

Anticancer and Antioxidant Activities of Some Algae from Western Libyan Coast

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Abstract

Seaweeds are considered as one of the largest biomass producers in marine environment that is rich in bioactive metabolites and a source of natural ingredients for functional foods. The potential antioxidant activity and the potential inhibition of Caco2 cell proliferation, of crude extracts of: Chlorophyta (*Ulva lactuca*, and *Codium tomentosum*), Phaeophyta (*Cystoseira crinita*, *Cystoseira stricta*, and *Sargassum vulgare*), and Rhodophyta (*Gelidium latifolium*, *Hypnea musciformis*, and *Jania rubens*) collected from western Libyan coast were evaluated *in vitro*. The antioxidant activity was determined by reducing power and DPPH assays while cell proliferation, morphological changes and the cell cycle arrest were assessed by MTT, inverted light microscope and flow cytometry methods respectively. The polyphenols and flavonoids rich extracts showed remarkable reducing power and antiradical properties. After exposure of Caco2 cells to; various concentrations of extracts (50, 100, 150 and 200 µg/mL) especially from brown algae for 72 h, significantly reduced cell proliferation. The antiproliferative effect of algae extracts was correlated with their polyphenol and flavonoid contents. Cell cycle analysis further showed that cells were arrested in G phases along with an increment in sub-diploidal cell population (sub-G) after extract application. These results imply that seaweeds which are rich in bioactive compounds may be in anticancer drug research programs. However, further investigations are essential to reveal the molecular mechanisms of the anticancer activities of these algae.

Keywords: polyphenols, flavonoids, seaweeds, antioxidant activity, anticancer activity

Introduction

Seaweeds are large and diverse groups of that are rich in active metabolites and a source of novel ingredients for functional foods. Nutritional studies on seaweeds indicate that brown, green and red seaweeds possess good nutritional quality and could be used as an alternative source of dietary fiber, protein, and minerals [1]. Also seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites, characterized by a wide range of biological activities such as antimicrobial [2-4], anti-inflammatory [5], anti-viral; as well as anti-tumoral activities [7,8]. Moreover, many studies show that some algal extracts display substantial antioxidant activities [9-12].

Antioxidant substances in seaweeds contribute to the endogenous defense mechanism against external stressful conditions [13]. Antioxidant properties of some red, brown and green algae extracts have shown that they vary proportion to the content of antioxidative compounds [14]. In fact, the antioxidant activity in algae acts via several processes and compounds such as lipophilic scavengers (carotenoids), enzymatic scavengers (catalase, superoxide dismutase and peroxidase), and polyphenols [15,16].

Many studies indicated a close relationship between anticancer activity of algae and their contents of antioxidant compounds such as polyphenols and flavonoids. Seaweed extracts contain substantial amounts of polyphenols such as catechin, epicatechin, epigallocatechin gallate, and gallic acid, as reported in *Halimeda* sp. (Chlorophyceae) [17]. In addition, the extract of *Ascophyllum* spp. had a higher polyphenol content compared with other seaweeds, whereas *Ulva* spp. had the lowest content of these compounds [18,19]. Polyphenolic compounds inhibit cancer cells by xenobiotic metabolizing enzymes that alter metabolic activation of potential carcinogens, while some flavonoids can also alter hormone production and inhibit aromatase to prevent the development of cancer cells [20]. The mechanism of action of anticancer activity of phenolics may also occur by disturbing cellular division during mitosis at the telophase stage. Phenolics reduce the amount of cellular protein and mitotic index, as well as colony formation during cell proliferation of cancer cells [21]. Early studies proved a close relationship between antioxidant activities and total phenolic content [22,23]. Further, edible seaweed extracts like *Palmaria palmate*

were shown to be effective antioxidants, capable of inhibiting cancer cell proliferation [16]. The alcoholic extract of the red alga *Acanthophora spicifera* exhibited tumoricidal activity on Ehrlich's ascites carcinoma cells developed in mice [24]. In addition, enzymatic and polysaccharides extracts from brown seaweeds strongly showed antioxidant potential with dose-dependent radical scavenging activities [25] and suppressed the *in vitro* proliferation of selected cancer cell lines [26]. Therefore, the aim of the present study was to determine the polyphenols content, antioxidant and anticancer activities of some marine algae from the Western coast of Tripoli (Libya).

Materials and Methods

2.1. Experimental materials

Seaweeds algal species including Chlorophyta (*Ulva lactuca*, and *Codium tomentosum*); Phaeophyta (*Cystoseira crinita*, *Cystoseira stricta*, and *Sargassum vulgare*), and Rhodophyta (*Gelidium latifolium*, *Hypnea musciformis*, and *Jania rubens*) were collected from the western coast of Libya in March, 2013. The algal samples were authenticated in the Botany Department, Faculty of Science, University of Tripoli. Human colorectal carcinoma (Caco2) and Human Corneal Epithelial Cells (HCEC) cell lines were obtained from the American Type Culture Collection (ATCC).

2.2. Reagents

Chemicals required for the assays including 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (Saint-Louis, MO, USA). All other utilized reagents were of the highest available commercial grade.

Methods

2.3. Algae extraction procedure

Seaweed sampling and extraction: Samples were collected from the western part of the Libyan coast, in March 2013. Fresh seaweeds were rinsed with tap water and polished to remove any associated epiphytes, salt, sand, microorganisms and other suspended materials. Then, the clean material was air dried in a shady place at room temperature (25 - 30°C) on absorbent paper, and then ground to a fine powder in an electrical coffee mill. The extraction was carried out according to Senevirathne et al.

(2006) [27] with some modifications. Briefly, seaweeds (20g) were extracted with methanol (100 mL) in a shaking incubator at 25°C for 72 h. The extracts were filtered with Whatman's No. 1 filter paper and re-extracted three times. The filtrate was concentrated under reduced pressure by using Rotary evaporator (Heidolph300 LabroRota, Germany). The oily residues were stored at -20°C until analysis.

2.4. Determination of Total Polyphenol and Flavonoid Content

The total phenolic content was determined by the Folin –Ciocalteu method using gallic acids (10-200 mg/mL) as a standard and the absorbance measured at 720 nm [28]. Phenolic content of the extract was calculated as mg gallic acid equivalent (GAE) per gram of dried powder.

