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Biochemical and Cytotoxicity Evaluation of Marine Algae *Sargassum Wightii* and *Gracilaria Edulis* Extracts

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Abstract

The present study evaluated ethanolic extracts of two seaweed species brown (*sargassum wightii*) and red (*Gracelaria edulis*), collected from Red sea, Egypt with respect to their antimicrobial, antioxidant, and anti-inflammatory activities as well as cytotoxicity. 'The *sargassum wightii* extract showed the highest antioxidant activity (IC_{50} values were 25 and 35 µg/mL, respectively), Meanwhile, (the scavenging % value determined by DPPH method , total phenolic content were (75.49 ± 0.12 and 70.08 ± 0.34 mg Gallic acid equivalent/ gram on dry weight, respectively). Cytotoxicity assay for *S. wightii* and *G.* edulis extracts showed strong activity against tested cell lines (Caco-2 and HepG2) cell lines compared to Doxorubicin. Significant differences were also observed in anti-inflammatory activity of ethanol extracts of various investigated seaweeds used. The human red blood cells (HRBC) membrane stabilization method, results showed the high effect of *G.* edulis than *S.wightii* as anti- inflammatory in range of concentrations (100-1000) µg/mL compared with the non-steroidal anti-inflammatory drugs (NSAID) as a standard anti-inflammatory drug. The LC MS/MS analysis showed that most biological activities were refer to the phenolic content (Ellagic acid, Naringin, Carvedilol, Bromophenol, Salicyclic acid) of both tested seaweeds. From these results, it could be concluded that the prominent bioactive compounds in tested seaweeds have the potential to treat a wide range of serious diseases. The potential activities of specific bio compounds may be valuable in the pharmaceuticals, cosmeceutical, and functional foods industries.

Keywords: Antioxidant, Cytotoxicity, anti- inflammatory, LC- MS/MS

1. Introduction

The researcher workers in the phytochemical studies have a considerable widening of the scope of plant metabolites and to investigate the bioactive compounds possessed marked pharmacological action. In recent years interest in investigating the plant kingdom as a potential source of new drugs and strategies for the fraction of plant extracts based on its biological active compounds rather than on a class or group of compounds. Phytochemical investigation of marine resources involves the authentication, extraction, and characterization of isolated compounds in both qualitative and quantitative ways the assessment of their pharmacological activities. Seaweeds are marine macro-algae growing in tropical, subtropical, and shallow seawater. Depending on their pigmentation and chemical composition, they are classified as rhodophyta (red algae), pheophyta (brown algae) or chlorophyta (green algae). Seaweeds are non-toxic and provide hundreds of organic compounds. Sargassum is a kind of brown macro-algae that belongs to the family sargassaceae and is rich in carotenoids, fatty acids, vitamins and polysaccharides. The non-nutritive photo chemicals like algae carrageenan and agar materials are extensively used in the pharmaceutical,

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textile, dairy and paper industries as gelling, stabilising and thickening agents [1]. *Gracelaria edulis* red algae, belong to family Gracilariaceae, contains a high content of flavonoids, iso-flavones, polysaccharides, fibers,etc. both of which are rich in phenolic compounds that act as a main support for antioxidant activity.

Very recently, scientists worldwide have paid much attention to both qualitative and quantitative evaluation of marine organisms as alternative sources of novel metabolites with interesting biological and pharmacological activities [2] Seaweeds have the capacity to synthesise diverse secondary metabolites with a wide range of biological activities. It has been reported that brown seaweeds contain important phyto-constituents that have the immense biological activities [3-4]

The Phytochemicals of seaweeds have multiple mechanisms of action, such as antioxidant, or stimulation of the immune system, rate of metabolism, and antimicrobial effects. *Sargassum wightii* is one of the brown algae that contains enormous secondary metabolites with immense biological, pharmaceutical, and pharmacological properties [5-6]. Hence, preliminary phytochemical analysis of seaweeds will be a glorious approach to estimating the bioactive secondary constituents.

Natural antioxidants, such as polyphenols from multiple natural sources, are effective in fighting oxidative stress processes associated with multiple health disorders with fewer side effects when compared to other synthetic antioxidants [7-8]. Phenolic compounds derived from both *sargassum wightii* (brown macro-algae) and *Gracilaria edulis* (red macro-algae) exhibit a very wide range of biological activities, including antioxidants [9] anti-microbial [10] and anti-inflammatory activities among others that have attracted the attention of the scientific community [11]. Phlorotannins, polyphenols exclusively produced by brown macroalgae, have been described as powerful antioxidant compounds with multiple health benefits and applications as nutraceuticals and pharmaceuticals [12].

