Phytochemical and pharmacological screening of *Sargassum vulgare* from Suez Canal, Egypt

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Received: 22 August 2017 / Revised: 3 January 2018 / Accepted: 18 January 2018 / Published online: 15 February 2018
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Abstract The current study investigates the phytochemical and pharmaceutical activities of *Sargassum vulgare* (SVE) collected from the Suez Canal. The prescreening using cytotoxicity was tested against hepatocellular carcinoma cell lines. Furthermore the SVE inhibit cell growth effectively with $IC_{50} = 20.8 \mu\text{g/ml}$. The pharmacological studies revealed high antioxidant capacity at all examined concentrations. On the meantime, anticancer assay carried out using tyrosine kinase (PTK) and sphingosine kinase 1 inhibitor screening assays revealed inhibition with 75.73 and 80.01%; respectively. Furthermore, the anti-inflammatory profiling revealed that the activities against COX1, COX2, IL6 and TNF were 77.39, 88.35, 75.38 and 71.24%; respectively. Additionally, the anti-Alzheimer results showed high activity at 1 mg with 76.33%. Finally the antiviral activities using reverse transcriptase inhibition assay give 92.24%. Consequently, it can be easily conclude that the SVE collected from the Suez Canal are excellent source of natural products for nutritional and pharmaceutical applications.

Keywords Seaweeds · Suez Canal · Cytotoxic · Anti-oxidant · Anti-Alzheimer · Anti-cancer · Anti-inflammatory · Anti-viral

Introduction

The marine environments are rich in pharmaceutically potent chemicals basically related to polyphenols and sulfated polysaccharides. Recently, significant increase in the number of marine natural products studies have been carried out to extract, isolate and identify new marine and marine-derived natural products [1–4]. Seaweeds are rich sources of protein, iodine, vitamins, and minerals so; their metabolites have shown promising activities as anti-cancer. Edible seaweed like *Palmaria palmata* is shown to be effective antioxidant, capable of inhibiting cancer cell proliferation. They also contain high amounts of polyphenols such as catechin, epicatechin, epigallocatechin gallate, and gallic acid. Moreover, low-molecular weight fucoidan isolated from *Ascophyllum nodosum* shows an anti-proliferative effect on both normal and malignant cells, including fibroblasts, sigmoid colon adenocarcinoma cells, and smooth muscle cells [5, 6].

Sargassum species which are one of the most distributed tropical and sub-tropical brown seaweed have great potential to be used in nutraceutical industrial and pharmaceutical areas as they are considered a very rich source of nutritious and bioactive compounds such as vitamins (A, B1, B2, B3, B12, C, D, E), carotenoids, dietary fibers, proteins, minerals, polyunsaturated fatty acids and amino acids [5, 6]. It has been reported that many pharmacologically biologically active compounds such as sterols, flavonoids, sargaquinoic acids, polyphenols, terpenoids, protein, pheophytine, sulfated polysaccharides, were also extracted, isolated and characterized from different *Sargassum* species [7, 8]. These compounds showed varieties of biological activities such as analgesic, anti-inflammatory, antioxidant, neuroprotective, anti-microbial, anti-tumor, fibrinolytic, immune-modulatory, anti-

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coagulant, hepatoprotective, anti-viral activity, induction of larval settlement of hydrozoan and inhibition of acetylcholine-esterase, and cell toxicity.

The main objective of the present study is to isolate, extract and explore the phytochemical, therapeutic potential, and health benefits of different compounds isolated from *Sargassum vulgare* as antioxidant, anti-inflammatory, anticancer, antiviral and anti-Alzheimer.

Materials and methods

Area of study

The Suez Canal is an artificial sea-level waterway in Egypt which connects the Mediterranean Sea with the Gulf of Suez, a northern branch of the Red Sea. It expands from the Port Said which located northern terminus to Port Tewfik at the city of Suez which located at the southern terminus. Its length is 193.30 km (120.11 mi), including its northern and southern access channels [Fig. 1(A)].

Sampling, identification and prescreening bioassays of the seaweeds

A *Sargassum vulgare* sample was collected from Suez Canal during spring, 2014 [Fig. 2(B)]. The sample was transferred directly to the laboratory in sterile polyethylene bags under reduced temperature (zero °C). The identification has been done by NIOF team from Hydrobiology lab at the Marine Environment Division.

Chemicals and solvents

Potassium ferricyanide, ferric chloride, sodium hydroxide, chloroform, glacial acetic acid, ferric chloride solution, sulphuric acid, folin–Ciocalteu, vanillin, methanol, hydrochloric acid, n-hexane, hydrogen peroxide, nitric acid, iron, zinc, cobalt, manganese, selenium. etc., β -carotene, catechin, (+)-quercetin, sodium nitrite, aluminum chloride and gallic acid were purchased from Sigma Aldrich.

Instruments

Atomic absorption (AAS and GFASHIMADZU), GC–MS (Thermo, USA).

Preliminary phytochemical screening of *Sargassum vulgare* marine extract

The ethyl acetate extracts of the *Sargassum vulgare* samples were subjected to different chemical tests for the detection of different tannins, phlobatannins, saponins, alkaloids, flavonoids, quinines, coumarin, terpenoids and cardiac glycosides phytoconstituents.

Quantitative chemotaxonomy profiling

Determination of total phenolic contents

Total phenolic compounds in the *Sargassum vulgare* extract were determined [9] as mg gallic acid equivalent in 1 ml of the extract using the standard curve of the gallic acid.

Fig. 1 The *Sargassum vulgare* and the location site, (A) The Suez Canal area of Investigation, (B) Brown algae in the genus *Sargassum vulgare*

Fig. 2 Mineral, elements and elemental analysis of *Sargassum vulgare* extract, **(A)** The mineral and elements results, **(B)** The elemental analysis result of *Sargassum*, Data analysis using one way ANOVA where $p < 0.0001$

Determination of total flavonoid contents

Total flavonoid contents were determined by a colorimetric method [10] and the results were expressed as mean ml of (+)-Quercetin equivalents.

Determination of total tannins

Tannins (proanthocyanidins) were determined [11] as mg (+)-catechin/g. All samples were analyzed in triplicate using a standard curve.

Determination of total carotenoid

Total carotenoid contents were measured [12]. The results were reported as percentage of total β -carotene equivalents per 100 mg extract using the standard curve.

Preparation and extraction for mineral and metal assessment (Fe, Zn, Co, Mn, Cu and Se)

Wet digestion

A 0.5 gm of dried sample of the marine extract was digested using 5 ml concentrated HNO_3 , the mixture was heating using hot plate for 1 h and getting semi dried 5 ml of concentrated HNO_3 and 2 ml of H_2O_2 was added and kept on hot plate for 1 h. The semi dried cooled residue, filtered by using Whatman filter paper and the volume was made up to 25 ml with 2 N HNO_3 .

Determination of minerals and heavy metals using atomic absorption

The determination of minerals and heavy metals concentrations of the powdered sample was determined spectrometry by using AAS (GFASHIMADZU atomic absorption spectrophotometer AA-6800) [13].

Elemental analysis

The total carbon and hydrogen contents of *Sargassum vulgare* marine extract were determined using CHNO Elemental Analyzer.

