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The Scavenging Activity and Safety Effect of Red Marine Algae *Acanthophora spicifera* Ethanol Extract

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Abstract

The red algae, *Acanthophora spicifera*, are major species grown in Asia and Pacific areas, especially along the coast of southern Thailand. They have the fast growth rate that leads to an abundant supply; thus, they are currently used for wastewater treatment. Furthermore, an ethanolic extract from *A. spicifera* has been used as a food supplement because of its perceived antioxidant property. Therefore, this study aims to ascertain and evaluate the antioxidant activity and safety of the ethanolic extract from *A. spicifera*. Safety tests showed that the extract did not have cytotoxicity and genotoxicity effects at concentrations less than 100 µg/mL. In term of antioxidant activity, the DPPH and ABTS assays showed a Trolox equivalent antioxidant capacity (TEAC) of 5.24±0.44 and 4.03±0.13 mg Trolox/g of the extract, respectively. In conclusion, *A. spicifera* extract is relatively safe and has a rather high efficacy in scavenging free radicals, which makes it suitable for use as food supplement and cosmetics. However, there are a number of caveats regarding its uses in terms of excessive concentrations and exposure duration to avoid causing cytotoxicity and genotoxicity.

Keywords: Red seaweed, *Acanthophora spicifera*, Antioxidant, Genotoxicology, Cytotoxicity, safety effect, Marine algae

Introduction

A. spicifera is a red seaweed of the family Rhodomelaceae (order Ceramiales). *A. spicifera* can be found in coastal areas of Pacific and Indian oceans. It is especially abundant in Hawaii, India, Vietnam, The Philippines, Indonesia, Malaysia, and southern Thailand. In the latter it has also been cultured by Phetchaburi Coastal Fisheries Research and Development Center, Department of Fisheries, and the culturing technology has been promoted and transferred to farmers since 1993. This seaweed was used for wastewater treatment, but its rapid growth led to an excessive quantity which turned into waste. Several studies have shown that various bioactive compounds derived from *A. spicifera* possess pharmacological activities, including antibacterial [1,2], antioxidant [3], antiviral [4], and antitumor [5] activities. Based on these findings, it is suggested that extracts from *A. spicifera*, which contain biologically active compounds, could be utilized as primary products for future applications in cosmetic, pharmaceutical, and food supplement industries, particularly for antiaging and prevention of age-associated diseases (AADs), leading to increased demand and value for this alga [6]. However, before extensive applications in the aforementioned fields, it is crucial to ensure the extracts' biological-pharmacological activities and their safety [7]. Since the main contributing factors that cause aging and AADs are oxidation from free radicals generated endogenously and exogenously and chronic inflammation, for pharmacological usages of any extracts for health purpose, especially for antiaging and preventing AAD [8], their antioxidation and anti-inflammation activities should be determined. For safety, the cytotoxicity effects of the extracts must be evaluated through various methods, such as cell viability, cell proliferation, and live-cell function assays, which can be used to monitor their safety [9,10]. To ensure the safety of the extracts towards genetic

materials, genotoxicity assays, such as the micronucleus test (MN) and comet assay, are commonly employed for monitoring the genotoxic effects of chemical substances contained within the extracts [11]. These cytotoxicity and genotoxicity tests, especially with regard to the risks of cellular and DNA damage, are essential before the extracts can be used in long-term pharmacological and nutraceutical applications.

The aims of the present study are to determine the antioxidant activity of the ethanolic extract of *A. spicifera* and to investigate its cytotoxic and genotoxic effects, so that the extract can be effectively and safely used as starting materials for nutraceuticals, cosmeticological, and medicinal products, which will increase the value of this red seaweed and, consequently, the income for farmers in Thailand.

Materials and methods

Plant materials and extraction

The red seaweed (*A. spicifera*) was cultured and harvested under the supervision of Ms. Montakan Tamtin, Director, Phetchaburi Coastal Fisheries Research and Development Center, Thailand [12]. The seaweed was washed with tap water and dried at room temperature, then the samples were ground and macerated with 95 % ethanol at room temperature for 7 days. The supernatant from the extraction was then filtered and evaporated by a rotary evaporator. The crude ethanolic extract from *A. spicifera* was dissolved in Dimethylsulfoxide (DMSO) at 1 mg/mL and kept as the stock solutions and stored at −20 °C.

