



Original article

Preliminary screening of antioxidant and cytotoxic potential of green seaweed, *Halimeda opuntia* (Linnaeus) Lamouroux

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ABSTRACT

Marine natural products have displayed numerous advantageous effects on biological activities, including antioxidants and cytotoxicity. The total lipids, carotenoids, chlorophyll *a* and *b* content, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of methanolic crude extract of the green seaweed *Halimeda opuntia* were all measured in this study. The TPC of the extracts was determined according to the Folin-Ciocalteu method, yielding a result of 55.04 ± 0.98 mg GAE/g of extract. As determined by the aluminium chloride colorimetric method, the TFC of the extract was 40.02 ± 0.02 mg QE/g of extract. Antioxidant activity was determined by using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay with different concentrations that ranged between 200 and 1000 $\mu\text{g/mL}$, noted *H. opuntia* as the highest in DPPH reduction (63.61 %) at 1000 $\mu\text{g/mL}$ concentration. Total antioxidant capacity (TAC) of the extract was 57.36 ± 0.004 mg AAE/g extract at concentration of 1.0 mg/mL. The cytotoxic activity of this seaweed was pre-screened against a panel of cell lines including estrogen receptor-positive human breast adenocarcinoma (MCF-7), estrogen negative human breast adenocarcinoma (MDA-MB-231), human colorectal adenocarcinoma (HT-29), human hepatocellular carcinoma (HepG2), and mouse embryonic fibroblast (3T3) using the MTT assay. The content of total lipids in *H. opuntia* was 1.60 ± 0.002 %. Total carotenoids were 115.57 ± 0.98 $\mu\text{g/g}$, while chlorophyll *a* and *b* were 148.73 ± 2.60 $\mu\text{g/g}$ and 290.83 ± 9.46 $\mu\text{g/g}$, respectively. In terms of cytotoxicity activity, methanolic extract of *H. opuntia* was found to be highly cytotoxic to MCF-7 cells, with an IC_{50} of 25.14 ± 1.02 g/mL, and slightly less so to 3T3 cells (IC_{50} 65.23 ± 0.25 $\mu\text{g/mL}$). This study's findings suggest that natural pigments (carotenoids and chlorophyll), phytochemicals like phenolic and flavonoid compounds found in this species may play an important role and could be used as a natural cancer treatment.

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1. Introduction

Cancer is one of the global health concerns that ranked second in death (about 10 million in 2020). Currently, cancer prevalence has soared progressively. National Cancer Registry Department, National Cancer Institute, Ministry of Health, Malaysia reports that

cancer is the fourth major cause of death with an increment of 11.3 % of new cases from 2007 to 2016. From 48,639 new cases in 2020, breast cancer was found to be the most common cancer type in Malaysia, with 17.3% incidence, followed by colorectum and lung cancer, with 13.6 and 10.6% of cases, respectively (The Global Cancer Observatory, 2021). In line with the National Strategic Plan for Cancer Control Programme (NSPCCP) 2016–2020 by the Malaysian government, other alternatives such as using natural anti-cancer medication derived from natural products might be among the imperative strategies for cancer deterrence. However, the assessments of the active elements corresponding to the biological activities versus these diseases require appropriate investigation.

The immense diversity of structurally unique compounds produced by marine seaweeds, predominantly those characterised

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by their toxicity, has sturdily enthused marine natural product research. Studies have suggested that seaweed possesses numerous beneficial properties biologically as anticancer, antioxidant, anti-angiogenic, and neuroprotection for the human body due to the photosynthetic pigments it possesses such as chlorophylls, carotenoids, and phycobilins (Pangestuti and Kim, 2011; Chen et al., 2017). Pigments from seaweed can scavenge free radicals and act as antioxidants that impede oxidative damage, leading to carcinogenesis (Ferdous and Yusof, 2021b; Vijay et al., 2018; (Castro-Puyana et al., 2017) ro-Puyana et al., 2016; Linnewiel-Hermoni et al., 2015). Thus, radical scavengers from seaweed can be exploited ultimately to diminish tumour formation and may play a significant role in cancer inhibition (Ferruzzi and Blakeslee, 2007; Pemmaraju et al., 2018; Vaňková et al., 2018).

Seaweeds comprise various antioxidants like polysaccharides, minerals, vitamins, essential amino acids, indigestible carbohydrates, dietary fiber, and carotenoids (Jimenez-Escrig et al., 2001; Burtin, 2003). *Halimeda opuntia* is a marine green seaweed found in shallow tropical marine environments. A few studies demonstrated that marine seaweeds from the genus *Halimeda* consisted of bioactive compounds such as polyphenols, diterpenes, fatty acids, and sterols, which showed significant cytotoxicity to cancer cells (Yoshie et al., 2002; Nazarudin et al., 2020). The purpose of the current exploration was to determine the total carotenoid and chlorophyll compositions along with total phenolic and total content in green seaweed, *H. opuntia*, and investigate the antioxidant activity of methanolic extract of *H. opuntia* by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay as well as total antioxidant activity and test its therapeutic efficacy *in vitro* against breast, liver, and colon cancer cell lines.