Total flavonoids content was assessed according to the method of Park et al. (2008) [29]. An aliquot of 0.3 mL of extracts was mixed with 3.4 mL of 30% methanol, 0.15 mL of 0.3 M AlCl₃-6H₂O and 0.15 mL of 0.5 M NaNO₂ in a test tube (10 mL), and then 1 mL of 1 M NaOH was added. Absorption was measured at 506 nm. Flavonoids content was estimated from the standard calibration curve of 10-100 mgmL⁻¹rutin.

2.5. Antioxidant activity Assays

For antioxidant assays, all extracts (1.0 mg/mL of extracts) were dissolved in 95% methanol and a series of concentration- dependent dilutions were made (40-300 µg/mL). Standard reagents were utilized for comparison for all antioxidant assays.

2.5.1. DPPH Free radical scavenging activity

Free radical-scavenging activities of extracts were measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as described by Dandlen et al., (2010) [30]. The percentage inhibition of the DPPH radical by the samples was calculated according to the following equation: % Inhibition = $(A_0 - A_1) / A_0 \times 100$, where A₀ is the absorption of the blank sample (t = 0 minutes) and A₁ is the absorption of the tested extract solution (t=60 minutes). All determinations were performed in triplicate. The sample concentration providing 50% inhibition (IC₅₀) was obtained by plotting the inhibition percentage against extracts concentrations.

2.5.3. Reducing power assay

The reducing power of extracts was investigated following the method of Oyaizu (1986) [31]. Extract solution (2 mL), was mixed with potassium ferricyanide (2 mL, 10 mg/mL) and phosphate buffer (2 mL, 0.2 M, pH 6.6) kept for 30 min at 45 °C. TCA (2 mL, 100 mg/l) was added to the reaction mixture. Two mL of distilled water and 0.4 mL of 0.1% (w/v) ferric chloride were mixed with 2mL of reaction mixtures in a test tube, after 10 minute reaction time the absorbance was measured at 700 nm. Increase in absorption by the mixture indicated a higher reducing power.

2. 6. Determination of anticancer activity

2.6.1. Preparation of extracts for anticancer experiments

The residues of algae extracts were individually dissolved in 1% dimethyl sulfoxide (DMSO, Sigma, St. Louis, USA) to a final concentration of 1mg/mL. For all experiments, the final concentrations of the tested compounds were prepared by diluting the stock with the culture medium.

2.6.2. Cell lines and culture conditions

Human colorectal carcinoma (Caco2) and Human Corneal Epithelial Cells (HCEC) cell lines were maintained in monolayer culture at 37°C and 5% CO₂ in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 0.5 % glutamine (20 mM, Gibco, Scotland, UK), 0.5 % penicillin (100 IU/mL), Gibco, Scotland, UK), and non-essential amino acids (1%). Stock cultures were sub-cultured every 7th day after harvesting the cells with trypsin EDTA and then seeded in a tissue culture flask to maintain in exponential phase.

2.6.3. Cytotoxicity assay

Inhibition of cell proliferation by algal extracts was measured using the MTT assay. Cells (2×10^4 /well) were plated in 96-well culture plates. After an additional 24 h, various concentrations of crude algae extracts were added to the wells to obtain final concentrations of 50, 100, 150 and 200 µg/mL and incubated for 72 h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4),

10 μ l/well (5mg/mL) of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) in phosphate buffered saline (PBS) were added to each well, and incubated for 4 h at 37 °C. The medium was removed and formazan was dissolved in DMSO and the optical density was measured at 492 nm using a bioassay reader (Biorad, USA). The effect of the extracts on the proliferation of cells was expressed as the % inhibition of growth. All experiments were performed at least twice in triplicate.

2.6.4. Cell morphological analysis

Caco2 cells (3×10^5) were seeded in each well of 40 mm culture dishes and allowed to proliferate for 24 hours. After that, cells were treated with algae extracts at 50, 100,150, and 200 μ g/mL. Control untreated cells were also included. Morphological changes of cells untreated and treated with algae were performed by inverted light microscope (Olympus, Tokyo, Japan) after 72 hours.

2.6.5. Cell cycle analysis by flow cytometry

To determine cell cycle distribution analysis, 1×10^6 cells were plated in 25 cm² tissue culture flasks, treated with extracts of all tested algae (200 μ g/mL) for 72 h. After treatment, the cells were collected by trypsinization, fixed in 70% cold ethanol, washed in PBS, resuspended in 1mL of PBS containing 1 mg/mL RNase. The cells were then incubated for 30 min at 37 ° C, after which, 5 μ L of propidium iodide (1 mg/mL) was added to the cells and vortexed. Measurements were carried out on flow cytometer, (BD Facs Canto) and the data were analysed with BD FacsDiva software; the results are expressed as a percentage of the cells in each phase [32].

2.7. Statistical analysis

The experiments were performed in triplicate and all data are expressed as mean \pm standard deviation. The values were analyzed by one-way ANOVA using SPSS version 16.0 software and individual comparisons were obtained by Tukey's method. P value ≤ 0.05 was considered statistically significant.

3. Results and discussion

3.1. Polyphenolics and flavonoids content

Phenolic compounds are commonly found in plants, encompassing seaweeds, and have been reported to have a wide range of biological activities including antioxidant and anticancer activities [29,33]. Nevertheless, in Libya, little information about the polyphenols and flavonoids concentrations in Libyan coast seaweeds is available. The current study showed for the time the amounts of polyphenols and flavonoids in tested algae as well as their anticancer activity.

Results (Table 1) revealed that the amount of total phenolic and total flavonoid contents in the alcoholic extracts of *C. crinita* and *J. rubens* were 800.28 ± 36.23 mg/g and 600.33 ± 31.53 mg/g dry weight expressed as gallic acid equivalents, and 474.72 ± 26.51 mg/g 435.79 ± 25.61 , expressed as rutin equivalents, respectively (Table 1); these levels were significantly higher than those reported for other seaweeds ($P < 0.05$) [17]. In addition, the total phenolic content and flavonoids contents in extracts of *U. lactuca* were markedly higher than in *C. tomentosum* ($P < 0.05$) (Table 1).

3.2. Antioxidant activity

Screening of potential antioxidant activities of methanolic crude extracts from eight species of seaweeds was performed using two antioxidant assays; reducing power and DPPH.

As a result of the presence of a high level of polyphenols including phenolic acids, flavonoids, isoflavones, cinnamic acid, benzoic acid, quercetin in algae, these algal extracts are reliable sources antioxidants [12].

Table 1. Total polyphenol and flavonoid contents of methanolic extracts of the tested algae.

