Anticancer and Antioxidant Activities of Some Algae from Western Libyan Coast

Rabia Alghazeer¹, Nazlin K. Howell², Mahboba B. El-Naili¹, Nuri Awayn¹

¹Department of Chemistry, Faculty of Sciences, University of Tripoli, Tripoli, Libya; ²Faculty of Health and Medical Sciences, School of Biosciences and Medicine, University of Surrey, Guildford, England, UK

Correspondence to: Rabia Alghazeer, Rabia_alghazeer@yahoo.com

Keywords: Polyphenols, Flavonoids, Seaweeds, Antioxidant Activity, Anticancer Activity

Received: May 29, 2018 Accepted: July 7, 2018 Published: July 10, 2018

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) were collected from western Libyan coast and 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, cell proliferation was reduced significantly. The antiproliferative effect of algae extracts was correlated with their polyphenol and flavonoid contents. Cell cycle analysis further showed that cells were arrested in G1 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 used in anticancer drug research programs. However, further investigations are essential to reveal the molecular mechanisms of the anticancer activities of these algae.

1. INTRODUCTION

Seaweeds are large and diverse groups of plants 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, pro-
tein, 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 [6]; as well as anti-tumoral activities [7, 8]. Moreover, many studies show that some algae 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 algae 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).

2. MATERIALS AND METHODS

2.1. Experimental Materials

Seaweeds algae 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 algae samples were authenticated in the Botany Department, Faculty of Science, University of Tripoli. Human colorectal carcinoma (Caco2) and Human Corneal Epithelial Cells (HCEC) cell 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.

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˚C - 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 (20 g) 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]. Total 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 mg∙mL−1 rutin.

2.5. Antioxidant Activity Assays

For antioxidant assays, all extracts (1.0 mg/mL) were dissolved in 95% methanol and a series of concentration-dependent dilutions were made (40 - 300 µg/mL). Standard reagents were utilized for comparison of 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., [30]. The percentage inhibition of the DPPH radical by the samples was calculated according to the following equation: % Inhibition = (A0 − A1)/A0 × 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.

2.5.2. Reducing Power Assay

The reducing power of extracts was investigated following the method of Oyaizu [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 2 mL 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 1 mg/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) 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 algae 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 incubation time, 10 μl/well (5 mg/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 μg/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 cm^2 tissue culture flasks, treated with extracts of all tested algae (200 μg/mL) for 72 h. After treatment, the cells were collected by trypsinization, fixed in 70% cold ethanol, washed in PBS, resuspended in 1 mL of PBS containing 1 mg/mL RNase. The cells were then incubated for 30 min at 37°C. After which, 5 μL of propidium iodide (1 mg/ml) PI staining dye was added and cells were kept on ice until analyzed using a BD FACs Canto flow cytometer (BD Biosciences, California, USA). A minimum of 10,000 events was acquired in list mode and analyzed at excitation and emission wavelengths of 535 nm and 617 nm, respectively. 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 ± 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 was 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 80.28 ± 3.62 mg GAE/g and 60.33 ± 3.15 mg GAE/g dry weight expressed as gallic acid equivalents, and 47.72 ± 26.51 mg rutin/g 43.79 ± 25.61 mg rutin/g, 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).
Table 1. Total polyphenol and flavonoid contents of methanolic extracts of the tested algae.

