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Evaluation of Toxicity and Antioxidant Activity of the Ethanolic Extract from *Ulva Lactuca*

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Abstract

Ulva lactuca (sea lettuce) is a popular food for consumers and has high nutritional value. It has also been found to be a source of health-promoting substances, including a high amount of proteins which can be utilized as a future plant-based functional ingredient in the food industry. However, before extensive applications in the aforementioned fields, it is crucial to ensure the extracts' biological-pharmacological activities and their safety. In this study, we evaluated the cytotoxicity, genotoxicity, and antioxidant activity of the ethanolic extract of the sea lettuce extract.We tested cytotoxicity by MTT assays on 5 cell lines, including fibroblast (L929), macrophages (RAW 264.7), hepatocytes (FL83B), keratinocytes (HaCaT) and normal colon (CCD-18co) cells. Genotoxicity of the extract was tested by comet and micronucleus assays on human lymphoblast cells (TK6). Our results demonstrated at a concentration lower than 50 µg/mL the extract did not show any cytotoxicity as well as genotoxicity. The extract was shown to possess high antioxidant capacity by DPPH and ABTS scavenging at the EC50 value of *U. lactuca* extracts was 631.84 ± 5.64 and 330.35 ± 19.49 µg/mL, respectively. Therefore, the extract may be safely used as an ingredient for food, that should help to ensure food security for humans in the future, as well as for cosmetic, pharmaceutical and other health-promoting products.

Keywords: Green seaweed, *Ulva lactuca*, Antioxidant activity, Genotoxicology, Cytotoxicity, Safety effect, Marine algae

Introduction

Ulva lactuca is a green macroalgae belonging to phylum Chlorophyta. *U. lactuca* contains compounds, such as alkaloids, steroids, triterpenoids, saponins, phenolic compounds, and flavonoids Amin [1], that have pharmacological actions in antibacteria, antioxidation, anti-inflammation, anticoagulation, and promotion of wound healing [2]. Polysaccharides from *U. lactuca* possess strong antioxidant activity as well as can induce the production of antioxidant enzymes in cells including glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) which inhibit the production of superoxide anion and lipid peroxidation [3]. *U. lactuca* contains a high amount of proteins, polysaccharides, vitamins, but low amount of lipid Liu *et al.* [4], and it has been used as a component of commercial feeds for shrimp and other aquatic animals [5]. In addition, *U. lactuca* has been utilized as a commercially valuable component of food, nutraceuticals, cosmetics, [6-8]. Increasingly, extracts from *U. lactuca* will be utilized for nutritional and pharmaceutical formulations for human consumption and usages. Thus, there is a need to verify its safety and biological activities, especially anti-oxdation and anti-inflammation which are the essential properties for any substances that are to be used for human nutrition and for promoting human health.

Information on the cytotoxicity and genotoxicity of *U. lactuca* as well as its anti-oxidation capacity are still limited. This research was, therefore, undertaken to investigate these aspects of the ethanolic extract of *U. lactuca* which is a commonly used starting material for nutritional and pharmacological products for human consumption and health applications. The aims of the present study are to determine the antioxidant activity of the ethanolic extract of *U. lactuca* and to investigate its cytotoxic and genotoxic effects.

Materials and methods

Plant materials and extraction

U. lactuca was pond-cultured by Ms. Montakan Tamtin, Phetchaburi Coastal Fisheries Research and Development Center, Thailand. The algae were extensively washed with tap water and dried at room temperature (RT). The dried *U. lactuca* was air-dried, milled and macerated successively with 95 % ethanol at RT for 7 days. The extract was then filtered and evaporated under a vacuum. The crude ethanol extract from the *U. lactuca* were dissolved in 100 % DMSO at 1 mg/mL and kept as a stock solution and stored at –20 °C.

Chemical composition analysis of *U. lactuca* extract

The chemical composition of the ethanolic extract from *U. lactuca* was determined by gas chromatography coupled to mass spectrometry (GCMS) using an Agilent 8890 gas chromatograph (Agilent Technologies, Inc, USA) equipped with an Agilent 5973 N inert mass selective (IMS) detector. A capillary column DBWAX (30 m × 0.25 mm ID with 0.5 µm film thickness) was employed for the analysis. Helium (He) was used as carrier gas at a flow of 1.0 mL/min in a splitless mode, and both the IMS detector and the injector port temperatures were 320 and 240 °C, respectively. The elution program started at a temperature of 100 °C held for 5 min, and then increased to 240 °C at a speed of 10 °C/min and was held for 45 min. MS ion source temperature 230 °C. The electron energy is 70 eV and the solvent delay is 3 min. The scan mode was evaluated at 35 - 1000 m/z, speeds 1.562 u/s, and a frequency of 1.6 scans/s. The results were evaluated by GCMS and semi-quantitative analysis was performed by peak area normalization. Compounds were identified by retention time, relative molecular weight, relevant literature and other information of reference comparison with MS spectra

from the National Institute of Standards and Technology (NIST) mass spectral library.