	Polyphenols content * (mg GAE/g DW)	Flavonoids content (mg Rutin/g DW)
Chlorophyta		
<i>U. lactuca</i>	440.50 ± 39.13^a	368.07 ± 25.72^a
<i>C. tomentosum</i>	300.17 ± 35.38^b	268.07 ± 25.72^c
Phaeophyta		
<i>C. crinita</i>	800.28 ± 36.23^c	474.72 ± 26.51^a
<i>C. stricta</i>	430.6 ± 30.13^a	356.59 ± 29.31^b
<i>S. vulgare</i>	350 ± 26.28^b	251.67 ± 25.5^d
Rhodophyta		
<i>H. musciformis</i>	256.44 ± 24.18^d	203.02 ± 24.07^d

<i>J. rubens</i>	600.33±31.53 ^e	435.79±25.61 ^a
<i>G. latifolium</i>	368.63±21.53 ^b	298.65±18.01 ^c

DW: dry weight; Results were recorded as (mean ± SD); *mg GAE/g DW: milligram gallic acid equivalent per gram dry weight; mg Rutin /g DW: milligram Rutin equivalent per gram dry weight. Each value is presented as mean ± SD (n = 3). Means within each column with different letters (a-f) differ significantly ($P < 0.05$).

3.2.1. Antioxidant scavenging activity

Much experimental data emphasizes that plants including seaweeds are rich sources of antioxidant compounds. The reactive oxygen species (ROS) attack biomolecules, producing unfavorable changes in DNA, lipids, and proteins and are implicated in the pathogenesis of many diseases. Any natural or synthetic compound with antioxidant properties might contribute towards the partial or total alleviation of this damage [34]. All algal extracts possessed radical scavenging activity, although *C. crinita* was more effective in scavenging DPPH with lowest IC₅₀ (Table 2).

The antioxidant activity is proportional to the concentration of polyphenols and flavonoids. The maximum scavenging effect was shown by the extract of *C. crinita*, *C. stricta* and *S. vulgare* with the IC₅₀ values of 50.5, 75.11 and 150 µg/mL respectively (Table 2), this is in a good agreement with previous findings that brown algae have higher than antioxidant activity red or green algae [10]. The lowest scavenging ability was shown by *C. tomentosum* and *G. latifolium* with higher IC₅₀ (300µg/mL). The present study showed that the green algae collected from Libyan coast have very low antioxidant power which is in line with the other reports on the other green algae reports [14, 35].

Table 2. Antioxidant activity of selected algae

	DPPH* IC ₅₀ µg/mL
Chlorophyta	
<i>U. lactuca</i>	230.50 ± 9.03 ^a
<i>C. tomentosum</i>	300.17 ± 35.38 ^b
Phaeophyta	
<i>C. crinita</i>	50.5 ± 3.20 ^c
<i>C. stricta</i>	75.11 ± 30.13 ^d
<i>S. vulgare</i>	150 ± 26.28 ^e

Rhodophyta

<i>H. musciformis</i>	200.33±24.18 ^a
<i>J. rubens</i>	130.5±31.53 ^c
<i>G. latifolium</i>	300.03±9.22 ^b
<i>Ascorbic acid</i>	156±12.06

*:DPPH, 2,2-diphenyl-1-picrylhydrazyl

3.2.1. Reducing power

Reducing capacity is considered as a significant additional indicator of potential antioxidant activity of a compound or sample [36].

Figure 1 shows the concentration-response curves for the reducing power of the algal extracts under investigation. In general, the reducing power of the extract also increased with an increase in concentration applied. There were significant differences between the reducing power of extracts (A, B and C) and that of ascorbic acid that was used as positive control ($P<0.01$) (Figure 1D).

The present study indicate that the alcoholic extracts of the brown algae *C. crinite*, *C. stricta* and *S. vulgare* possessed good reducing power, followed by red algae *H. musciformis* and *G. latifolium* (Figure 1 B &C) showing a steady increase in reductive potential of the brown seaweed with an increase in the absorbance in a concentration-dependent manner. On the other hand, green algae (*C. tomentosum* and *U. lactuca*) extracts showed low reducing power (Figure 1A). The results obtained correlate with the total phenolic and flavonoids contents (Table 1), indicating that these algae could potentially be a good source of natural and easy extractable antioxidants for pharmaceutical, dietary and cosmetic purposes.

