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Enteromorpha Compressa, *Gelidium pulchrum* Macro algae Exhibit Potent Anticancer, Antioxidant and anti-inflammatory Activities

Faten Abou-Elella[1] , Mahgoub M Ahmed [2]

*Corresponding author:

Faten Abou-Elella

[1]Biochemistry Department Faculty of Agriculture Cairo University - 12613 Giza, Egypt
[2]Molecular Drug Evaluation Department, National Organization for Drug Control and Research, 12613 Giza , Egypt

Abstract

Over the past several decades, algae and their extracts have generated an enormous amount of interest in the pharmaceutical industry as a fresh source of bioactive compounds. The aim of this work was to evaluate anticancer, antioxidant and anti-inflammatory properties of absolute and aqueous (50%) methanol extracts of both *Gelidium pulchrum* and *Enteromorpha compressa* algae. *In vitro* cytotoxicity of the extracts was tested by MTT and trypan blue technique against two malignant cell lines (HeLa and EACC). The effect of methanol extracts on caspase-3 and Bax gene protein expression levels was evaluated as indicator of apoptosis induction in treated- Hela cells. The antioxidant activity was evaluated using three methods,(DPPH, Reducing power and ABTS) andanti-inflammatory activity measured by two systems (*in vivo and in vitro*). The obtained results showed that all studied extracts expressed significant *in vitro* cytotoxic activity toward HeLa, and EACC cell lines. The data revealed up regulation of both Bax and caspase-3 protein expressions levels in treated-HeLa cell line. In DPPH assay aqueous MeOH extracts of *Gelidium pulchrum* exhibit maximum inhibition (IC50= 51±2.3). The highest reducing power was 179.3±4.7μg/mL GAE in aqueous MeOH extract of *Gelidium pulchrum*. The data also showed that all tested extracts have anti-nflammatory activity against carrgeenan
induced paw oedema. While absolute MeOH extract of *Gelidium pulchrum* has maximum protection (71.7±1.7 %) in membrane stabilization test. Data obtained indicate the potential of these algal extracts for the antitumor through induction of apoptosis in cancer cells in addition to, the antioxidant and anti-inflammatory actions. This biological action is interesting for further isolation and identification of new natural anticancer and antioxidant compounds.

Keywords: *Gelidium pulchrum*,*Enteromorpha compressa,* Anticancer, Apoptosis, Antioxidant and Anti- inflammatory Activities

Introduction

Cancer is a dreadful human disease, increasing with changing of life style, nutrition, and global warming. Cancer treatments do not have potent medicine as the currently available drugs are causing side effects in some instances. In this context, the natural products derived from medicinal plants have gained significance in the treatment of cancer. Natural products and their derivatives represent more than aqueous of all the drugs in clinical use of the world.

For many generations, marine algae have been extensively used as food, organic fertilizers and traditionally medicine. The high protein, lipid, and vitamin content of marine algae have encouraged their cultivation and use as a food source in many parts of the world [1[. They are rich source of pharmacologically active metabolites 2,4 having antimicrobial, antineoplastic, antiviral anti-inflammatory and immunostimulant activities [5-7].They were not exploited so far for their antiproliferating properties till today. Only few reports are available regarding the anticancer activities of algae. *Spirulina, Anabaena* and *Aphanizomenon,* members of *Cyanophyceae* (blue green algae) were reported to induce apoptosis in HL-60 and MCF7 cell lines [8-10]. According to our knowledge reports regarding the anticancer properties of *Gelidium pulchrum* (red algae) *Enteromorpha compressa* (green algae) are spare

It is now well recognized that apoptosis is a mode of cell death used by multi-cellular organisms to eradicate cells in diverse physiological and pathological settings. Recent evidence also shows that suppression of apoptosis by tumor promoting agents in pre-eoplastic cells is an important mechanism in tumor promotion11. In this context, it is noteworthy that apoptosis-inducing ability seems to have become a primary factor in considering the efficacy of chemopreventive agents. Among the positive and negative regulators of apoptosis, p53, the tumor suppressor gene, is an important defense against cancer as it suppresses tumor growth through two mechanisms, cell cycle arrest and apoptosis [12-14]. Bax, the pro-apoptotic member of Bcl-2 family, is a p53 target and is transactivated in a number of