2. Materials and methods

2.1. Materials

Seaweeds were collected from Red sea in Egypt, All solvents and chemicals (DPPH, Methanol, Ethanol) used were HPLC grades and obtained from Sigma Chemical Co. USA.

Normal (Vero cells) human liver carcinoma; (HepG2), Colon carcinoma cell line (Caco-2) and human breast cancer cell line (MCF7), were obtained from Vacsera Vaccination Centres and preserved in the National Cancer Institute (NCI), Cairo, Egypt laboratory. Cells

2.2. Extraction procedures

Seaweeds were collected then washed by deionized water, dried on blotting paper followed by oven (40 °C) and then ground to a particle size using feature. The extraction of polyphenols was carried out using ethanol; Different methods were carried out for the extraction of polyphenols as follows: brown seaweed powder (70g) was refluxed and kept in a shaker, either in acidic ethanol (1% HCL) overnight an ultrasonic extractor was used to extract bioactive compounds from the brown algae sample for 30 min. After extraction, the mixture was collected and the ethanol was removed at 45 °C using a rotary vacuum evaporator [13].

2.3. **Methods of Analysis**

2.3.1 Total Phenolic Content (TPC)

According to [14] the Folin-Ciocalteau colorimetric technique is used to determine the TPC of *Sargassum wightii* and *Gracilaria edulis* ethanol extracts. The results were determined and represented as mg gallic acid equivalents (mg GAE/g extract).

2.3.2 Antioxidant activity

2.3.3. DPPH free radical scavenging assay

Sargassum wightii and *Gracilaria edulis* extracts at different concentrations (25, 50, 100, 200, 400, 800, and 1000 µg/ml) were tested for their ability to inhibit free radicals using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay according to [15] .Ascorbic acid was used as a standard. The IC_{50} of all extracts was calculated. The DPPH scavenging activity (%) was calculated as inhibition percent by using the following equation:

% Inhibition = [(A control – A sample) /A control] x 100%.

Where:

A control represents the absorbance of the control at zero time.

A sample represents the absorbance of the samples after 30 minutes.

2.3.4 In vitro cytotoxicity activity.

Cytotoxicity of *Sargassum wightii* and *Gracilaria edulis* ethanol extracts was determined by viability assay (MTT protocol) according to [16] and carried out in Faculty of Science, Al-Azhar University, Egypt.

In vitro cell line Propagation:

Using 4 cell lines; normal (Vero cells) human liver carcinoma; HepG2), Caco-2 (Colon carcinoma cell line) and human breast cancer cell line (MCF7).

The 96 well tissue culture plate was injected with 1 X 10^5 cells/ml (100ul/well), which were treated with different concentrations (31.25, 62.5, 125, 250, 500, and 1000 µg/ml) of tested seaweed ethanol extracts and the drug doxorubicin (as a standard) at different

Concentrations (31.25, 62.5, 125, 250, 500, and 1000 µg/ml) separately and incubated in 5% CO_2 at 37°C for 24 hours. After adding 0.5 mg/ml MTT, the plate was incubated for 4 hours at 37°C with 5% CO_2. Formazan (MTT metabolic product) should be resuspended in DMSO. At 570 nm, the absorbance was measured to evaluate the number of viable cells and the concentrations required to kill 50% of cancer cells (IC_{50}) were calculated. By using the following formula, the percentage of cell viability was calculated:

Cell viability (%) = [(Control OD – Sample OD)/Control OD] X 100

2.3.5. Anti-inflammatory effects

The anti-inflammatory effects were carried out by using the human red blood cell (HRBC) membrane stabilization method in the Regional Centre for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt, using hypotonic solution-induced erythrocyte haemolysis. The tested sample consisted of stock erythrocyte (RBCs) suspension (0.50 ml) mixed with 5 ml of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing the extract (1000-7.81 µg/ml) or indomethacin. The control sample consisted of 0.5 ml of RBC mixed with a hypotonic-buffered saline solution alone. The mixtures were incubated for 10 min at room temperature and centrifuged for 10 min at 3000 g. In 96 well plates, the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of haemolysis or membrane stabilization was calculated according to the modified method described by [17] and assuming the haemolysis produced in the presence of distilled water was 100%. The present inhibition of haemolysis by the extract was calculated using the following equation:

% Inhibition of haemolysis= 1-((OD2-OD1)/ (OD3-OD1)) *100

Where OD1 = absorbance of test sample in isotonic solution

OD2 = absorbance of test sample in hypotonic solution

OD3 = absorbance of control sample in hypotonic solution

The IC_{50} value was defined as the required concentration of the sample to inhibit 50% RBCs haemolysis under the assay conditions. All determinations were carried out in triplicate manner.