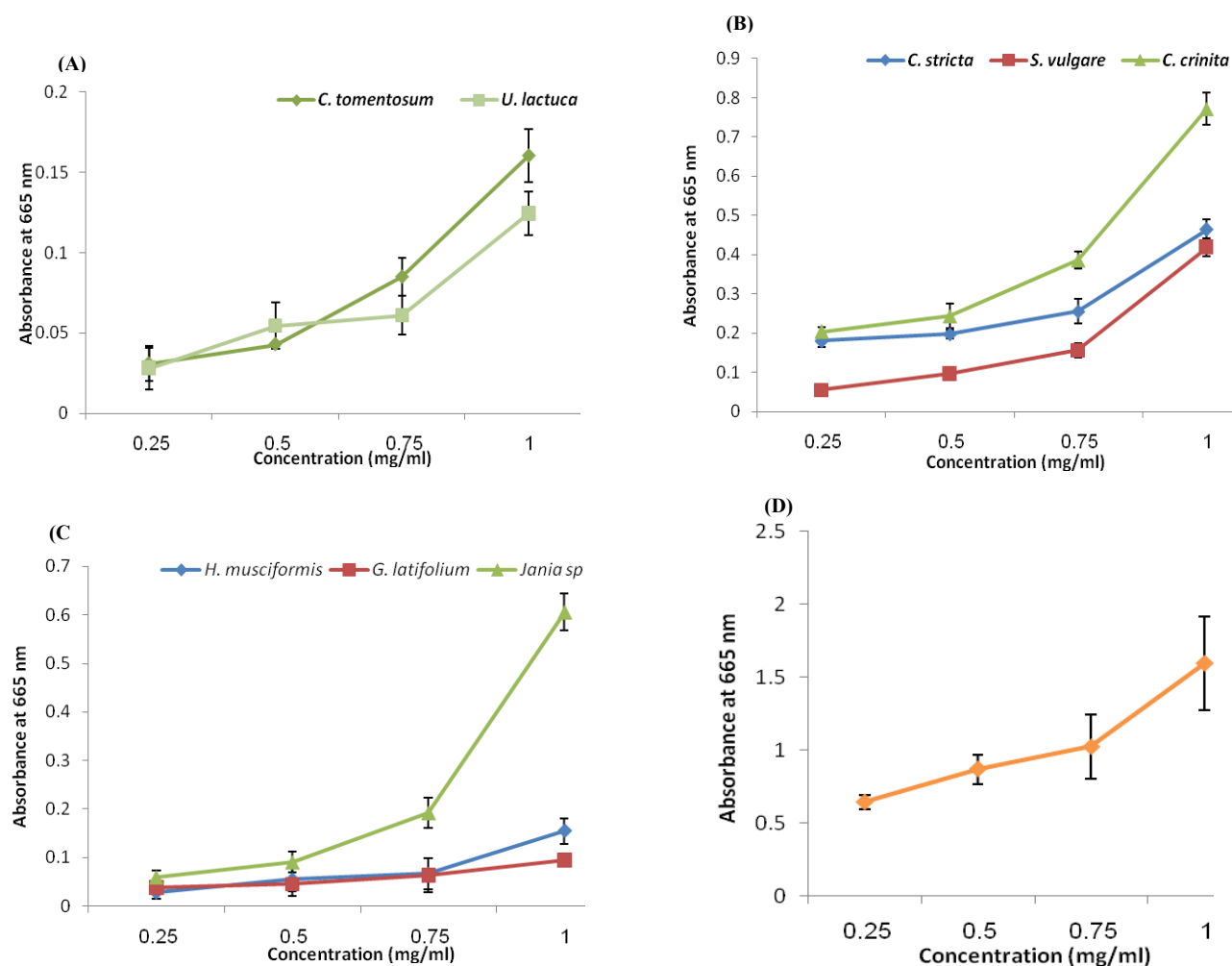


Figure 1. Reducing potential of crude algae extracts determined by reducing power assay. Data are mean \pm SD. (A) green algae; (B) brown algae, (C) red algae extracts and (D) ascorbic acid (positive control).

3.3. Anticancer activity

3.3.1. Cytotoxic activity of crude algae extracts

Cytotoxicity is an activity that is consistent with anticancer activity, the major advantage of cytotoxicity assays is that all potential mechanisms of cellular proliferation can be monitored simultaneously. In the present study, colon cancer cell line (Caco2) was used to determine the cytotoxic activity of crude algae extracts at various concentrations (50, 100, 150 and 200 μ g/mL) (Figure 2). Under the same experimental conditions, the extracts were additionally tested using Human Corneal Epithelial Cells (HCEC) cell lines in order to examine their cytotoxic effect on normal cells (Figure 3).

Figure 2, shows the percentage changes in the growth inhibition of cancer cells treated with algae extracts. The tested algae extracts especially *C. crinita* extract, showed a strong selective cell proliferation inhibition of the cancer cell line. This might be due to high polyphenols and flavanoids contents of the 200 $\mu\text{g}/\text{mL}$ extract, which showed had maximum growth inhibition (87.05%). At the same concentration, cells treated with *C. tomentosum* extract containing a low content of flavonoids and polyphenols, exhibited the lowest growth inhibition (46.2%). The experimental observation indicted that cell death was a concentration-dependent process, hence the number of non-viable cells increased with increasing concentration of algal extracts.

Among brown algae, the *C. crinita* extract induced highly significant cytotoxic effect on the Caco2 cells after 72 h exposure; the percentage of inhibition was 87% at 200 $\mu\text{g}/\text{mL}^{-1}$ compared to lower inhibition (%) of extracts of *C. stricta* and *S. vulgare* ($P<0.01$) (Figure 2B); this was in agreement with observations of previous study [9]. The extensive research on the crude extracts of various brown algae against different cancer cell lines shows promising anticancer potential [37].

In red algae extracts, *G. latifolium* extract displayed a substantial inhibition effect (85%) at 200 $\mu\text{g}/\text{mL}$, in comparison with cells treated with *H. musciformis* extract (48%) (Figure 2C). In comparison with tested green algae extracts, *U. lactuca* caused significant cytotoxicity at the very low concentration (50 $\mu\text{g}/\text{mL}$; 55%) ($P<0.01$) and higher cytotoxic effect, in a dose-dependent manner in the range 50–200 $\mu\text{g}/\text{mL}^{-1}$ (55, 60, 70 and 77%) (Figure 2B). In contrast, all tested algae displayed a non-significant cytotoxic effect on human normal HCEC cell line where the percent of inhibition did not exceed 24% at 200 $\mu\text{g}/\text{mL}$ (Figure 3).

IC_{50} obtained against Caco2 cell line in the presence of the crude extract of *C. crinita*, *C. stricta*, *S. vulgare* were; >50 $\mu\text{g}/\text{mL}$, 120 $\mu\text{g}/\text{mL}$ and 150 $\mu\text{g}/\text{mL}$ respectively. In contrast, the IC_{50} s obtained against Caco2 cell line in the presence of the crude extracts of *H. musciformis*, *J. rubens*, *G. latifolium* were; >200 $\mu\text{g}/\text{mL}$, 50 $\mu\text{g}/\text{mL}$ and 120 $\mu\text{g}/\text{mL}$ respectively. Moreover IC_{50} obtained against Caco2 cell line in the presence of the crude extract of *U. lactuca*, *C. tomentosum* were; 50 $\mu\text{g}/\text{mL}$, and >200 $\mu\text{g}/\text{mL}$ respectively. The low IC_{50} values of *C. crinita*, *J. rubens* and *U. lactuca* indicate promising anti-proliferation activity of their extracts.

Recent phytochemical studies, confirm the presence of bioactive compounds such as saponins, flavanoids, tannins and polyphenolic components in most tested algae [4, 38]. Therefore, the cytotoxic effect of algae, via the inhibition of the proliferation of Caco2 cells is likely to be related to their content of these compounds especially polyphenolics and flavonoids [39]. For example, quercetin shows antioxidant activity that is believed to have a cytoprotective role against oxidative stress [40]. In addition, the presence of 2,3-double bond in flavonoid molecules correlates with mitochondrial damage and cancer cell death [41].

A good correlation was observed between the total polyphenol contents and proliferation activity in seaweed extracts ($R^2 = 0.686$) (Figure 4). The anticancer activity of polyphenols could be induced via multiple anticancer pathways such as interaction with key enzymes in cellular signaling pathways, cell cycle, apoptosis and metastasis [42,43].