Cell culture

Cell lines (Mouse fibroblast (L929), mouse macrophages (RAW 264.7), mouse hepatocyte (FL83B), keratinocytes (HaCaT), and human colon cells (CCD-18Co)) were purchased from American Type Culture Collection (ATCC). Briefly, L929, FL83B, and HaCaT cells were grown in DMEM culture medium with 1 g/L D-glucose, L-glutamine, 110 mg/L sodium pyruvate, penicillin G (10 U/mL), streptomycin (10 ug/mL), and 10 % fetal bovine serum. RAW 264.7 and CCD-18Co cells were grown in RPMI 1640 medium with L-glutamine, penicillin G (10 U/mL), streptomycin (10 ug/mL), and 10 % fetal bovine serum. All cell lines were cultured at 37 °C in a humidified incubator with 5 % CO_2 atmosphere. The culture media were changed every 2 - 3 days. The cells at logarithmic phase were harvested immediately before performing each experiment.

MTT assay

The cytotoxicity effect of *U. lactuca* extract was determined by MTT assay. Briefly, each of L929, RAW 264.7, FL83B, HaCaT and CCD-18Co cells was incubated at 8×10^3 cells/well in the presence of *U. lactuca* at the concentrations of 40, 60, 80, 100, 200, 300 and 400 ug/mL in the culture medium, and 1 % DMSO diluted in the culture medium as a negative control for 24 and 48 h at 37 °C. MTT salt was dissolved in PBS (stock solution of 5 mg/mL). After treatments, the reaction medium was removed, the MTT was dissolved in culture medium (working solution: 0.5 mg/mL), added to each well and incubated for 3 h at 37 °C. Finally, the MTT was removed, and 100 µl of DMSO was added to solubilize formazan crystals. The plates were gently shaken for at least 15 min, and the OD were then read with a microplate spectrophotometer (VersaMax microplate reader) at a wavelength of 690 nm (background signal) and 570 nm. The percentage of viable cells was calculated after normalization with the negative control (1 % DMSO), which was considered to have 100 % cell viability. The conditions were repeated 3 times for each test.

Comet assay

Comet assay was carried out to measure the DNA damage. TK6 cells were seeded at 2×10^5 cells/ mL in 24-well plate, the plate was maintained in a humidified incubator with 5 % CO_2 and 37 °C for 24 and 48 h. Cells were incubated in the presence of *U. lactuca* extract at the concentrations of 40, 60, 80, 100, 200, 300 and 400 ug/mL in the culture medium, and in 10 µM hydrogen peroxide-H_2O_2 as a positive control, and in 10 % FBS in RPMI medium as a negative control, for 24 and 48 h at 37 °C. After incubation, the 100 µl of TK6 cell suspension was used for performing comet assay. For single gel format, cell suspension was centrifuged at 4,500 rpm for 5 min. Then the supernatant was discarded, and cells resuspended with 100 µl PBS pH 7.4. The cell suspension was mixed with 1 % low melting point (LMP) agarose at 1:10 ratio at 37 °C. And the same volume was deposited on a previously prepared thin layer 1 % normal melting point (NMP) agarose, and then covered with a cover glass. The cover glasses were removed, and the slides were immersed in a freshly prepared lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris (pH 10), supplemented with 1 % Triton X-100) for overnight, at 4 °C in the dark. The slides were then transferred to the unwinding solution (300 mM NaOH, 1 mM EDTA, pH > 13) which detected single and double stranded breaks, for 30 min at RT in a dark room and then electrophoresis was performed (20 v; 300 mA; 1 Vcm^{-1}) for 30 min. The slides with single gel were neutralized in 0.4 M Tris pH 7.5 for 5 min and stained with SYBR Green, in the dark. The samples were examined with a fluorescence microscope (Olympus

with 40X magnification) immediately and analysed with the Score 2.0 software for determining DNA damage. At least 50 randomly non-overlapping cells per culture were examined in double blind observations. These cells were scored based on tail and head (nucleus) sizes. Following parameters were recorded: tail length, % of tail DNA, and tail moment, intensity of the fluorescence DNA. Tail length was the length of the comet tail which indicated the extent of DNA damage. Percent of tail DNA was the intensity of fluorescence caused by damaged DNA in the comet tail, and the tail moment was calculated as (tail length) × (% tail DNA).