2.3.6 Identification of polyphenols

The analysis of the sample was performed using liquid chromatography–Electrospray Ionization–Tandem Mass Spectrometry (LC-ESI-MS/MS) with an ExionLC AC system for separation and SCIEX Triple Quad 5500+ MS/MS system equipped with electrospray ionization (ESI) for detection [18].

Negative mode:

The separation was performed with a Ascentis® C18 Column (4.6×150 mm, 3 µm). The mobile phase adopted for the separation of antioxidant compounds was consisted of two eluents (A): 0.1% formic acid; (B): acetonitrile (LC grade). The following elution gradient was used for separation: 10 % (B) at 0-20 min, 10-90 % (B) from 20-30 min, 90% (B) from 30-35 min, 10% at 36.1, and 10% from 30-45 min. the flow rate was 0.7 ml/min and the injection volume was 10 µl. For MS/MS analysis, negative ionization mode was applied with a scan (EMS-IDA-EPI) from 100 to 1000 Da for MS1 with the following parameters: curtain gas: 25 psi; Ion Spray voltage: -4500; source temperature: 500°C; ion source gas 1 & 2 were 45 psi and from 50 to 1000 Da for MS2 with a declustering potential: -80; collision energy: -35; collision energy spread: 15. Compounds' identification was performed using MS-DIAL software version 4.70.

2.3.7. Statistical Analysis

Numerical data is presented as means ± S.D., and the obtained data was analysed using a one-way analysis of variance (ANOVA). For determining differences between groups, the Duncan test was used. All p values < 0.05 were significant [19].

3. Results and discussion.

3.1. Total phenolic contents of selected seaweeds

Phenolic compounds, have gained particular attention in the human diet because of their specific bioactivities. Due to the well-known antioxidant properties of brown seaweeds; current research focused on finding newer sources for phenolics. Brown and red seaweed phenolic content, are shown in (Fig.1). From these results it could be concluded that seaweed are rich in phenols the *S.wightii* ethanol extract has the higher TPC than *G. edulis* where it reached (75.49 ± 0.12) mg GAE/g, while *G.edulis* recorded (70.08 ± 0.34) mg GAE/g extract. These results are in agreement with [20] who mentioned that brown macro-algae are well known to be rich in polyphenol compounds, which potentially contribute to its antioxidant capacity. Also, are in the line with [21] who mentioned that the higher content of phenols. Was in *S.wightii* (brown algae) compared to *G.edulis* (red algae).

3.2. Antioxidant activity

3.2.1. DPPH free radical scavenging assay

The antioxidant activity of the seaweed ethanol extracts was evaluated using *in-vitro* DPPH radical scavenging activity compared to the IC_{50} of Ascorbic acid as standard. The antioxidant activity of *S.wightii* exhibited good antioxidant activity when compared to *G.edulis*, were shown in Table (1). From the

results, it could be observed that the IC_{50} value was found to be 25 ppm and 35 ppm, respectively, which indicates that the antioxidant activity of brown and red is relatively strong [22]. Also Thanh T. Dang [23] confirmed that earlier studies reported that brown seaweeds have generally higher DPPH radical scavenging than red seaweeds. With agreement of present study the extract of *S.wightii* recorded a high antioxidant activity 51.90^{a}±0.14 while *G. edulis* reached antioxidant activity 48.9^{a}± 0.14.

