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Chemical Assessment and Anticancer Activity Study of Diverse Marine Organisms From Red Sea Riviera

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Abstract

The chemical investigation and *in vitro* cytotoxic, caspase and apoptotic activities of extracts/n-hexane fractions of eleven diverse marine organisms, collected from the Red Sea at the Egyptian coasts, were studied against the human hepatocarcinoma (HepG2). The organisms were identified as *Thalassia hemprichii* [SP1], *Sargassum arnaudianum* [SP2], *Echinodictyum flabelliforme* [SP3], *Dendronephthya hemprichi* [SP4], *Rumphella* sp. [SP5], *Iotrochota purpurea* [SP٦], un-identified [SP7], *Sarcophyton glaucum* [SP8] and *Sarcophyton* sp. [SP9]), *Ircinia* sp. [SP10] and *Ircinia echinate* [SP11]). Phytochemical profiling by GC-MS analysis of the hexane components obtained from the eleven organisms revealed their unique diversities, reporting altogether 114 divers compounds. According to anticancer profiling the n-hexane fractions of the reported organisms showed diverse potent cytotoxicity against HepG2: the soft coral *Dendronephthya hemprichi* [SP4] showed cytotoxic activity at IC_{50} = 25 and 12.5 µg/mL after 24 and 48 h, respectively, meanwhile the sponge [SP6] showed less cytotoxicity of IC_{50} = 100 and 50 µg/mL after 24 and 48 h, respectively), accompanied with moderate induction of non-apoptotic caspase-activity. The unidentified soft coral [SP7] had time-independent cytotoxicity (IC_{50}= 20 µg/mL) after 24 and 48 h, accompanied with high-induction of caspase–dependent early apoptosis. *Sarcophyton glaucum* [SP8] was potentially cytotoxic (IC_{50}= 12.5 µg/ml after 24) without induction of caspase-activity or apoptosis, meanwhile the soft coral [SP9] showed induction of caspase–independent apoptosis. Finally, the brown alga [SP2] showed high induction of non-apoptotic caspase-activity after 48 h. This recognized the high potentiality of the marine organisms, [SP2], [SP4], [SP7], [SP8] and [SP9] as talented sources of drug leads with significant biological activities, encouraging our future research planning to isolate and structurally identify such corresponding potentially active compounds.

Keywords: Diverse Marine Organisms; Red Sea; GC-MS Analysis; Cytotoxicity; Apoptosis; Hepatocellular Carcinoma

1. Introduction

The marine environment provides a broad range of diverse habitats from which novel sources of natural products can be derived. The Oceans, which are called as the 'mother of origin of life', covering more than 70% of the world's surface, are rich sources of biological and chemical diversity. [1-3] Marine floras include flowering plants (Seagrass, mangroves and other halophytes), macroalgae (seaweeds), [1,4,5] microflora (bacteria, actinobacteria, cyanobacteria and fungi) and microalgae. [1,6-8] Marine invertebrates including Porifera (sponges), Coelenterate (soft corals), Molluscs, Echinoderms, Crustaceans, etc. represent prolific sources of diverse chemical structures and unique biological properties as well due to their exposing to extreme marine environmental conditions. [1,9-14].

Alternatively, Sponges are the most studied marine invertebrates in terms of secondary metabolites. Up till now, more than 5300 different chemically potent and bioactive substances classified into alkaloids, lipids, steroids, terpenoids, poly-acetylenic lipid derivatives, glycerol ethers and linear alcohol, have been discovered from sponges. Many of these compounds have pharmaceutical activities against human diseases like malaria, AIDS, and

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cancer. [15-17] Many out such secondary metabolites are cytotoxic to human gastric tumor cells, KB-16 human cell line, ovarian sarcoma cell line, pancreatic cancer cell line, colorectal adenocarcinoma cell line. [18,19] Other several secondary metabolites were also identified to have anti-inflammatory action in human neutrophils and possess inhibitory activities against α-Glucosidase. [19]. Sponges were also found out to contain natural products having antioxidant, and immunomodulatory activities. [20]

The Red Sea is an important natural source of bioactive compounds. Distinctive features that have intrigued natural products chemists to investigate marine organisms in the Red Sea include its great seasonal fluctuations of air and water temperatures and its great marine biodiversity. [21] For example, of the 180 soft corals species identified world-wide, approximately 40% are native to the Red Sea. [22] This vast marine floral and invertebrate's resources offer a great way for discovery of new drugs which can fight deadly diseases like cancer, acquired immune deficiency syndrome (AIDS), arthritis, etc. [1].

Alternatively, more than 30 000 diseases have been clinically described, however, less than one-third of these can be treated symptomatically and only a few can be cured. [1] Up to date and based on the recent chemical and biological advances (structure identification, synthetic chemistry, and bioassays) novel anticancer agents have been investigated from marine organisms. [23] For example, bryostatin I [24] and didemnin B were considered as promising antitumor drugs. Bryostatin 1 which interferes with protein kinase C is currently in phase II clinical trials, [25] meanwhile, didemnin B, a depsipeptide inhibits the synthesis of RNA, DNA, and proteins in various cancer cell lines [26]. The latter shows anti-viral and immunosuppressive activities and an effective agent in the treatment of leukaemia and melanoma as well. However, it was withdrawn from phase II clinical trials due to its toxicity. [27,28] The increasing threat of cancer has initiated a renewal of interest in research for novel anticancer agents, especially that the cancer disease is responsible for 7.6 million deaths yearly worldwide. [29] Therefore, new therapeutic agents and anticancer/sources are urgently necessary to treat medical needs that are currently unmet**.**

In the present article, the phytochemical profiling by GC-MS analysis and *in vitro* anticancer inhibitory effects of different extracts from eleven marine organisms were studied. The marine organisms are belonging to four categories; Seagrass [Sp1], brown algae [Sp2], four sponges ([SP3], [SP6], [SP10], and [SP11]) and five soft corals ([SP4], [SP5], [SP7], [SP8] and [SP9]), which were obtained from the Red Sea at the Egyptian coasts. The outcomes of these potentials are hereby revealed and discussed.