3.3.2. Cell morphology study by inverted light microscope

The ability of algal extracts to induce cell death was estimated by analyzing its effect on cell morphology (Figure 5). The observation of Caco2 cells under a phase contrast microscope showed that after 48 h of treatment with 200 $\mu\text{g}/\text{mL}$ extracts, detectable changes were found, including altered cell morphology, cell shrinkage and membrane blebbing, the characteristic features of apoptotic cell death (Figure 5).

It is vital to maintain the homeostasis between cell proliferation and cell death in normal mammalian tissues; therefore, the process in which the rate of cell proliferation exceeds that of cell loss in tumor cells might be suppressed or perturbed [44]. In the present study, the cell cycle phase distribution of Caco2 cells treated with 200 $\mu\text{g}/\text{mL}$ algal extracts for 72 hours is represented in Figure 6. Crude extracts blocked proliferation of Caco2 cells by arresting the cell cycle. Flow cytometric analysis indicated G2-M block in algae-treated cells along with significant increase in the sub-diploid cell population (sub-G1). Onset of G2-M cell cycle arrest along with increase in the sub-diploid cell population (sub G1) suggests that the extracts were potent enough to induce both G2-M phase cell cycle arrest and apoptosis (Figure 6).

Previous studies reported that some algae extracts inhibited cell growth in a dose- and time-dependent manner, by arresting cell-cycle progression and by promoting apoptosis in the HCT-116 colon cancer cell line [45]. Figure 7 shows cell cycle phase distribution of control and treated cells with green, brown and red algae extracts (200 $\mu\text{g}/\text{mL}$) for 72 hours. The data indicated that the treated Caco2 cells in the G1 phase decreased with a concomitant increase in the sub-G peak

The brown algae mainly *C. crinita* extracts arrested the cells in a post G1 and G phases, and the numbers of sub G and G cells gradually increased significantly from 3.9%, 37.2% to 37.2% to 51.6% respectively after treatment ($P < 0.05$) (Figure 7b), in accord with previous findings [37]. Among tested red algae, most cells treated with *H. musciformis* extract, arrested in G phase (57.6%) (Figure 7c), whereas cells treated with *U. lactuca* extract showed a decrease to 53.4% (Figure 7a). These results suggest that algae extracts especially brown algae are promising candidates for further investigation .

Many chemotherapeutic agents are found to be selectively toxic to tumor cells because they increase oxidant stress and enhance these already stressed cells beyond their limit [46]; in contrast, the anticancer activity of plant compounds may be attributed to their high affinity to the target, little loss of entropy when they bind to a protein and their bioavailability. Moreover, plant compounds are considered to have conformational flexibility in aqueous and lipophilic environments [47] and may act as good alternative anti-cancer agents.

There is growing need for the development and or discovery of highly potential bioactive compounds from natural sources due to the resistance to chemical drugs.

4. Conclusion

The present study elucidated for the first time the antioxidant and anticancer properties of eight Libyan seaweeds. The results reveal that among tested algae, *C. crinita*, *C. stricta*, *J. rubens* and *U. lactuca* extracts possess high antioxidant and antiproliferative activities which might be helpful in preventing or slowing the progress of various oxidative stress related disorders. However, further investigation is needed to assess the molecular mechanisms of the potential anticancer activities of

these algae extracts as well as to identify of the bioactive compounds in the algae extracts and their commercial potential and applications in medicine, food production and in the cosmetic industry.