2. Materials and methods

2.1. Plant material and samples preparation

The marine green seaweed, *H. opuntia*, used in this study was collected in March 2021 in Port Dickson, Negeri Sembilan, Malaysia (2° 26' N, 101° 51' E), as shown in Fig. 1. Cross-referencing with taxonomic books, monographs, and reference herbaria was used for taxonomic identification. Seaweed specimens were initially



Fig. 1. Green seaweeds, *Halimeda opuntia* (Linnaeus) Lamouroux (ID: IBS-SW5).

rinsed thoroughly with seawater to eradicate debris, sand, epiphytes, pebbles, and shells. The cleaned samples were brought to the research facilities in transparent polyethylene bags. At the laboratory, the collected samples were further rinsed with distilled water and stored at -80°C (Thermo Scientific, USA). Frozen seaweed was subjected to lyophilization and then ground to a fine powder using a blender to pass through a 200- μm -sized sieve. Finally, the seaweed powder was stored in a sealed bag in a -80°C freezer until further use.

Lyophilized fine-powdered seaweed sample was mixed and dissolved with 50 mL of methanol (in the ratio of 1:20, w/v) for 30 min in a sonicator bath (Power Sonic 505, Korea). The crude extract was passed through a Whatman paper filter and subsequently concentrated by rotary evaporation (N-1001S-WD, with EYELA Water Bath OSB-2000, Japan) under a partial vacuum at 40°C . The concentrated extract was transferred into amber bottles and then stored at -20°C before subsequent analysis.

2.2. Lipid extraction and total carotenoids and chlorophyll content determination

For the lipid content of *H. opuntia*, the sample was extracted according to the modified method of Regal et al., 2020. An amount of 0.5 g of lyophilized seaweed was mixed homogeneously (Power Sonic 505, Korea) for fifteen minutes in 20 mL of chloroform:methanol (2:1, v/v) and then filtered by using a paper filter. The filtrates were mixed in 10 mL of sodium chloride (9 %) before being centrifuged at 2000 rpm for 8 min (Eppendorf Centrifuge 5810R, Germany), resulting in two layers being separated. The bottom layer consisting of chloroform was collected, subsequently concentrated, and left to dry in a 40°C vacuum oven (Memmert, USA) until the weight became constant for gravimetric quantification of the seaweed total lipid content.

The dried seaweed lipid extract was diluted with methanol. In the meantime, the determination of total carotenoids, chlorophyll *a* and *b* were carried out following the method of Nazarudin et al., 2020, by measuring the absorbance at 470, 665.2, and 652.4 nm, respectively, using a UV-spectrophotometer (Shimadzu UV 1601, Japan) and then calculated using the Lichtenthaler equations. All the values were articulated as $\mu\text{g/g}$ dry weight ($\mu\text{g/g}$ DW).

$$C_{(x+c)}(\mu\text{g/g}) = (1000 A_{470} - 1.63 C_a - 104.96 C_b)/221$$

$$C_a(\mu\text{g/g}) = 16.72 A_{665.2} - 9.16 A_{652.4}$$

$$C_b(\mu\text{g/g}) = 34.09 A_{652.4} - 15.28 A_{665.2}$$

Where, $C_{(x+c)}$ = total carotenoids, C_a = chlorophyll *a*, C_b = chlorophyll *b*, A_{470} = absorbance at 470 nm, $A_{665.2}$ = absorbance at 665.2 nm, $A_{652.4}$ = absorbance at 652.4 nm.

2.3. Total phenolic content (TPC) assay

Total phenolic content for *H. opuntia* was determined using the Folin-Ciocalteu methods (Nazarudin et al., 2020). To determine TPC, approximately 100 μL of Folin-Ciocalteu's reagents and 80 μL 7.5 % (w/v) of sodium carbonate were added to 20 μL (1 mg/ml) of aqueous extract in a well of a 96-well plate. The 96-well plate was incubated at room temperature for 30 min. Using an ELISA plate reader (Thermo multiskan go), the absorbance was read at 765 nm against a blank (solvent without extract). A standard curve of gallic acid (0–1 mg/mL) was plotted. The results are expressed as milligram gallic acid equivalent (mg GAE)/gram dry weight of samples using the following equation.

$$C = c \times V / m$$

Where;

C: Total phenolic content (mg GAE/g plant extract)
 c: concentration of gallic acid (mg/mL)
 V: volume of extract (mL)
 m: weight of extract