2. Experimental

2.1. Collection and morphological identification of the marine organisms

The eleven organisms were collected in February 2016 near Mahmieat of the Red Sea about ~1 km off the coast of Hurghada, east Egypt, at a depth between 10 and 18 m (geographical coordinates: Latitude 27° 15' 26 N, Longitude 33° 48' 46 E) using SCUBA method. The collected organisms were stored immediately in a freezer until working up and extraction. Morphologically, the organisms were identified by Dr. Mohamed A Ghani, Red Sea Parks, Hurghada, Egypt. The marine samples were photographed *in situ* using an underwater camera. The identification was made on the bases of microscopic examination of skeletons and siliceous and calcareous spicules. Category and size of the spicule were examined and detected using a binocular microscope with a range of 400X magnification as described by Borges *et al.* [30,31]. Brief morphological description and adequate documentation about locality, depth, and habitat were also used in identification [31,32]. They were accordingly classified into: *Thalassia hemprichii* [Sp1], *Sargassum arnaudianum* [Sp2], *Echinodictyum flabelliforme* [Sp3], *Dendronephthya hemprichi* [Sp4], *Rumphella sp.* [SP5], *Lotrochota purpurea* [SP6], Unidentified soft coral [SP7], *Sarcophyton glaucum* [SP8], *Sarcophyton sp.* [SP9], *Ircinia sp.* [SP10] and *Ircinia echinata* [SP11].

2.2. Extraction and working up

The methodology of extraction of the marine organisms [SP1] (500 g), [SP2] (250 g), [SP3] (205 g), [SP4] (400 g), [SP5] (500 g), [SP6] (500 g), [SP7] (250 g), [SP8] (450 g), [SP9] (200 g), [SP10] (400 g) and [SP11] (280 g)] and working up was carried out as similar as our previously reported work [13,33] delivering the following corresponding weights of extracts (g): 13.14, 4.65, 6.50, 40.68, 42.53, 120, 37.16, 34.16, 11.90, 18.94 and 4.50, respectively.

2.3. n-Hexane Extracts of the marine organisms

100 mg from each sample of the obtained dried marine extracts were individually applied to soaking and washing with n-hexane (3 × 5 mL) followed by decantation. The afforded hexane extracts were then dried over anhydrous sodium sulphate, followed by filtration and concentration *in vacuo*, resulting in the corresponding un-polar hexane fractions (mg): 40.0, 20.0, 41.0, 25.0, 60.0, 22.0, 60.0, 46.0, 1.0, 8.0 and 9.0, respectively.

2.4. GC-MS analysis of the n-hexane extracts

The GC/MS analysis was performed using a Thermo Scientific, Trace GC Ultra / ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30 m, 0.251mm, 0.1 mm film thickness). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used, Helium was used as the carrier gas at a constant flow rate of

1mL/min. The injector and MS transfer line temperature was set at 280 °C. The oven temperature was programmed at an initial temperature 50 °C (hold 2 min) to 150 °C at an increasing rate of 7 °C /min, then to 270 at an increasing rate 5 °C /min (hold 2min) then to 310 as a final temperature at an increasing rate of 3.5 °C /min (hold 10 min). The quantification of all the identified components was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY library data of the GC/MS system. Characterization of individual components by GC-MS was performed in triplicate.

2.5. Anticancer activity

2.5.1. Cell Line propagation and treatment

Human hepatocellular carcinoma cell line (HepG2) was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). Cells were cultured in RPMI-1640 medium (Gibco BRL, Gaithersburg, Md.) supplemented with 10 % fetal bovine serum (Gibco BRL, Gaithersburg, Md.), 1% penicillin/ streptomycin (5 mg/ml each) and 2 % L-glutamine (2 mM) at 37 °C in a humidified atmosphere of 5 % CO_2. Cells at approximately 80% confluence were trypsinized, seeded and incubated at 37ºC and 5 % CO_2 overnight. Treatments were dissolved in RPMI-1640 medium. All non- cytotoxic treatments were tested for caspase activity in a single concentration = 100 µg/mL. The cytotoxic treatments were tested for caspase activity and for apoptosis detection (annexin test) in concentrations equal to the values of the IC_{50}s.

2.5.2. MTT assay

Cytotoxicity against HepG2 cells was assessed by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay according to Hwang et al. [34] Cells were seeded in 96 well microplates (3×10^3 cells/well) in 100 µl RPMI-1640 culture medium and incubated at 37 ºC and 5 % CO_2 overnight. Cells were treated and re-incubated for 24 h, 100 µl MTT (0.5 mg/ml) solution were added to each well and incubated until purple formazan crystals appeared. The medium was discarded and 100 µl of DMSO was added to dissolve the crystals. The optical density (OD) of solubilized formazan was measured at 592 nm using an automatic microplate reader. The absorbance is related to the relative number of viable cells. Results are expressed as percent of untreated control.

2.5.3. Caspase activity

Caspase-Glo® Assay was used to measure caspase 2, 3/7 activity according to manufacturer/ protocol. 50 µl of Caspase-GloR 2. The reagent was added to each well of 96 well plate containing 50 µl of blank, control (treated cells). The plate was gently mixed and incubated at room temperature for 2 hours. Luminescence was recorded with a plate reading Luminometer.

2.5.4. Apoptosis detection

Annexin test was made for the n-hexane fractions that exhibited cytotoxic activity against HepG2 cells after the treatment for 72 h then collected and resuspended at a concentration of 1.5 × 105 cells in 500 µL of 1 X binding Buffer. Five microliters of annexin V-FITC and 5 µL of propidium iodide (PI 50 µg/mL) were added, and then incubated at room temperature for 5 min in the dark. Analysis of annexin V-FITC binding by flow cytometry (Ex = 488 nm; Em = 530 nm) was performed using FITC signal detector and PI staining by the phycoerythrin emission signal detector according to the method described by Lu et al. [35]

2.5.5. Statistical analysis

Statistical analysis was done using SPSS (version 16) one-way ANOVA Tukey analysis, where $P<0.05$ was considered to indicate a statistically significant difference. [36]

3. Results and Discussion

3.1. Phytochemical profiling

Chemical assessment of the nonpolar organic extracts (n-hexane) of the eleven marine organisms was performed using GC-MS analysis (Table 1, structures of the whole identified compounds are shown in supplementary data). Accordingly, from the seagrass *Thalassia hemprichii* [SP1], nine compounds were detected among them seven phthalic acid esters along with 1,1',2,2'-tetrahydro-1,1'-dimethoxy lycopene (**8**) and octylpalmitate. Seagrass represents a part of a critical, albeit fragile, eco-system, which is widely distributed along temperate and tropical coastlines of the world. It is the only marine flowering plant which often lives entirely submerged and completes its life cycle in seawater. The plant has been reported as rich source of secondary metabolites, particularly phenolic compounds which are interesting for their biological activities. [37] So far, there are no previous studies in the literature on the chemical constituents and biological activities of our Seagrass species studied herein.