Micronucleus test (MN)

TK6 cells were seeded at 2×10^5 cells/mL in 24-well plate, then the plate was maintained in a humidified incubator with 5 % CO_2 at 37 °C for 24 h. And then, cells were incubated in the presence of *U. lactuca* extract at concentrations of 40, 60, 80, 100, 200, 300 and 400 ug/mL in the culture medium, in 10 ug/mL mitomycin as a positive control, and in 1 % DMSO as a negative control. After incubation, 6 ug/mL cytochalasin B was added into each well to obtain dividing binucleated cells for a period of 19 h. The cells were collected and 500 uL of TK6 cells were centrifuged at 4,500 rpm for 5 min, and the supernatant was discarded, cells resuspended in 500 uL PBS pH 7.4 and then centrifuged at 4,500 rpm for 5 min. A 500 uL of hypotonic solution (0.075M KCl) was added into each pellet and incubated at 37 °C for 30 min and centrifuge at 4,500 rpm for 5 min. Then the pelleted cells were fixed in methanol and acetic acid solution with the ratio 3:1. Finally, the slides were prepared by cytospin, stained with 10 % Giemsa, and immediately examined in a light microscope (Olympus with 40X magnification). At least 50 cells were counted for each experimental and control sample, and the numbers of binucleated cells with micronucleus were recorded.

DPPH assays for antioxidant activity

The DPPH reagent (2,2–diphenyl–1–picrylhydrazyl) was dissolved in ethanol at a concentration of 0.5 mM as the stock solution and further diluted to 0.02 mM as the working solution. The *U. lactuca* extract was dissolved in absolute ethanol at concentrations of 100, 200, 400, 600, 800, and 1000 µg/mL. Next, the working solution of the DPPH reagent was mixed with *U. lactuca* extract (sample A) and absolute ethanol (control A) at a 1:1 ratio in 96-well plates. Trolox was used as a positive control at concentrations of 1.63, 3.13, 6.25, 12.5, 25, and 50 µM. They were incubated for 20 min and measured at 518 nm. The results were calculated using free radical scavenging activity from the DPPH assay and then used in the equation to determine the Half Maximum Effective Concentration (EC50) and calculate the Trolox equivalent antioxidant capacity (TEAC) in mg Trolox/g extract. the percentage of free radical scavenging /antioxidant activity was calculated according to this equation.

$$\%\ scavenging\ activity = \frac{A\ control - A\ sample}{A\ control} X\ 100 \quad (1)$$

ABTS assays for antioxidant activity

The ABTS reagent (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)) was dissolved in distilled water at a concentration of 7 mM and mixed with 2.45 mM of potassium persulfate. The mixture was incubated for 16 h at RT in the dark. The ABTS solution was diluted to an absorbance range of 0.7 - 0.75 at 734 nm. The working solution of ABTS was mixed with Trolox and *U. lactuca* extract at the same concentrations and ratio as in the DPPH assay. The mixture was incubated for 6 min, and the free radical scavenging activity was calculated from the ABTS assay and used in the equation to determine the EC50 value and to calculate the Trolox equivalent antioxidant capacity (TEAC) in mg Trolox/g extract.

the percentage of free radical scavenging /antioxidant activity was calculated according to this equation.

$$\%\ scavenging\ activity = \frac{A\ control - A\ sample}{A\ control} X\ 100 \quad (2)$$

Statistical analysis

The experimental data presented as mean ± standard deviation (SD) and analyzed by GraphPad Prism 7 software (version 7.0). Comparisons between the extracts and controls were conducted using a one-way analysis of variance (ANOVA) followed by Duncan's post hoc comparisons. Differences were considered statistically significant at $p < 0.001$.

Results and discussion

Chemical composition of the ethanolic extract from *Ulva lactuca*

The chromatogram obtained from the GCMS analysis of the ethanolic extract from *U. lactuca* extract showed 4 chromatographic peaks from 20 to 40 min and 4 components were identified (**Figure 1**, **Table 1**). A total of 4 fatty acids amounting 51.83 % were identified. Among these, 78.80 % were saturated fatty acids and 21.20 % unsaturated fatty acids. The main component was palmitic acid (36.36 %). From the **Table 1**, the relative levels of 4 significant parameters; oleic:linoleic acid (O/L) ratio, total saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) were analyzed. These results increase the knowledge on the phytochemical composition of *U. lactuca* ethanol extract and it should add to the understanding of the product pharmacological properties.

Table1 Chemical composition of the ethanolic extract from *Ulva lactuca* analysis by the GC-MS method.