Cell culture

Cell lines (mouse hepatocyte (FL83B), mouse fibroblast (L929), mouse macrophages (RAW 264.7), keratinocytes (HaCaT), and human colon cells (CCD-18Co)) were purchased from American Type Culture Collection (ATCC) and used for genotoxicity and cytotoxicity assays. These cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) culture medium. The lymphoblast cell line (TK6 cells) were purchased from American Type Culture Collection (ATCC) and cultured in RPMI-1640 medium. Both culture media contained 1 g/L D-glucose, L-glutamine, 110 mg/L sodium pyruvate, penicillin G (10 U/mL), streptomycin (10 µg/mL), and 10 % fetal bovine serum. All cell lines were cultured at 37 °C with a 5 % CO_2 atmosphere, and culture media were changed every 2 - 3 days. All chemical reagents, including DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid), Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid), and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) reagent were purchased from Sigma-Aldrich Co.

MTT assay

In the MTT assay, FL83B, L929, HaCaT, RAW 264.7, and CCD-18Co cells were utilized. The cells were cultured until they reached approximately 80 % confluence and then transferred to 96-well plates at a density of 8×10^3 cells per well. After 24 h, a stock solution of the extract was diluted with culture medium to concentrations of 40, 60, 80, 100, 200, 300, and 400 µg/mL (sample solutions). A negative control solution was prepared by diluting DMSO with a culture medium to a concentration of 1 %. MTT reagent was dissolved in a culture medium at a concentration of 0.5 mg/mL. The cells were treated with the sample solutions and negative control solution for 24 and 48 h. After the treatment period, the culture medium was replaced with an MTT working solution and incubated for 2 h at 37 °C. The MTT reagent was then discarded, and the cells were washed with DMSO for 15 min. The absorbance of the MTT pellet was measured using a spectrophotometer, with the absorbance of the background (690 nm) subtracted from the sample absorbance (at 570 nm). The percentage of cell viability was calculated using the following equation.

$$\%\ cell\ viability = \frac{Absorbance\ of\ cells\ treated\ with\ the\ extract}{Absorbance\ of\ cells\ treated\ with\ negative\ control} \times 100 \quad (1)$$

The half maximal inhibitory concentration (IC50) of the extract was calculated by a line equation that indicated the concentration causing 50 % cell viability.

Micronucleus test (MN)

Micronuclei are small nuclei that are fragmented from the main nuclei following mitotic division, which are used for determining genotoxicity. TK6 cells were seeded and cultured in 24 well-plates at 2×10^5 cells/well for 24 h. After incubation, cells were treated with positive control solution (10 µg/mL mitomycin), negative control solution (1 %DMSO), and *A. spicifera* extract at various concentrations (40, 60, 80, 100, 200, 300 and 400 µg/mL) for 24 h. Cytochalasin B was added to the culture medium at 6 µg/mL for 24 h. Then, cells were harvested and centrifuged at 4,500 rpm for 5 min. The cell pellets were

resuspended and washed in PBS and centrifuged at 4,500 rpm 2 times for 5 min. The cell pellets were treated with hypotonic solution (0.075M of KCl) for 30 min at 37 °C and centrifuged at 4,500 rpm for 5 min. The solution was discarded, and cell pellets were fixed in methanol and acetic acid solution (3:1). The cytospin was used for transferring cells to glass slides. The slides were stained with 10 % Giemsa. The cells bearing micronuclei were observed and counted in a light microscope at 40× magnification. For each sample, at least 50 TK60 cells including those bearing micronuclei were counted. The data were compared between extract-treated or negative control versus positive control according to the following equation.

$$\%\ cells\ with\ micronucleus = \frac{Binucleated\ cells\ with\ micronucleus}{Total\ binucleated\ cell} \times 100 \quad (2)$$