Identification and phytochemical screening using gas chromatography-mass spectrometry (GC-MS)

The *Sargassum vulgare* marine extract was analyzed by GC-ITQ-MS using a Thermo Trace GC Ultra TM gas chromatograph system (Thermo Scientific, USA), equipped with a 30 m $\times$ 0.25 mm i.d., 0.25 μm film thickness, non-polar TR-5MS fused silica capillary column, connected to an ion trap quadrupole (ITQ) mass selective detector (unit mass resolution). Split was 1:50, with helium as carrier gas at a flow rate of 1 ml/min, while the damping gas flow was 0.3 ml/min. The initial oven temperature was set to 40 $^\circ\text{C}$ for 1 min. The GC oven temperature program was as follows: 40 $^\circ\text{C}$ to 220 $^\circ\text{C}$, by ramping at 3 $^\circ\text{C}$, and held at 220 $^\circ\text{C}$ for 20 min. The injector temperature was 220 $^\circ\text{C}$ and the transfer line was held at 220 $^\circ\text{C}$. The screening of bioactive compounds were complete using Thermo ITQ 900TM mass spectrometer with the EI mode (ionization energy of 70 eV, ion source temperature of 180 $^\circ\text{C}$,

emission current of 220 μA). The acquisition was made in full scanning mode (mass range 50–900 m/z ; 3 scans/s). Maximum ionization time was 25 ms. A solvent delay time of 5 min (set off) was used to avoid overloading the mass spectrometer with hexane. Data collection, analysis and integration were performed using the software XCaliburTM (version 2.0.7). Areas were recorded for all detectable peaks, and percent composition was calculated by taking area of peak divided by total chromatogram area $\times 100$.

Identification of phytochemicals

The identification and interpretation of the marine extract results of mass-spectrum GC–MS was conducted using the database of the National Institute Standards and Technology (NIST), which having more than 62,000 patterns. The spectrums of the unknown bioactive compounds were identified and compared in the NIST library with the spectrum of known components stored. The name, molecular weight and structure of the components of the test materials were investigated [14].

Prescreening bioassays using *in vitro* cytotoxicity using cell lines

Different concentrations of different marine extracts $\mu\text{g/ml}$ from all samples were tested for each cell line. Samples were dissolved in DMSO and further diluted with cell culture medium. The final DMSO concentration used was 1% of total volume of the medium in all treatments, including the control group. Cells with no treatment were examined as negative and positive controls, respectively [15].

Primary screening assay

2,2'-Diphenyl- α -picrylhydrazyl (DPPH) radical scavenging assay

DPPH radical scavenging assay of the total marine extract was performed [16]. Assays were performed in flat bottom polystyrene 96 well microtiter plates. To 100 μL of each sample (1–6 mg/ml) in EtOH 25 μL DPPH (1 mM) in ethanol was added. The resultant mixture was briefly shaken and maintained in the dark at room temperature, for 30 min. At the end of this period the absorbance (A) of the mixture was measured at 490 nm, using ELISSA. Scavenging ratio of DPPH assay calculated as follows:

$$\% \text{Scavenging} = [\text{A control} - \text{A sample}] / \text{A control} \times 100.$$

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) ABTS radical scavenging assay

The ABTS⁺ free radical decolorization assay [17]. The inhibition percentage of free radicals were measured at absorbance of 734 nm and calculated for each point and the antioxidant capacity of the test samples was expressed as percent inhibition (%). The percentage scavenging of ABTS⁺ was calculated by the following formula:

$$\text{Scavenging activity (\%)} = (\text{A}_0 - \text{A}_x) / \text{A}_0 \times 100$$

A_x and A_0 were the absorbance at 734 nm of samples with and without extract, respectively.

Specialized screening assays

Acetylcholine esterase inhibition (AChEI) assay

Inhibition of AChE by *Sargassum vulgare* extract was evaluated [18]. The increase in absorbance value due to the spontaneous hydrolysis of the substrate was corrected by subtracting the ratio of the reaction before adding the enzyme from the rate after the enzyme addition.

Percentage inhibition by extracts were calculated using the equation below

$$\text{Inhibition (\%)} = (1 - \text{Sample reaction rate}) / (\text{Blank reaction rate}) \times 100.$$

Determination of tyrosine kinase inhibitory activity (PTK)

Sample preparation

The dimethylsulfoxide (DMSO) sample solution of the appropriate extract was diluted with H₂O (1:1 v/v) to yield corresponding sample solutions (1 mg/mL). Tyrosine kinase (TK) inhibitory activity was determined using a commercial test kit (Tyrosine kinase Assay Kit, non-radioactively, Takara Cat.# MK410). PTK activity of sample was calculated on the basis of the prepared standard curve. The color intensity is stable for 1 h after addition of stop solution at room temperature in a light room.

$$\% \text{Inhibition} = \frac{[\text{Initial activity} - \text{inhibitor}]}{\text{Initial activity}}.$$

Determination of sphingosine kinase 1 inhibitor screening assay (SHK1)

Sphingosine kinase inhibitory activity of the crude extract was determined by using the colorimetric sphingosine kinase 1 inhibitor screening assay kit from Cayamen. The plate was covered and the fluorescence was measured using an excitation wavelength between 530 and 540 nm and an emission wavelength between 580 and 590 nm.

$$\% \text{Inhibition} = \frac{[\text{Initial activity} - \text{inhibitor}]}{\text{Initial activity}}.$$

Determination of cyclooxygenase 1 (COX1) and cyclooxygenase 2 (COX 2) inhibitor screening assays

Cyclooxygenase inhibitory activity of the crude extract was determined by using the colorimetric COX (Ovine) inhibitor screening assay kit from Cayamen. The absorbance was measured at 590 nm using a plate reader.

$$\% \text{Inhibition} = \frac{[\text{Initial activity} - \text{inhibitor}]}{\text{Initial activity}}.$$

Determination of tumor necrosis factor alpha (TNF- α) assay

Tumor necrosis factor alpha (TNF- α) inhibitory activity of the crude extract, was determined by using the KOMA BIOTECH INC colorimetric kit. The absorbance was measured at 450 nm.

Determination of interleukin 6 (IL-6) assays

An Interleukin 6 (IL-6) inhibitory activity of the crude extract was determined by using the KOMA BIOTECH INC colorimetric kit. The absorbance was measured at 450 nm.

Determination of reverse transcriptase enzyme inhibitor screening assay

Reverse transcriptase (RT) inhibitory activity of the crude extract against a purified recombinant HIV1-RT, was determined by using Roche colorimetric kit [19]. HIV-1 protease enzyme and the substrate which is a synthetic peptide that contains a cleavage site Tyr-Pro for HIV protease as well as two covalently modified amino acids for the detection of cleavage. The blank treatment consists of an assay buffer with only the substrate, untreated control of enzyme and substrate was also included where the positive control for HIV inhibition was Acetyl pepstatin (AP). The absorbance was measured at 450 nm.

Statistical analysis

All statistical analyses were performed using the statistical software prism version 6.

Results and discussion

Chemical profiling

Elemental analysis

The results of elemental analysis in 50 mg of marine extract including H%, N%, C%, S%, C/H and C/N [Fig. 2(B)], showed highest ratios of H%, N%, C%, S%, C/H% and C/N%. On the other hand, Fe, Mn, Co, Se, Cu, and Zn which are known to be very important for various metabolic processes in the human body as they are known to be closely linked to growth and health of human being [4]. The results of elemental analysis cleared out high contents of sulfur and selenium, i.e. 1.469 ppm and 27,062%; respectively which are well known to be antioxidant agents that ameliorate oxidative damage. They also have very effective role in activation and regeneration of antioxidants enzymes [4]. The results of mineral and metals analysis the marine extract [Fig. 2(A)] high concentrations of Fe, Cu and Co. Additionally high sulfur and nitrogen contents were also measured.