Peak no.	Retention time (min)	Compounds		Peak area (%)[a]
1	20.632	Myristic acid	C14:0	4.48
2	25.973	Palmitic acid	C16:0	36.36
3	33.952	Oleic acid	C18:1 (ω-9)	6.78
4	36.163	Linoleic acid	C18:2 (ω-6)	4.21
	Identified components (%)			51.83
	Unidentified components (%)			48.17
	Total saturated fatty acid (%)			78.80
	Total unsaturated fatty acid (%)			21.20
	Monounsaturated fatty acids (%)			13.08
	Polyunsaturated fatty acids (%)			8.12
	Oleic: Linoleic acid (O/L) ratio (ω-9/ω-6)			1.61

[a]Comparison of mass spectra with those listed in the NIST2017 libraries and with published data.

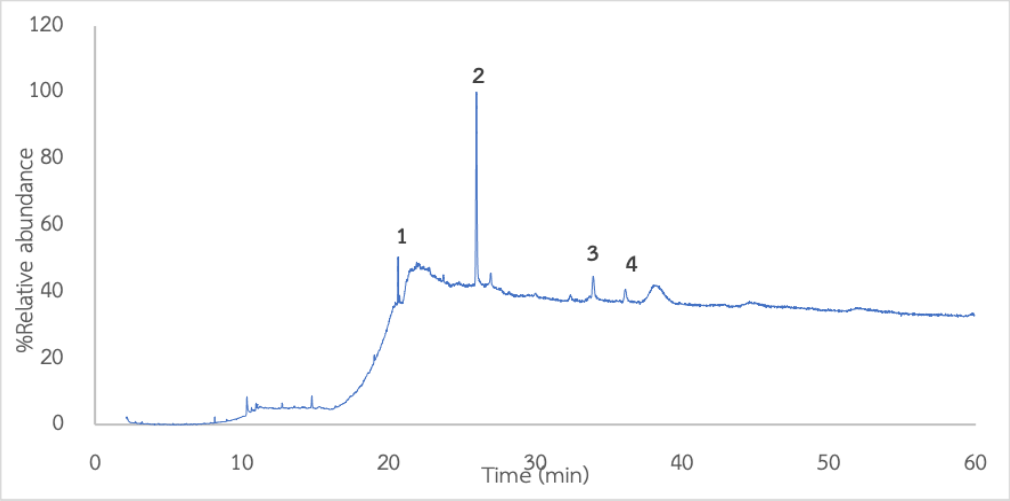

Figure 1 GC-MS chromatogram of chemical composition of the ethanolic extract from *Ulva lactuca.* Peaks of chemical composition were separated by time point. The peaks and peak areas were compared to mass spectra with those listed in the NIST2017 libraries and with published data (%). The chemical compounds of the *Ulva lactuca* were as follows: 4.48 % Myristic acid (1), 36.36 % Palmitic acid (2), 6.78 % Oleic acid (3), and 4.21 % Linoleic acid (4).

U. lactuca is a plant that contains high amount of nutritious component especially protein and carbohydrates that can be used as a raw material in the food industry and cosmetics for developing into future food health products. In addition, previous research found that *U. lactuca* contains secondary metabolites such as alkaloids, triterpenoids, steroids, saponins, phenolic compounds, and flavonoids Shalaby and Amin [9]; and these active compounds have shown antibacterial, anti-inflammatory, antioxidant, antihyperlipidemic, antitumor and anticoagulant activities [10]. However, at high concentrations a mixture of these compound in the extract may be toxic. Thus, toxicity test is required first to know the safe concentration of the extract that can be used for further health product development. The seaweed is generally extracted by various organic solvents, such as acetone, methanol-toluene, ether, or chloroform-ethanol, as using organic solvents generally leads to higher yield and increased activity [11].

Cytotoxicity of the ethanolic extract from *U. lactuca* by MTT assay

Cytotoxic effect *U. lactuca* ethanolic extract at 24 and 48 h, as measured by MTT assays at different concentrations (ranging from 40 to 400 µg/mL) against 5 cell lines, were presented in **Figure 1**. The cell viability and the IC50 of L929 and FL83B cells were more than 400 µg/mL compared with the negative control at 24 and 48 h. And the IC50 of RAW264.7 cell at 24 and 48 h was more than 400 and 396.11 µg/mL for 48 h, respectively. For HaCaT cells and CCD-18co cells revealed a dose-dependent decrease in cell viability with increased concentration of the extracts after treatment for 24 and 48 h incubation. The IC50 was at 277.7 and 123.71 µg/mL for HaCaT cells and 362.15 and 87.81 µg/mL for CCD-18co cells, respectively. These results indicated that *U. lactuca* extracts were not cytotoxicity to L929, FL83B, and RAW264.7 cells, while HaCaT and CCD-18co cells showed cytotoxic effect at concentrations of the extract higher than 300 µg/mL at 24 h and 100 µg/mL at 48 h.