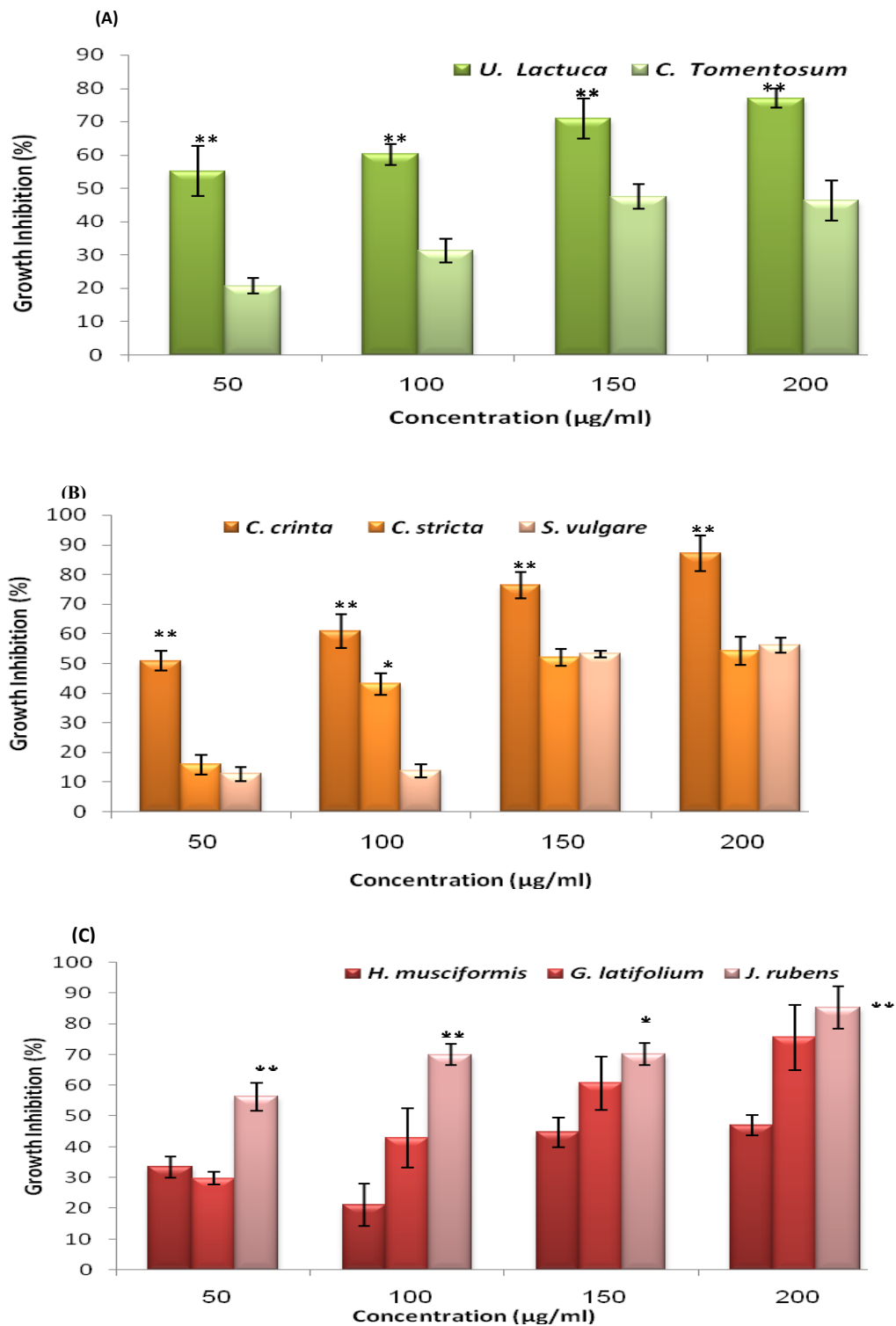


Figure 2. antiproliferative effects of selected Libyan seaweeds: (A) **Chlorophyta algae** (*U. lactuca*, *C. tomentosum*), (B) **Phaeophyta algae** (*C. crinite*, *C. stricta*, *S. vulgare*) and (C) **Rhodophyta algae** (*H. musciformis*, *J. rubens*, *G. latifolium*) extracts on Caco2 cells. The cells were treated with increasing concentration of algae extracts for 72 hours. Cytotoxicity activity of the extracts was evaluated by MTT assay. Results were reported as mean (n = 6) percent of inhibition of cell growth with error bars showing the standard deviation. Asterisks indicate significant cytotoxicity relative to the control (* $P < .05$, ** $P < 0.01$).

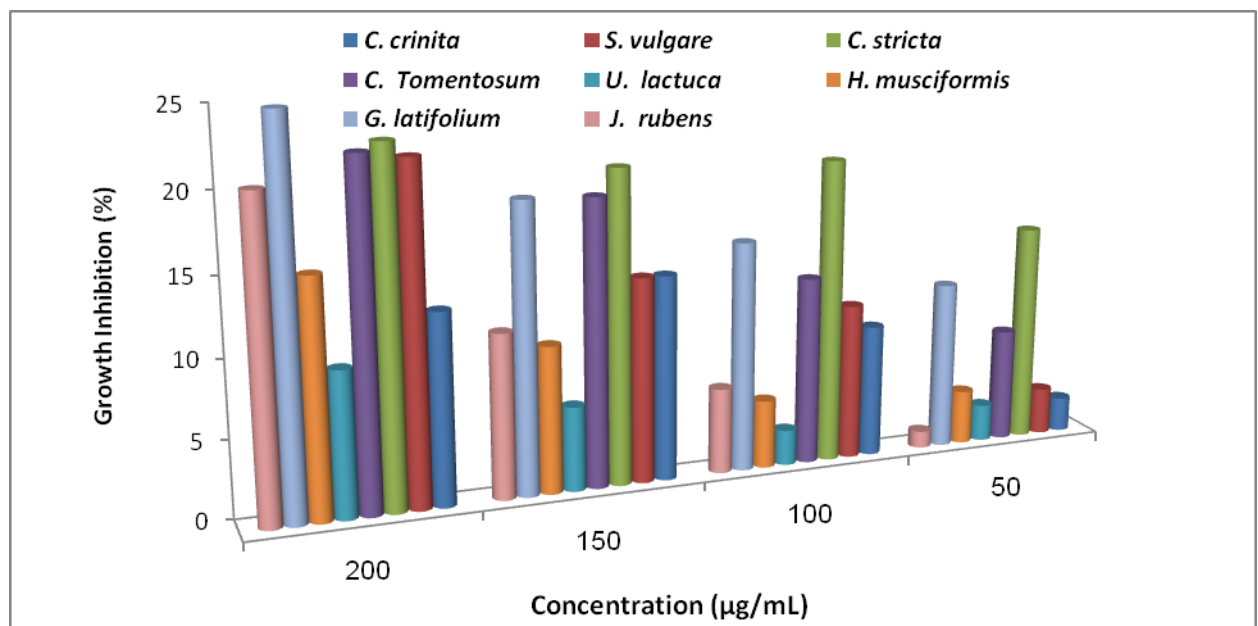


Figure 3: Antiproliferative effects of selected Libyan seaweeds (*U. lactuca*, *C. tomentosum*, *C. crinite*, *C. stricta*, *S. vulgare* and *H. musciformis*, *J. rubens*, *G. latifolium*) **extracts on HCEC cells line.** The cells were treated with increasing concentration of algae extracts for 72 hours.

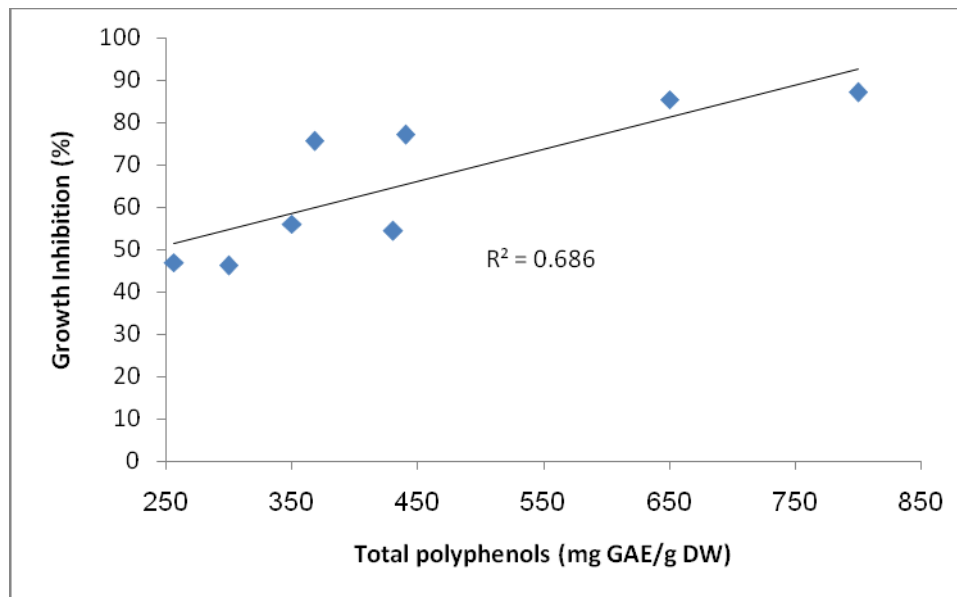


Figure 4: Correlation between the contents of total phenols in seaweeds and anticancer activity of extracts.