2.4. Total flavonoid content (TPC) assay

Total flavonoid content was determined using the aluminium chloride colorimetric method (Vongsak et al., 2013). The methanolic extract (500 g/mL) was mixed with 2 % (w/v) of aluminium chloride solution in methanol. The mixtures were incubated at room temperature for 10 min. The absorbance of the mixtures was measured using a microplate reader (Thermo Multiskan GO) at 415 nm against a blank. The standard curve of quercetin (0–0.05 mg/mL) was plotted and the results were expressed as milligrams quercetin equivalent (mg QE)/gram extract using the following equation:

$$C = c \times V / m$$

Where;

C: Total flavonoid content (mg QE/g plant extract)
 c: concentration equivalent to quercetin (mg QE/mL)
 V: volume of extract (mL)
 m: weight of extract (g)

2.5. Antioxidant activity

2.5.1. DPPH radical scavenging

The DPPH radical scavenging activity of methanolic extracts of seaweed was determined according to the method adopted by Li et al. (2012) that has been described by Karthivashan et al. (2013) with minor modifications. For preparing the DPPH solution, 1.95 mg of DPPH powder was dissolved into 50 mL of methanol and used immediately. The stock solution of methanolic extracts was prepared by dissolving 5 mg of extract into 1 mL of methanol. Following that, series dilutions were performed to produce 200, 400, 600, 800, and 1000 µg/mL with a working volume of 500 µL. Ascorbic acid was prepared as a positive control, while for the negative control, 500 µL of methanol was added into 500 µL of DPPH solution. Then, 500 L of DPPH solution was added to each sample and left in a dark room for 30 min. A volume of 100 µL for each sample was transferred into 96-well plates, and the absorbance was measured at a wavelength of 517 nm by using a microplate reader (Tecan Sunrise Basic, Groedig, Austria). The antioxidant activity was calculated by using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = [(A_0 - A_1/A_0) \times 100]$$

Where A_0 = absorbance of control, A_1 = absorbance of sample

2.5.2. Total antioxidant capacity (TAC)

The total antioxidant capacity was assessed using the phosphomolybdenum method by Prieto et al. (1999) with some modifications. The reagent solution was prepared by mixing 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate with the same amount of each. Then, 0.3 mL of extract with a concentration of 1.0 mg/mL was mixed with 3 mL of reagent solution. The test was done in a dark environment since it was a light-sensitive test. The mixture was shaken and incubated in a water bath at 95 °C for 90 min. After they cooled at room temperature, the absorbance of each mixture was measured at 695 nm using a microplate reader (Thermo Multiskan GO). Blanks were run using solvent without extract while quercetin was used as a positive control. A standard curve of ascorbic acid with a concentration of (0–0.5 mg/mL) was plotted, and the total antioxidant capacity was expressed as milligrammes of ascorbic acid equivalent (mg AAE)/gram extract using the following equation:

$$C = c \times V / m$$

Where;

C: Total antioxidant capacity (mg AAE/g plant extract)
 c: concentration equivalent to ascorbic acid (mg AAE/mL)
 V: volume of extract (mL)
 m: weight of extract (g)

2.6. Cell lines and culture condition

The cell lines used were MCF-7 cells (human breast adenocarcinoma cell line, estrogen receptor-positive), MDA-MB-231 cells (human breast adenocarcinoma cell line, estrogen receptor-negative), HT-29 cells (human colorectal adenocarcinoma cell line), HepG2 cells (human liver hepatocellular carcinoma cell line) and 3T3 cells (mouse embryonic fibroblast cells). All cell lines were maintained in RPMI 1640 medium, supplemented with 10% fetal calf serum (FCS). The media was then treated with penicillin (100 units/ml) and streptomycin (0.1 mg/ml) and kept at 37 °C in a 5% CO₂ (95% humidity) atmosphere for 24 h.

2.7. MTT assay

The cytotoxicity of *H. opuntia* methanolic extracts was assessed using an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay developed by Nazarudin et al., 2020. About 100 µL of cell culture (1×10^5 cells/mL) was plated onto 96-well plates. The cells were incubated at 37 °C in an atmosphere of 5% CO₂ (95% humidity) for 24 h. The diluted extracts were added to each well by adjusting the final concentrations to 1.56, 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL and incubated for 24 h. Subsequently, 2 mg/ml of MTT solution was added to each well and incubated in the dark for 1 h. Finally, the optical density (OD) was measured by using a microplate reader (Thermo Multiskan GO) at 570 nm. The cytotoxicity (IC₅₀) was recorded using the formula given below:

$$\text{Cell viability} = \frac{\text{OD sample (mean)}}{\text{OD control (mean)}} \times 100$$

2.8. Clonogenic survival assay

MCF-7 cells (1×10^3 cells/well) were seeded into a 6-well plate and incubated overnight at 37°C in a 5% CO₂ atmosphere. The seeded cells were treated into a 6-well plate and incubated at 37 °C and 5% CO₂ atmosphere overnight. Following incubation, 3 mL of test compounds at various concentrations were added to the culture media and incubated for another seven days. Upon completion, cells were washed with PBS and fixed with 4% paraformaldehyde for 15 min followed by cell staining with 0.5% (w/v) crystal violet for 20 min. The resultant plates were then washed with tap water and dried at room temperature. Upon drying, the plate image was photographed.