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systems during p53-mediated apoptosis [15]. The up-regulation of Bax expression and down regulation of Bcl-2 has been well demonstrated during apoptosis[16]. All these reports indicate that a cross talk between these pro- and anti-apoptotic proteins is one of the important factors deciding the fate of a cell. One set of molecules that appear to be modulated by the presence of Bax are the caspases [17,18]. Caspases, a family of cysteine proteases implicated in the commitment and execution of apoptotic cell death, exist as proenzymes until cleaved in response to apoptotic stimuli [19]. However, the role of algaein regulating the balance between these pro- and anti-apoptotic factors in tumor cells is not yet revealed. The objective of this the present study, is evaluated the cytotoxic potential of methanol extracts of two algae *Gelidium pulchrum* (red algae) and *Enteromorpha compressa* (green algae) against two cancer cell lines (EACC and cervical cancer cell line (HeLa). Caspase-3 and Bax expressions were studied to investigate their role in inducing cell death of HeLa cell line. Also total phenols, flavonoids, antioxidant and anti-inflammatory activities of methanol extracts of *Gelidium pulchrum* (red algae) and *Enteromorpha compressa* were evaluated.

Materials and Methods

Chemicals

All chemicals used were of the highest analytical grade.

Algal Collection and authenticated

Enteromorpha compressa and *Gelidium pulchrum* were harvested in April from Abu Quir bay at Alexandria-Egypt during 2014. Adhered sand was removed from the algae by washing with seawater and the material was transported to the laboratory of Biochemistry Department,Faculty of Agriculture, Cairo University. A small amount of algal sample was preserved in the Herbarium and in 4% formalin for subsequent formal identification by Dr. S. Shanab (Prof. of Botany, Faculty of Science). Algae were thoroughly washed sequentially with tap water and distilled water then allowed to air dry at 25±◦C away from direct light. The dried material was ground (using electric morter, moulinex) to a fine powder and transferred to labeled brown bottles until required.

Extraction

Twenty fifty grams of each *Enteromorpha compressa* green algae and *Gelidium pulchrum* red algae were extracted with 250 ml both of absolute methanol (100%) and aqueous methanol(50%).. Each extract was evaporated using a rotary vacuum evaporator at 45˚C. The obtained extracts were kept in light-protected containers at 18 C until use.

Measurement of Total Phenolic Content

The total phenolics content of four methanol extracts was determined with the Folin-Ciocalteu reagent [20] . Gallic acid standards were prepared using various weights (0-500 µg). To 50 µl of each sample (three replicates), 2.5 ml diluted Folin-Ciocalteau's reagent (1/100) and 2 ml of Na2CO3 (7.5%, w/v) were mixed and incubated at 45oC for 15 min. The absorbance of all samples was measured at 765 nm using a UV–Vis spectrophotometer (GAT UV-9100). A standard curve was plotted using different concentrations of gallic acid and the results were expressed as gallic acid equivalent.

Measurement of Total Flavonoids Content

The total flavonoid contents of the methanol extracts was evaluated [21]. Rutin standards were prepared (10-100µM). A standard curve was plotted using different concentrations of rutin and the results were expressed as rutin .

Viability of Ehrlich Ascites Carcinoma Cells (EACC).

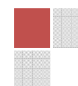
A line of Ehrlich Ascities Carcinoma was obtained from National Cancer Institute (NCI) Cairo, Egypt. The tumor cell line was maintained in female Swiss albino mice by weekly intraperitoneal (ip) transplantation of 2.5 106 cells/ mouse.
Cancer cells (2x 104 /ml) were incubated with various tested methanol extracts (50, 100 and 200µg /ml) as control for 2 h then the viability was determined by the modified cytotoxic trypan blue-exclusion technique [22].

MTT Assay

The culture medium was prepared using RPMI 1640 media with 1.2 g/l sodium carbonate and L-glutamine (Gibco, Grand Island, USA), 10% inactivated fetal bovine serum (Gibco), and 100 units/ ml penicillin and 100 mg/ ml streptomycin were added. The anticancer effect of the different 4 methanol extracts on HeLa cell line was determined by the MTT assay [23]. Cells at exponentially growing phase were used. Five thousand cells per well (100 µl) were plated in 96-well plates in the presence of various concentrations of the extracts (50, 100 and 200µg /ml) for 24h at 37 C in 5% CO2 incubator. The activity of mitochondrial succinic dehydrogenase was measured by incubation for 4 h in the presence of 0.5 mg/ml of MTT (3-(4,5-dimethylthiozol-2-yl)-2,5-diphenyltetrazolium bromide). Absorbance reflects the viable cell number and was measured at 570 nm. Percentage of Cell death was calculated using the following formulas24.
% Cell death = (Control OD –Sample OD)/Control OD x 100.