The brown alga *Sargassum arnaudianum* [SP2] revealed the presence of ten divers compounds: three sterols (**11,16,18**), an alkaloid, 2-morpholinophenazine (**12**), an ingol derivative (**13**), 7,3',4'-trimethoxy-qurcetine (**14**), 1,3-dioxolane derivative (**15**), 3-hydroxy-3-(spiro-cyclopropyl)-chromane-2-carboxylic acid methyl ester (**17**) and bis-(vinyloxiryl)-cyclobutane derivative (**19**). Marine algae are rich sources of potentially bioactive compounds, and seaweeds have traditionally been

used as food and in folk medicine as well, especially by coastal peoples. Recently**,** much attention has been paid to the antitumor activity of seaweeds. [38]

Alternatively, the marine sponges *Echinodictyum flabelliforme* [SP3], *Iotrochota purpurea [*SP6], *Ircinia sp.* [SP10] and *Ircinia echinate* [SP11] revealed the presence of fourteen, seventeen, fifteen and seventeen bioactive metabolites, respectively. The sponge *Echinodictyum flabelliforme* [SP3] is rich with five steroidal analogues (**21, 22, 24-27, 29**), the congener of colchicine, colchifoleine (**28**), the dihydrocardenolide glycoside, corchorside B (**23**), the bufadienolide marinobufagin (**32**) along with spherodenone (**30**), vinyl 2-ethylhexanoate (**20**) and propane-1,2,3-triyl trioctanoate (**31**). On the other hand, *Iotrochota purpurea* [SP6] is abundant with two monoterpenes, 1-limonene (**52**), (-)-carvone (**53**), three phathalates, seven sterols (**57-63**), 7,3',4'-trimethoxy quercetin (**14**), isochiapin B (**56**), and several fatty acid esters. From the sponge *Ircinia* sp. [SP10], several hydrocarbons (**91-92,99,102**) along with three sterols (**98,100,101**), four fatty acids-aldehydes/alcohols (**93-97**) and isochiapin B (**56**) were detected. Finally, the sponge *Ircinia echinate* [SP11] is highly abundant with nine monoterpenes (**64, 103-110**) and several fatty acid esters/phthalates, three sterols (**58, 113, 114**) and 7,3',4'-trimethoxy-quercetin (**14**).

On the other hand, extracts of the five soft corals [SP4], [SP5], [SP7], [SP8] and [SP9] revealed the presence of fourteen, eleven, seventeen, thirteen and seven diverse compounds, respectively. Predominantly, form the soft coral *Dendronephthya hemprichi* [SP4], three fatty acids (**33, 41, 42**), seven sterols (**21, 36-39, 43,44**), quercetin-7,3',4'-trimethoxy (**14**), rhodoxanthin (**40**), oxatricyclo analogue (**35**) and a phthalic acid derivative (**34**) were detected. The soft coral *Rumphella* sp. [SP5] showed the presence of spherodenone (**30**), zeaxanthin (**46**), 9-dodecyltetradecahydro-phenanthrene (**45**), three sterols (**21,47,49**), and lycopene analogue (**8**), meanwhile the unidentified soft coral [SP7] is abundant with four fatty acids, three sterols (**74-76**), one monoterpene (**64**), seven sesquiterpenes (**65-71**) among them the polyoxygenated xanthumin (**69**) and chiapin B (**71**). From *Sarcophyton glaucom* [SP8], one monoterpene (**64**), vitamin A alcohol (**79**), ledene oxide (II) (**67**), three phthyl esters, three fatty acid esters, retinoyl glucuronide analogue (**80**) and two sterols (**74,81**) were reported, while *Sarcophyton* sp. [SP9] is abundant with n-hexadecanoic acid, heptacosane, nonacosane and 11-decyl-docosane (**89**), squalene (**86**), decylpropargyl fumarate (**88**) and the pyrrole-poly aromatic alkaloid lamellarin K (**90**); the latter was isolated by Bowden and coworkers in 1993 from *Didemnum* sp. collected from South West Cay, Australia. [39]

Based on our tentative phytochemical profiling using GC-MS analysis, the reported marine organisms represent as very rich sources of diverse bioactive compounds, and a future talent/ promising resource for drug exploring supporting the scientists in overcoming the newly investigated human diseases and curing them.

Table 1: GC-MS analysis of the n-hexane fractions from the 11 marine organisms

Compound Name	**Rt (min)**	**A (%)**	**MF**	**MWt**
1: Seagrass: *Thalassia hemprichii* [SP1]				
Diisooctyl phthalate (**1**)	43.60	95.97	$C_{24}H_{38}O_4$	390
Bis (3,5,5-trimethylhexyl) phthalate (**2**)	46.25	0.54	$C_{26}H_{42}O_4$	418
1,2-Benzenedicarboxylic acid, dinonyl ester (**3**)	46.50	0.58	$C_{26}H_{42}O_4$	418
1,2-Benzenedicarboxylic acid, diisononyl ester (**4**)	46.61	0.99	$C_{26}H_{42}O_4$	418
1,2-Benzenedicarboxylic acid, bis(7-methyloctyl) ester (**5**)	46.70	0.34	$C_{26}H_{42}O_4$	418
Phthalic acid, nonylpentadecyl ester (**6**)	47.11	0.56	$C_{32}H_{54}O_4$	502
1,4-Benzenedicarboxylic acid, dihexyl ester (**7**)	48.70	0.14	$C_{20}H_{30}O_4$	334
Lycopene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy, all-trans (**8**)	56.34	0.11	$C_{42}H_{64}O_2$	600
Palmitic acid, octadecyl ester (**9**)	58.73	0.12	$C_{34}H_{68}O_2$	508
Total sum.		**99.35**		
2: Brown Alga: *Sargassum arnaudianum* [SP2]				
1,2-Benzenedicarboxylic acid, dioctyl ester (**10**)	43.98	25.5	$C_{24}H_{38}O_4$	390
18-Norcholest-17(20),24-dien-21-oic acid,16-acetoxy-4,8,14-trimethyl-3,11-dioxo, methyl ester (**11**)	49.63	5.27	$C_{32}H_{46}O_6$	520
2-Morpholinophenazine (**12**)	51.70	5.53	$C_{16}H_{15}N_3O$	260
9-Desoxo-9-xacetoxy-3,8,12-tri-O-acetylingol (**13**)	54.18	12.82	$C_{28}H_{40}O_{10}$	536
Qurecetin-7,3',4'- trimethoxy (**14**)	55.50	8.53	$C_{18}H_{16}O_7$	344
1,3-Dioxolane, 2-(1-methylethyl) (**15**)	55.77	5.94	$C_6H_{12}O_2$	116
Androst-4-ene-3,20-dione,11,16,22-triacetoxy- (**16**)	56.23	5.63	$C_{27}H_{36}O_8$	488
Methyl-3,4-dihydro-6-hydroxyspiro[2H(1,4)b-enzoxathiine-3,1'-	57.09	10.67	$C_{12}H_{12}O_4S$	255