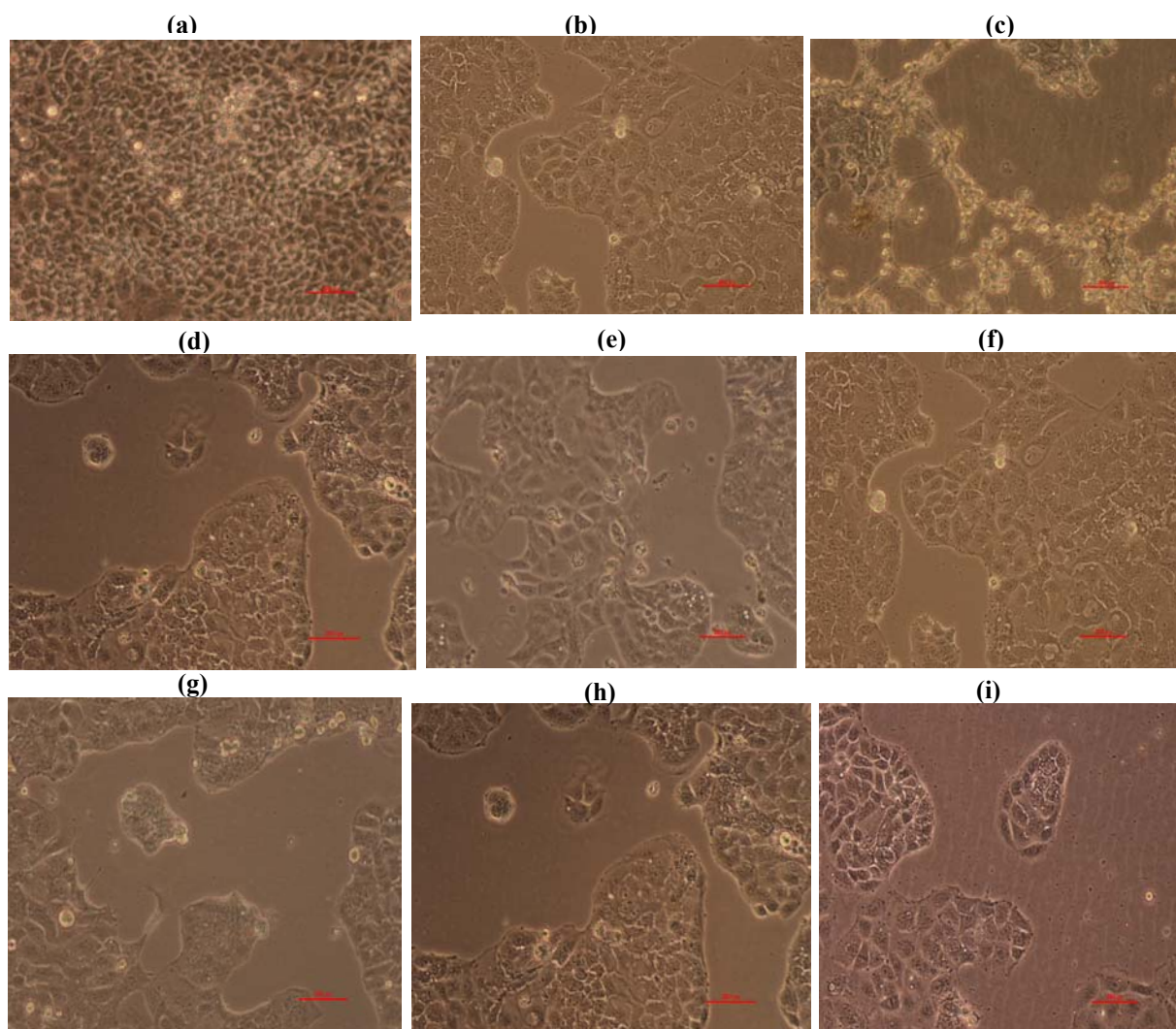


Figure 5. Morphological changes of cells untreated and treated with seaweeds extracts observed under an inverted light microscope (Olympus, Tokyo, Japan). Caco-2 cells were incubated for 48 h in the absence (a) and presence of (200 µg/mL) of *S. vulgare* (b), *C. crinita* (c), *C. stricta* (d), *H. musciformis* (e), *J. rubens* (f), *G. latifolium* (g), *C. tomentosum* (h), *U. lactuca* (i) extracts. Control cells appeared healthy and confluent while (b, c and d) treated cells were unwell and most cells were detached. Mag. X100.