Comet assay

TK6 cells were used for measuring the genotoxicity of *A. spicifera* by comet assay. The cells were cultured and expanded to around 80 % confluent, and then seeded in 24 well-plates at 2×10^5 cells/well. The cells were cultured in a complete medium for 24 h. After that, the cells were treated with *A. spicifera* extract at concentrations 40, 60, 80, 100, 200, 300, and 400 µg/mL and negative control solution for 24 and 48 h. Then, the treated and positive control cells were treated with 10 µM of hydrogen peroxide (H_2O_2) in a culture medium. TK6 cells were harvested and centrifuged at 4,500 rpm for 5 min. The cell pellets were resuspended in 100 µL phosphate-buffered saline (PBS), then each cell suspension was mixed with 100 µL of 1 % low melting point (LMP) agarose at 37 °C and transferred onto a glass slide. After that, a thin layer of 1 % normal melting point (NMP) agarose in PBS was added as a top layer on a glass slide. The agarose slides were soaked in lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, and 1 % Triton X-100; pH 10) for 16 h at 4 °C in the dark and transferred to the unwinding solution (300 mM NaOH, 1 mM EDTA; pH >13) for 30 min at room temperature. The slides were electrophoresed and neutralized with 0.4 M Tris pH 7.5 for 5 min. Finally, the cells were observed and evaluated under a fluorescence microscope using SYBR Green stain and analyzed with the Score 2.0 software for determining DNA damage by measuring tail length, % tail DNA, and tail moment (tail length x % tail DNA).

Determination of antioxidant activity by DPPH and ABTS assays

The antioxidant activity of the extract was determined by DPPH and ABTS assays. For positive control of the antioxidant assay Trolox was used at 1.63 - 50 µM in the DPPH assay and 0.82 - 25 µM in the ABTS assay. The DPPH stock solution was prepared by dissolving the compound in absolute ethanol and the concentration was adjusted to 5 mM. The DPPH working solution was obtained by diluting the stock solution to 0.02 mM with ethanol. Similarly, ABTS stock solution was prepared by dissolving ABTS with absolute ethanol and concentration adjusted to 7 mM. The ABTS working solution was obtained by mixing ABTS stock solution with 2.45 mM potassium persulfate and diluted with ethanol to obtain the absorbance of 7.0 - 7.5 at 734 nm. The extract was diluted with absolute ethanol and mixed with DPPH or ABTS working solution at the final concentrations of 200, 400, 600, 800, and 1,000 µg/mL and incubated for 20 or 6 min in DPPH or ABTS assay. After incubation, DPPH and ABTS scavenging (antioxidant) activities were determined by measuring the absorbances at 518 and 734 nm. Finally, the percentage of free radical-scavenging (antioxidant) activity was calculated according to the equation below.

$$\%\ scavenging\ activity = \frac{A\ control - A\ sample}{A\ control} \times 100 \quad (3)$$

A control (Absorbance of absolute ethanol mixed with ABTS or DPPH working solution),

A sample (Absorbance of AS extract diluted with ethanol and mixed with ABTS or DPPH working solution)

The scavenging activity of each concentration was plotted as a line equation and half maximum effective concentration (EC50) was calculated. The Trolox equivalent antioxidant capacity (TEAC) was calculated by comparing mg of Trolox /g extract at the same % scavenging effect.

Statistical analysis

The data of the experiments were presented as mean ± standard deviation (SD) and analyzed by GraphPad Prism 7 software (version 7.0). The results were compared between the extracts and controls 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

The cell viability after treatment with *A. spicifera* extract

The MTT assay revealed that exposure to the extract resulted in a decrease in cell viability when the concentration of the extract was increased (**Figure 1**). The IC50 values of all treated cell types, when exposed to the extract for 24 h, were higher than 400 µg/mL. In contrast, the IC50 values of the cells exposed to the extract for 48 h were lower at 100 µg/mL. L929, FL83B, and HaCaT cells exhibited a significant decrease in cell viability at 100 µg/mL concentrations. In comparison, CCD-18Co cells showed a significant decrease in cell viability at 60 µg/mL after exposure to the extract for 48 h. However, no significant decrease in cell viability was observed when the cells were exposed to the extract for 24 h, except for FL83B and HaCaT cells at a concentration of 300 and 400 µg/mL. On the other hand, the extract increased cell viability of RAW264.7 cells at low doses (40 and 60 µg/mL) while these cells only showed a significant decrease of viability at 400 µg/mL at 48 h.