cyclopropane]- 2-carboxylate (**17**)				
5à-Pregn-16-en-20-one, 3á,12à-dihydroxy, diacetate (**18**)	63.42	7.88	$C_{25}H_{36}O_5$	416
Cyclobutane,1,3bis[2(2-isopropyl-3,3 dimethyloxiran-2-yl) ethenyl] 2,4diacetyl (**19**)	63.68	9.03	$C_{26}H_{40}O_4$	416
Total sum.		**96.80%**		
3*:* Sponge: *Echinodictyum flabelliforme* [SP3]				
Vinyl 2-ethylhexanoate (**20**)	35.02	8.55	$C_{10}H_{18}O_2$	170
Diisooctyl phthalate (**1**)	43.94	8.40	$C_{24}H_{38}O_4$	390
Ethyl isoallocholate (**21**)	46.92	7.42	$C_{26}H_{44}O_5$	436
Cholestan-3-one, cyclic -1,2 –ethanediylacetal (**22**)	47.86	5.98	$C_{29}H_{50}O_2$	430
Corchorside B (**23**)	49.18	4.60	$C_{29}H_{42}O_8$	398
Cholestanol, 5,6-eposy, acetate (ester) (**24**)	49.87	6.84	$C_{29}H_{48}O_3$	444
Cholest-5-ene-16,22-dione, 3á,26-dihydroxy, 3-acetate(20S,25R) (**25**)	50.15	5.80	$C_{29}H_{44}O_5$	472
Ergosta-5,22-dien3-ol,acetate(3á,22E) (**26**)	50.48	6.70	$C_{30}H_{48}O_2$	440
Ergost-5-en-3-ol,22,23-dimethylacetate (**27**)	50.69	5.78	$C_{32}H_{54}O_2$	470
Colchifoleine (**28**)	52.36	5.05	$C_{21}H_{23}NO_7$	401
Stigmastan-3,5-diene (**29**)	53.04	12.02	$C_{29}H_{48}$	396
Spherodenon (**30**)	54.82	6.78	$C_{41}H_{58}O_2$	582
Propane-1,2,3-triyl trioctanoate (**31**)	55.03	7.88	$C_{27}H_{50}O_6$	470
Marinobufagin (**32**)	58.17	6.72	$C_{24}H_{32}O_5$	400
Total sum.		**98.52%**		
4: Soft coral*: Dendronephthya hemprichi* [SP4]				
Octadecanoic acid, 2-hydroxyethyl ester (**33**)	35.11	11.88	$C_{20}H_{40}O_3$	328
1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (**34**)	43.97	16.68	$C_{24}H_{38}O_4$	390
3-Oxatricyclo [20.8.0.0(7,16)]triaconta(22),7(16),9,13,23,29-hexaene (**35**)	47.86	8.51	$C_{29}H_{42}O$	406
Pregn-9(11)-en-3-one, 18,20-dihydroxy,cyclic 1,2-ethanediylacetal, (5à,20R) (**36**)	49.17	5.77	$C_{23}H_{36}O_4$	376
Ergosta-5,22-dien-3-ol, acetate,(3á,22E) (**37**)	49.33	5.36	$C_{30}H_{48}O_2$	440
Cholest-5-en-3-ol (3á),tetradecanoate (**38**)	49.91	4.68	$C_{41}H_{72}O_2$	596
Quercetin - 7,3',4 '-trimethoxy (**14**)	52.76	9.58	$C_{18}H_{16}O_7$	344
9,19-Cyclolanostan-24-one, 3-acetoxy-25-methoxy (**39**)	53.13	4.81	$C_{33}H_{54}O_4$	514
Ethyl iso-allocholate (**21**)	53.87	4.81	$C_{26}H_{44}O_5$	436
Rhodoxanthin (**40**)	53.99	4.72	$C_{40}H_{50}O_2$	562
Octadecanoic acid, eicosyl ester (**41**)	55.05	6.95	$C_{38}H_{76}O_2$	496
Hexadecanoic acid, 2-hydroxy1,3-propanediyl ester (**42**)	55.88	4.74	$C_{35}H_{68}O_5$	568
25-Nor-9,19-cyclolanostan-24-one, 3-acetoxy-24-phenyl (**43**)	57.83	4.76	$C_{35}H_{50}O_3$	518
17-(1,5-Dimethylhexyl) 10,13-dimethy-l-3-styrylhexadecahydrocyclopenta[a]phenanthren-2-one (**44**)	64.17	4.76	$C_{35}H_{52}O$	488
Total sum.		**98.01%**		
5: Soft coral: *Rumphella* sp. [SP5]				
1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) Ester (**34**)	44.00	36.22	$C_{24}H_{38}O_4$	390
Spherodenon (**30**)	49.27	4.79	$C_{41}H_{58}O_2$	582
9-dodecyltetradecahydro-phenanthrene (**45**)	49.67	2.76	$C_{26}H_{48}$	360,
Zeaxanthin (**46**)	51.76	6.38	$C_{40}H_{56}O_2$	568
5à-Cholestan-7à-yl 4-(à-Hydroxyphenylmethyl)phenylacetate 25,à-ether (**47**)	52.19	5.81	$C_{42}H_{58}O_3$	610
2[(2-'ethylphenyl)(2''-isopropylphenyl) methoxy]ethanol (**48**)	54.21	3.96	$C_{20}H_{26}O_2$	298
Propanoic acid, 2(3-acetoxy4,4,14-trimethylandrost-8-en-17-yl) (**49**)	55.66	5.96	$C_{27}H_{42}O_4$	430
Pentaacetayl D-Xylitol, (**50**)	55.77	3.42	$C_{15}H_{22}O_{10}$	362
5,9,23-Tricontatrienoic acid, methyl ester (**51**)	56.39	14.74	$C_{31}H_{56}O2$	460
Ethyl isoallocholate (**21**)	57.81	6.33	$C_{36}H_{48}O_2$	512
Lycopene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy, all-trans] (**8**)	60.81	7.17	$C_{42}H_{64}O_2$	600
Total sum.		**97.54%**		