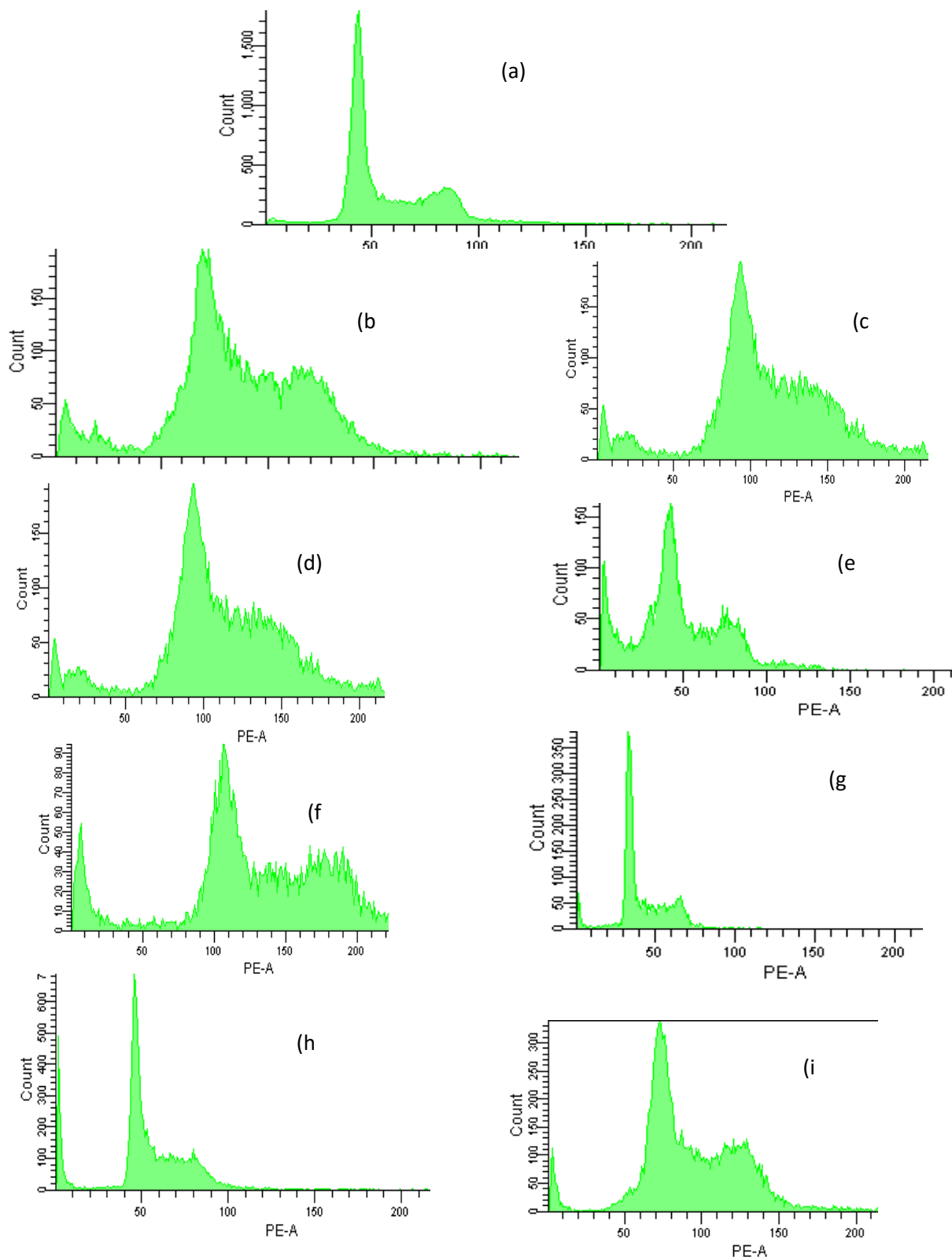


Figure 6. Cell cycle analysis of CaCo2 cancer cells treated with 200µg/mL of algae extracts for 72 hours. Caco-2 cells were cultured with control (a) and presence of *C. crinita* (b), *C. stricta* (c), *S. vulgare* (d), *J. rubens* (e), *H. musciformis* (f), *G. latifolium* (g), *U. lactuca* (h), *C. tomentosum* (i).

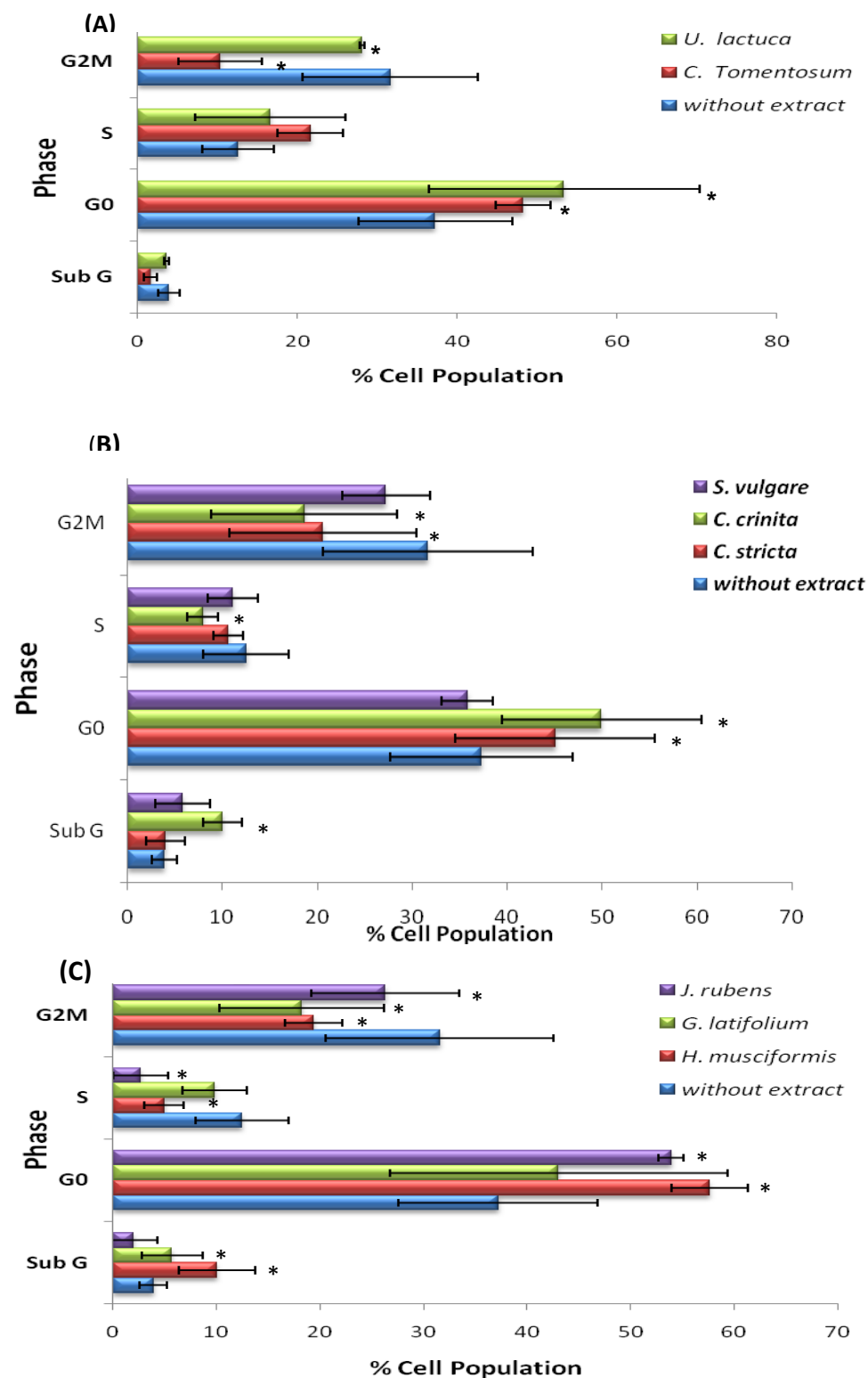


Figure 7. Effect of algal extracts on Caco2 Cell Cycle after 72 hours incubation. Cells were fixed with ethanol and stained with propidium iodide, and then cell cycle distribution was analyzed by flow cytometry. Bar charts representing the percentage of cell populations in Caco2 cells treated with 200 µg/mL extracts of (A) green algae; and (B) brown algae; (C) red algae. The asterisk indicates a significant difference between control and algal-treated cells, (* $P < 0.05$).

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Conflict of interest

None declared.

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