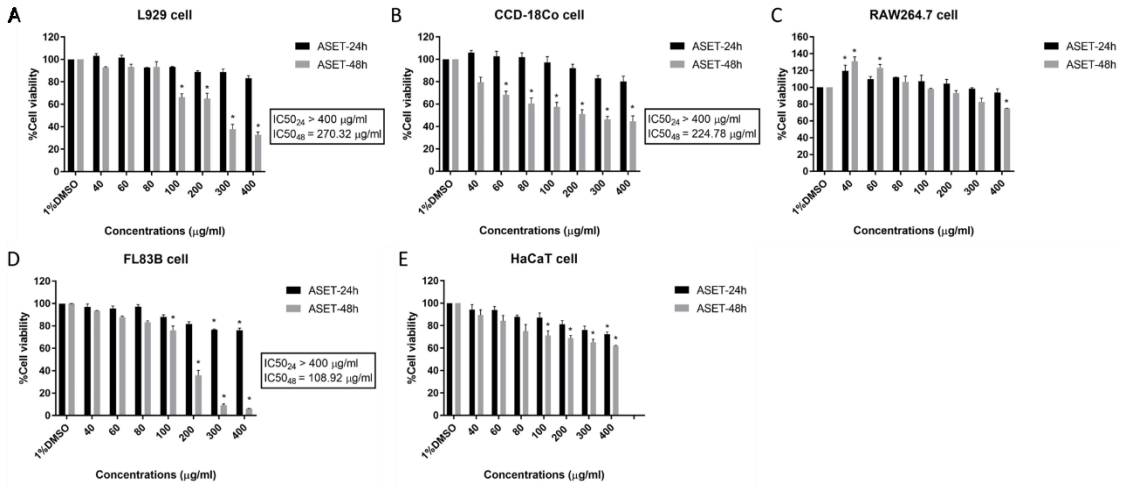

Figure 1 The percentage of cell viability after cells were exposed to *A. spicifera* extract for 24 and 48 h. A) mouse fibroblast; L929, B) human colon; CCD-18Co, C) mouse macrophages; RAW 264.7, D) mouse hepatocyte; FL83B, and E) keratinocytes; HaCaT. *Significant difference as compared to 1 %DMSO (control group) at P 0.05 (± SEM, n = 3).

Table 1 The percentage of TK6 cells with micronucleus when treated with negative control, positive control, and various concentrations of *A. spicifera* extract.

Treatment	Micronucleus (%)
Negative control	4
Positive control	24
A.spicifera extract	
40 µg/mL	8
60 µg/mL	10
80 µg/mL	10
100 µg/mL	10
200 µg/mL	11
300 µg/mL	10
400 µg/mL	18

The effect of *A. spicifera* extract on genotoxicity by micronucleus test

During cell division, damaged chromosomes are fragmented and tiny extra-nuclear bodies are formed at the anaphase stage. For positive control, TK6 cells were treated with 10 µg/mL mitomycin C for 24 h,

which caused chromosome breakage. Twenty four percent of the treated cells showed micronucleus while normally dividing cells are binucleated. When the cells were treated with the extract, the percent of cells with micronucleus were significantly lower than positive control but gradually increased from 8 % at 40 µg/mL to 18 % at 400 µg/mL. For untreated negative control cells, the percentage of cells with micronuclei was low at 4 % (**Table 1**).