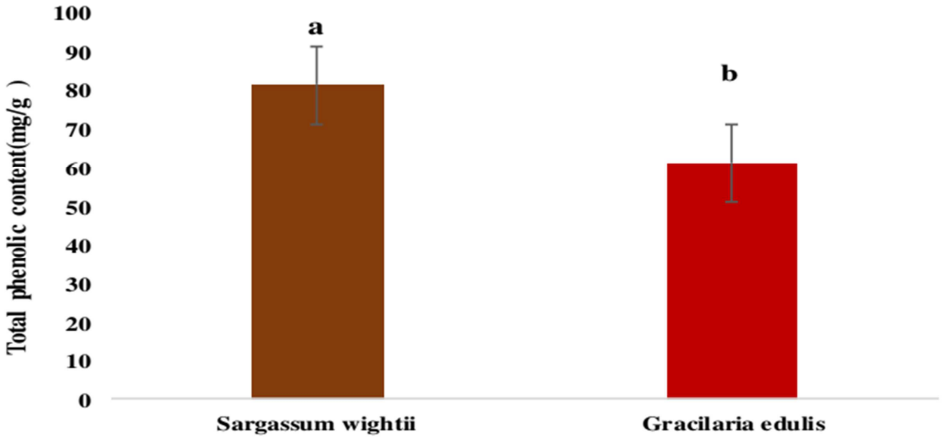

Figure (1): Total phenolic content of *Sargassum wightii* and *Gracilaria edulis* [a,b]The values in small letters above bars with differing superscripts indicate a significant difference P < 0.05

Table (1): Antioxidant activity (%) at different concentrations of tested seaweeds extracts determined by DPPH free radical scavenging

Concentrations (ppm)	*Sargassum wightii*	*Gracilaria edulis*
100	44.66^{c}± 2.63	41.55^{d}± 0.09
150	50.37^{ab}± 0.23	44.38^{c}± 0.14
200	51.90^{a}± 0.14	45.61^{b}± 0.09
250	45.77^{c}± 0.14	48.9^{a}± 0.14
IC_{50} of DPPH (ppm)	25.12	35.05
LSD 5%	1.93	0.42

The data is provided as mean ± SD (n=3). The mean values within the same column with different superscript letters are significantly different at P ≤ 0.05, The IC_{50} values were determined from dose – effect curves by linea

3.3. *In vitro* anti-inflammatory activities

The chronic or acute inflammation was caused by lysosomal enzyme and resulted in several health disorders. Many researchers worked on the human red blood cells (HRBC) membrane to determine the stability of extracts to predict the anti-inflammatory activity, because the lysosomal membrane is similar to erythrocyte membrane [24].

Table (2): Membrane stabilization (%) for *S.wightii* and *G. edulis*

Concentrations (µg/mL)	*Gracilaria edulis*	*Sargassum wightii*
100	24.6^{a}	16.1^{b}
200	28.3^{a}	20.2^{b}
400	38.4^{a}	27.6^{b}
600	55.2^{a}	36.7^{b}
800	71.4^{a}	47.8^{b}
1000	89.6^{a}	65.2^{b}

The mean values within the same column with different superscript letters are significantly different at P ≤ 0.05,

Flavonoids like quercetin have effective acute inflammatory inhibitory activity due to degrading of several enzymes like protein tyrosine kinases, proteinkinase C, phosphodiesterases, and phospholipase A2 [25]. The HRBC membrane stability of seaweed extracts were demonstrated in Table 2. It was depicted that maximum inhibition was 89.6 and 65.2 % at a concentration of 1000 µg/ml for ethanol extract of *S.wightii* and *G.edulis* extracts, respectively.

3.4. *In vitro* cytotoxicity assay.

The cytotoxicity activity of the selected seaweed extracts was tested using the MTT protocol against three cancer cell lines : human liver carcinoma; HepG2), $Caco_2$ (Colon carcinoma cell line) and human breast cancer cell line (MCF7), compared to untreated cells (normal cell line -Vero) isolated from the kidney of African green monkey and drug doxorubicin as a positive control (DOX) at different concentrations (3.125, 6.25, 12.5, 25, 50, and 100 µg/ml, In (Table 3 and Fig.2). The results revealed that the two ethanol seaweed extracts had a strong activity against Caco2 and McF7. This was in accordance with [26] with IC_{50} (4.32±0.23, 3.21±0.14) µg/m. of *S.wightii* at concentration 3.125, 6.25 µg/ml while the *G.edulis* with IC_{50} (4.94±0.18, 2.95±0.14) µg/ml when compared with Vero cells with IC_{50} (10.79±0.17) µg/m in *S.wightii* and IC_{50} (15.15±0.62) in *G.edulis* respectively, but it was found that both seaweeds haven't effect on HepG2 because they recorded high IC_{50} than Vero (normal cell). Where the results showed the following, in case of *S.wightii* IC_{50} of HepG2 was (14.11±0.17) and Vero (10.79±0.17) on the other hand IC_{50} of HepG2 in *G.edulis* (50.02±2.23) µg/m also higher than IC_{50} of Vero(15.15±0.62) µg/m. However, when comparing the Doxorubicin as a reference for the same cell lines (in Table 3), Therefore, the seaweed extracts have the same effect as the Doxorubicin on cell lines Caco-2 and McF7 with IC_{50} (5.22±0.04 and 2.93±0.18) compared with IC_{50} of Vero (11.21±0.29) µg/m, *Such* finding is in the same line of [27] who explained the reason of anticancer activity referring to the presence of Cinnamic acid.