Bax and Caspase-3 expressions analysis

Protein expression level of Bax was measured in HeLa cell line exposed to methanol extracts of *Enteromorpha compressa* and *Gelidium pulchrum* (100µg/mL)/24h using Abcam's Human Bax *in vitro* ELISA kit, while protein expression level of Caspase-3 of treated-HeLa cell line was measured using CASP3 ELISA kit. Concentrations of Caspase 3 and Bax were determined and analyzed according to the manufacturer's protocols (Wkea Med Supplies Corp, China and Sun Red, Biotechnology Company, China, respectively).



Measurement of DPPH free-radical Scavenging Activity

DPPH radical scavenging activity of methanol extracts were evaluated [25]. The reaction mixture contained (50 and 100 µg/mL) of test samples (or 80% MeOH as a blank) and 5 ml of a 0.04% (w/v) solution of DPPH in methanol. Standard antioxidants butylated hydroxytoluene (BHT) was used as a positive control. Discoloration was measured at 517 nm after incubation for 30. Measurements were performed in triplicate.

Determination of Reducing Power

The reducing power of all methanol extracts was determined [26]. 100 µg/mL were added to 1mL of distilled water and mixed with phosphate buffer (2.5 mL, 36 0.2 mol/ L, pH 6.6) and potassium ferricyanide [K3 Fe(CN)6] (2.5 mL, 1%). The mixture was incubated at 50 Co for 20 min. 2.5 ml of trichloroacetic acid (TCA, 10%) were added to the mixture, which was then centrifuged at 3000 rpm (MSE Mistral 2000, UK) for 10 min. The reaction was initiated by the addition 200µl FeCl3 (0.1%) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The value is expressed as gallic acid equivalent (GAE).

ABTS assay

The antioxidant activity of the methanol extracts was also measured by ABTS assay [27] with some modification. ABTS•+ was produced by reacting 7 mmol/L ABTS aqueous solution with 2.45 mmol/L potassium persulphate in darkness for 12-16 hours at room temperature. Prior to assay, this solution was diluted in aqueous and equilibrated at 30oC. Sample of 0.2 mL was mixed with 3.0 mL of diluted ABTS cation radical solution. The absorbance at 734 nm was measured using spectrophotometer. The percentage inhibition was calculated of the control absorbance at 734 nm.

Anti-inflammatory activity by Carrageenan test

Thirty six male adult rats weighing 120-135 g maintained on balanced diet were fasted for 16 hours starting the experimental and divided into 6 groups each comprised of six rats. Before any treatment, the thickness of the back paw of each animal was determined using Vernier calipers (Italy). Samples were prepared by suspending them in 0.5% carboxymethyl cellulose (CMC) solution. Rats were administered orally with samples at dose 100 mg/kg, while control group received 10 mg/kg of 0.5% CMC. Indomethasin (10mg/kg) was used as standard. One hour after the administrations, each rat received in its right back paw a subplanter injection of 1% carrageenan suspension (0.1 mL / animal)28. The thickness of the back paw of each rat was measured at 1 till 4 hours after the injection of the inflammatory agent. The results were presented as the paw thickness (mm). The anti-inflammatory potency of all treatments was determined relative to (%) animals receiving 1% carrageenan solution.

The HRBC membrane stabilization assay

HRBC membrane Stabilization assay was performed [29]. Assay system consisted of 100 µg\mL of the test methanol extract, 1 mL phosphate buffer (0.15 M, pH 7.4), 2 mLof hyposaline (0.36%) and 0.5 ml of HRBC suspension. Standard was prepared by using Diclofenac sodium (50mg/mL). Control consisted of distilled water instead of hyposaline. The assay systems were incubated at 37oC for 30 min and centrifuged at 3000 rpm for 20 min. The hemoglobin (Hb) content of the supernatant solution was estimated spectrophotometrically at 560 nm. The percent age stabilization of HRBC membrane was calculated by using the formula:
Absorbance control-absorbance sample/ absorbance control X 100

Statistical Analysis

The direction and magnitude of correlation between variables were done using analysis of variance (ANOVA) and quantified by the correlation. The P-values less than 0.05 were considered statistically significant.