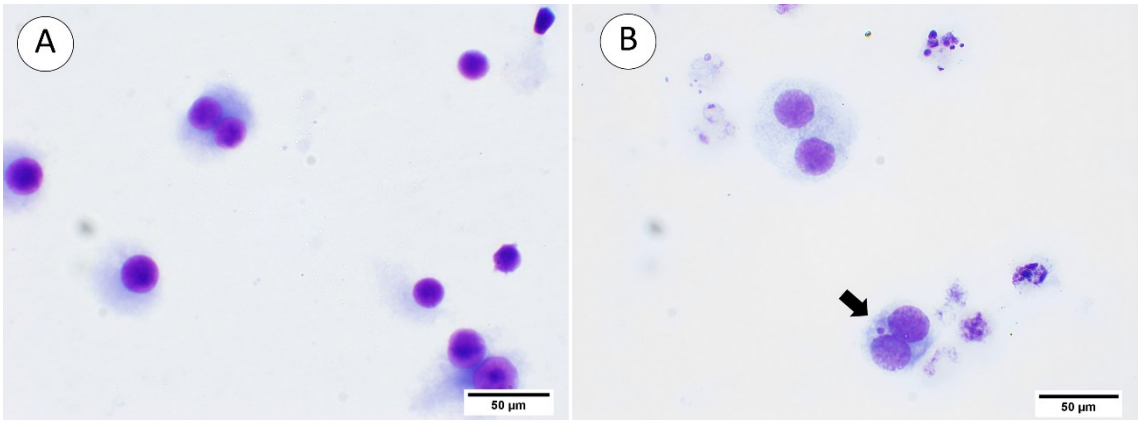

Figure 2 Chromosome damage was determined by micronucleus assay. TK6 cells in mitosis stages were stained with Giemsa stain: A) normally dividing cells appear as binucleated cells while abnormally dividing cells B) cells also contain micronucleus (arrow). The micrographs were captured at 40× magnification, and at least 50 cells were counted in each field of view and the percentages of cells with micronucleus were calculated.

Phytochemical compounds are essential components that could be used to improve the quality of food supplements, cosmetics, and drugs because of their beneficial antioxidant as well as other activities, particularly anti-inflammation, but they must be tested for toxicity to ensure their safety before use. The commonly used tests for estimating toxicity include cytotoxicity and genotoxicity assays [13]. Cytotoxicity tests are used to determine the toxicity of a substance to cells by assessing mitochondrial function, while genotoxicity tests are used to determine the toxicity of the extracts in damaging chromosomes and DNA [14]. The *A. spicifera* is a red macroalgae that contains many biochemical compounds, especially a flavone, apigenin, which showed antioxidant, antinociceptive, and anti-inflammatory activities in rats with carrageenan-induced paw edema by suppressing the expression of IL-1β, TNF-α, IL-6, and PGE2 [15].

The cytotoxicity of *A. spicifera* ethanolic extract was evaluated by MTT assay. The MTT is a soluble yellow tetrazolium salt, which reacts with NAD(P)H catalyzed by oxidoreductase enzymes of mitochondria in viable cells to form insoluble formazan crystals, which can be dissolved in DMSO, a non-polar solvent [16,17]. Five types of cells, including mouse hepatocyte (FL83B), mouse fibroblast (L929), mouse macrophages (RAW 264.7), keratinocytes (HaCaT), and human colon cells (CCD-18Co), representing liver, skin, immune system, and colon, were used for testing the cytotoxic effects of the ethanolic extract, since these are the main organs exposed to chemicals from cosmetics, drugs, and the environment [18,19]. The results of the experiment showed the extract at a concentration lower than 400 µg/mL did not affect the viability of all cell types except for hepatocyte (FL83B) at 200 µg/mL with 24 h incubation and colon cells at concentrations lower than 100 µg/mL with 48 h incubation. These results suggest maximum doses (concentration and duration of exposure) for each type of cell to avoid the cytotoxic effects of the extract, which agreed with a previous report [20]. However, the dose of the extract may vary when used with different cell types and animals [21].