Phytochemical screening assays

Qualitative and Quantitative phytochemical screening assays including different tests for phlobatannins, saponins, flavonoids, alkaloids, quinones, coumarin, terpenoids and cardiac glycosides are shown in Table 1. The results of *Sargassum vulgare* marine extract give + vulgare marine extract for all tests. The results of quantitative total β -

Table 1 The qualitative analysis of *Sargassum*

Test	Aqueous Extract
Test for tannins	++
Test for phlobatannins	++
Test for saponins	+
Test for flavonoids	+++
Test for alkaloids	+++
Test for quinines	++
Test for coumarin	++
Test for terpenoids (Salkowski test)	++
Test for cardiac glycosides (Keller–Kiliani test)	+

Fig. 3 The phytochemical analysis of *Sargassium* using qualitative and quantitative assay. **(A)** The Quantitative result of phytochemicals compounds, Data analysis using one way ANOVA where $p < 0.0001$

(B) The GC-MS profiling percentage distribution of bioactive compounds, **(C)** The GC-MS chromatogram of *Sargassium vulgare* extract

carotene and total flavonoids content, total tannins content and total phenolic in 100 mg [Fig. 3(A)] revealed the presence of high β -carotene and flavonoids contents in the *Sargassium vulgare* marine extract.

Qualitative GC-MS screening

A number of marine natural products have been characterized by using modern chromatography techniques, namely; GC-MS, LC-MS, and IC based on mass-charge ratio (m/z), and retention time of ion spectra of GC-MS. They were further accurately matched with standard data of library NIST [4, 7, 8, 20]. In addition, HPLC-MS was also used to identify the biodegradation products from metabolites. Modern GC-MS, LC-MS-MS and other quantitative assays were applied in the present study for identification of bioactive compounds such as alkaloids, flavonoids, phenols, glycosides, and saponins. The GC-MS

profiling of *Sargassium vulgare* marine extract are shown in Table 2, and are illustrated in Fig. 3(C). The results revealed the presence of different bioactive compounds, such as fatty acids, alkaloid terpenoids, vitamins, hormones, polyphenolic acids, amino acids,...etc. The results revealed also the presence of a chemical diversity and bioactivity [Fig. 3(B), 4(B), Table 2] which are in good agreement with the results of many other researchers [21]. In the present study, a number of bioactive compounds such as tannins, saponins, coumarin, and flavonoids were identified. There are many medicinal applications for these compounds. For example, tannin-containing drugs are used in medicine as astringent. They are also used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns, piles and as antidote. Furthermore, tannins have been found to have antiviral, antibacterial, antiparasitic effects. In addition to their anti-inflammatory, antiulcer and antioxidant properties for possible therapeutic applications. This

Table 2 The bioactive and GC-MS profiling of *Sargassum vulgare*

Compound name	RT	Prob.	Area	Area %	Compound name	RT	Prob.	Area	Area %
(E)-13-Docosenoic acid	0.07	7.20	358,084.25	0.10	Corynan-17-ol, 18,19-didehydro-10-methoxy-, acetate (ester)	1.36	6.16	137,212.27	0.04
9,10-Secocholesta-5,7,10(19)-triene-1,3-diol, 25-[(trimethylsilyl)oxy]-, (3á,5Z,7E)-	0.17	6.64	320,935.48	0.09	Ethyl iso-allocholate	1.52	5.48	116,195.34	0.03
Octadecanoic acid, 4-hydroxy-, methyl ester	0.17	6.64	320,935.48	0.09	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, trans-	1.89	18.65	160,341.24	0.04
Spiculesporic acid	0.33	7.58	29,624.78	0.01	Phorbol 12,13-dihexanoate	2.05	9.08	224,162.33	0.06
Ursodeoxycholic acid	1.78	18.54	129,937.60	0.03	Heptanoic acid, docosyl ester	2.28	9.35	136,712.49	0.04
Chenodiol	1.78	12.72	129,937.60	0.03	2-(2-Azepan-1-yl-2-oxoethyl)-1-hydroxy-1-phenyl-octahydro-pyrido[1,2-a]azepin-4-one	4.72	15.28	356,081.28	0.10
Glucobrassicin	0.75	7.06	440,260.95	0.12	EPPS	4.72	11.09	356,081.28	0.10
Retinoic acid, methyl ester	0.82	5.39	1,621,521.96	0.44	2-(Cyanomethylene)-3,5-dihydroxy-4-methoxycyclohexyl)-á-glucoside	4.77	12.38	175,012.00	0.05
Morphinan-4,5-epoxy-3,6-di-ol, 6-[7-nitrobenzofurazan-4-yl]amino-	2.23	10.08	44,151.57	0.01	Benzaldehyde, 4-methyl-, oxime	4.84	20.85	67,640.79	0.02
11-Heneicosanone	1.21	11.85	210,142.95	0.06	Heptadecane, 9-hexyl-	6.76	27.94	840,216.97	0.23
5,8,11,14-Eicosatetraynoic acid	3.98	5.82	83,212.10	0.02	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	6.76	18.04	840,216.97	0.23
Pregn-4-ene-3,20-dione, 17,21-dihydroxy-, bis(O-methylloxime)	4.08	30.27	81,015.18	0.02	2,5-Furandione, dihydro-3-octadecyl-	6.90	7.86	36,299.75	0.01
1-Oxaspiro[4.4]non-8-ene-4,7-dione, 9-hydroxy-6-(3-methyl-2-butenyl)-2-(1-methyl-ethyl)-8-(3-methyl-1-oxobutyl)-	4.08	6.10	81,015.18	0.02	9,19-Cyclolanostan-3-ol, 24,24-epoxymethano-, acetate	6.90	4.29	36,299.75	0.01
2-Chloro-1,3-bis(4-methylpiperazin-1-yl)-4-nitrobenzene	4.19	14.99	379,086.59	0.10	à-N-Normethadol	6.93	13.07	218,379.53	0.06
Dodecane, 5,8-diethyl-	4.34	13.93	337,434.19	0.09	9,10-Secocholesta-5,7,10(19)-triene-1,3-diol, 25-[(trimethylsilyl)oxy]-, (3á,5Z,7E)-	7.06	7.14	98,889.56	0.03
2,5-Furandione, dihydro-3-isooctadecyl-	4.65	7.13	295,458.80	0.08	Propionitrile, 3-[1-[4-[1-(2-cyanoethoxy)cyclohexyl]buta-1,3-dienyl]cyclohexyloxy]-	7.14	6.74	249,560.47	0.07
9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3á,5Z,7E)-	3.11	21.68	50,947.59	0.01	L-Lysine, N6-acetyl-N2-[N-[N-(N2-acetyl)-N,N,N2-trimethyl-L-asparagyl)-N-methyl-L-phenylalanyl]-N-methyl-L-phenylalanyl]-N,1-dimethyl-L-tryptophyl]-N2,N6-dimethyl-, methyl ester	7.22	4.81	149,066.26	0.04
4-Heptadecyne, 1-chloro-	3.11	11.18	50,947.59	0.01	Prost-13-en-1-oic acid, 9-(methoxymino)-11,15-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester, (8.xi.,12.xi.)-	7.58	13.82	5,675,895.92	1.52
Morphinan-4,5-epoxy-3,6-di-ol, 6-[7-nitrobenzofurazan-4-yl]amino-	3.19	6.63	1,059,592.03	0.28	Octasiloxane, 1,1,3,3,5,5,7,9,9,11,11,13,13,15,15-hexadecamethyl-	9.31	15.52	6,731,734.92	1.81
à-N-Normethadol	3.26	25.19	344,333.02	0.09	Remoxipride	9.98	6.59	582,702.43	0.16
2-Methyl-thiobenzamide	4.84	6.13	67,640.79	0.02					

