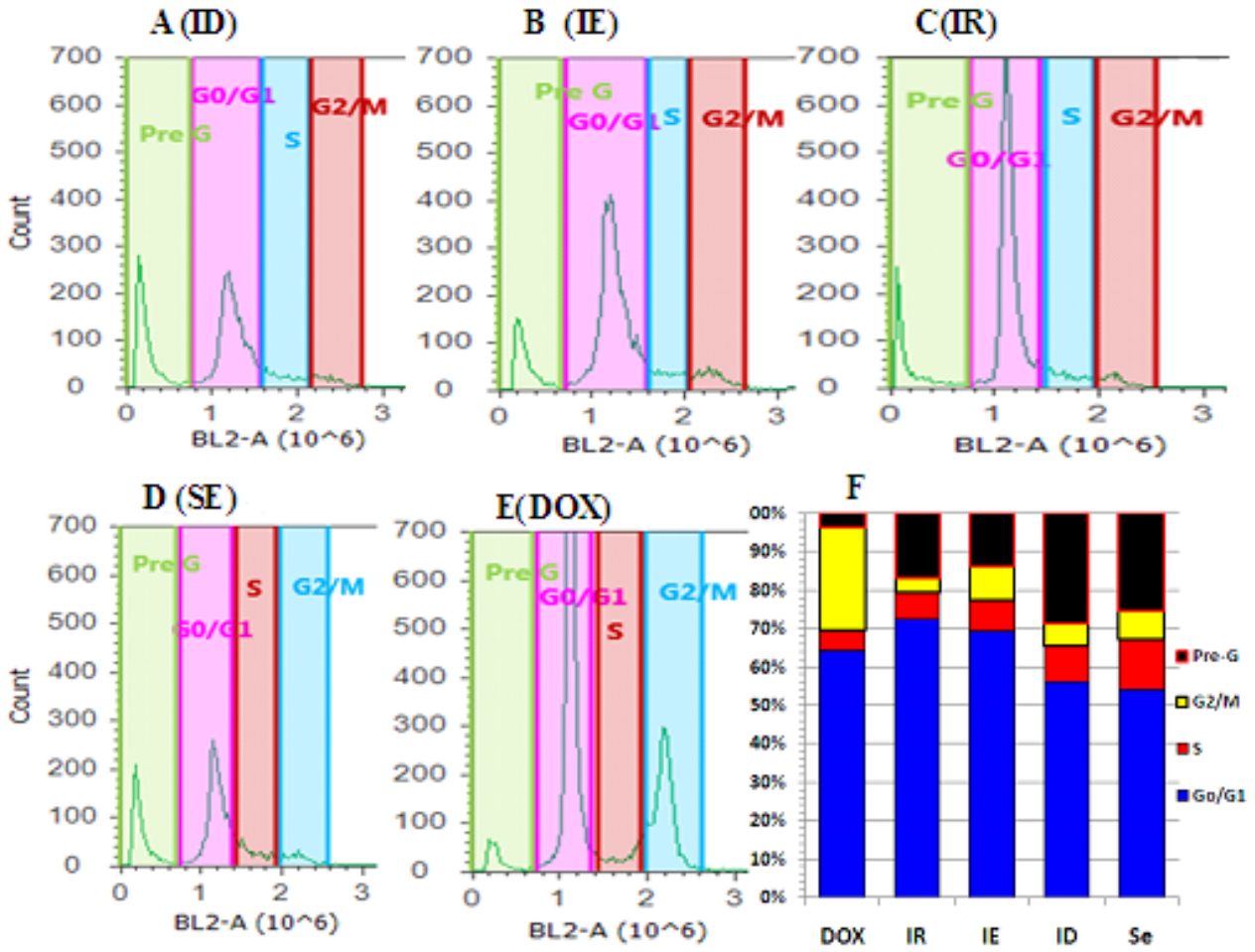


Fig. 1: Location of collection of the studies marine samples

Table 3 and fig. 2 indicate that extracts of ID, IE, IR and SE enhanced accumulation of cell population in S-phase in a range 5.06-12.5%. These values were comparable to that of DOX (7.1%). Further, they increased the non-



HepG2 cancer cells were exposed to *Dictoyota dichotoma* (ID, A), *Hyrtios erectus* (IE, B), *Rumphella aggregata* (IR, C) and *Sarcophyton glaucum* (SE, D) for 24 h and compared to Doxorubicin (DOX, E) and Cell cycle distribution was determined using DNA cytometry analysis and different cell phases were plotted (F) as percent of total events (n=3).