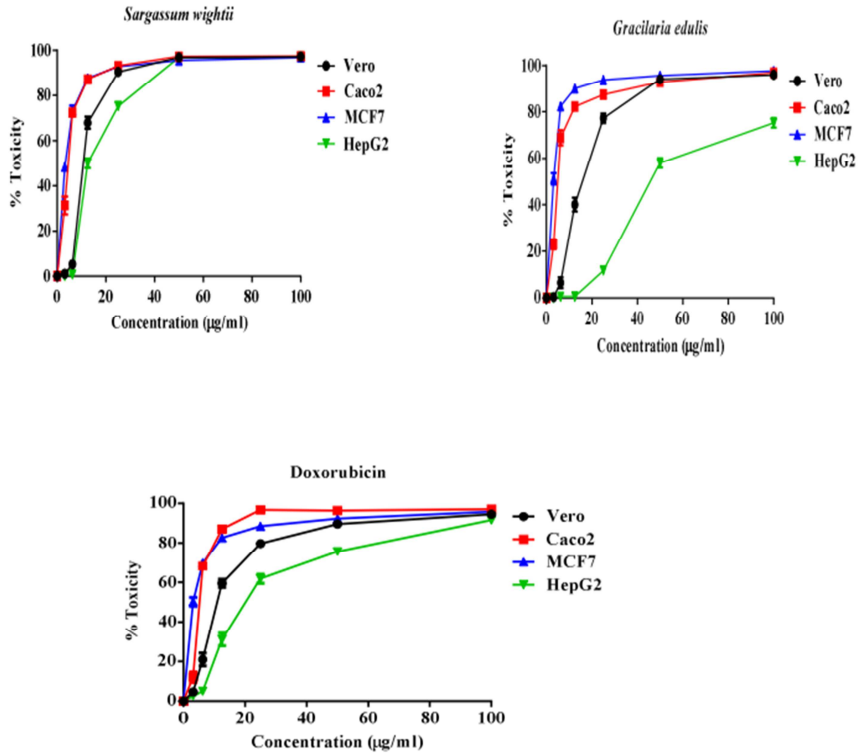

Figure 2: Inhibition ratios % (IC_{50}) of different cancer cell lines and Vero cells with tested algae and Doxorubicin at different Concentration

Table (3): Toxicity percentage of ethanol extracts of *S. wightii* and *G.edulis* on Caco.2(human Colon carcinoma cell line) and human breast cancer cell line (MCF7and human liver carcinoma cell line (HepG2)