The genotoxic effect of *A. spicifera* extract estimated by comet assay

The comet assay was used to test the genotoxicity of the extract, particularly the damage to DNA, by estimating the tail length and % tail DNA of comet cells which represent the extent of DNA damage caused by toxins or extracts. The tail length and % tail DNA of comet cells from the positive control cells and cells treated with the extract at concentrations of 100 - 400 µg/mL were significantly higher than the negative control. In addition, the percentage of a tail moments of extract-treated groups with concentrations of 200

- 400 µg/mL were significantly higher than that of the negative control cells. These results were consistent for both 24 h and 48 h incubations. However, the extract induced significantly much lower levels of DNA damage in the treated cells at all doses than the positive control cells treated with 10 µg/mL of mitomycin whose levels of tail length was about 46 %, % tail DNA about 50 %, and tail moment about 25 % (**Table 2**).

Table 2 The effect of *A. spicifera* extract on DNA damage in TK6 cells as determined by tail length, % tail DNA, and tail moment.

Treatment	Time (h)	Tail Length ± SD	% Tail DNA ± SD	Tail moment ± SD
Negative control	24	0.79±0.61	0.00±0.00	0.00±0.00
	48	0.86±0.60	0.00±0.00	0.00±0.00
Positive control	24	46.08±7.92	55.28±11.27	25.61±7.12
	48	46.93±9.85	49.83±12.48	23.05±6.39
40 µg/mL	24	0.86±0.73	0.00±0.00	0.00±0.00
	48	0.54±0.83	0.00±0.01	0.00±0.00
60 µg/mL	24	0.54±0.73	0.00±0.00	0.00±0.00
	48	0.78±0.84	0.15±0.70	0.00±0.01
80 µg/mL	24	1.43±1.65	0.52±1.93	0.03±0.14
	48	1.23±1.76	2.63±7.27	0.12±0.54
100 µg/mL	24	4.55±4.68*	10.27±14.11*	1.00±1.92
	48	4.88±3.20*	17.76±16.89*	1.04±1.73
200 µg/mL	24	6.70±5.68*	18.82±17.37*	1.87±2.64*
	48	7.95±9.42*	22.26±17.14*	2.63±5.04*
300 µg/mL	24	12.88±9.35*	33.41±20.31*	5.55±6.38*
	48	15.42±5.43*	27.91±14.33*	4.63±3.21*
400 µg/mL	24	16.02±10.09*	26.60±15.33*	5.21±6.23*
	48	24.41±7.65*	25.69±14.10*	6.59±4.49*

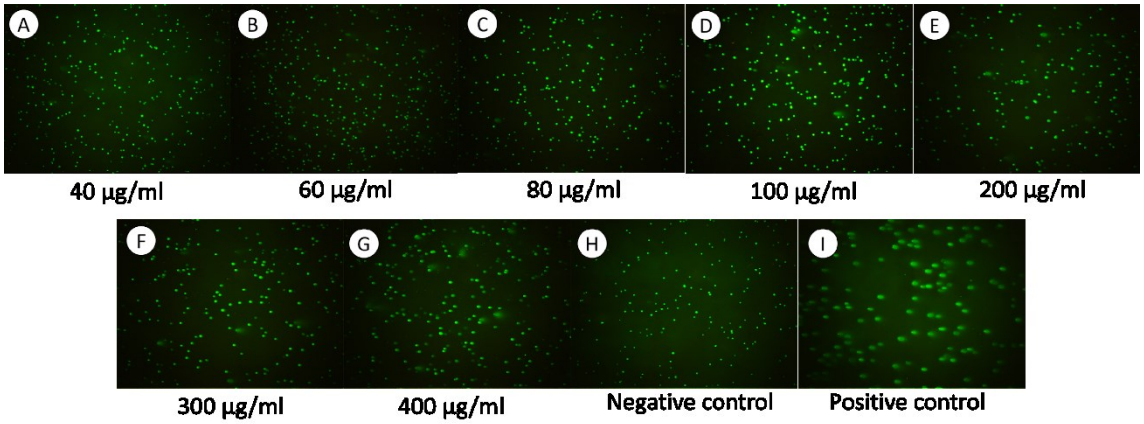

Figure 3 The DNA damage was evaluated by comet assay. The percentages of tail length, tail DNA and tail moment were measured by Score 2.0 software. The TK6 cells were exposed to *A. spicifera* extract at various concentrations at 24 and 48 h: A) 40 µg/mL, B) 60 µg/mL, C) 80 µg/mL, D) 100 µg/mL, E) 200 µg/mL, F) 300 µg/mL, G) 400 µg/mL, and I) Representatives of positive control cells that were exposed to hydrogen peroxide for 24 h and stained with SYBR Green.