Results

Total phenolic compounds (Y= 0.002 X + 0.004 at R2 = 0.887)
Table 1 recorded the total phenols concentration of aqueous and absolute methanol extracts of both *Gelidium pulchrum* and *Enteromorpha compressa* algae. Total phenolics in the aqueous and absolute methanol extracts of *Gelidium pulchrum* was computed to be (281.4±8.4 and 107.5±5.5) µg/mL as GAE respectively while it was 73.5±4.3 and74.0±4.0 µg/mL as GAE in the aqueous and absolute methanol extracts of *Enteromorpha compressa*.
Total flavonoids compounds (Y = 0.001x + 0.056 at R = 0.99)
Table 1 recorded Total flavonoids in the aqueous and absolute methanol extracts of *Gelidium pulchrum* and *Enteromorpha compressa.* Total flavonoids was computed to be (2219.0±14.4 and 8679.0±2.9) µM rutin equivalents in *Gelidium pulchrum* methanol extracts while it was (8097.3±294.2, 338.0±1.7) µM rutin in the aqueous and absolute methanol extracts of *Enteromorpha compressa* .

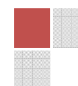


Table 1 Total phenolic and total flavonoids contents

Algal Methanol extracts	Total phenolic as GAE (µg/ml)	Total Flavonoids as rutin (µM)
Aqueous MeOH *Gelidium P*	281.4±8.4	2219.0±14.4
Absolute MeOH *Gelidium P*	107.5±5.5	8679.0±2.9
Aqueous MeOH *Enteromorpha C*	73.5±4.3	8097.3±294.2
Absolute MeOH *Enteromorpha C*	74.0±4.0	338.0±1.7

Each value represents the mean of three replicates ± S.E. GAE; gallic acid equivalent.

Trypan blue-exclusion assay

Trypan blue-exclusion assay was used for the evaluation of anticancer activity of four methanol extracts of two algae *Gelidium pulchrum* and *Enteromorpha compressa* against EACC Table 2. The maximum detectable anticancer activity on EACC cell line was observed in the three methanol extracts (MeOH) of *Gelidium pulchrum* and absolute and aqueous, MeOH extracts of *Enteromorpha compressa* which reached to 100 % anticancer activity at concentration of 200µg/ ml .

Table 2: *In vitro* anticancer effect of tested methanol extracts on EACC cell line

Treatment	Concentrations µg/mL			
	50	100	200	IC50
Control	0.0	0.0	0.0	
Aqueous MeOH *Gelidium P*	8.0±1.2b	22.3±1.5c	78.6±2.4b	143.7±2.4
Absolute MeOH *Gelidium P*	16.7±1.7a	82.0±2.0b	98.3±1.7a	95.6 ± 1.7
Aqueous MeOH *Enteromorpha C*	16.0±2.0a	98.3±1.7a	99.0±1.0a	86.3±1.7
Absolute MeOH *Enteromorpha C*	16.7±1.7a	98.3±1.7a	99.7±0.3a	85.76±0.3

Each value represents the mean of three replicates ± S.E. Values in the same column with the same letter are not significantly at (P 0.05).

MTT assay

MTT assay was used for the evaluation of anticancer activity of four methanol extracts against HeLa cell line Table3. The Data showed that all tested methanol extracts have significant anticancer activity against HeLa cell line (80%) and more than positive control. The present findings exhibited a concentration dependent inhibition of cancer cells by the all tested methanol extracts (Table 3).

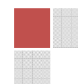


Table (3) In vitro anticancer effect of tested methanol extracts on HeLa cell line

Treatment	Conc.µg/mL 50	100	200	IC_{50}
Control	0.0	0.0	0.0	0.0
Aqueous MeOH *Gelidium P*	73.3±2.3c	81.7±0.3b	82.6±0.6b	92.59±0.6
Absolute MeOH *Gelidium P*	82.0±1.2a	87.3±0.3a	86.3±0.7a	87.2 ± 0.7
Aqueous MeOH *Enteromorpha C*	83.7±0.7a	84.3±0.6b	87.3±0.3a	87.4 ±0.3
Absolute MeOH *Enteromorpha C*	85.0±0.6a	86.6±0.7a	87.7±1.7a	86.2 ±0.7
Fytoside	77.3±0.3b	79.3±1.8 b	78.3±0.3c	95.6± 0.3

Each value represents the mean of three replicates ± S.E. Values in the same column with the same letter are not significantly at (P 0.05).

Bax and caspase protein expression analysis

Table 4 recorded the protein expressions level of Caspase-3 and Bax in HeLa cell line after treatment with tested methanol extracts. Caspase-3 expression was found to be significantly up regulated in all treated samples which was more pronounced in aqueous, absolute methanol extracts of *Gelidium pulchrum* (aqueous and 52% respectively). Also Bax expression level was significantly up regulated in all treated samples, which was more pronounced in two methanol extracts of *Gelidium pulchrum* (56 and 49%) than two methanol extracts of *Enteromorpha compressa* (46 and 42 %) Table 4.