6: Sponge: *Iotrochota purpurea* [SP6]				
1-Limonene (**52**)	6.92	3.27	$C_{10}H_{16}$	136
(-)-Carvone (**53**)	14.14	0.79	$C_{10}H_{14}O$	150
11-Octadecenoic acid, methyl ester (**54**)	37.49	1.91	$C_{19}H_{36}O_2$	296
1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (**34**)	46.44	61.80	$C_{24}H_{38}O_4$	390
1,2-Benzenedicarboxylic acid, bis(7-methyloctyl) ester (**5**)	49.85	0.99	$C_{26}H_{42}O_4$	418
1,2-Benzenedicarboxylic acid, dinonyl ester (**3**)	50.90	4.50	$C_{26}H_{42}O_4$	418
Cyclopropanedecanoic acid, à(acetyloxy)2-hexyl, methyl ester (**55**)	51.25	1.09	$C_{22}H_{40}O_4$	368
Isochiapin B (**56**)	51.69	0.70	$C_{19}H_{26}O_6$	350
Quercetin 7,3',4'-trimethoxy (**14**)	52.03	9.58	$C_{18}H_{16}O_7$	344
Ethyl isoallocholate (**21**)	52.73	0.79	$C_{26}H_{44}O_5$	436
Desmosterol (**57**)	56.02	1.03	$C_{27}H_{44}O$	384
Cholest-5-en-3-ol(3á) (**58**)	56.64	2.19	$C_{27}H_{46}O$	386
Dihydrocholesterol (**59**)	56.81	1.55	$C_{27}H_{48}O$	388
26-Homo-25-hydroxycholesterol (**60**)	57.28	2.32	$C_{28}H_{48}O_2$	416
5-Cholestene-3-ol,24-methyl (**61**)	58.37	0.87	$C_{28}H_{48}O$	400
Stigmasterol (**62**)	58.72	0.70	$C_{29}H_{48}O$	412
β-Sitosterol (**63**)	59.63	2.25	$C_{29}H_{50}O$	414
Total sum.		**96.33%**		
7: Soft coral (un-identified) [SP7]				
Cyclohexene,1-methyl-4-(1-methylethenyl) (**64**)	6.94	4.56	$C_{10}H_{16}$	136
Longipinocarveol, trans (**65**)	34.05	2.33	$C_{15}H_{24}O$	220
Bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9-(Prop-1-en-3-ol-2-yl) (**66**)	34.47	2.36	$C_{15}H_{24}O_2$	236
Ledene oxide (II) (**67**)	35.80	2.04	$C_{15}H_{24}O$	220
Menthol, 1'-(Butyn-3-one-1-yl),(1S,2S,5R) (**68**)	37.30	2.23	$C_{14}H_{22}O_2$	222
11-Octadecenoic acid, methyl ester (**54**)	37.50	2.98	$C_{19}H_{36}O_2$	296
Xanthumin (**69**)	37.81	2.54	$C_{17}H_{22}O_5$	306
3-Oxoisocositic acid (**70**)	39.47	2.11	$C_{15}H_{20}O$	248
Chiapin B (**71**)	39.62	1.26	$C_{19}H_{26}O_6$	350
10,12-Tricosadiynoic acid, methyl ester (**72**)	40.02	1.85	$C_{24}H_{40}O_2$	360
1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) Ester (**34**)	46.43	61.22	$C_{24}H_{38}O_4$	390
Myrisitic acid, hexadecyl ester (**73**)	57.33	1.07	$C_{30}H_{60}O_2$	452
Ergost-5-en--3ol (**74**)	58.34	1.40	$C_{28}H_{48}O$	400
Stigmast-5,22-dien-3-ol (**75**)	58.72	1.14	$C_{29}H_{48}O$	412
Stigmast-5-en-3-ol (3β,24S) (**76**)	59.66	3.66	$C_{29}H_{50}O$	414
Palmitic acid, tetradecyl ester (**77**)	60.31	3.71	$C_{30}H_{60}O_2$	452
Palmitic acid, hexadecyl ester (**78**)	63.49	1.49	$C_{32}H_{64}O_2$	480
Total sum.		**97.95%**		
8: Soft coral: *Sarcophyton glaucum* [SP8]				
Cyclohexene,1-methyl-4 (1-methylethenyl) (**64**)	6.93	4.27	$C_{10}H_{16}$	136
Vitamin A alcohol (**79**)	33.49	0.61	$C_{20}H_{30}O$	286
Ledene oxide (II) (**67**)	35.79	2.65	$C_{15}H_{24}O$	220
11-Octadecenoic acid, methyl ester (**54**)	37.48	1.25	$C_{19}H_{36}O_2$	296
1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (**34**)	46.44	63.60	$C_{24}H_{38}O_4$	390
1,2-Benzenedicarboxylic acid, bis(7-methyloctyl)ester] (**5**)	49.85	5.50	$C_{26}H_{42}O_4$	418
1,2-Benzenedicarboxylic acid, dinonyl ester (**3**)	49.96	0.84	$C_{26}H_{42}O_4$	418
Palmitic acid, octadecyl ester (**9**)	50.41	10.65	$C_{26}H_{42}O_4$	418
Retinoyl-á-glucuronide -6',3'- lactone (**80**)	52.09	1.78	$C_{26}H_{34}O_7$	458
Androstan-17-one, 3-ethyl-3-hydroxy (**81**)	52.22	0.91	$C_{21}H_{34}O_2$	318
Tetradecanoic acid, tetradecyl ester (**82**)	57.31	2.01	$C_{28}H_{56}O_2$	424
Ergost-5-en-3-ol (**74**)	58.32	1.36	$C_{28}H_{48}O$	400
Eicosanoic acid, octadecyl ester (**83**)	68.00	0.63	$C_{38}H_{76}O_2$	564
Total sum.		**96.06%**		
9: Soft coral: *Sarcophyton* sp. [SP9]				
n-Hexadecanoic acid (**84**)	35.01	9.43	$C_{16}H_{32}O_2$	256