2.9. Cell death analysis

Cell death analysis of MCF7 cells was monitored using double staining of Hoechst 33,342 (Biotium, USA) and propidium iodide (PI) (Sigma, USA). Initially, 120,000 cells per well were seeded in a 6-well plate overnight. Then, the cells were treated with methanolic extract of *H. opuntia* at a concentration of previously identified IC₅₀ values for 72 h. Then, the cells were harvested, washed with PBS, and centrifuged at 1500 rpm for 5 min. The supernatant was removed prior to the addition of ice-cold ethanol for cell fixation, and the fixed cells were incubated at 4 °C for 30 min. After a series of washing and centrifugation steps, Hoechst

33,342 was added to the cell suspension and mixed thoroughly prior to incubation at 37 °C for 15 min. The washing and centrifugation steps were then repeated before PI was added to the cell suspension and mixed thoroughly. Next, the cells were incubated at room temperature for 15 min in a light-protected area. Finally, the stained cells were washed with PBS and analysed by an Olympus CKX41 Zeiss Axio Vert.A1 inverted microscope equipped with ZEN Lite software (Zeiss, Germany) for the colour intensity exhibited by both cell lines.

2.10. Gas chromatography-mass spectrometry analysis

To characterise and quantify the bioactive components of *H. opuntia*, the sample was subjected to gas chromatography-mass spectrometry (GC-MS) analysis using the GC-MS QP2010 Ultra (Shimadzu Co., Japan) system on a DB-1 (0.25 µm film × 0.25 mm ID × 30 m length) column according to the method by Nazarudin et al., 2020. The metabolites were detected by assessing the spectral patterns acquired with those of mass spectral archives (NIST3208 and WILEY libraries).

2.11. Data analysis

Data were presented as mean values from three replications for total carotenoids, chlorophyll *a* and *b*, total phenolic, total flavonoid content, DPPH radical scavenging, total antioxidant assay and six replicates for MTT assay with standard deviations. One-way ANOVA was used to check the differences that were considered significant at $P < 0.05$.

3. Results

3.1. Content of total lipids, carotenoids and chlorophylls

The total lipid recorded in *H. opuntia* was 1.60 ± 0.002 % DW. The results of total carotenoids, chlorophyll *a* and *b* content (in µg/g) were tabulated in Table 1. The total carotene content found in *Halimeda opuntia* was 115.57 ± 0.98 g/g. Chlorophyll *a* content in *H. opuntia* was detected at 148.73 ± 2.60 g/g, while chlorophyll *b* was recorded higher than chlorophyll *a* at 290.83 ± 9.46 g/g.

3.2. Total phenolic content

The total phenolic content (TPC) of *H. opuntia* was determined, expressed in mg GAE/g of extract, and shown in Table 2. The total phenolic content found in *H. opuntia* was 55.71 ± 0.98 mg GAE/g of extract.

3.3. Total flavonoid content

The total flavonoid content (TFC) of *H. opuntia* was determined, expressed in mg QE/g of extract, and shown in Table 2. The total flavonoid content found in *H. opuntia* was 40.02 ± 0.02 mg QE/g of extract.

Table 1
Pigment content analysis in *Halimeda opuntia*.

Seaweed Species	Total carotenoids (µg/g)	Chlorophyll a (µg/g)	Chlorophyll b (µg/g)
<i>Halimeda opuntia</i>	115.57 ± 0.98	148.73 ± 2.60	290.83 ± 9.46

Values are expressed as Mean ± standard deviation, n = 3, Dry weight basis.

Table 2
Total phenolic and flavonoid content of *H. opuntia*.

Seaweed Species	TFC (mg QE/g)	TPC (mg GAE/g)
<i>Halimeda opuntia</i>	40.02 ± 0.02	55.71 ± 0.98

Values are expressed as Mean ± standard deviation, n = 3.

3.4. Antioxidant activity

3.4.1. DPPH radical scavenging activity

The free radical scavenging capacity of *H. opuntia* methanolic extract was evaluated at various concentrations and compared with ascorbic acid as the standard, as shown in Fig. 2. The graph pattern shows that the seaweed extract took part in the DPPH reduction as the concentration increased. This present study reveals that *H. opuntia* methanolic extract showed the DPPH reduction ranged from 56.29 to 63.91 %, and 50 % of inhibition was achieved at a concentration of 200 µg/mL.