Table 4 Caspase 3 and Bax protein expression levels in treated-HeLa cell line

Treatment	Caspase 3 ng/ml	% Caspase 3 Exp.	Bax ng/ml	% Bax Expr.
Control	7.38±0.04e		2.06±0.01e	
Aqueous MeOH *Gelidium P*	14.80±0.20b	50±0.20b	4.63±0.04a	56±0.04a
Absolute MeOH *Gelidium P*	15.33±0.12a	52±0.12a	4.09±0.03b	49±0.03b
Aqueous MeOH *Enteromorpha C*	12.28±0.19c	40±0.19c	3.82±0.06 c	46±0.06 c
Absolute MeOH *Enteromorpha C*	9.89±0.08d	25±0.08d	3.54±0.08 d	42±0.08 d

Each value represents the mean of three replicates ± S.E. Values in the same column with the same letter are not significantly at (P 0.05).

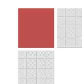
Antioxidant activity using the DPPH method

Table 4 recorded the antioxidant activity of methanol extracts of two tested algae using DPPH assay. The antioxidant was more pronounced in the aqueous methanol extracts of *Gelidium pulchrum* algae (94.0±2.3 %) which were more than positive control BHT (92.0±5.8%). Other three methanol extracts gave antioxidant activity less than positive control. A direct positive relationship between antioxidant activity and increasing concentration of the all tested methanol extracts.

Table (5) DPPH radical-scavenging activities of tested methanloic extracts

Treatment	Conc. µg/mL 50		100		
	30min	60 min	30min	60min	IC_{50}
Aqueous MeOH *Gelidium P*	12.0±1.0d	54.0±2.3c	58.3±0.9b	94.0±2.3a	51±2.3
Absolute MeOH *Gelidium P*	8.0±1.2e	25.0±2.8d	13.7±2.9d	64.0±1.7b	81.6±1.7
AqueousMeOH *Enteromorpha C*	35.0±2.9b	53.3±3.3c	45.3±3.5c	60.0±4.0b	72.1±4.0
Absolute MeOH *Enteromorpha C*	20.0±1.5c	68.7±2.7b	44.7±5.8c	67.0±2.0b	61.6 ±2.0
Positive control (BHT)	74.3±6.3a	86.0±5.8a	65.0±2.9a	92.0±5.8a	46.3±5.8

Each value represents the mean of three replicates ± S.E. Values in the same column with the same letter are not significantly at (P 0.05).



Reducing power and ABTS radical scavenge activity

The reducing power of the tested methanol extracts of *Gelidium pulchrum* and *Enteromorpha compressa* was determined by relation to that of the gallic acid from the standard curve with the linear equation Y = 0.003x at R2 = 0.728. The reducing power was 179.3±4.7 and 156.3±3.3 µg/ml in aqueous and absolute MeOH extracts of *Gelidium pulchrum* and 151.3±5.7 and160.4±5.2b µg/mL GAE in aqueous and absolute MeOH extracts of *Enteromorpha compressa*. It was evident that the aqueous MeOH of *Gelidium pulchrum* extract had a higher reducing power than the methanol extracts of *Enteromorpha compressa* Table 6.

Table 6 illustrated ABTS radical scavenge activity of tested methanol extracts. The data showed that all tested methanol extracts had ABTS scavenging activity more than 80%. ABTS scavenging of the aqueous and absolute methanol extracts of *Gelidium pulchrum* were computed to be (89.7±1.7 and 86.7±2.3 respectively) while it was (87.0±3.2 and 88.7±4.3) in the aqueous and absolute methanol extracts of *Enteromorpha compressa* Table 6.

Table (6) Reducing power and ABTS scavenging of tested methanol extracts

Alga Extracts	Reducing power as GAE (µg/g)	ABTS Scavenging %
Aqueous MeOH *Gelidium P*	179.3±4.7a	89.7±1.7a
Absolute MeOH *Gelidium P*	156.3±3.3b	86.7±2.3a
Aqueous MeOH *Enteromorpha C*	151.3±5.7b	87.0±3.2a
Absolute MeOH *Enteromorpha C*	160.4±5.2b	88.7±4.3a

Table 7 Anti-inflammtory activity of tested methanol extracts by Carrageenan

Treatment	Swelling (thickness) (mm)			
	1h	2h	3h	4h
Carrageenan	10.25±0.48a	11.25±0.47a	16.00±0.70a	19.00±0.40a
Indomethacin	6.50±0.29b	7.00±0.41b	7.25±0.63d	8.00±0.41b
Aqueous MeOH *Gelidium P*	7.75±0.48c	8.75±0.75c	11.00±0.40b	11.70±0.47c
Absolute MeOH *Gelidium P*	9.75±0.25a	10.50±0.65ac	13.00±0.40c	15.75±0.85d
Aqueous MeOH *Enteromorpha C*	10.00±0.71a	10.00±0.71ac	12.75±0.47c	15.25±0.63d
Absolute MeOH *Enteromorpha C*	10.25±0.25a	10.25±0.63ac	14.75±0.48a	16.50±0.65 d

Each value represents the mean of three replicates ± S.E. Values in the same column with the same letter are not significantly at (P 0.05).