Table 2 continued

Compound name	RT	Prob.	Area	Area %	Compound name	RT	Prob.	Area	Area %
à-N-Normethadol	4.93	19.97	362,160.62	0.10	Cyclohexane, 1,1'-dodecylidenebis[4-methyl-	11.10	4.13	1,235,479.08	0.33
L-Serinamide, 1-methyl-5-oxo-L-prolyl-N,1-dimethyl-L-histidyl-N,1-L-tryptophyl-N,N,N ₂ ,O-tetramethyl-	4.93	5.23	362,160.62	0.10	[5,9-Dimethyl-1-(3-phenyl-oxiran-2-yl)-deca-4,8-dienylidene]-(2-phenyl-aziridin-1-yl)-amine	11.16	10.90	354,576.81	0.10
1-Oxaspiro[4.4]non-8-ene-4,7-dione, 9-hydroxy-6-(3-methyl-2-butenyl)-2-(1-methylethyl)-8-(3-methyl-1-oxobutyl)-	5.15	6.31	248,651.71	0.07	Benzene, [3-(2-cyclohexylethyl)-6-cyclopentylhexyl]-	11.16	5.62	354,576.81	0.10
Heptadecane, 9-hexyl-	5.27	6.58	1,297,029.32	0.35	Cholestane-3,7,12,25-tetrol, tetraacetate, (3à,5à,7à,12à)-	13.12	6.40	1,192,314.28	0.32
Hexadecane, 1,1-bis(dodecyloxy)-	5.27	4.91	1,297,029.32	0.35	Ursodeoxycholic acid	13.39	3.71	838,378.12	0.23
3-Pentanone, 1,5-diphenyl-	5.35	10.71	351,525.71	0.09	Glucobrassicin	13.77	7.55	84,398.25	0.02
[5,9-Dimethyl-1-(3-phenyl-oxiran-2-yl)-deca-4,8-dienylidene]-(2-phenyl-aziridin-1-yl)-amine	5.35	5.52	351,525.71	0.09	5,8,11,14-Eicosatetraynoic acid	11.96	17.64	554,245.64	0.15
4-Hexyl-1-(7-methoxycarbonylheptyl)bicyclo[4.4.0]deca-2,5,7-triene	5.47	8.19	66,532.26	0.02	Gibberellic acid	11.96	10.69	554,245.64	0.15
9,10-Secosterosta-5,7,10(19)-triene-3,24,25-triol, (3à,5Z,7E)-	5.76	11.58	601,347.56	0.16	Trilostane	12.19	11.74	1,276,758.21	0.34
7-Chloro-4-[1-methyl-3-[N-methyl-N1-bromoacetylhydrazinyl]propyl]aminoquinoline	5.88	11.14	350,029.26	0.09	l-Galactopyranose, 6-deoxy-1,2-bis-O-(trimethylsilyl)-cyclic methylboronate	14.02	5.08	64,690.61	0.02
.psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	6.36	10.52	45,278.33	0.01	1,3-Dioxocane, 2-pentadecyl-	15.79	4.64	870,991.45	0.23
Photodioldrin	7.84	27.29	172,556.63	0.05	.psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	15.16	9.29	585,030.76	0.16
Ethynodiol Diacetate	8.17	5.63	128,311.73	0.03	8,11,14-Eicosatrienoic acid, methyl ester, (Z,Z,Z)-	16.56	5.77	189,229.87	0.05
Docosanoic acid, 1,2,3-propanetriyl ester	8.17	4.54	128,311.73	0.03	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	16.56	5.55	189,229.87	0.05
Ursodeoxycholic acid	9.57	7.16	263,257.19	0.07	Curan-17-oic acid, 2,16-didehydro-20-hydroxy-19-oxo-, methyl ester	16.67	4.94	537,003.63	0.14
Gibberellic acid	9.66	7.18	529,204.53	0.14	à-N-Normethadol	16.74	13.79	437,226.78	0.12
Phorbol	8.44	17.21	338,593.24	0.09	Phorbol 12,13-dihexanoate	18.39	12.16	600,446.20	0.16
Dodecane, 5,8-diethyl-	8.55	4.64	297,081.43	0.08	Norvenlafaxine	17.18	5.36	388,506.99	0.10
Monorden	8.85	35.41	173,918.06	0.05	Heptadecane, 9-hexyl-	17.18	4.32	388,506.99	0.10
Androstan-17-one, 3-ethyl-3-hydroxy-, (5à)-	10.53	20.25	111,628.74	0.03	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	17.28	5.93	275,978.22	0.07
Benzene, 1,1'-[3-(3-cyclopentylpropyl)-1,5-pentanedyl]bis-	10.53	10.44	111,628.74	0.03	Ethyl iso-allocholate	17.28	4.78	275,978.22	0.07
Methoxyphenamine	10.65	29.36	8,624,803.65	2.32	Corynan-17-ol, 18,19-didehydro-10-methoxy-, acetate (ester)	17.80	27.42	214,480.23	0.06
Scytalone	10.65	21.90	8,624,803.65	2.32	Retinoic acid, methyl ester	19.22	11.99	496,739.98	0.13
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	10.85	12.98	4,105,007.91	1.10	L-Serinamide, 1-methyl-5-oxo-L-prolyl-N,1-dimethyl-L-histidyl-N,1-L-tryptophyl-N,N,N ₂ ,O-tetramethyl-	19.32	22.09	450,905.21	0.12