3.4.2. Total antioxidant capacity

The total antioxidant capacity was determined using the phosphomolybdenum method to evaluate the antioxidant potential of the extract. In the presence of antioxidant compounds, Mo (VI) would be reduced to Mo (V) forming a green phosphomolybdenum complex at acidic pH. From the test, the TAC of green seaweed *H. opuntia* was 57.36 ± 0.004 mg AAE/g as shown in Table 3.

3.5. Cytotoxicity activity

The cytotoxic activity of *H. opuntia* methanolic extract against five cancer cell lines determined in this study is tabulated in Table 4. The extract exhibited the ability to cytotoxicity act on all cell lines tested. As shown in Table 4, the extract showed an IC₅₀ of 25.14 ± 1.02 µg/mL against the MCF-7 cell line, which is the most cytotoxic among all cell lines. Besides, the extract displayed less toxicity towards normal cells (3T3) with an IC₅₀ of 65.23 ± 0.25 µg/mL.

3.6. Colony formation assay

The treatment of seaweed was found to inhibit colony formation of MCF-7 cells using the identified IC₅₀ on MCF-7 cells as shown in Fig. 3. A control of 5 µM tamoxifen was also used as a control to observe the inhibition effect between the commercial drug and the treatment.

3.7. Induction of *H. Opuntia* by citral in breast cancer spheroids

Apoptosis induction was determined using double staining with Hoechst 33,342 and propidium iodide, which allows for the distinct visualisation of viable, necrotic, and apoptotic cells. To be more specific, viable cells were intensely blue, whereas apoptotic cells showed signs of nuclear condensation and were red (Fig. 4). As shown in Fig. 4, treatment of *H. opuntia* in both breast cancer spheroids gave rise to a mixture of cells that were normal and undergoing apoptosis and necrosis, which caused an increase in pink-stained cells that represent late apoptosis or secondary necrosis.

3.8. GC-MS analysis and metabolites identification

The GC-MS analysis of *H. opuntia* methanolic extracts revealed numerous components, as tabulated in Table 5, with the main chemical constituents found in high percentages being stigmast-5-en-3-ol-(3β) (54.74%), 1-dodecanol, 3,7,11-trimethyl (16.82%), and neophytadiene (8.47%). Other metabolites discovered included

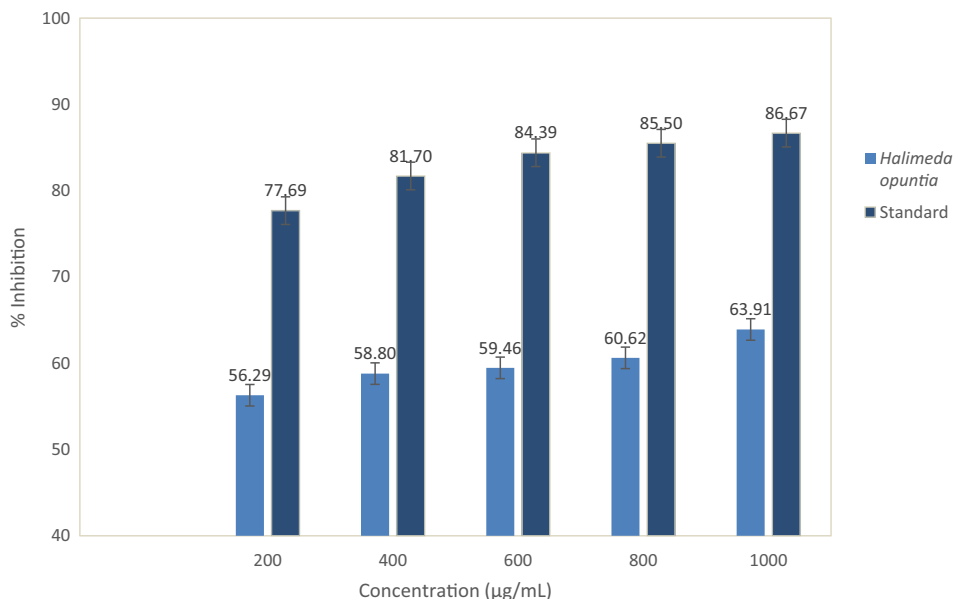


Fig. 2. DPPH scavenging activity of *H. opuntia* methanolic extract and ascorbic acid as standard, at different concentrations (200–1000 µg/mL). Results are expressed as mean ± SE of triplicate measurement and are significantly different ($P < 0.05$).

Table 3

Total antioxidant capacity of *H. opuntia* extract at 1.0 mg/mL.

Solvent	TAC (mg AAE/g extract)
Methanol	57.36 ± 0.004

Value is presented based on Mean ± standard deviation, n = 3.

Table 4

IC₅₀ value (µg/mL) of the methanolic extract of *H. opuntia* against cancer cell line. Data were presented as mean ± SD and significantly different at $P < 0.05$.