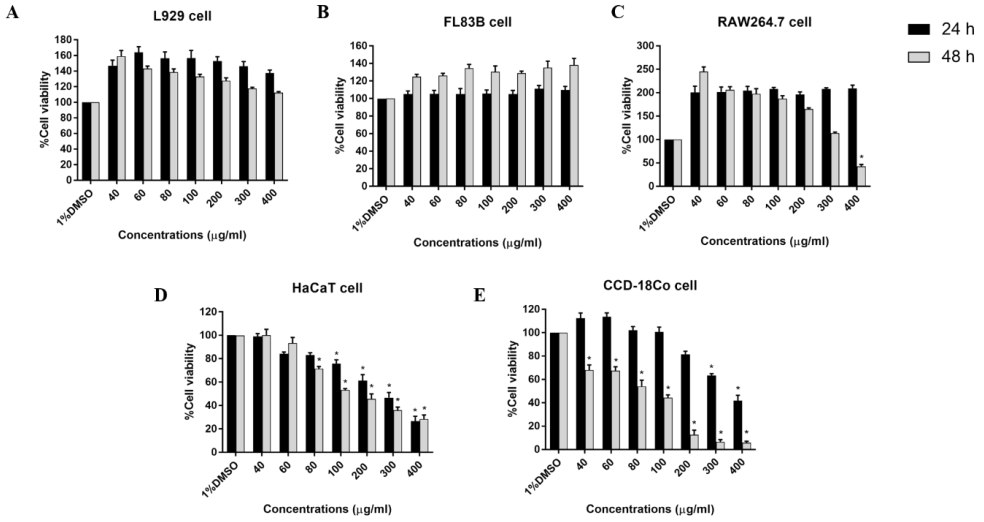

Figure 2 Cell viability of 5 cell types treated with ethanolic extract of *U. lactuca* for 24 and 48 h, assessed by the MTT assay. (A) L929 cell line; (B) RAW 264.7 cell line; (C) FL83B cell line; (D) HaCaT cell line; (E) CCD-18co cell line. Data (percentage of cell viability) were expressed as mean ± standard deviation and compared with 1 % DMSO (assigned to have cell viability at 100 %), $^{*}p < 0.001$.

U. lactuca ethanolic extract from low (40 µg/mL) to high (400 µg/mL) concentrations was tested by the standard MTT assay to determine its cytotoxicity in 5 cell lines which represented the skin (keratinocyte-HaCaT), the immune cells (macrophage-RAW 264.7), liver (hepatocyte-FL83B) and the colon (normal human colonic cell-CCD-18Co), as theseorgan are directly exposed to dietary supplements and cosmetics. The MTT assay measures cellular metabolic activity as an indicator of cell viability, proliferation, and cytotoxicity. This colorimetric assay is based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) to purple formazan crystals by metabolically active cells. Hence, the MTT assay depends on mitochondrial respiration and indirectly assesses a cellular energy capacity and viability [12]. Our MTT assay showed high cell viability among fibroblast (L929), macrophages (RAW 264.7), and hepatocyte (FL83B). For keratinocytes (HaCaT) and human colon cells (CCD-18Co) the concentration equal to and lower than 300 µg/mL at 24 and 48 h were safe. Previous studies also reported that *U. lactuca* extract by ethanol had some cytotoxic effect with IC50 at 25 µg/mL for T lymphoblast cells [13]. And another study that tested cytotoxicity in colon cancer cells found that cell viability IC50 was 1 g/mL [14]. Taken together, it can be seen that the degree of toxicity depends on the type of cells being tested and concentration of the extract. However, the extract was considered safe at concentrations lower than 300 µg/mL in L929, FL83B, and RAW264.7 cells but in HaCaT and CCD-18co cells lower than 100 µg/mL which indicated a relatively low cytotoxicity.

Genotoxicity of the ethanolic extract from *U. lactuca* determined by comet assay

The comet assay evaluated the effects of *U. lactuca* extracts on DNA damage in the lymphoblast cell line (TK6), and the results are shown in **Figure 3**. After treatment, the 3 parameters were compared between experimental and the negative control groups. The results showed Tail length and % Tail DNA increased significantly at concentrations more than 40 µg/mL at 24 and 48 h. As well, all Tail moments were increased significantly at concentrations more than 100 µg/mL at 24 h and more than 40 µg/mL at 48 h (**Figure 3**). However, *U. lactuca* extract showed low

genotoxicity compared to the negative control group at 24 h. These results suggested that the ethanolic extract of *U. lactuca* at low concentrations (< 40 µg/mL) were safe when tested by comet assay for DNA damage.