The tests of genotoxicity were necessary for determining the toxicity to genes of the extract, which can induce mutation and cancer in organisms [22]. The combined micronucleus and comet assays are convenient and effective methods for estimating genotoxicity through the abnormalities induced at both the chromosome and DNA levels. The comet assay is used to determine DNA damage, such as DNA strand breaks and alkali-labile sites in a single cell [23], while the micronucleus assay is a widely used method for studying the effects of extracts on chromosome fragmentation, as the formation of micronucleus occurs when chromosomes are damaged by toxic substances [24]. Normally, there are cell cycle checkpoints that are controlled by cyclin-dependent kinase inhibitors in the S, G1, and G2 phases, which activate DNA repair in case of minor damage or programmed cell death in case of major damage [25], and these could be affected by the extract. In our study, a lymphoblast cell line (TK6 cell) was used to evaluate the genotoxicity of *A. spicifera* extract. The results of the comet assay showed that the *A. spicifera* extract did not induce DNA damage at concentrations lower than 100 µg/mL in TK6 cells. However, the micronucleus assay showed an increased percentages of micronuclei formation when exposed to higher concentrations of the extract, but these percentages were still much lower those that of the positive control. In our previous study, the ethanolic extract of red macroalgae *H. durvillei* also showed slight DNA damage in comparison to hexane, butanol, and ethyl acetate extracts by comet and micronucleus assays [26].

The free radical-scavenging activities of *A. spicifera* extract

The *A. spicifera* extract was evaluated for its free radical-scavenging activity using the DPPH and ABTS assays. The percentage of DPPH and ABTS scavenging effects of the extract ranged from 3.61±0.16 to 22.08±3.44 and 11.05±2.69 to 52.41±1.36, respectively (**Figure 4**) The EC50 values were calculated to be 2,563.15±302.18 and 950.05±29.40 µg/mL for DPPH and ABTS, respectively (**Table 3**). A Trolox, a vitamin E derivative in the water-soluble form used as a standard reagent for measuring the antioxidant activity, showed the EC50 value of DPPH scavenging activity at about 13.38±0.71 µg/mL and ABTS scavenging activity at about 3.82±0.03 µg/mL. The antioxidant effect of the extract was compared with the standard reagent to determine the TEAC. After the calculation, the TEAC values of the extract was found to be 5.24±0.44 and 4.03±0.13 mg Trolox/g extract.

Table 3 The free radical-scavenging (antioxidation) effect of *A. spicifera* extract determined as EC50 value and mg Trolox/g by DPPH and ABTS assay.

Samples	EC50 (µg/mL)		mg Trolox/g extract	
	DPPH assay	ABTS assay	DPPH assay	ABTS assay
Trolox (Positive control)	13.38±0.71	3.82±0.03	-	-
A. spicifera extract	2,563.15±302.18	950.05±29.40	5.24±0.44	4.03±0.13