Cancer cell lines	IC ₅₀ value (µg/mL)
MCF-7	25.14 ± 1.02
MDA MB 231	53.60 ± 0.67
HT 29	45.23 ± 0.41
Hep G2	47.16 ± 0.52
3T3	65.23 ± 0.25

Values are Mean ± standard deviation, (n = 6).

cholest-4-en-3-one (3.83%), heptadecane (3.42%), hexadecanoic acid (2.96 %), and 1-eicosanol (1.87 %).

4. Discussion

Seaweed diverges in morphological, physiological, and chemical compositions with terrestrial plants due to their antagonistic

appearances. Due to its photosynthetic pigment, seaweed may comprise a large quantity of lipid, protein, and carbohydrates (Ozcimen and Inan, 2015). In terms of nutritional aspects, lipid extract contains various types of fatty acids, namely polyunsaturated, polysaturated, monosaturated, and monounsaturated, which will act as a catalyst for cytotoxic activities (Goecke et al., 2010). A study found that methanolic extract of different seaweed yielded n-hexadecanoic acid (a common saturated fatty acid established in seaweed), which showed cytotoxicity against different cancer cells significantly (Nazarudin et al., 2020). In this study, the total concentration of carotenoids in *H. opuntia* was slightly lower than in *H. macroloba*, with a total carotenoid of 117.36 ± 1.30 µg/g DW, and higher than in *Caulerpa lentillifera*, with a total carotenoids of 63.5 ± 13.0 µg/g DW (Othman et al., 2018; Nazarudin et al., 2020), where the difference reported that siphonaxanthin had growth-inhibitory activity against HL-60 cells (Ganesan et al., 2011). The total chlorophyll results in this current study agreed with previous reports that the highest chlorophyll *a* and *b* content was in green algae (Gordillo et al., 2006).

Methanol was used as the solvent to determine the total phenolic content. The methanolic extract has the highest TPC value compared to hexane extract, acetone extract, and chloroform extract (Nazarudin et al., 2020). The study shows that various extraction solvents result in different total phenolic content due to their chemical compositions. Polar solvents such as methanol and etha-

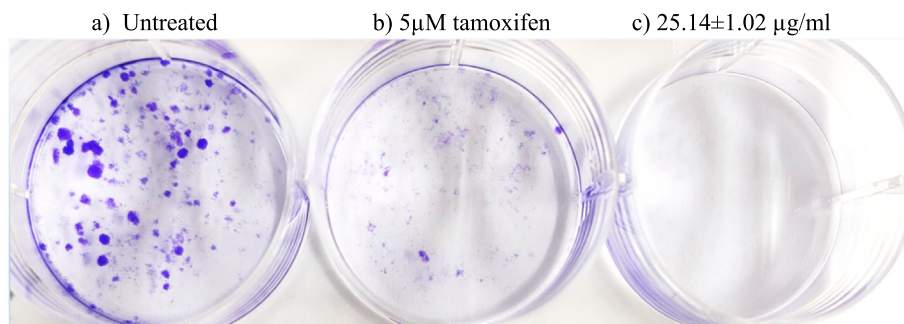


Fig. 3. Effects of *H. opuntia* colony formation at 7 days post-treatment.

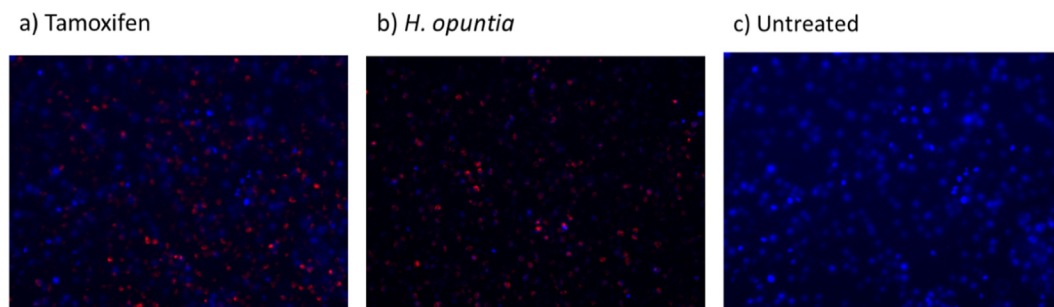


Fig. 4. Scanning inverted microscopic images of MCF-7 cells stained with double staining of Hoechst 33,342 and propidium iodide: (a) tamoxifen (control) (b) *H. opuntia* (c) untreated.