Anti-inflammatory activity by Carrageenan-induced paw oedema test.

Intraplantar injections of carrageenan in rats led to a time-dependent increase in paw volume after administration of carrageenan (Table 7). The mean paw thickness of the carrageenan group reached its peak at the fourth hour. However, carrageenan-induced paw oedema was significantly reduced in a dose-dependent manner by treatment with methanol extractsof *Gelidium pulchrum and Enteromorpha compressa* algae. Indomethacin (10 mg/kg) profoundly reduced the carrageenan-induced oedema in the right hind paws of rats over the 4 h period of measurement Table 7.

Anti-inflammatory activity by HRBC membrane stabilization test

Table 8 summarized the HRBC membrane stabilization effect of 50 and absolute MeOH extracts of two algaeGelidium pulchrum and Enteromorpha compressa. According to our knowledge, this is the first report on the in vitro membrane stabilization potential of two methanol algae ofGelidium pulchrum and Enteromorpha compressa extracts. In this study the maximum anti-inflammatory activity was found in absolute methanol extracts of Gelidium pulchrum algae at a concentration of 100 µg/ml (71.7±1.7) as standard drug Diclofenac which showed 72.0±2protection. The other three methanol extracts have weak anti-inflammatory activity Table 8.

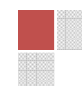


Table (8) *in vitro* Anti-inflammtory activity of tested methanol extracts by membrane stabilization test

Alga Extracts (100µg/mL)	Anti- inflammatory %
Aqueous MeOH *Gelidium P*	12.0±1c
Absolute MeOH *Gelidium P*	71.7±1.7a
Aqueous MeOH *Enteromorpha C*	20.7±1.7b
Absolute MeOH *Enteromorpha C*	18.0±1.2b
Sodium declofanic	72.0±2a

Each value represents the mean of three replicates ± S.E. Values in the same column with the same letter are not significantly at (P 0.05).

Discussion

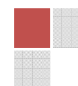
Total phenols of *Gelidium pulchrum* and *Enteromorpha compressa* methanol extracts was determined. The values of total phenols indicated a higher level of phenols in the aqueous and absolute MeOH of *Gelidium pulchrum* algae extracts. These results may give an indication on the potential effect of the roles played by phenolic compounds in the activity of these algae. Phenols are very important plant constituents. There is a highly positive relationship between total phenols and antioxidant activity of many plant species, because of the scavenging ability of their hydroxyl groups30. In this concern, Phenolic compounds, may exerts antioxidant effects as free radical scavengers by hydrogen donating sources or singlet oxygen quenchers and metal ion chelating [31]

The results of total flavonoids indicated a higher level of flavonoids in the, absolute MeOH of *Gelidium pulchrum* algae extract and aqueous methanol (50%) extract of *Enteromorpha compressa*. These results give an indication on the potential effect of the roles played by flavonoids compounds in the activity of these algae. Flavonoids are regarded as one of most spread groups of natural constituents found in plants. It has been revealed that flavonoids actively participate in antioxidant scavenging and the mechanism of action of flavonoids is through scavenging [32] or chelating process which terminates free radical. The study showed that the extracts have the proton-donating ability and could serve as free radical scavenger, acting possibly as primary antioxidant.

The results of anticancer activity revealed that the maximum detectable anticancer activity on EACC cell line was observed in the three methanol extracts, absolute MeOH of *Gelidium pulchrum* and aqueous, absolute MeOH extracts of *Enteromorpha compressa* which reached to 100 % anticancer activity.