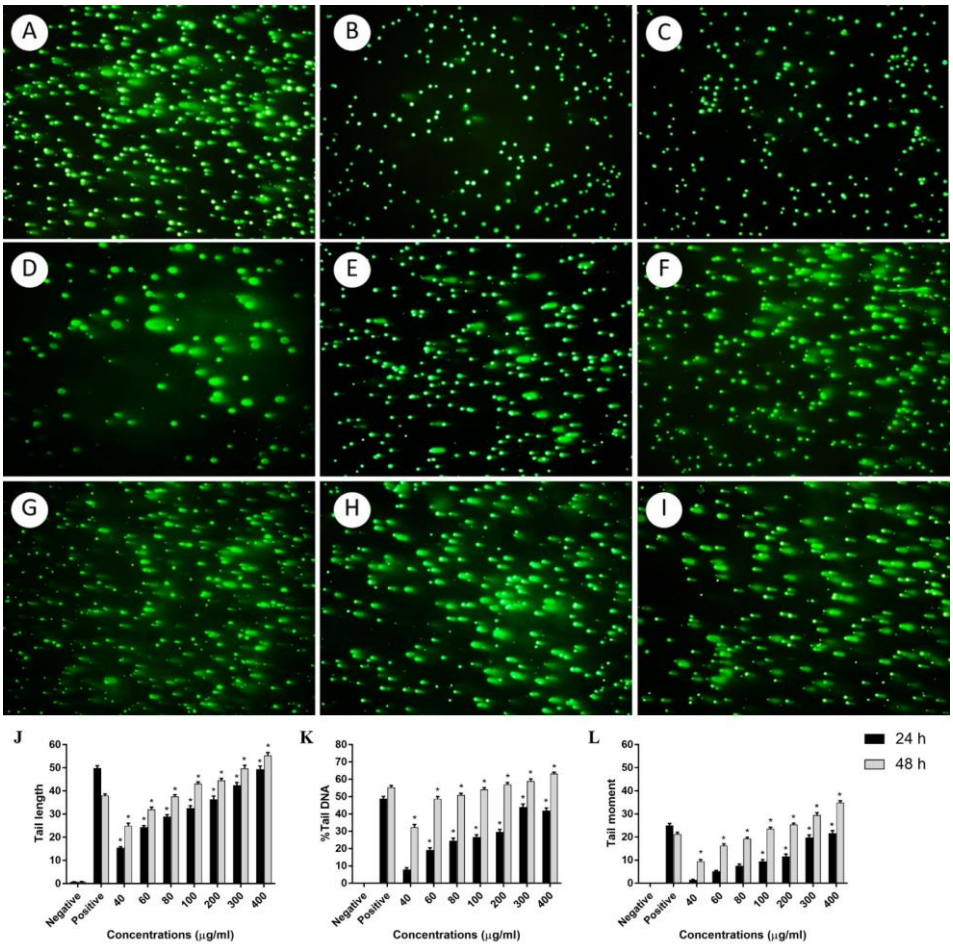

Figure 3 Genotoxicity estimated by comet assay was performed using TK6 cells. The DNA damage is expressed in a comet pattern due to strand breaking and alkylating agents as analyzed by Comet Score 2.0 software. (A) Positive control; (B) Negative control; pictures of comets 40X magnification: (C) 40; (D) 60; (E) 80; (F) 100; (G) 200; (H) 300; (I) 400 µg/mL; histograms of the 3 comet parameters: (J) Tail length; (K) % Tail DNA; (L) Tail moment.

Genotoxicity of the ethanolic extract from *U. lactuca* determined by micronucleus test

The genotoxic effect of the *U. lactuca* extract as tested on the lymphoblast cell line (TK6) by micronucleus test showed that the positive control cells, treated with mitomycin C, showed chromosome damage indicated by the presence of binucleated cells containing micronucleus at 32 % (**Figure 4**(**A**), **Table 2**), while the untreated negative control cells comprised binucleated cells with micronucleus at 2 % (**Figure 4**(**B**), **Table 2**). For the cells treated with *U. lactuca* ethanolic extract the percentages of binucleated cells with micronucleus ranged from low at 10 % (when treated with 40 µg/mL) to moderate at 14 % (when treated with 200 µg/mL) to significantly higher percentage at 18 and 46 % when treated with the extract at 300 and 400 µg/mL (**Table 2**). These results suggested that at concentrations lower than 200 µg/mL the *U. lactuca* ethanolic extract is safe.