Table 2 continued

Compound name	RT	Prob.	Area	Area %	Compound name	RT	Prob.	Area	Area %
Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandro-8-en-17-yl)-	10.85	8.64	4,105,007.91	1.10	Digoxigenin	19.32	6.61	450,905.21	0.12
Hexadecanoic acid, ethyl ester	10.95	7.08	2,279,096.83	0.61	.psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	19.38	3.40	124,040.26	0.03
Oxiraneoctanoic acid, 3-octyl-, cis-	10.95	6.53	2,279,096.83	0.61	3-Oxatricyclo[20.8.0.0(7,16)]triacenta-1(22),7(16),9,13,23,29-hexaene	19.51	6.03	134,068.87	0.04
Oleic Acid	11.10	5.25	1,235,479.08	0.33	L-Lysine, N6-acetyl-N2-[N-[N-(N2-acetyl-N,N,N2-trimethyl-L-asparaginy)-N-methyl-L-phenylalanyl]-N-methyl-L-phenylalanyl]-N,1-dimethyl-L-tryptophyl]-N2,N6-dimethyl-, methyl ester	19.56	7.24	430,323.73	0.12
2-Naphthalenamine, 1,2,3,4-tetrahydro-N-(1-methylethyl)-N-(phenylmethyl)-	18.31	5.90	324,536.57	0.09	Pregn-4-ene-3,20-dione, 17,21-dihydroxy-, bis(O-methylloxime)	23.70	14.02	282,327.29	0.08
17a-Methyl-3a-methoxy-17a-aza-D-homoandro-5-ene-17-one	18.39	22.29	600,446.20	0.16	2,7-Diphenyl-1,6-dioxypyridazino[4,5:2',3']pyrrolo[4',5':d]pyridazine	23.75	8.87	202,300.27	0.05
5-Aminovaleramide, N-methyl-N-[4-(1-pyrrolidinyl)-2-butyryl]-N'-boc-	18.76	11.02	491,379.41	0.13	Corynan-17-ol, 18,19-didehydro-10-methoxy-, acetate (ester)	23.75	4.57	202,300.27	0.05
Retinoic acid, methyl ester	19.22	11.99	496,739.98	0.13	Gona-1,3,5(10)-trien-17-one, 3-methoxy-, (13a)-	23.81	6.74	195,523.47	0.05
Pentacosane, 13-phenyl-	20.85	7.40	166,888.41	0.04	Gona-1,3,5(10)-trien-17-one, 3-methoxy-, (13a)-	23.81	6.74	195,523.47	0.05
Cyclopropanoic acid, 2-octyl-, methyl ester, cis-	20.90	6.65	35,392.22	0.01	Tricyclo[4.2.1.1(2,5)]decan-9-one, 10-cyano-10-trimethylsilyloxy-	23.91	4.12	250,250.95	0.07
Eicosanebioic acid, dimethyl ester	20.90	6.39	35,392.22	0.01	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	23.91	4.12	250,250.95	0.07
Corynan-17-ol, 18,19-didehydro-10-methoxy-, acetate (ester)	19.71	15.57	186,504.69	0.05	N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid]	24.12	28.24	77,633.59	0.02
Heptadecane, 9-hexyl-	19.71	8.50	186,504.69	0.05	Benzene, (3-octylundecyl)-	24.12	11.16	77,633.59	0.02
Heptanoic acid, docosyl ester	19.77	20.15	401,368.59	0.11	Triazolam	24.18	17.00	130,833.04	0.04
Cholestane-3,7,12,25-tetrol, tetraacetate, (3a,5a,7a,12a)-	20.08	5.51	91,682.18	0.02	Cyclohexane, 1,1'-dodecylidenebis[4-methyl-	23.00	3.23	441,978.85	0.12
Oleic Acid	20.45	3.72	371,362.44	0.10	1-Naphthaleneopropanol, a-ethyldecahydro-4-hydroxy-a,2,5,5,8a-pentamethyl-, [1S-[1a(R*),2a,4a,4a,8a]]-	23.07	7.41	721,998.42	0.19
Folic Acid	20.76	12.01	560,147.31	0.15	Chromane-3-carbonitrile, 2,3-dehydro-5,6,7,8-tetrahydro-2-amino-5-oxo-4-(3-pyridyl)-	23.07	5.97	721,998.42	0.19
Pregna-6,16-diene-11,20-diol, 3,9-epoxy-18-[N-methyl-N-[14-(2'-epoxyethyl)]amino]-	22.36	18.07	164,845.77	0.04	Cholan-24-oic acid, 3,6-bis(acetyloxy)-, methyl ester, (3a,5a,6a)-	23.22	9.27	308,515.76	0.08
Azafrin	22.42	6.83	509,634.27	0.14	Corynan-17-ol, 18,19-didehydro-10-methoxy-, acetate (ester)	23.22	8.55	308,515.76	0.08
Pentacyclo[12.3.0.0(1,13).0(2,10).0(5,9)]heptadecan-6-ol-15-one, 5-methyl-13-methoxycarbonyl-	22.42	5.23	509,634.27	0.14	Cholestan-26-oic acid, 3,7,12-trihydroxy-, (3a,5a,7a,12a)-	23.31	23.59	144,600.45	0.04
Stibine, triethyl-	22.47	22.19	1,259,213.58	0.34	Quinolin-5(6H)-one, 7,8-dihydro-2-dimethylamino-6-dimethylaminomethylene-7-phenyl-	23.51	6.09	233,774.93	0.06

Table 2 continued

Compound name	RT	Prob.	Area	Area %	Compound name	RT	Prob.	Area	Area %
Undecanoic acid, 11-bromo-ethyl ester	22.47	16.99	1,259,213.58	0.34	Picrotoxin	23.51	4.91	233,774.93	0.06
Photodieldrin	22.64	12.92	83,344.14	0.02	Androstan-17-one, 3-ethyl-3-hydroxy-, (5 α)-	24.66	6.26	59,251.95	0.02
1-Heptatriacotanol	22.64	10.91	83,344.14	0.02	Androst-5,7-dien-3-ol-17-one	24.71	8.54	382,511.96	0.10
Triazolam	24.01	11.45	89,581.89	0.02	Morphinan-4,5-diol-6-one, 1-bromo-	24.79	4.52	174,046.98	0.05
Clenbuterol	24.01	9.23	89,581.89	0.02	Ursodeoxycholic acid	24.79	3.46	174,046.98	0.05
Propane-1,1,2,2-tetracarbonitrile, 3-(4-acetyl-2,5-dimethyl-3-furanoyl)-	24.05	13.26	157,137.45	0.04	Oxiraneoctanoic acid, 3-octyl-, methyl ester	24.87	12.55	203,789.31	0.05
5,8,11,14-Eicosatetraynoic acid	24.05	8.83	157,137.45	0.04	Azafrin	24.87	11.09	203,789.31	0.05
Galactitol, 2,3:4,5-bis-O-(phenylmethylene)-	22.96	32.06	241,328.02	0.06	Sumatriptan	24.93	40.27	71,681.46	0.02
Cholan-24-oic acid, 3,12-bis(acetyloxy)-7-oxo-, methyl ester, (3 α ,5 α ,12 α)-	22.96	7.43	241,328.02	0.06	Dodecanoic acid, hexadecyl ester	25.98	5.11	212,740.69	0.06
8,14-Seco-3,19-epoxyandrostane-8,14-dione, 17-acetoxy-3 α -methoxy-4,4-dimethyl-	23.00	3.23	441,978.85	0.12	Nonanedioic acid, dibutyl ester	26.06	20.75	111,213.16	0.03
1,3,2-Dioxaphosphorinane, 2-(3-indolyl)(4-methoxyphenylamino)methyl-, 2-oxide	26.65	8.55	1,029,948.09	0.28	8,14-Seco-3,19-epoxyandrostane-8,14-dione, 17-acetoxy-3 α -methoxy-4,4-dimethyl-	26.06	5.21	111,213.16	0.03
Morphinan-6-one, 7,8-didehydro-4,5 α -epoxy-14-hydroxy-3-methoxy-17-methyl-, cyclic ethylene acetal	26.72	6.60	260,410.88	0.07	cis-1,2-Bis(aminomethyl)cyclohexane	26.15	8.64	1,154,504.76	0.31
N-(3-Dimethylamino-2-(4-chlorophenyl)-thioacryloyl)-dimethylformamidine	26.72	6.34	260,410.88	0.07	Cyclopentane, chloro-	26.15	3.67	1,154,504.76	0.31
Ethanthiol, 2-(3-(3-chloro-2-pyridyloxy)propyl)amino-, hydrogen sulfate	26.96	10.47	1,838,357.47	0.49	Cyclohexane, 1,1'-dodecylidenebis[4-methyl-	26.30	21.05	286,657.53	0.08
α -D-Glucopyranoside, methyl 2-(acetylamino)-2-deoxy-3-O-(trimethylsilyl)-, cyclic butylboronate	25.68	7.47	284,387.17	0.08	Cyclohexane, 1,3,5-trimethyl-2-octadecyl-	26.30	4.96	286,657.53	0.08
16-Nitrobicyclo[10.4.0]hexadecan-1-ol-13-one	27.26	21.01	334,106.57	0.09	Docosanoic acid, 17-oxo-, methyl ester	26.44	7.53	928,767.60	0.25
Methapyriline	27.26	4.79	334,106.57	0.09	5-[2-(2-Chloro-6-methyl-phenyl)-vinyl]-3-methyl-4-nitro-isoxazole	26.44	6.66	928,767.60	0.25
5,8,11,14-Eicosatetraynoic acid	27.42	6.12	1,964,239.46	0.53	There is no signature data to report.				
Butylaldehyde, 4-benzoyloxy-4-[2,2-dimethyl-4-dioxolanyl]-	24.18	7.23	130,833.04	0.04	9,10-Secocholesta-5,7,10(19)-triene-1,3-diol, 25-[(trimethylsilyloxy)-, (3 α ,5Z,7E)-	26.65	18.78	1,029,948.09	0.28
Gibberellic acid	25.88	21.01	234,012.32	0.06	Linoleic acid ethyl ester	27.42	5.64	1,964,239.46	0.53
α -D-Galactopyranoside, methyl 2,6-bis-O-(trimethylsilyl)-, cyclic butylboronate	25.94	7.78	225,967.69	0.06	Oxiranedodecanoic acid, 3-octyl-, cis-	27.57	8.75	294,024.74	0.08
Cyclohexane, 1,1'-dodecylidenebis[4-methyl-	25.98	15.25	212,740.69	0.06	Retinol	27.78	8.94	598,590.48	0.16
2H-Pyran, tetrahydro-2-(12-pentadecyloxy)-	27.03	8.99	1,062,203.87	0.29	10,13-Octadecadiynoic acid, methyl ester	27.78	8.60	598,590.48	0.16
5 α -Cholestane-3 α ,7 α ,12 α ,24,25,26-hexol hexa-TMS	28.84	9.34	459,133.72	0.12	2-(5-Cyano-4,4,5-trimethyl-pyrrolidin-2-ylidene)-malonic acid, dimethyl ester	27.84	15.63	329,717.56	0.09
Withaferin A	28.84	7.89	459,133.72	0.12	Heptadecane, 9-hexyl-	29.39	10.50	188,333.64	0.05