<table>
<thead>
<tr>
<th></th>
<th>Polyphenols content <em>(mg GAE/g DW)</em></th>
<th>Flavonoids content (mg Rutin/g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chlorophyta</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>U. lactuca</em></td>
<td>44.50 ± 39.13^a</td>
<td>36.07 ± 2.57^a</td>
</tr>
<tr>
<td><em>C. tomentosum</em></td>
<td>30.17±35.38^b</td>
<td>26.07 ± 2.57^c</td>
</tr>
<tr>
<td><strong>Phaeophyta</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. crinita</em></td>
<td>80.28 ± 36.23^c</td>
<td>47.72 ± 2.651^a</td>
</tr>
<tr>
<td><em>C. stricta</em></td>
<td>43.6 ± 30.13^a</td>
<td>35.59 ± 2.931^b</td>
</tr>
<tr>
<td><em>S. vulgare</em></td>
<td>35 ± 26.28^b</td>
<td>25.67 ± 2.55^d</td>
</tr>
<tr>
<td><strong>Rhodophyta</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. musciformis</em></td>
<td>25.44 ± 4.18^d</td>
<td>20.02 ± 2.40^d</td>
</tr>
<tr>
<td><em>J. rubens</em></td>
<td>60.33 ± 31.53^*</td>
<td>43.79 ± 2.56^a</td>
</tr>
<tr>
<td><em>G. latifolium</em></td>
<td>36.63 ± 21.53^b</td>
<td>29.65 ± 1.80^c</td>
</tr>
</tbody>
</table>

DW: dry weight; *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 same superscripted letters^a-f are not significantly different at p = 0.05.

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.

The presence of a high level of polyphenols including phenolic acids, flavonoids, isoflavones, cinnamic acid, benzoic acid, quercetin in algae, nominate these algae extracts as reliable sources of antioxidants [12].

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 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 algae extracts possessed radical scavenging activity, although *C. crinita* was more effective in scavenging DPPH with lowest IC$_{50}$ (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 IC$_{50}$ 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 antioxidant activity than red or green algae [10]. The lowest scavenging ability was shown by *C. tomentosum* and *G. latifolium* with higher IC$_{50}$ (300 µg/mL). The present study showed that the green algae collected from Libyan coast have very low antioxidant power which is consistent with the other reports on the green algae [14, 35].

3.2.2. 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 algae extracts under investigation. In general, the reducing power of the extract was concentration dependent. There were
Table 2. Antioxidant activity of selected algae.

<table>
<thead>
<tr>
<th>Algae</th>
<th>DPPH* IC&lt;sub&gt;50&lt;/sub&gt; µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chlorophyta</strong></td>
<td></td>
</tr>
<tr>
<td><em>U. lactuca</em></td>
<td>230.50 ± 9.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>C. tomentosum</em></td>
<td>300.17 ± 35.38&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Phaeophyta</strong></td>
<td></td>
</tr>
<tr>
<td><em>C. crinita</em></td>
<td>50.5 ± 3.20&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>C. stricta</em></td>
<td>75.11 ± 30.13&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>S. vulgare</em></td>
<td>150 ± 26.28&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Rhodophyta</strong></td>
<td></td>
</tr>
<tr>
<td><em>H. musciformis</em></td>
<td>200.33 ± 24.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>J. rubens</em></td>
<td>130.5 ± 31.53&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>G. latifolium</em></td>
<td>300.03 ± 9.22&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Ascorbic acid</em></td>
<td>156 ± 12.06</td>
</tr>
</tbody>
</table>

*: DPPH, 2,2-diphenyl-1-picrylhydrazyl. Each value is presented as mean ± SD (n = 3). Means with same superscripted letters a-f are not significantly different at p = 0.05.

Figure 1. Reducing potential of crude algae extracts determined by reducing power assay. Data are mean ± SD. (a) green algae; (b) brown algae, (c) red algae extracts and (d) ascorbic acid (positive control).
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 1(d)).

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) & Figure 1(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 1(a)). 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.

3.3. Anticancer Activity

3.3.1. Cytotoxic Activity of Crude Algae Extracts

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 µg/mL) (Figure 2(b)), while HCEC cells were used to determine the extract cytotoxic effect against a normal cell (Figure 2(a)).

Figure 2(b) shows the percentage changes in the growth inhibition of cancer cells treated with algae extracts. The tested algae extracts especially $C. crinite$ extract, showed a strong selective cell proliferation inhibition of the cancer cell line (87.05%). This might be due to high polyphenols and flavonoids contents (200 µg/mL) extract. 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 indicated that cell death was a concentration-dependent process, hence the number of non-viable cells increased with increasing concentration of algae extracts.