nol are more suitable extraction solvents than semi-polar and non-polar solvents. The result from this study is higher than other green seaweed within the same *Halimeda* genus. In diethyl ether extract, *H. discoidea* has a total phenolic content of 21.74 ± 0.19 mg GAE/g (Supardy et al., 2011), while *H. gracilis* has a 3.8 mg GAE/g (Suganya et al., 2019). In comparison with other types of seaweed, the total phenolic content of green seaweed (*H. opuntia*) is slightly higher. *Acanthophora spicifera* has a total phenolic content of 40.583 ± 1.161 mg GAE/g (Zakaria et al., 2011), while *Fucus vesiculosus* has a total phenolic content of 46.7 mg GAE/g (Tibbetts et al., 2016). Due to the different cellular mechanisms and genetic codification, the production and diversity of phenolic compounds are intimately tied to the seaweed taxonomic group and individual species. Numerous factors affect the quality and quantity of the phenolic compounds. For example, seaweed geolocation, ecological characterization, season, biotic factors (herbivory or direct competition with other benthic organisms), and abiotic factors (salinity, temperature, light incidence, pH, and water nutrient composition) all have effects on the total concentration of phenolic compounds (Lomartire et al., 2021).

Flavonoids are the largest group of phenolic compounds in plants, which are flavanols, flavones, and isoflavonoids, and they exert strong anticancer activities (Johnson et al., 2019; Kopustinskiene et al., 2020; Ferdous and Yusof, 2021a). The present study shows that this green seaweed species has the potential to become a natural drug that can be used in cancer treatment as a higher total flavonoid content of *H. opuntia* was recorded compared to other green seaweeds in the *Halimeda* genus. The TFC of *H. gracilis* in ethanol extract was 8.3 mg QE/g. To compare the total flavonoids content with brown seaweed *Sargassum wightii*, the TFC recorded was 10 mg QE/g (Suganya et al., 2019), which is lower than the TFC of *H. opuntia*.

The DPPH assay is regarded as a reliable, accurate, simple, and cost-effective method of assessing antioxidant radical scavenging activity. The DPPH method takes into account not only the antioxidant concentration, but also the time it takes for the scavenging reaction to reach a plateau. In this study, the DPPH radical scavenging activity of seaweed extract shows that as concentration

increases, the DPPH decreases. This result suggests that the seaweed possesses antioxidant properties as it is known to have a high content of natural bioactive compounds like carotenoids, phenolics, and flavonoids (Leelavathi and Prasad, 2014). According to the current study findings, *H. opuntia* had a higher TAC value than other *Halimeda* genus, which were 5.23 ± 0.03 mg AAE/g and 4.11 ± 0.004 mg AAE/g for *H. tuna* and *H. macroloba*, respectively, in methanol extract (Sivaramakrishnan et al., 2017), and 35.9 mg AAE/g for *H. gracilis* in ethanol extract (Suganya et al., 2019). *Hypnea pannosa* red seaweed was 17.31 mg AAE/g, *S. coriifolium* brown seaweed was 28.08 mg AAE/g in methanol extract (Sobuj et al., 2020), and 47 mg AAE/g for *S. wightii* in ethanol extract (Suganya et al., 2019). Compared with other brown and red seaweed, the TAC of *H. opuntia* was the highest. The antioxidant activity of *H. opuntia* may be associated with the presence of the phenolic compounds in the extract as high TPC has been recorded based on Table 2. This relationship can be supported by a previously observed positive correlation between TPC and antioxidant activity (Sobuj et al., 2020) and has also been suggested by Neethu et al., 2017 in their study. In addition, phenolic compounds play a vital role as a defence mechanism against invading bacteria, ecological stress, and ultraviolet radiation (Klepacka et al., 2011).

In this study, a methanolic extract of marine green seaweed (*H. opuntia*) demonstrated cytotoxicity against various cancer cell lines. Previously, other *Halimeda* species have been reported to show cytotoxicity activity against several cancer cell lines. Methanolic extracts of *H. tuna* and *H. macroloba* show cytotoxic activity against MCF-7 cancer cell lines with IC_{50} of 0.02719 and 37.25 μ g/mL, respectively, which is comparable to our result with IC_{50} of 25.14 ± 1.02 μ g/mL for the same cell line. *H. macroloba* extract has been reported to have cytotoxicity towards HT 29 cells with an IC_{50} of 21.32 μ g/mL (Kurt et al., 2014; Nazarudin et al., 2020). It is shown that these edible seaweeds have the potential to be an alternative to being used as a source for more anticancer drugs. The concentrations of seaweed pigments (total carotenoids, chlorophyll *a* and *b*) in this study revealed that marine macroalgae-derived pigments are predominant radical scavengers to combat cancer development.

Table 5
Chemical components in *Sargassum ilicifolium* extract.