Table 2 continued

Compound name	RT	Prob.	Area	Area %	Compound name	RT	Prob.	Area	Area %
D-Streptamine, O-2-amino-2-deoxy-à-D-glucopyranosyl-(14)-O-[O-2,6-diamino-2,6-dideoxy-à-L-idopyranosyl-(13)-à-D-ribofuranosyl-(15)]-2-deoxy-	28.96	10.05	1,102,529.23	0.30	N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid]	29.43	6.11	50,722.74	0.01
Curan, 16,17-didehydro-, (20.xi.)-	29.29	9.28	1,124,671.49	0.30	Oxytetracycline	29.43	4.93	50,722.74	0.01
5,8,11,14-Eicosatetraynoic acid	29.29	4.78	1,124,671.49	0.30	Gibberellic acid	27.99	5.99	219,057.62	0.06
Octadecane, 3-ethyl-5-(2-ethylbutyl)-	29.39	11.88	188,333.64	0.05	Morphinan-4,5-epoxy-3,6-di-ol, 6-[7-nitrobenzofurazan-4-yl]amino-	28.59	8.03	102,975.61	0.03
Androst-4-en-9-thiocyanomethyl-11-ol-3,17-dione	30.00	11.26	128,123.12	0.03	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-en-1-yl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	28.59	8.03	102,975.61	0.03
Androst-4-en-11-ol-3,17-dione, 9-thiocyanato-	30.00	9.51	128,123.12	0.03	Morphinan-6-one, 7,8-didehydro-4,5à-epoxy-14-hydroxy-3-methoxy-17-methyl-, cyclic ethylene acetal	29.82	12.45	436,445.33	0.12
DL-Cystine	31.64	6.79	253,797.69	0.07	L-Serinamide, 1-methyl-5-oxo-L-prolyl-N,1-dimethyl-L-histidyl-N,1-L-tryptophyl-N,N ₂ O-tetramethyl-	32.53	16.04	395,700.81	0.11
Pentacosane, 13-phenyl-	32.15	14.25	1,946,316.27	0.52	5,16,20-Pregnatrine-3beta,20-diol diacetate	32.53	7.79	395,700.81	0.11
5,8,11,14-Eicosatetraynoic acid	30.86	9.37	236,753.27	0.06	N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid]	32.62	16.66	352,442.46	0.09
à-D-Galactopyranoside, methyl 2,3-bis-O-(trimethylsilyl)-, cyclic methylboronate	31.10	5.32	244,969.67	0.07	Remoxipride	32.62	7.59	352,442.46	0.09
9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	31.10	4.70	244,969.67	0.07	16-Nitrobicyclo[10.4.0]hexadecan-1-ol-13-one	32.73	10.78	306,089.94	0.08
1,4,7-Androstatrien-3,17-dione	31.15	12.70	264,326.84	0.07	Pregn-4-ene-3,20-dione, 17,21-dihydroxy-, bis(O-methylxime)	34.29	12.96	313,980.28	0.08
Androst-5,7-dien-3-ol-17-one, acetate	31.15	12.21	264,326.84	0.07	Z,Z-3,15-Octadecadien-1-ol acetate	34.29	5.12	313,980.28	0.08
2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine	31.21	9.02	75,022.27	0.02	à-D-Galactopyranoside, methyl 2,6-bis-O-(trimethylsilyl)-, cyclic butylboronate	35.10	15.12	515,659.92	0.14
Octadecanedioic acid	32.73	6.96	306,089.94	0.08	à-D-Galactopyranoside, methyl 2,6-bis-O-(trimethylsilyl)-, cyclic butylboronate	35.10	11.28	515,659.92	0.14
N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid]	32.99	24.72	1,785,383.42	0.48	6-(2-Nitro-1-phenylethyl)-1,4-dithiaspiro[4.5]decane	39.66	6.90	619,045.87	0.17
L-Serinamide, 1-methyl-5-oxo-L-prolyl-N,1-dimethyl-L-histidyl-N,1-L-tryptophyl-N,N ₂ O-tetramethyl-	32.99	18.93	1,785,383.42	0.48	Podocarpa-1,12-diene-è14,à-acetic acid, 7-hydroxy-8,13-dimethyl-3-oxo-, è-lactone	39.88	3.96	515,660.54	0.14
Benzene, 1,1'-(chloroethylenidene)bis[4-ethyl-	34.44	4.46	177,021.32	0.05	Acetamide, N-[2-(acetylony)-2-[4-(acetylony)phenyl]ethyl]-	38.47	9.78	528,379.27	0.14
9-Anthracenepropanoic acid, 10-carboxy-9,10-dihydro-, cis-	34.44	3.24	177,021.32	0.05	Oxiraneoctanoic acid, 3-octyl-, cis-	38.70	4.25	501,003.03	0.13
Allo-cassaic acid methyl ester	33.81	10.20	226,266.92	0.06	2-Octadecenoic acid, methyl ester	39.05	5.79	758,454.84	0.20