Concentration (µg/ml)	100	50	25	12.5	6.25	3.125	IC_{50} (µg/ml)
	***Sargassum wightii* (% Toxicity)**						
Caco2	97.55±0.23^{a}	97.35±0.23^{a}	93.24±0.64^{b}	87.24±1.38^{a}	72.47±1.00^{a}	31.33±3.96^{a}	4.32±0.23^{a}
HepG2	97.29±0.08^{a}	96.99±0.17^{a}	75.26±1.04^{a}	48.00±2.11^{a}	0.72±1.37^{b}	0.24±0.05^{b}	14.11±0.17^{b}
McF7	96.95±0.09^{b}	95.58±0.37^{a}	92.86±0.37^{b}	87.70±1.17^{a}	73.20±2.31^{b}	48.27±1.76^{a}	3.21±0.14^{a}
Vero	97.33±0.29^{a}	96.99±0.17^{a}	90.57±1.06^{a}	67.94±2.69^{a}	5.23±1.51^{b}	1.00±1.32^{b}	10.79±0.17^{b}
	***Gracilaria edulis* (% Toxicity)**						
Caco2	96.90±0.31^{b}	92.79±0.42^{c}	87.34±1.21^{c}	82.28±1.70^{b}	68.87±3.27^{a}	22.97±2.22^{b}	4.94±0.18^{a}
HepG2	75.26±2.08^{c}	58.07±2.13^{c}	11.68±1.26^{c}	0.60±1.07^{c}	0.52±0.45^{b}	0.08±1.41^{b}	50.02±2.23^{a}
McF7	97.62±0.20^{a}	95.65±0.46^{a}	93.77±0.74^{a}	90.04±0.92^{a}	82.40±1.20^{a}	51.13±2.52^{a}	2.95±0.14^{a}
Vero	96.08±0.35^{b}	94.07±0.35^{b}	77.29±2.08^{b}	40.12±3.03^{c}	6.46±2.36^{b}	0.33±1.26^{ab}	15.15±0.62^{a}
	Doxorubicin (% Toxicity)						
Caco2	96.93±0.11^{b}	96.30±0.38^{b}	96.63+0.32^{a}	86.94±1.38^{a}	68.17±1.70^{a}	12.11±2.71^{c}	5.22±0.04^{a}
HepG2	91.51±0.77^{b}	75.58±1.12^{b}	61.85±2.28^{b}	31.01±3.20^{b}	4.94±1.05^{a}	2.73±1.47^{a}	21.16±1.23^{b}
McF7	95.78±0.09^{c}	92.21±0.45^{b}	88.44±0.55^{c}	82.60+1.63^{b}	69.70±1.78^{c}	50.22±2.15^{a}	2.93±0.18^{a}
Vero	94.49±0.24^{c}	89.56±1.07^{c}	79.63±0.95^{b}	59.54±2.12^{b}	20.98±3.13^{a}	4.68±1.26^{a}	11.21±0.29^{b}

Mean ± SD: Mean of triplicate determinations ± standard division. The IC_{50} values were determined from dose – effect curves by linear regression

3.5: Identification of Bioactive components fraction using LC MS/MS.

LC-MS / MS have been widely used for the characterization of the phenolic profiles of different plant and marine samples to evaluate their potential application as functional ingredients [28]. A qualitative analysis of the phenolic compounds from different seaweed extracts was achieved by LC-ESI-MS/MS analysis in negative ionization mode (Tables 4-5). Phenolic compounds present in two different seaweeds were tentatively identified from their m/z values. From Fig's (3, 4) some of these compounds were tentatively identified and their characteristics are shown in Tables (3-4), 24 phenolic compounds, including 19 phenolic acids, 3 flavonoids, followed by 2 Iso flavones were detected. Phenolic acids have been reported as the most abundant phenolic compounds in red, green and brown algae [29]. In the present work, four sub-classes of phenolic acids were detected, including hydroxybenzoic acids, hydroxycinnamic acids, hydroxyphenylpentanoic acids and hydroxyphenylacetic acids. The presence of hydroxybenzoic acid and other phenolic acids in seaweeds was reported by, [30] who evaluated the phenolic composition of selected algae species, including brown seaweeds. In addition, [31] also detected peaks corresponding to Gallic acid 4-O-glucoside which were identified as hydroxyl-benzoic acid derivatives and presented peak5 (RT 8.25 min) molecular ion of m/z 331.09 This compound has been reported to have therapeutic activities in gastrointestinal, neuropsychological, metabolic, and cardiovascular disorders [32]. Also, some compounds, or more correctly phenolic acids, have been identified that have a therapeutic effect and important biological effects for human health, such as Chlorogenic acid identified at peak 28 (RT 20.79), molecular ion of m/z 353.20, peak 8 (RT1.63 min) at [M − H]− m/z 244.89 was tentatively identified as Sinapic acid and peak 11 was suggested as Ferulic acid at (RT11.50 min) with m/z (194.05) respectively , The presence of such compounds in brown algae extract enhances its value, especially, Ferulic acid is relatively non-toxic and exhibits a wide range of physiological properties, such as anti-inflammatory, antibacterial, anti-cancer (including lung, breast, colon, and skin cancer), anti-arrhythmic, and antithrombotic activity [33].