In this study we assessed the expressions of caspase-3, Bax proteins in treated-HeLa cell line. From our data it was found significant up regulation of both caspase-3 and Bax expression in treaed- HeLa cell line indicating a cell death process mediated by apoptosis (through caspase 3 and Bax activation). Various findings suggest that algal extracts and its active metabolites possess anticancer effect. Algal metabolites [33,34] are the key-active components for the inhibition of bax gene and activation of caspase-3 thereby account for the antitumor promotion effects of algal extracts against HeLa cell line. On the other hand, Cytotoxic effect of various doses of two algae was also observed *in vitro* on EAC cells and HeLa cell lines. Results of the present study suggest that two algal extracts have apoptogenic effect on HeLa cell line. In response to apoptotic signals (DNA damage/stress) pro-apoptotic Bcl2 family protein Bax becomes activated resulting in mitochondrial membrane permeability. As a result, cytochrome C and APAF-1 (apoptotic protease activating factor 1) are released from the inter-membrane space and activate caspase-9 through cleavage [35]. Caspase 9 generates a signaling cascade of caspase cleavage that results in DNA fragmentation into 180 base pairs and its multiplications, the effecter caspase is caspase-3. P53 has a major role in cell survival, in healthy cells the nuclear amount of p53 is very low due to binding of adapter protein MDM2, P53 is subsequently exported to cytosol and degraded. Whereas, in the cells with damaged DNA, P53 becomes phosphorylated. As MDM2 cannot recognize phosphorylated P53, the nuclear P53 is stabilized and induce pro-apoptotic proteins (Bax, Puma and Noxa etc.) [36]. It is now well recognized that whether a cell becomes committed to apoptosis partly depends upon the balance between proteins that mediate growth arrest and cell death, e.g. P53, P21, Bax and proteins that promote cell viability, e.g. Bcl-2. Among these proteins, Bax has been found to facilitate apoptosis in various cell types. Antiproliferative activity of algal extracts in cancer cell lines, may be due to water-soluble polysaccharides, such as laminarans and fucoidans, which are representative anticancer substances extracted from seaweeds [37]. Aqueous extracts of *Gracilaria corticata* (red algae) [38] inhibited the proliferation of human leukemic cell lines. Both ethanol [39] and methanol [40] extracts of *Gracilaria tenuistipitata* reportedly had anti-proliferative effects on Ca9-22 oral cancer cells and were involved in cellular apoptosis, DNA damage, and oxidative stress. Methanol extracts of two green algae, *Enteromorpha intestinalis* and *Rizoclonium ripariumon* inhibited proliferation of HeLa cells [41].

Similarly, caspase-dependent apoptosis induced by a methanol extract of *Plocamium telfairiae* has been demonstrated using HT-29 colon cancer cells [42]. Among green algae, a hot water extract of *Capsosiphon fulvescens* that contained polysaccharides induced the apoptosis of gastric cancer cells [43]. Dimethyl sulfoniopropionate, a tertiary sulfonium metabolite found in green algae and other algae species, exhibited anti-cancer effects in mice with Ehrlich ascites carcinoma [44] and HeLa cell line [45]. All these results together with this study suggested that the active substances interact with special cancer-associated receptors or



cancer cell special molecule, thus triggering apoptosis that cause cancer cell death [46].

Antioxidant activities have been identified in various marine algae, including red, green, and brown algae species [47] and in their enzymatic extracts [48,49]. The antioxidant properties of methanol extracts and bioactive components of two algae are evaluated. Antioxidant activity of methanol extracts of two algae was determined using DPPH assay . A direct positive relationship between antioxidant activity and increasing concentration of the all tested methanol extracts. These results are in agreement with the results obtained by Plaza et al., [50] and Shanab et al.,[51]who reported that, the absence of structural damage in the algae leads to the consideration that these organisms are able to generate the necessary compounds to protect themselves against oxidation. In this respect, algae can be considered as an important source of antioxidant compounds that could be suitable also for protecting our bodies against the reactive oxygen species formed by our metabolism or induced by external factors (as pollution, stress, and UV radiation, etc.). There is a strong positive and significant correlation between DPPH radical scavenging and phenolic + flavonoid contents. Therefore, they represnt the main contributors of antioxidant activity in these two algae [52].

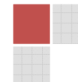
In the present study, the reducing power of the tested methanol extracts of two algae *Gelidium pulchrum* and *Enteromorpha compressa* was determined. It was evident that the aqueous MeOH of *Gelidium pulchrum* extract had a highest reducing power. In the reducing power assay, the presence of antioxidants (reductants) in the sample would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. The reducing capacity of compound may serve as a significant indicator of its potential antioxidant activity. This would have the effect of converting free radicals to more stable products and thus terminating free radical initiated chain reaction [53]. The reducing capacity of extracts may serve as significant indicators of its potential antioxidant activity. The reducing power assay is correlated with the results obtained by the DPPH assay.These activities are due to the presence of phenolics and flavonoids compounds [54,55].