Table 2 continued

Compound name	RT	Prob.	Area	Area %	Compound name	RT	Prob.	Area	Area %
Normorphine	33.81	7.61	226,266.92	0.06	4,6-Androstadien-3 α -ol-17-one, acetate	40.43	7.90	340,211.09	0.09
α -D-Galactopyranoside, methyl 2,3-bis-O-(trimethylsilyl)-, cyclic methylboronate	35.16	6.96	360,983.64	0.10	2,5-Furandione, dihydro-3-octadecyl-	39.19	5.90	268,893.08	0.07
.psi.,.psi.-Carotene, 3,3',4,4'-tetrahydro-1,1',2,2'-tetrahydro-1,1'-dimethoxy-2,2'-dioxo-	35.16	6.69	360,983.64	0.10	Glucobrassicin	39.92	6.87	322,380.12	0.09
Androst-4-en-9-thiocyanomethyl-11-ol-3,17-dione	35.24	4.72	705,798.37	0.19	2-[(Benzo [1,3] dioxole-4-carbonyl)-amino]-3-hydroxy-propionic acid	40.18	10.30	822,937.54	0.22
Isodihydrohistrionicotxin 285a	35.24	4.54	705,798.37	0.19	Ethyl 5,8,11,14,17-icosapentaenoate	40.43	11.19	340,211.09	0.09
Picrotoxinin	35.55	15.93	964,361.50	0.26	α -N-Normethadol	40.55	7.46	355,655.68	0.10
Galactopyranoside, methyl 2-acetamido-2-deoxy-3,4,6-tri-O-methyl-, α -D-	37.76	3.38	75,953.49	0.02	α Carotene	38.11	3.14	202,675.99	0.05
Phorbol 12,13,20-triacetate	37.86	12.51	739,177.80	0.20	L-Serinamide, 1-methyl-5-oxo-L-prolyl-N,1-dimethyl-L-histidyl-N,1-L-tryptophyl-N,N ₂ ,O-tetramethyl-	39.73	13.54	225,351.65	0.06
5,6-Dicarbadeborane(12)	37.86	6.45	739,177.80	0.20	Ethyl 5,8,11,14,17-icosapentaenoate	39.88	11.83	515,660.54	0.14
Ajmalan-16-carboxylic acid, 19,20-didehydro-1-demethyl-17-hydroxy-, methyl ester, (2 α ,17S,19E)-	39.66	8.17	619,045.87	0.17					

has been confirmed in the present study (Figs. 4, 5). Saponins and coumarin compounds have, on the other hand, been observed in *Sargassium vulgare* marine extract. Saponins are known to produce antimicrobial, anti-inflammatory, anti-feeding, and hemolytic effects. Coumarin, on the other hand, has been used as anti-coagulant drugs and to treat lymphedema [22]. On the meantime, flavonoids which are the major group of phenolic compounds were reported for their antimicrobial, antiviral and anticancer activity [4]. Flavonoids ability of scavenging hydroxyl radicals, superoxide anion radicals and lipid proxy radicals highlights many of the flavonoids health-promoting functions in organisms. These abilities are important for prevention of diseases associated with oxidative damage of membrane. Proteins and DNA are also identified in the present study (Figs. 4, 5).

Biochemical and pharmacological assays

The biochemical and pharmacological bioassay screening assays were performed on different phases including pre-screening, primary screening and specialized screening for *Sargassium vulgare* marine extract.

Broad biochemical drug discovery bioassay

Prescreening: cytotoxicity of marine sargassium

In the present study *Sargassium vulgare* marine extract was screened against human cancer cell line using the MTT cytotoxicity assay. The cytotoxicity of the marine extract and the inhibitory activity against Hepatocellular carcinoma cells were detected with $IC_{50} = 20.8 \mu g$. The results were presented in Fig. 5(A). The use of marine natural products as therapeutic and cure tools has been started since 1997 with many discoveries [1–7]. The results of the present study revealed that marine derived *sargassium* from the Suez Canal, especially those belong to genus *vulgare*, are rich source of structurally novel and biologically potent natural products. This is in agreement with recent studies which have shown that many seaweeds extracts from the Egyptian marine environment along the Mediterranean Sea displayed significant cytotoxic and apoptotic activity [5, 6]. The deep-sea anoxic brines of the Red sea are one of the most extreme environments on the earth in comparison to overlying seawater. The anoxic brines are characterized by high salinity (increased from 4% up to 26%), high temperature (up to 70 °C), increased concentration of heavy metals, and decrease in O₂ levels [23] Therefore, marine-derived seaweeds bioactive secondary metabolites have the ability to tolerate and survive in these extreme conditions, since they are uniquely equipped with some self-defenses [1–4, 20].

Fig. 4 The specialized screening using different screening assay, (A) The Anti-Alzheimer using acetyl cholinesterase inhibition of different marine extracts of *Sargassum vulgare* extract, (B) The Anti-

cancer, anti-inflammatory and anti-HIV of different marine extract of *Sargassum vulgare* extract

Primary screening assays

Total antioxidant capacity (TAO) of different marine extracts using DPPH and ABTS inhibition assays

The results of total antioxidant capacity at different concentrations of *Sargassum vulgare* marine extract have been done using DPPH and ABTS inhibition Fig. 5(B), (C). The present study cleared out that the inhibition assay give high inhibition ratio with 93.33 and 90.67% of both

DPPH and ABTS, respectively by the marine extract at 1 mg. At concentrations from 50 mg up to 6.25 mg the *Sargassum vulgare* extract showed complete inhibition (100%). Different kinds of radicals are generated in the normal metabolic activities and sometimes the antioxidant capacity of the body is inadequate to cope with them. Therefore, there is a growing interest on the discovery of natural antioxidants which is more safer than synthetic chemicals because they reduce the risk of developing chronic disease such as cancer [6, 7, 20].

	50 mg	25 mg	12.5 mg	6.25 mg	1 mg
Mean	100.0	100.0	100.0	93.67	90.67
Std. Deviation	0.0	0.0	0.0	1.155	0.5774

(B)

	50 mg	25 mg	12.5 mg	6.25 mg	1 mg
Mean	100.0	100.0	100.0	97.33	93.33
Std. Deviation	0.0	0.0	0.0	0.5774	0.5774

(C)

Fig. 5 The specialized screening using different screening assay, (A) The cytotoxicity using Hepatocellular carcinoma cells line of *Sargassum vulgare* extract, (B) The Total antioxidant capacity using DPPH of different marine extracts concentrations of *Sargassum*

vulgare extract, (C) The Total antioxidant capacity using ABTS of different marine extracts concentrations of *Sargassum vulgare* extract

Specialized screening bioassays

The anti-alzheimer activity of different marine extracts using acetyl cholinesterase inhibition assay