Fig. 2: FACSCAN of the marine organisms extracts

proliferating cell fraction (G_0/G_1-phase) in range of 51.6-70.9%. It is noteworthy to mention that these values were also comparable to that exhibited by DOX (62.2%). The extracts decreased the G2/M in range 3.9-7.1% while DOX showed an increase by 25.9. Finally, all extracts significantly enhanced the non-proliferative phase Pre-G phase which is representative of an apoptotic effect in a range of 13.8-28.4%, compared to a value of 4.7% exhibited by the standard reference compound (DOX).

DISCUSSION

Soft corals belong to the genus *Sarcophyton* (Family Alcyoniidae) are forty-six species with common name toadstool coral. They are rich source of macrocyclic cembrane-type diterpenoids. More than 300 cembranoids were identified (Duffy *et al.*, 2012). Cembranes are large family of diterpenes with different characteristic functional groups (Wang *et al.*, 2011; Duffy *et al.*, 2012; Xi *et al.*, 2013). Cytotoxicity is a notable property of this class of diterpenoids. Thus, these active constituents can give support to the observed cytotoxicity in the current study. However, our data indicate a pro-apoptotic activity as well. The typical basic chemical feature of cembranoids is 14-membered carbon skeleton. However, unconventional derivatives contain a 12-, 13-membered carbon nucleus (Sauleau *et al.*, 2006).

Members of the genus *Hyrtios* (Demospongiae class, Dictyoceratida order, Thorectidae family) (Hooper *et al.*, 2002) are rich source of biologically active metabolites. These include sesterterpenes (Pettit *et al.*, 2005) sesquiterpenes (Salmoun *et al.*, 2000), macrolides, indole and β-carboline alkaloids (Sauleau *et al.*, 2006). Indole containing metabolites have diversity of biological activities including; anticancer activity (Sugiyama *et al.*, 2009) This might help explain the observed activity against the different tumor cell lines in our study. For instance; heteronemin is a sesterterpene isolated from *Hyrtios sp.* It showed significant effects against

myelogenous leukemic cells. DNA micro array profiling was used to determine its molecular mechanisms in TNF alpha-treated cells. It was shown to modulate cell cycle, apoptosis, mitogen-activated protein kinases (MAPKs) pathway and the nuclear factor kappa B (NF-kappaB) signaling cascade (Schumacher *et al.*, 2010).

The Gorgonian corals of the genus *Rumphella* were the subject of several chemical studies, which have focused on the lipids and steroid components (Rod'kina *et al.*, 2005). In addition to these studies, the ecological and medical effects had been investigated (Imbs *et al.*, 2009) which led to discovering of nor-sesquiterpenoids of caryophyllane skeleton (Berge *et al.*, 2005; Puglisi *et al.*, 2002; Sung *et al.*, 2007; Chuang *et al.*, 2007). There are several studies indicated that brown algae of the genus *Dictyota elaborate* secondary metabolites displaying diversity of biological activities including cytotoxicity (Amico *et al.*, 1980). The diterpene amijiol acetate is the main metabolite of *Dictyota dichotoma* and responsible for its effect on cell cycle of the different cancer cells.

In conclusion, extracts of *Dictoyota dichotoma, Hyrtios erectus, Rumphella aggregate* and *Sarcophyton glaucum* exhibit potent anti-proliferative activity against HepG2 cells. These effects can be attributed, at least partly, to their pro-apoptotic activity as evidenced by their enhancing accumulation of cell population in the Pre-G phase. The current results warrant further investigations.

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