Heptacosane (**85**)	38.57	11.75	$C_{27}H_{56}$	380
Squalene (**86**)	45.45	13.47	$C_{30}H_{50}$	410
Nonacosane (**87**)	49.19	13.86	$C_{29}H_{60}$	408
decylpropargyl fumarate (**88**)	52.58	10.45	$C_{17}H_{26}O_4$	294
Docosane, 11-decyl (**89**)	59.52	9.90	$C_{32}H_{66}$	450
lamellarin K (**90**)	63.59	30.10	$C_{29}H_{25}NO_9$	531
Total sum.		**98.96%**		
10: Sponge: *Ircinia* sp. [SP10]				
Tetratetracontane (**91**)	33.54	0.85	$C_{44}H_{90}$	618
Ethyl isoallocholate (**21**)	34.43	0.63	$C_{26}H_{44}O_5$	436
Nonadecene (**92**)	34.95	0.62	$C_{19}H_{38}$	266
Octadecanoic acid (**93**)	35.03	0.78	$C_{18}H_{36}O_2$	284
Pentadecanoic acid, 14-methyl,methyl ester (**94**)	38.17	0.53	$C_{17}H_{34}O_2$	270
Nonadecanoic acid, methyl ester (**95**)	38.96	1.19	$C_{20}H_{40}O_2$	312
1-Heneicosyl formate (**96**)	39.47	1.62	$C_{22}H_{44}O_2$	340
1-Heneicosanol (**97**)	43.68	1.26	$C_{21}H_{44}O$	312
Diisooctyl phthalate (**1**)	46.45	82.77	$C_{24}H_{38}O_4$	390
Isochiapin B (**56**)	47.66	0.56	$C_{19}H_{22}O_6$	346
Drebyssogenin f (**98**)	49.90	1.51	$C_{28}H_{42}O_8$	506
Dotriacontane (**99**)	51.25	1.85	$C_{32}H_{66}$	450
Cholesta-3,5-diene (**100**)	52.77	1.54	$C_{27}H_{44}$	368
Cholesta-5,7,9-(11)-trien-3β-ol (**101**)	53.21	1.66	$C_{27}H_{42}O$	382
17-Pentatriacontene (**102**)	54.61	1.57	$C_{35}H_{70}$	490
Total sum.		**98.94%**		
11: Sponge: *Irciniae chinata* [SP11]				
Pinene (**103**)	4.55	2.83	$C_{10}H_{16}$	136
Camphene (**104**)	4.95	1.10	$C_{10}H_{16}$	136
Myrcene (**105**)	5.84	1.19	$C_{10}H_{16}$	136
Phellandrene (**106**)	6.33	1.25	$C_{10}H_{16}$	136
Cyclohexene,1-methyl-4-(1-methylethenyl) (**64**)	6.93	48.37	$C_{10}H_{16}$	136
Eucalyptol (**107**)	7.04	4.31	$C_{10}H_{18}O$	154
Terpinene (**108**)	7.77	5.61	$C_{10}H_{16}$	136
Thujone (**109**)	9.36	1.40	$C_{10}H_{16}O$	152
Camphor (**110**)	10.72	1.09	$C_{10}H_{16}O$	152
Hexadecanoic acid, methyl ester (**111**)	33.42	0.67	$C_{17}H_{34}O_2$	270
9-Octadecenoic acid (Z), methyl ester (**54**)	37.48	3.86	$C_{19}H_{36}O_2$	296
Phthalic acid, di(2-propylpentyl)ester (**112**)	46.44	15.24	$C_{24}H_{38}O_4$	390
Anthraergostatetraenol benzoate (**113**)	53.80	0.92	$C_{35}H_{46}O_2$	498
Cholest-5-en-3-ol-(3á) (**58**)	56.71	2.99	$C_{27}H_{46}O$	386
Quercetin 7,3',4'-trimethoxy (**14**)	57.32	1.53	$C_{18}H_{16}O_7$	344
25-Homo-24-ketocholesterol (**114**)	59.71	3.02	$C_{28}H_{46}O_2$	414
Palmitic acid, octadecyl ester (**9**)	60.32	2.77	$C_{34}H_{68}O_2$	508
Total sum.		**98.15%**		

3.2. Anticancer Activity Studies

The anticancer activity of the eleven marine organisms reported in this investigation, was basically studied depending on their cytotoxic (Table 2 and Figures 1-2) and apoptotic activities (Figures 3 and 4) via caspase enzyme and annexin assays against liver cancer cell line (HepG2 cells). Accordingly, the total extracts of almost marine organisms were non- cytotoxic after the treatment for 24 and 48 h, except for the unidentified soft coral [Sp7] which showed weak cytotoxicity (IC_{50} = 100 µg/ml) after 24 h, but it diminished after 48 h. Because of their neither cytotoxic activity nor induction of the caspase activity, extracts of ([SP1], [SP2], [SP3], [SP9] and [SP10]) were excluded from annexin test.

On the other hand, n-hexane fractions for almost marine organisms were cytotoxic with diverse IC_{50} values after the treatment for 24 and 48 h. Particularly, the n-hexane fraction of the soft coral *Dendronephthya hemprichi* [SP4] showed a cytotoxicity of IC_{50} = 25 and 12.5 µg/ml after 24 and 48 h, respectively, however with slight inhibition of caspase activity. N-hexane fraction of the sponge

Iotrochota purpurea [SP6] showed time-dependent cytotoxic activity of IC_{50} = 100 and 50 µg/ml after 24 and 48 h, respectively, with moderate induction of non-apoptotic caspase activity (i.e. no apoptosis induction). N-hexane fraction of the unidentified soft coral [SP7] exhibited time-independent cytotoxicity (IC_{50}= 20 µg/ml) after 24 and 48 h with high induction of caspase–dependent early apoptosis, meanwhile the n-hexane fraction of *Sarcophyton glancum* [SP8] was cytotoxic (IC_{50} = 12.5 µg/ml) after 24, but non-cytotoxic after 48 h. The *Sarcophyton* sp. [SP9] n-hexane fraction was non-cytotoxic after 24 h, meanwhile it was cytotoxic after 48 h (IC_{50} = 30 µg/mL) with an induction of caspase–independent apoptosis.

The unpolar fractions of the sponges *Ircinia sp.* [SP10] and *Ircinia echinata* [SP11] were non-cytotoxic after 24 h, but they exhibited cytotoxic effect after 48 h (IC_{50} = 70 and 60 µg/mL, respectively). The brown alga *Sargassum arnaudianum* [Sp2] hexane fraction was non-cytotoxic after 24 h, while it exhibited weak cytotoxicity (IC_{50} = 100 µg/ml) with high induction of non- apoptotic caspase activity after 48 h. Based on this reported anticancer activity study, the marine organisms, [SP2], [SP4], [SP7], [SP8] and [SP9] have been recognized as talented sources of drug leads with significant biological activities, encouraging our future research planning to isolate and structurally identify the corresponding biologically active compounds, and test their anticancer activities broadly.