Examinations of *Sargassum vulgare* extract as anti-Alzheimer using acetyl cholinesterase inhibition assay [Fig. 4(A)] cleared out that the inhibition ratio was 76.33% at a concentration of 1 mg of the marine extract. Different concentrations examined (50 mg, 25, 12.5 and 6.25 mg) gave inhibition ratio of 100%. Restoring acetylcholine levels by inhibiting AChE has become the primary treatment for the cognitive deficits of AD. The inhibition of AChE is beneficial not only to the enhancement of

cholinergic transmission in the brain, but also to reduce the aggregation of β -amyloid and the formation of the neurotoxic fibrils in AD. In recent decades, researchers have been devoted to developing new AChE inhibitors, especially the so-called “multifunctional AChE inhibitors” with additional efficiency in vascular dementia treatment [24]. There have been plenty of phytochemicals that found to be effective in inhibiting AChE, which mainly consist of alkaloids, cannabinoids, curcumins, stilbenes, and flavonoids. Among them, flavonoids have attracted more and more interest for their high inhibitory activity and low toxicity. Moreover, their diverse activities such as anti-oxidation, inhibition on advanced glycation products, and cardio-cerebrovascular protection give them extra

advantages to be the potential multifunctional therapeutic agents for aging related diseases [25]. The highest inhibition ratio of marine sargassum extract as anti-alzheimer is in agreement with previous studies which indicated that marine natural products specifically marine seaweeds produces variety of secondary metabolites and these secondary metabolites serve as source of bioactive compounds for use in human therapies as they thrive in harsh oceanic climate [4–7]. Moreover, Natarajan et al. [26] reported that methanolic extract of *Sargassum* showed revealed strong inhibition on Cholinesterase activity. Two farnesyl acetone derivatives were isolated from the Korean brown alga *Sargassum sagamianum* showed moderate acetylcholinesterase and butyrylcholinesterase inhibitory activities. Moreover, two plastoquinones were isolated from *Sargassum sagamianum* [22].

The results of anti-cancer, anti-viral, anti-inflammatory

The anti-viral effect (anti-HIV) using reverse transcriptase inhibitor assay (RTE)

The activities of *Sargassum* extracts as anti-HIV using reverse transcriptase inhibitor assay [Fig. 4(B)] showed the highest antiviral activity (92.51%) which is in a good agreement with many previous studies [16]. Seaweeds, especially the genus *Gracilaria* and *sargassum* are the most attractive candidate that has the anti-HIV activities [23] because of its stability to achieve high yields and producing commercially valuable extracts. It has been concluded that sessile marine organism (sponge and seaweeds) contain substances capable of potent biological activity which has also been demonstrated against different types of cancer and HIV/AIDS [27]. Two major types of HIV have been identified so far, namely, HIV-1 and HIV-2. HIV-1 is the cause of the worldwide epidemic and is most commonly referred to as HIV. The basic biological processes in the HIV-1 life cycle are now well established. Natural compounds targeting specific steps in this life cycle can be found [28]. According to the different targets, anti-HIV agents are classified under several categories: (1) HIV entry inhibitors, including attachment and fusion inhibitors, and chemokine receptor antagonists [chemokine receptor subtype 5 (CCR5) and CXCR-specific receptor subtypes 4 (CXCR4) antagonists] [29]; (2) HIV reverse transcriptase (RT) inhibitors, including nucleotide RT inhibitors and non-nucleotide RT inhibitors; (3) HIV integrase (IN) inhibitors; (4) HIV protease (PR) inhibitors; and (5) other HIV inhibitors, such as maturation inhibitors. Most clinical anti-HIV drugs are HIV RT and PR inhibitors, including the various combinations known as highly active antiretroviral therapy. In the last decade (2002–2011), 132

anti-HIV natural products were obtained from marine organisms. It has been stated that more than half of anti-HIV bioactive marine natural products were derived from marine sponges [1–4].

Anti-cancer effect of Sargassum marine extract

The inhibitory activities of *Sargassum* marine extract as anticancer using SKH1 inhibition assay [Fig. 4(B)] showed that the highest activity (77.14%) was observed by the *Sargassum* marine extract. In the last two decades, cancer was the most common cause of increased mortality rate in both genders in the world. Many researchers have developed different anticancer drugs, and subsequently applied in clinical trials [28]. It is important to note that the anticancer results of the present study are matching well with previous results [28]. Recently, many researchers cleared out that seaweeds are important sources of bioactive metabolites for the pharmaceutical industry in drug development. Many of their compounds are used to treat diseases like cancer acquired immune deficiency (AIDS), inflammation, pain, arthritis as well as viral, bacterial and fungal infection [30].

The anti-inflammatory activities using COX 1, Cox2, IL6 and TNF interleukin inhibition assays

The anti-inflammatory activities of *Sargassum* marine extract using ant-COX 1 and anti-COX 2 assays [Fig. 4(B)] cleared out that the extract of *Sargassum vulgare* has the highest anti-COX1 and anti-COX2 activity of 77.89 and 88.35%; respectively. On the meantime, the anti-inflammatory activities of *Sargassum* marine extract against IL6 and TNF [Fig. 4(B)] cleared out that the anti-inflammatory activity using IL-6 inhibition was found to be 76.33%. At the same time, the *Sargassum vulgare* marine extract indicated an anti-TNF value of 71.24%. Many papers reported that the seaweeds in general and specifically *Sargassum* extract show impressive potent anti-inflammatory effect and that through different mechanism of action [31]:

1. Indirect effect through the antioxidant capacity. The sulfated polysaccharide and sulfated polyphenolic show potent antioxidant effect [32].
2. Through high contents of minerals and metals as sulfur, halide compound such as bromine, iodine and chlorine which are very unique compounds for marine habitat. This finding was confirmed in the present study as represented in Fig. 3.

Inflammatory response is normal process and is consider as one of the body defense action mechanisms to recover the damaged process occur when chemical and physical

stimuli produced. *Sargassum* extract showed direct potent anti-inflammatory activity by suppressing the activation of the NF- κ B pathway [33] and through also the effect of different bioactive compounds found in the present study in the *Sargassum vulgare* marine extract such as Carotenoid, polyphenolic, flavonoids, marine polyunsaturated fatty acids (PUFAs) [34]. Worth mentioning that the mechanism of action as anti-inflammatory in case of marine polyunsaturated fatty acids (PUFAs) is through reducing or substituting the arachidonic acid. Arachidonic acid and Eicosapentaenoic acid is a major intermediate derived from n-6 fatty acids and they competes for the same metabolic pathway. When Eicosapentaenoic acid are consumed in high levels, arachidonic acid levels are reduced and substituted by Eicosapentaenoic acid [35]. Macrophages are activated by different cytokines and stimuli produced by immune cells to induce inflammatory responses. Macrophages secrete the inflammation-mediated cytokines as IL-6 and tumor necrosis factor- α (TNF- α). The formation of these inflammatory mediators' would cause fatal consequences where arachidonic acid is changed into leukotrienes, thromboxanes, and prostaglandins through the action of cyclooxygenase (COX) and mass generation of nitric oxide (NO). Additionally, nuclear factor-kappa B (NF- κ B) is a transcription factor that controls the synthesis of different growth factor chemokines and cytokine. It has been reported that NF- κ B is responsible for the synthesizes COX-2, iNOS, and different inflammation-related cytokines [36].

In conclusion, the present study highlighted the highly significance of *Sargassum vulgare* marine extract collected from Suez Canal as reservoir of new therapeutic and tremendous agents. It revealed the importance of the biodiversity of new chemical structures with biological activities. Today seaweeds are considered to be responsible for the biosynthesis of many of these agents. The present study revealed that seaweeds from the Egyptian marine environment represent a promising source of bioactive compounds resource which needs to be biotechnologically explored.

Acknowledgements I appreciate the effort for all authors to complete this work and their valuable contributions.

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