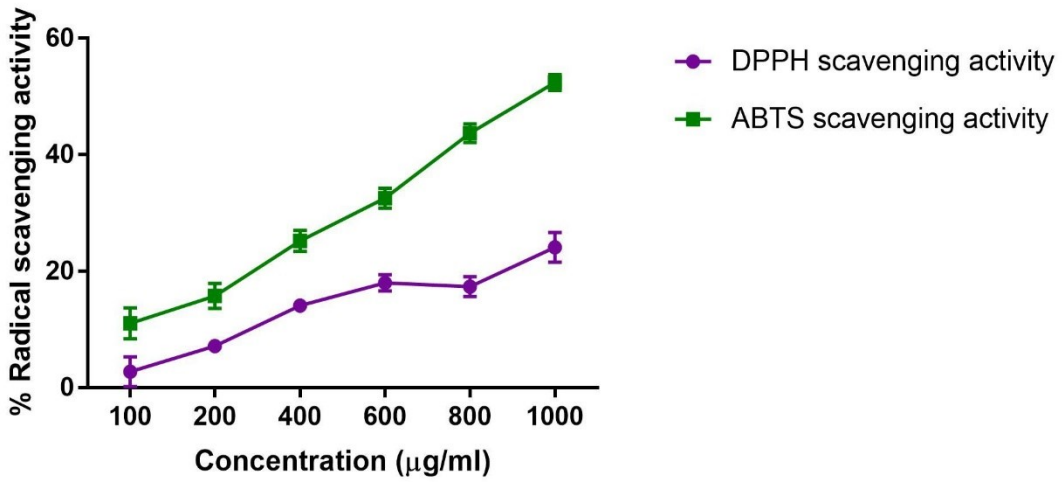

Figure 4 The percentage of DPPH and ABTS free radical-scavenging activity of *A. spicifera* extract (± SD).

The *A. spicifera* is a natural source of antioxidants that has been cultured in many countries, such as Hawaii, Mexico, and India. However, this macroalgae cultivated in different environments may exhibit varying antioxidant properties due to differences in their nutrients and exposure to sunlight. Generally, macroalgae live in harsh environments, and those with limited access to nutrients or extreme temperatures tend to produce substances with higher antioxidant activity [27]. In this study, we tested the ethanolic extract of *A. spicifera* cultured in Thailand, which showed high antioxidant activity, with EC50 values of 2,563.15±302.18 and 950.05±29.40 µg/mL by DPPH and ABTS assays. When compared to the antioxidant activity of Trolox, the extract showed 5.24±0.44 and 4.03±0.13 mg Trolox/g extract. In comparison, the antioxidant activity of a similar extract from *A. spicifera* cultured and harvested in Fiji was at 3.2 mg Trolox/g by ABTS assay [28]. By contrast, it was reported that ethyl acetate extract of *A. spicifera* cultured in India had the highest antioxidant activity of 44.4±9.35 %, which was higher than the crude extract with an antioxidant activity of 32.5±6.50 % [29,30]. In a study on the antioxidant effect of extracts from *A. spicifera* cultured in Malaysia, it was found that the extracts fractionated by various solvents revealed a range of DPPH scavenging percentages from 20.75±0.42 to 46.83±0.68 extracts with solvents with higher polarity, such as hexane, ethyl acetate, chloroform, and diethyl ether, exhibited higher antioxidant activity due, possibly, to their higher total phenolic content [2]. However, despite having higher antioxidant efficacy, extracts in non-polar solvents also have higher cytotoxicity and genotoxicity effect than ethanolic extract [27]. The hydro-ethanolic and hydro-methanolic extracts exhibited reasonably high antioxidant activity because the aqueous-organic solvent can extract greater total phenolic content [27]. In addition, there are various other methods for extracting active compounds apart from maceration with ethanol, including ultrasound, heating plate, and reflux. The reflux method produces the highest antioxidant and free radical-scavenging activity [31]. However, the maceration method is the easiest and can be performed in larger quantities, even though it may yield a lower amount of antioxidative compounds. This method is still considered the most cost-effective for the extraction of natural compounds with reasonably high antioxidant activity [32,33], which can be used as starting material for cosmetics, food supplements, and other health-related products.

Conclusions

The ethanolic extract of *A. spicifera* showed potent antioxidant activity and safety, when used in moderation, suggesting that it has the potential for use as a natural antioxidant. It is thus a promising source of phytochemicals that can be generated from easily cultured *A. spicifera*, which is currently used for wastewater treatment. This resource can be used to improve food supplements, manufacturing of drugs, and cosmetics products in the future. However, further studies are needed to evaluate the safety of the extract, particularly at higher concentrations and longer exposure times in animals and humans.

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