As matched with reported biological activities in literature, the organic extract of the seagrasses *Thalassia hemprichii* [SP1] was reported displaying moderate anticancer activity with less undesirable side effects. [40] The Soft coral *Dendronephthya hemprichi* [SP4] extract reported toxicity with LC_{50} of 28.18 ppm. [41] Based on its cytotoxic activity evaluation reported by Abdel-Lateff et al., 2017, the marine sponge *Rumphella sp*. [SP5] was confirmed by to exhibit high anticancer activity. [42] The alkaloidal compounds, itampolin A and matemone, isolated from the sponge *Iotrochota purpurea*. [SP6], were found to have anticancer activity against numerous cancer cell lines. [43,44] Reported results by Al Baqami et al., 2017 showed that the *Sarcophyton glaucum* [SP8] are capable to inhibit cancer development in the tested animals, establishing their potentiality to fight cancer diseases. [45] The greatest anti-cancer activity of malformin A, kuanoniamine D, hymenialdisine and gallic acid obtained from the soft coral *Sarcophyton* sp. [SP9] extract deduced its anticancer potentiality and capability. [46] The cytotoxic activity of the compounds isolated from the marine sponge *Ircinia sp*. [SP10] reported their mild anticancer activity, however with a decrease in selectivity in the investigated cancer cell lines. [47] Finally, the alcoholic extract of *Irciniae* mutans have a noticed cytotoxic activity. [48] Alternatively, the anticancer activity of the marine sponge *Irciniae chinata* [SP11] reported herein by us is to first time so far.

Table 2: *In vitro* cytotoxicity (IC_{50}) of studied marine organisms ([SP1-SP11]) against HepG2 cells at 24 and 48.

Marine Organism	Total extract IC_{50} (µg/mL)		N-hexane fraction IC_{50} (µg/mL)	
	24h	48h	24h	48h
Seagrass: *Thalassia hemprichii* [SP1]	-	-	-	-
Brown alga: *Sargassum arnaudianum* [SP2]	-	-	-	100
Sponge: *Echinodictyum flabelliforme* [SP3]	-	-	100	100
Soft coral*: Dendronephthya hemprichi* [SP4]	-	-	25	12.5
Soft coral: *Rumphella* sp. [SP5]	-	-	-	100
Sponge: *Iotrochota purpurea* [SP6]	-	-	100	50
Soft coral (un-identified) [SP7]	100	-	20	20
Soft coral: *Sarcophyton glaucum* [SP8]	-	-	12.5	-
Soft coral: *Sarcophyton* sp. [SP9]	-	-	-	30
Sponge: *Ircinia* sp. [SP10]	-	-	-	70
Sponge: *Irciniae chinata* [SP11]	-	-	-	60

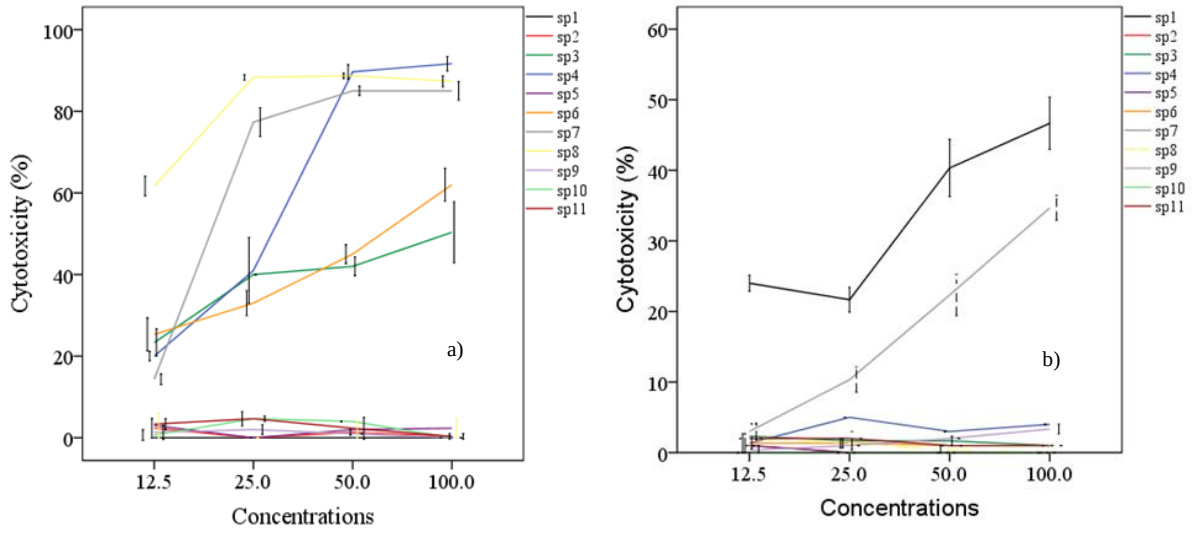

Figure 1*:* a) Cytotoxic activity of the total extracts of the 11 marine organisms against HepG2 cells after 24 h treatment *in vitro*; and b) after 48 h treatment *in vitro*. Data were expressed as percent of control ± SE (n=3).

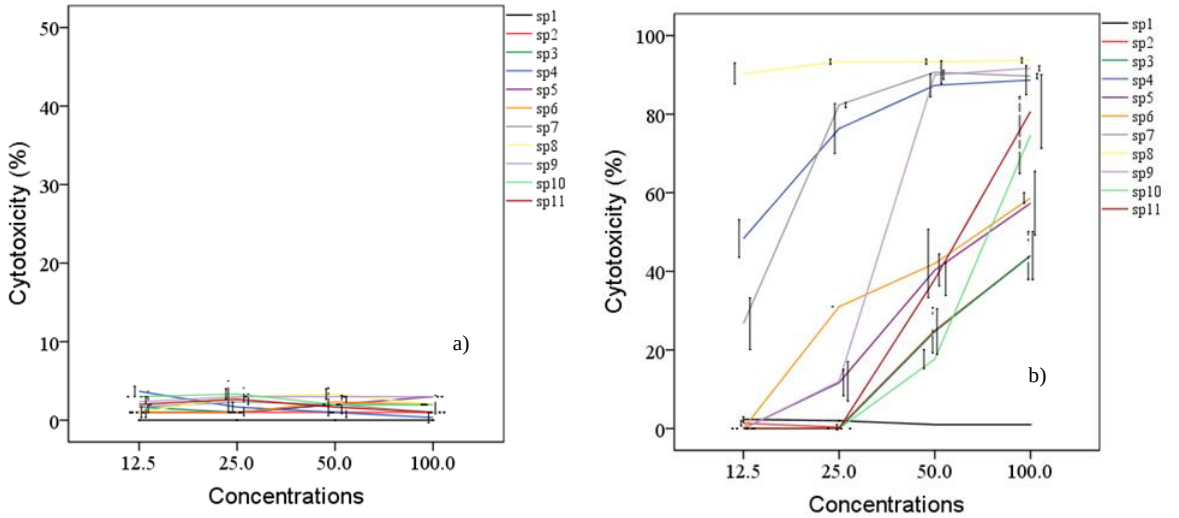

Figure 2*:* a) Cytotoxic activity of the n-hexane fractions of the 11 marine organisms against HepG2 cells after 24 h treatment *in vitro*; and b) after 48 h treatment *in vitro*. Data were expressed as percent of control ± SE (n=3).