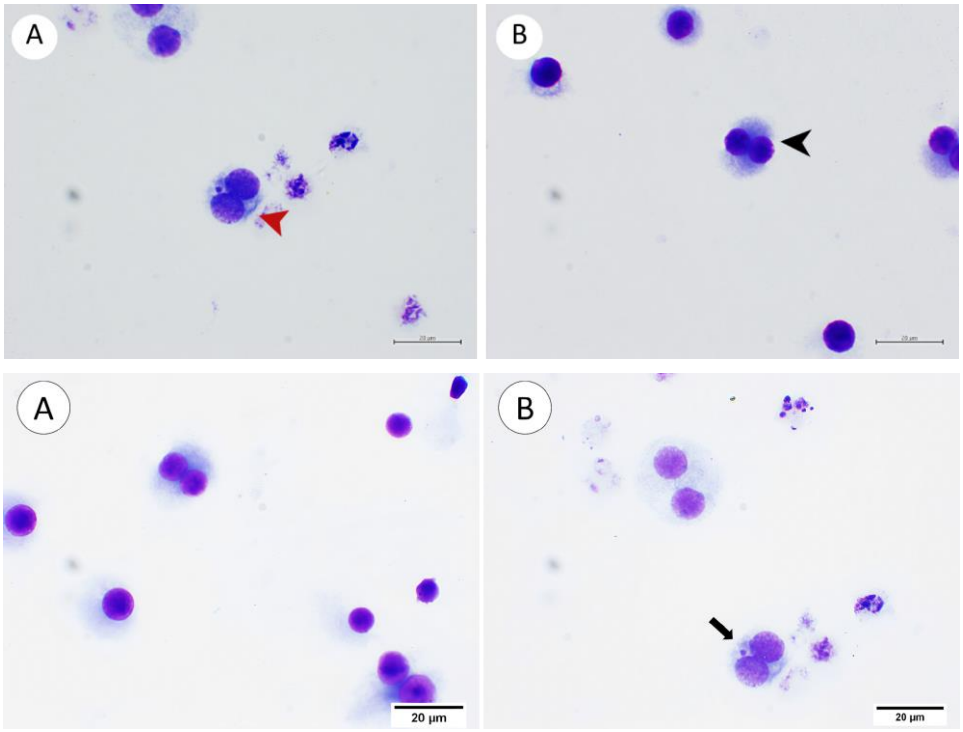

Figure 4 Genotoxicity as measured by micronucleus test indicated chromosome damage. (A) Positive control: Red arrow head indicates binucleated cells with micronucleus; (B) Negative control: Black arrow head indicates binucleated cells with no micronucleus (40x magnification)

Table 2 Genotoxicity of the ethanolic extract of *U. lactuca* measured by micronucleus assay showing % micronucleus in TK6 cells treated with extract for 24 h. ($^{*}p < 0.001$).

Treatment UA	Cells with micronucleus (%)
Negative	2
Positive control (10 µg/ml Mitomycin C)	32
40 µg/mL	10
60 µg/mL	10
80 µg/mL	12
100 µg/mL	12
200 µg/mL	14
300 µg/mL	18
400 µg/mL	46

Comet assay estimated DNA strand breakage while micronucleus assay assessed chromosomal damage in cells, and both are the most common tests for genotoxicity. TK6 is a well-accepted cell for evaluating DNA damage in comet assay due to its high accuracy (90 %) Bajpayee *et al.* [15] Bajpayee *et al.* [16] which is recommended by the FDA guidelines and the ICH S2B guidance for a small molecule [17]. Also, these evaluations are important early steps in the safety assessment of any chemicals to be used as ingredients for food, cosmetics, pharmaceuticals, and other industrial products. Our data from the comet assay

showed no genotoxicity at concentrations lower than 60 µg/mL for 24 and 48 h, while micronucleus assay showed no genotoxicity at concentrations lower than 300 µg/mL. There are similar previous studies on the genotoxicity of the total carotenoids extract from green algae *Chlorococcum humicola* was the concentration more than 300 µg/mL showed micronucleus frequencies were significantly increased in human lymphocyte culture [18].

Antioxidant activity of the ethanolic extract as determined by DPPH and ABTS assays

The free radicals-scavenging or antioxidant activity of *U. lactuca* extract was calculated and expressed as EC50 values from DPPH and ABTS assays as shown **Figure 5**. The antioxidant activity of the *U. lactuca* ethanolic extract at concentrations from 100 to 1000 µg/mL ranged from 13.94 ± 0.35 to 54.95 ± 1.54 in DPPH assay and 26.42 ± 1.42 to 79.32 ± 2.64 in ABTS assay (**Figure 4**). The EC50 value of *U. lactuca* extracts was 631.84 ± 5.64 and 330.35 ± 19.49 µg/mL, respectively. Trolox (Vitamin E), a standard reagent for measuring the antioxidant activity, showed EC50 at 13.60 ± 0.63 µg/mL by DPPH and 3.71 ± 0.19 µg/mL by ABTS assays. When comparing the antioxidant activity of the extract with the standard reagent, it can be seen that the TEAC values for DPPH and ABTS scavenging activity of the extract were 21.53 ± 0.19 and 11.26 ± 0.66 mg Trolox/g (**Table 3**).