The suggested compound for P19 (RT18.65 min) with high peak area (8.01 %) was rosmanol, the appearance of these compounds in brown macro algae is consistent with [34]. Fucophlorethol is one of the important and distinctive compounds of algae presented at peak 22 (RT 12.23) with (m/z 623.03), it is derived from fucoxanthin, xanthophyll component and is known as a secondary metabolite produced in brown seaweed chloroplasts [35]. The compound has been widely investigated due to its tremendous biological protective properties for human health and has been industrially produced from seaweeds. *S.wightii* extract is still the richest in containing various compounds, which have an important role for human health in terms of food and medicine.

On the other hand, *G.edulis* extract contains more flavonoids than *S.wightii*. For example, the compound of peak 13 (RT 12.90) was tentatively identified as Kaempherol with (m/z 286.15) and peak 3 act as Naringin at (RT 4.81) with molecular ion (m/z 580.99). It was also noted that Kaempherol has anti-inflammatory and antioxidant activity [36],

Also, naringin has shown potential to become an alternative as traditional anti-inflammatory drugs, because it exerts fewer side effects than chemically synthesized compounds [37]. Both of them have a considerable % area (8.95 and 4.35), respectively. Peak 11 (RT 11.82 min) data is suggested as a quinic acid derivative as probable compound due to the parention of m/z 398.05 and fragments of ([quinic acid − H]−). These results are in the same line of [36]. The isoflavone peak 24 (RT 17.54) proposed as Diadzein with (m/z 251.12) contributes to the fact that the *G.edulis* extract has anti-inflammatory activity compared to *S.wightii* extract this has been previously experimentally proven.

Finally, there were some peaks for both seaweed extracts that could not be identified (unknown compounds) based on the mass spectrum Similar difficulties for the identification of phenolic compounds were reported by other Researchers [38]; in the characterization of the phenolic composition of several vegetable matrices.

Table 4: LC -MS/MS Characterization of The main components of *sargassum wightii*

Peak NO.	Proposed compounds (Identification)	RT (min)	(%) Peak Area	Molecular formula
1	Coumaric acid	1.91	3.144	$C_9H_8O_3$
2	6,7-Dihydroxycoumarin	5.98	0.918	$C_9H_6O_4$
4	Salicyclic acid	7.30	0.800	$C_7H_6O_3$
5	Gallic acid 4-O-glucoside	8.25	2.80	$C_{13}H_{16}O_{10}$
7	4-Isopropylbenzoic acid	10.32	0.285	$C_{10}H_{12}O_2$
8	Bromophenol	10.28	0.293	$C_{19}H_{10}Br_4O_5S$
9	Genistein	11.36	0.478	$C_{15}H_{10}O_5$
10	Protocatechuic acid 4-O-glucoside	11.98	0.319	$C_{13}H_{16}O_9$
11	Ferulic acid	11.50	2. 079	$C_{10}H_{10}O_4$
12	Sinapic acid	11.68	1.649	$C_{11}H_{12}O_5$
14	Syringetin-3-O-galactoside	14.83	5.46	$C_{23}H_{24}O_{13}$
15	3,4-Dihydroxyphenylglycol	15.73	1.44	$C_8H_{10}O_4$
19	Rosmanol	18.65	8.01	$C_{20}H_{26}O_5$
20	Ellagic acid	18.81	4.91	$C_{14}H_6O_8$
22	Fucophlorethol	12.23	12.912	$C_{30}H_{22}H_{15}$
27	Gallocatechin	22.41	1.544	$C_{15}H_{14}O_7$
28	Chlorogenic acid	20.79	0.736	$C_{16}H_{18}O_9$
30	Licoflavone B	25.11	3.64	$C_{25}H_{26}O_4$

Compound was detected in more than one seaweed samples, data presented in this Table were detected in negative [M- H] - mode of ionization while only single mode data was presented. RT = stands for "retention time"