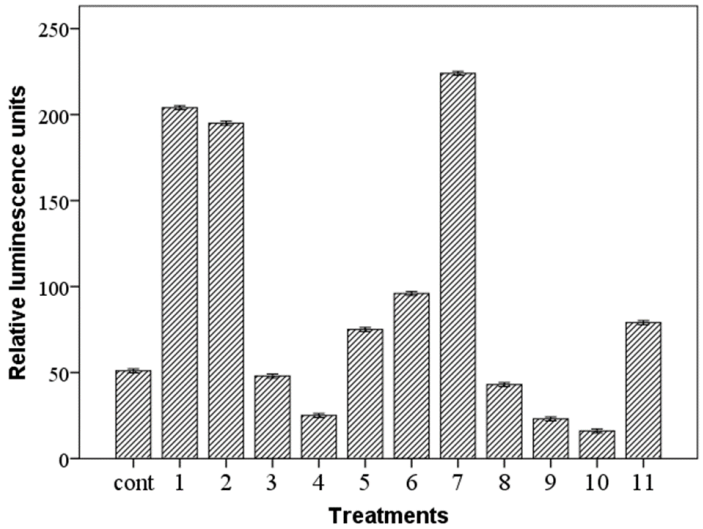

Figure 3*:* Caspase activity of HepG2 cells in response to treatment with n-hexane fraction of 11 selected marine organisms. Values are means ± SE (n=3).

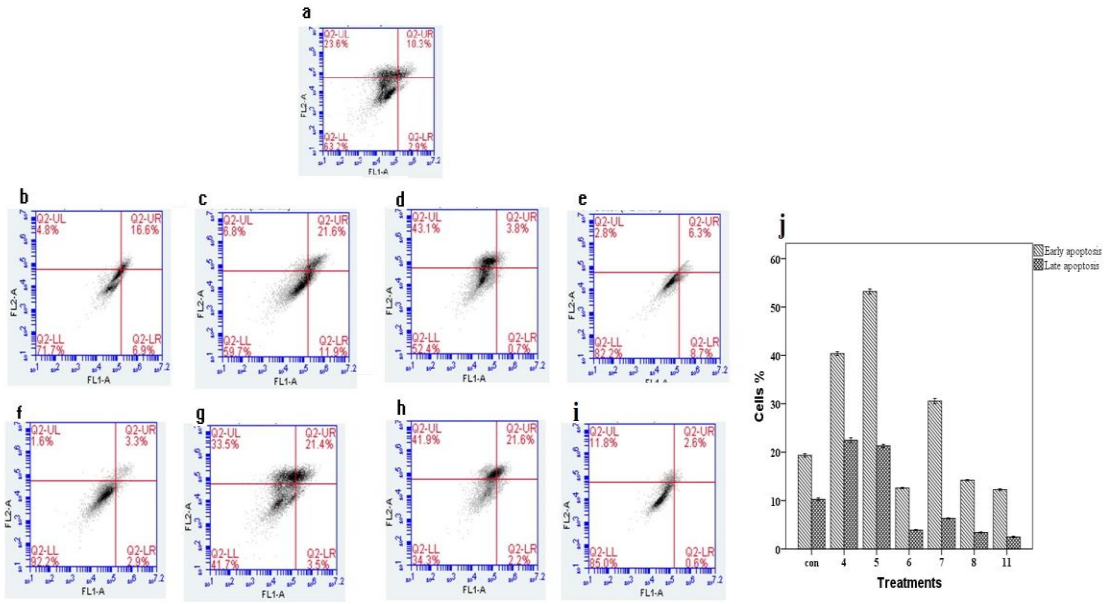

***Figure 4**:* Apoptotic activity of HepG2 cells in response to treatment with the n-hexane fraction of 6 selected marine organisms. a–i: Annexin V-FITC/PI double staining analysis of apoptosis, J: Annexin percentages of apoptosis in HepG2 cells. Statistical analysis was conducted using One-way analysis of variance (ANOVA). Values are presented as means±standard error (SE) (n = 3).

4. Conclusions

The phytochemical profiling of diverse eleven marine organisms collected from the Red Sea at Egyptian coasts was investigated revealing the presence of altogether 114 compounds of different structures/categories classified mainly into: sterols, terpenes, hydrocarbons, carrotenoids, phthylesters, and fatty acid triglycerides. The *in vitro* anticancer activity of the main crude extracts and corresponding unpolar fractions were comparatively studied against the human hepatocarcinoma (HepG2) including cytotoxic, caspase and apoptotic activities.

According to the anticancer profiling studied, the n-hexane fractions showed diverse promising cytotoxicity: the soft coral *Dendronephthya hemprichi* [SP4] showed cytotoxic activity at IC_{50} = 25 and 12.5µg/mL after 24 and 48 h, respectively, meanwhile the sponge [SP6] showed less cytotoxicity of IC_{50} = 100 and 50 µg/mL after 24 and 48 h, respectively), accompanied with moderate induction of non-apoptotic caspase-activity. The unidentified soft coral [SP7] had time-independent cytotoxicity (IC_{50}= 20 µg/mL) after 24 and 48 h, accompanied with high-induction of caspase–dependent early apoptosis. *Sarcophyton glaucum* [SP8] was potentially cytotoxic (IC_{50}= 12.5 µg/ml after 24) without induction of caspase-activity or apoptosis, meanwhile the soft coral [SP9] showed induction of caspase–independent apoptosis. Finally, the brown alga [SP2] showed high induction of non-apoptotic caspase-activity after 48 h. This recognized the high potentiality of the marine organisms, [SP2], [SP4], [SP7], [SP8] and [SP9] as talent sources of drug leads with significant biological activities, encouraging our future research planning to isolate and structurally identify such corresponding potentially active compounds, and examine their anticancer activities broadly.

Conflicts of interest

The authors declared no conflict of interest.

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Authors' contributions

All the participant researchers contributed to do this work. All authors read and approved the final manuscript.

List of abbreviations

AIDS: Immune Deficiency Syndrome
HepG2: Human hepatocarcinoma cell line
RNA: ribose nucleic acid
DNA: Deoxy ribose nucleic acid
GC-MS: Gas chromatography–mass spectrometry
MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
ANOVA: One-way analysis of variance.
DMSO: Dimethyl sulfoxide
IC_{50}: The half-maximal inhibitory concentration.

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