Among brown algae, the $C. crinite$ extract induced cytotoxic effect on the Caco2 cells after 72 h exposure significantly; the percentage of inhibition was 87% at 200 µg·mL$^{-1}$ compared to lower inhibition (less than 50%) of extracts of $C. stricta$ and $S. vulgare$ ($P < 0.01$) (Figure 2(b)). This finding 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 µg/whereas cells treated with $H. musciformis$ extract showed 48% inhibition (Figure 2(b)). In comparison with tested green algae extracts, $U. lactuca$ caused significant cytotoxicity at the very low concentration (50 µg/mL; 55%) ($P < 0.01$) and higher cytotoxic effect, in a dose-dependent manner in the range 50 - 200 µg·mL$^{-1}$ (55, 60, 70 and 77%) (Figure 2(b)). 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 µg/mL (Figure 2(a)).

IC$_{50}$ obtained against Caco2 cell line in the presence of the crude extract of $C. crinite$, $C. stricta$, $S. vulgare$ were; >50 µg/mL, 120 µg/mL and 150 µg/mL respectively. In contrast, the IC$_{50}$ obtained against Caco2 cell line in the presence of the crude extracts of $H. musciformis$, $J. rubens$, $G. latifolium$ were; >200 µg/mL, 50 µg/mL and 120 µg/mL respectively. Moreover IC$_{50}$ obtained against Caco2 cell line in the presence of the crude extract of $U. lactuca$, $C. tomentosum$ were; 50 µg/mL, and >200 µg/mL respectively. The low IC$_{50}$ values of $C. crinite$, $J. rubens$ 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 proliferation inhibition of Caco2 cells is likely to be related to their content of these compounds especially polyphenols 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 3). The anticancer activity of polyphenols could be induced via
Figure 2. 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 (a) and Caco2 cells (b). The cells were treated with increasing concentration of algae extracts for 72 hours. Cytotoxicity activity of the extracts was evaluated by MTT assay.

Figure 3. Correlation between the contents of total polyphenols in seaweeds and anticancer activity of extracts.

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 algae extracts to induce cell death was estimated by analyzing its effect on cell morphology (Figure 4). The observation of Caco2 cells under a phase contrast microscope showed that after 48 h of treatment with 200 µg/mL extracts, detectable changes were found, including altered cell morphology, cell shrinkage and membrane blebbing, the characteristic features of apoptotic cell death (Figure 4).

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 lead to cell suppression or Perturbation [44]. The cell cycle phase distribution of Caco2 cells treated with 200 µg/mL algae extracts for 72 hours is represented in Figure 5. 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
Figure 4. 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. musci-formis (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 was unwell and most cells were detached. Mag. X100. Arrows indicate.

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 5).

Previous studies reported that some algae extracts inhibited cell growth in a dose- and time-dependent manner, by arresting cell-cycle progression and/or promoting apoptosis in the HCT-116 colon cancer cell line [45]. Figure 6 shows cell cycle phase distribution of control and treated cells with
Figure 5 Cell cycle analysis of CaCo2 cancer cells treated with 200 µg/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 algae 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 algae-treated cells, (*P < 0.05).

green, brown and red algae extracts (200 µg/mL) for 72 hours. Obtained 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% and 51.6% respectively after treatment (*P* < 0.05) (Figure 6(b)), in consistent with previous findings [37]. Among tested red algae, most cells treated with *H. musciformis* extract, arrested in G phase (57.6%) (Figure 6(c)), whereas cells treated with *U. lactuca* extract showed a decrease to 53.4% (Figure 6(a)). 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 oxidative 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 to evaluate 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 the bioactive compounds in the algae extracts and their commercial potential and applications in medicine, food production and in the cosmetic industry.

ACKNOWLEDGEMENTS

This work was financially supported by the University of Tripoli, Tripoli, Libya.

CONFLICT OF INTEREST

None declared.

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