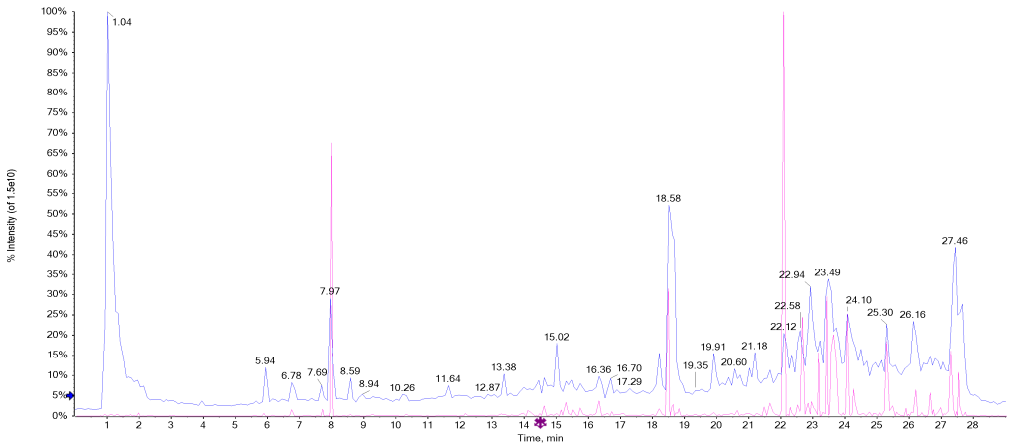

Figure 3: Representative chromatogram of phenolic compounds from *S.wightii* ethanol extract (S.W.E) obtained by LC (MS/MS

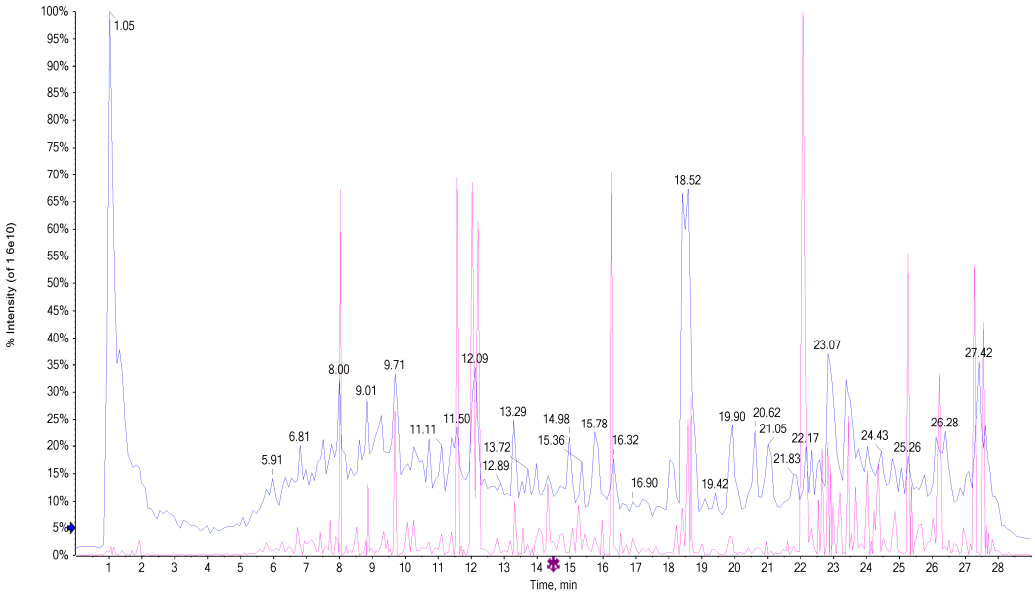

Figure 4: Representative Chromatogram of phenolic compounds from *G.edulis* ethanol extract (G.E.E) obtained by LC (MS/MS)

Table 5: LC -MS/MS Characterization fractions of the main components of *Gracilaria edulis*

Peak NO.	Proposed compounds (Identification)	RT (min)	(%) Peak Area	Molecular formula
1	Coumaric acid	1.91	2.18	$C_9H_8O_3$
2	Protocatechuic acid	4.16	0.311	$C_7H_6O_4$
3	Naringin	4.81	4.35	$C_{27}H_{32}O_{14}$
5	Benzoic acid	2.64	0.712	$C_7H_6O_2$
11	3-Sinapoylquinic acid	11.82	10.21	$C_{18}H_{22}O_{10}$
13	Kaempferol	12.90	8.95	$C_{15}H_{10}O_6$
24	Daidzein	17.54	6.27	$C_{15}H_{10}O_4$
26	Ellagic acid	18.81	1.46	$C_{14}H_6O_8$

Conclusion

A crude natural product extract is, in general, a highly complicated blend of numerous compounds possessing variable chemical and physical properties and habitually having opposing pharmaceutical properties. It is interesting that among the screened seaweeds, *S. wightii* and *G. edulis* ethanol extracts exhibit effective anticancer and anti-inflammatory activity and seem to have low toxicity in normal cells. Further investigations are required to isolate, characterise, compound, and it can be implicated as an effective therapeutic tool against liver and caco2.

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