The data of ABTS radical scavenging activity revealed that all tested methanol extracts had ABTS scavenging activity more than 80%. It was clear now that the Algal methanol extracts contain various compounds including pigments (Chlorophyll a, b and carotenoids), alkaloids flavonoids and phenolic compounds, as well as essential oils which can participate in the obtained great antioxidant activity [51].

Concerning to anti-inflammtory activity, results showed that the tested algal extracts inhibit carrageenan-induced paw oedema in rats. Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow [56]. Inflammatory processes are the physiological response of the organism to different stimuli such as trauma, infections or immunological mechanisms. The arachidonic acid cascade is highly activated during inflammation, resulting in the formation of eicosanoids and it is mediated by cyclooxygenase and 5-lipoxygenase enzymes [57]. The involvement of histamine, 5-hydroxytrptamine, bradykinin, prostaglandin and nitric oxide in carrageenan-induced paw oedema was reported [58]. Indomethacin has been shown to produce its anti-inflammatory effect by inhibiting the enzyme, cyclooxygenase, thus inhibiting prostaglandin synthesis [59]. The versatility of the functions of algae may derive from their abundant bioactive metabolites [34,60] brominated phenols, brominated oxygen heterocyclics, nitrogen heterocyclics, kainic acids, guanidine derivatives, phenazine derivatives, amino acids + amines, sterols61 and sulfated polysaccharides [62-64]. Therefore, it is possible, to say that phenols and flavonoids may also be contributing to the anti-inflammatory activities of methanol extracts of two algae *Gelidium pulchrum and Enteromorpha compressa* used in this study.

Data of anti-inflammatory activity by HRBC membrane stabilization demonstrated that absolute methanol extract of Gelidium pulchrum algae inhibits heat-induced hemolysis of erythrocytes. This indicated that this extract possess biological membrane stabilization properties preventing stress-induced destruction of the plasma membrane. Stabilization of lysosomal membrane is important in limiting the anti-inflammatory response by preventing the release of lysosomal constituents of activated neutrophils such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extracellular release [65]. The erythrocyte membrane is analogous to lysosomal membrane66 and its stabilization implies that the extract may as well stabilize lysosomal membranes. However, anti-inflammatory properties have been reported for two species, *G. verrucosa* and *G. textorii*. Evidence of anti-inflammatory properties in other species of red algae is also increasing. Crude extracts and purified components of some green algae species are also known to have anti-inflammatory properties [2-4].

The present findings exhibited a relation between antioxidant, anticancer and anti-inflammatory activities of methanol extracts of Gelidium pulchrum and Enteromorpha compressa algae. In this concern, the antioxidant compounds play an important role in the later stages of cancer development. There is increasing evidence that oxidative processes promote carcinogenesis, although the mechanisms are not well understood. The antioxidants may be able to cause the regression of premalignant lesions and inhibit their development into cancer. Several herbs and spices in addition to plenty of other medicinal plants are reportedly exhibiting antioxidant activity [66-69]. Majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins. In addition, vitamins C and E, β-carotene, and -tocopherol present in natural foods, are known to possess anticancer potential [70-72]. Thus, potential antioxidant and anticancer properties of plant extracts (or isolated products of plant origin) can possibly be explored for developing the anticancer drugs. The mechanism of inflammation injury is attributed in part to release ROS from activated neutrophil and macrophages. This over release leads to tissueinjury by damaging the macromolecule and lipid peroxidation of membranes. ROS propagate inflammation by stimulating the



release of the cytokines such as tumor necrosis factor ,interleukine 1,which stimulate recruitment of additional neutrophil and macrophages. Thus free radicals are important mediators that provoke or sustain inflammatory processes and consequently their neutralization by antioxidants and radical scavengers can attenuate inflammation [73]. Many marine natural products that contain antioxidants and anticancer compounds are known to have anti-inflammatory effects [74-76].

Conclusion

Based on the results of anticancer activity, it was revealed that there is no difference between both absolute and aqueous methanol extracts of both Gelidium pulchrum and Enteromorpha compressa which have potential in vitro anticancer against different cancer cell lines. The data of anticancer activity was paralleled with data obtained from capase-3 andB expressions levels. Potent antioxidant activity was pronounced in aqueous methanloic extracts of both Gelidium pulchrum and *Enteromorpha compressa*. They have *in vitro* potential as antioxidant using different systems. . Anti-inflammation activity using membrane stablization test was pronounced in absolute methanol extracts of *Gelidium pulchrum*. These biological activities are due to the presence of various phytoconstituents. Also, the results revealed that all the tested extracts showed dose dependent activity. Further study on isolation and identification of the active compound(s) from these two algal extracts are needed.

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