Table 3 Antioxidant activity of *U. lactuca* ethanolic extract expressed as EC50 value and mg Trolox/g by DPPH and ABTS assays.

Samples	DPPH scavenging activity		ABTS scavenging activity	
	EC50 (µg/mL)	mg Trolox/g	EC50 (µg/mL)	mg Trolox/g
Trolox (ug/mL)	13.60 ± 0.63	-	3.71 ± 0.19	-
U. lactuca extract	631.84 ± 5.64	21.53 ± 0.19	330.35 ± 19.49	11.26 ± 0.66

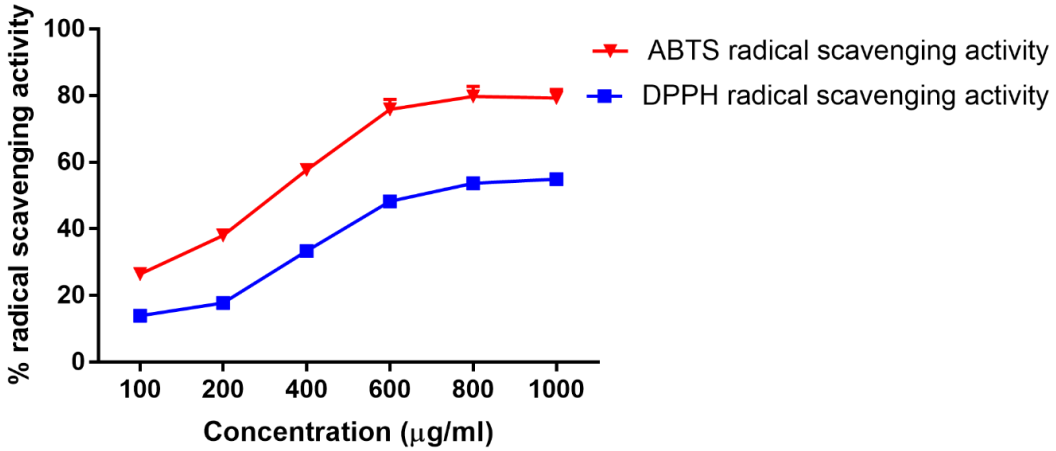

Figure 5 The free radical-scavenging activity (anti-oxidant activity) of *U. lactuca* ethanolic extract by DPPH and ABTS assays. Each value represents mean ± standard error (n=3). The data were used to generate trendlines and calculate the EC50 value.

DPPH and ABTS are free radical scavenging compounds widely used to determine the antioxidant activity of any extracts. The DPPH and ABTS used for assessing the potential of substances to serve as hydrogen providers or free-radical scavengers (FRS) [15]. Our study showed that the ethanolic extract of *U. lactuca* possessed relatively high antioxidant activity with the TEAC values for DPPH and ABTS scavenging activity as high as 21.53 ± 0.19 and 11.26 ± 0.66 mg Trolox/g, Previous studies showed that several phytochemicals are present in *U. lactuca* ethanolic extract, including flavonoids, saponins, alkaloids, and cardiac glycosides [19]. In particular, flavonoids exhibit antioxidant activity *in vitro* because of their ability to effectively scavenge free radicals [20]. Moreover, saponins contain unique, residue-like 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP), which can scavenge superoxide by forming hydroperoxide that prevents biomolecular damage [21]. Cardiac glycoside from the castor plant also exhibits antioxidant activity as determined by DPPH assay [22]. Our observation also agreed with the results reported elsewhere [23,24]. Moreover, U. Lactuca exhibited the antioxidant activity and antitumor property. Qiu *et al.* [25] In addition, a previous study on *U. lactuca* from Egypt showed a very high antioxidant activity, especially in the seaweed harvested during summer Khairy and El-Sheikh [26], which indicated that this seaweed might produce more antioxidative substances as mentioned above during the period of high temperature and UV light. Taken together, we believe that the ethanolic extract from *U. lactuca* has beneficial properties as it is relative safe at low concentration and has especially high antioxidant activity.

Conclusions

This investigation demonstrated that the ethanolic extract from *U. lactuca* cultured in Thailand, at moderate concentration, has very low cytotoxicity, genotoxicity, and very high antioxidant activity. Thus, it can be safely used for consumption or as a raw material for producing food, cosmetic, pharmaceutical and other health-related products. It will increase the value of this green seaweed and, consequently, the income for farmers in Thailand.

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