Compounds	Molecular formula	Retention Time	Area (%)	Molecular weight (g/mol)	Compound class
Heptadecane	C ₁₇ H ₃₆	41.56	3.42	240	Hydrocarbon
Neophytadiene	C ₂₀ H ₃₈	46.68	8.47	278	Hydrocarbon
Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	50.91	2.96	256	Fatty acid
1-Dodecanol, 3,7,11-trimethyl	C ₁₅ H ₃₂ O	55.84	16.82	228	Fatty Alcohol
1-Eicosanol	C ₂₀ H ₄₂ O	66.72	1.87	298	Fatty alcohol
Stigmast-5-en-3-ol, (3 β)	C ₂₉ H ₅₀ O	86.82	54.74	414	Sterol
Cholest-4-en-3-one	C ₂₇ H ₄₄ O	90.69	3.83	384	Sterol

Several studies have reported that carotenoids, chlorophylls, and their derivatives are the prime phytonutrients for impeding tumor development and proliferation and can initiate apoptosis in cancer cells, including breast cancer cells (MCF-7), colon cancer cell lines (HT-29), T-cell leukemia, colon cancer cells (Caco-2, HT-29 and DLD-1), prostate cancer cells (PC-3, DU 145 and LNCaP), fibroblast (L929) cell lines, and pancreatic adenocarcinoma cell lines (PaTu-8902). (Vijay et al., 2018; Castro-Puyana et al., 2017; Linnewiel-Hermoni et al., 2015; Pemmaraju et al., 2018; Vaková et al., 2018). Due to their structurally unique bioactive compounds and cytotoxicity properties, marine natural products have earned an encouraging acceptance to be used as chemopreventive mediators in cancer therapy as a supplement. Some studies have reported that marine natural products, which contain an abundance of biologically active substances with novel chemical structures, can be exploited to stop many disease progressions and protect against various cancers (Taleró et al., 2015; Ryu et al., 2013). Algal bioactive compounds can engender cytotoxicity in numerous ways, including inhibition of tumour growth, invasion, and metastasis, and through apoptosis in cancer cells (Farooqi et al., 2012). Seaweed secretes various types of antioxidants as they respond to environmental stresses (Lesser, 2006). Antioxidants help to remove free radicals, also known as reactive oxygen species (ROS). Superoxide (O_2^-), hydroxyl (HO^-), and hydrogen peroxide (H_2O_2) are the three components of ROS (Boonchumi et al., 2011). Free radicals and anticancer drugs can initiate tumour formation in the human body. Hence, marine algae-derived radical scavengers can be an alternative to fighting cancer formation (Pangestuti and Kim, 2011). Carotenoids and chlorophylls can prevent oxidative damage caused by free radicals and play a crucial role in the inhibition of cell propagation, making them useful for tumbling the peril of cancer (Rowles and Erdman, 2020). Chlorophylls and their derivatives have *in vitro* anti-mutagenic effects against numerous dietary and environmental mutagens (Ferruzzi and Blakeslee, 2007) and have anticancer properties as well (Chernomorsky et al., 1999; Donaldson, 2004). However, the antioxidant and cytotoxicity activity would not be closely connected with a specific compound, like carotenoid, for instance, but with a mixture of compounds, as this mixture of compounds can act synergistically (Plaza et al., 2010).

The chemical composition of the extract could be investigated using GC-MS analysis to identify active components responsible for various biological activities. A total of seven (7) different bioactive metabolites were successfully identified. Stigmast-5-en-3-ol, (3β) (54.74 %), a phytosterol compound found in plants, was abundant in *H. opuntia*. According to Nazarudin et al. (2020), phytosterol functions chemically as a compound with high antioxidant activity and a moderate radical scavenger. The extracts also contained a high concentration of neophytadiene, a member of the sesquiterpenoids class of compounds (Mujeeb et al., 2014). It is said to have powerful antioxidant and anti-inflammatory properties (Venkataraman et al., 2012). Meanwhile, 1-dodecanol, 3,7,11-trimethyl found in *H. opuntia* methalonic extract may be responsible for biological effects such as anti-tumor and antimicrobial effects (Esghaei et al., 2018). Cholest-4-en-3-one, a metabolite found in *H. opuntia* methalonic extracts, has been reported to have antitumor activity, decreasing breast cancer cell viability (Elia et al., 2019). Hexadecanoic acid is a fatty acid found in high concentrations in *Halimeda* species (Nazarudin et al., 2020). It has been reported to have antioxidant properties and to be cytotoxic to human colorectal carcinoma cells (HCT-116) (Mujeeb et al., 2014; Ravi and Krishnan, 2017). Meanwhile, heptadecane and 1-eicosanol have previously been studied for bactericidal activity and have proven to be effective antibacterial agents against a variety of Gram positive and Gram-negative bacteria (Kubo et al., 1993; Ozdemir et al., 2004).

5. Conclusion

In conclusion, this study discovered that *H. opuntia*, the green seaweed, possesses a high lipid content, carotenoids, and chlorophyll *a* and *b*. As for antioxidant activity, the entire species exhibits encouraging results by showing high antioxidant activity due to its high phenolic and flavonoid content. It can be applied as a practical approach to treating a wide range of chronic diseases, such as cancer. Therefore, this finding indirectly shows that seaweed has a wide range of benefits for humankind.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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