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Full Length Research Paper

Anti-inflammatory, anti-proliferative and anti-oxidant activities of organic extracts from the Mediterranean seaweed, *Cystoseira crinita*

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This study was conducted to evaluate the anti-inflammatory, the anti-proliferative, total phenolic content (TPC) and the anti-oxidant activity of the chloroformic ($CHCl_3$), the ethyl acetate (AcOEt) and the methanolic (MeOH) extracts of *Cystoseira crinita*. These extracts were assayed on carrageenan induced rat paw oedema assay. They exhibited significant anti-inflammatory activity in a dose dependent manner. The anti-proliferative activity of organic extracts of *C. crinita* was evaluated on cancer cell lines (A549, MCF7 and HCT15) by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. $CHCl_3$ and AcOEt extracts showed important anti-proliferative activity against both HCT15 and MCF7 cell lines. Organic extracts of *C. crinita* showed important TPC. Moreover, they exhibited significant radical scavenging activity and displayed the highest reducing power. Organic extracts of *C. crinita* might be used as a significant potential source of natural compounds with anti-inflammatory, anti-proliferative and anti-oxidant activity. It could have a promising role in future medicine.

Key words: *Cystoseira crinita,* anti-inflammatory, anti-proliferative, 2,2-diphenyl-1- picrylhydrazyl radical scavenging activity, total polyphenol content.

INTRODUCTION

Over the years, marine algal species offer the biological diversity for sampling in discovery-phase of new drug development (Munro et al., 1999). Recently, a great deal of interest has been developed to isolate novel bioactive compounds from marine resources because of their numerous health beneficial effects. Therefore, it is clearly documented that, pre-clinical pharmacological research with new marine compounds continued to be extremely active in recent history, and numerous macroalgae have shown potent cytotoxic activity (Mayer and Gustafson, 2006; Mayer and Hamann, 2004). Moreover, a large number of algae are reputed to have excellent medicinal value, and are in use for the treatment of several ailments. In medicine, various indigenous drugs are used, in single and/or in combined forms, for treating different types of inflammatory and arthritic conditions, with considerable success. Search for alternative anti-inflammatory drugs and medicines among marine seaweed, natural marine products having both anti-inflammatory and anti-oxidant activities, have received extra attention due to their potential pharmacological activities (Lim et al., 2006). Certain authors have suggested consuming algae as a chemopreventive agent against several cancers (Yuan and Walsh, 2006). Consumption of brown marine algae is thought to ameliorate some inflammatory disorders, breast cancer and high cholesterol level (Fitton, 2003). The strong association between the increasing of the consumption of these natural products and human diseases prevention has

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been explained by the content of the phytonutrients (Halliwell and Gutteridge, 1997). These phytonutrients include alkaloids (homoharringtonine), flavonoids (resveratrol, Kaempferol and quercetin), coumarins (scopoletin and scopolin), and steroids (beta sitosterol). The *in vitro* and *in vivo* antioxidant property, as well as protective effects and anticancer activity of these molecules have been demonstrated (Gupta et al., 2004).

Cystoseira is a genus of worldwide distribution with about 80% of the species. Secondary metabolites from Mediterranean species of this genus have been widely studied (Amico et al., 1990). A number of diterpenes and sterols have been isolated from the brown algae belonging to the genus *Cystoseira* and many diterpenoids as the linear diterpenes or the acyclic and cyclic meroditerpenoids (Culioli et al., 2004; Valls et al., 1995). Despite the diversity in quality and quantity of the Mediterranean Tunisian coast marine flora, with its large contains of seaweeds, most of them have not yet been investigated for anti-inflammatory, anti-proliferative and anti-oxidant potencies. Therefore, the objective of this research was to evaluate the anti-inflammatory, the anti-proliferative and the anti-oxidant activities of organic extracts from *C. crinita,* respectively: chloroformic ($CHCl_3$), ethyl acetate (AcOEt) and methanolic (MeOH) extracts.

MATERIALS AND METHODS

Samples collection

C. crinita a brown algae was collected from the Mediterranean in various areas of the coastal region of Monastir (Tunisia), in June 2007, at a depth between 1 and 3 m. After collection, the seaweeds were rinsed with fresh water to remove associated debris and epiphytes. The cleaned material was then air dried to dryness in the shade at 30°C. The dried samples were finely powdered and stored at – 20°C until use. Identification of specimens was carried out in the National Institute of Marine Sciences and Technologies (Salamboo, Tunisia). A voucher specimen has been deposited in the Department of Pharmacology, Monastir University.

Chemicals and reagents

Carrageenan (BDH Chemicals Ltd Poole England), acetylsalicylic of lysine (ASL), dimethylsulfoxide (DMSO), Dulbecco's modified Eagle's minimum essential medium (DMEM), fetal bovine serum (FBS), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT), penicillin and streptomycin, were purchased from Sigma Chemical (Berlin, Germany). The following chemicals used for antioxidant activity were purchased from Sigma-Aldrich. Chemical Co (St. Louis, MO, USA): 2, 2- diphenyl-1- picrylhydrazyl (DPPH) Folin- Ciocalteu reagent, gallic acid (GA), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were obtained from Sigma Chemical. Analytical grade methanol, ethyl acetate, and chloroform were obtained from Merck.

Preparation of extracts

For extraction of bioactive in shade dried seaweeds, 600 g of finely powdered algal material were packed in small bags (5 x 10 cm) of Whatman filter paper # 1 and all bags were sealed and soaked three times in an organic solvent bath for steeping during 24 h. The extraction was carried out, separately, with different organic solvents in the order of increase polarity: chloroform, ethyl acetate and methanol. The organic extracts were concentrated to solvent free by evaporation in a rotary vacuum evaporator (Buchi, B- 480) at 40°C. The residues obtained were finally dried in a vacuum dessicator.

Anti-inflammatory activity

Animals

Male adult Wistar rats weighing 150 to 170 g of both sexes were obtained from Pasteur Institute (Tunis, Tunisia). They were housed in polypropylene cages and were left for two weeks for acclimatization to animal room maintained under controlled conditions: (a 12 h light–dark cycle at 22 ± 2°C), on standard pellet diet and water *ad libitum*. Before the day of assay, Wistar rats were fasted overnight with the free access to water. Housing conditions and *in vivo* experiments were approved according to the guidelines established by the European Union on Animal Care (CFE Council (86/609). The rats were used for the anti-inflammatory evaluation of the extracts testing.

Carrageenan induced rat paw oedema

Wistar rats were divided into groups of six animals. Oedema was induced by injecting 0.05 ml of 1% carrageenan subcutaneously into the sub-plantar region of the left hind paw (Winter and Nuss, 1962). Chloroform, ethyl acetate and methanol extracts of *C. crinita* (25 or 50 mg kg^{-1}) were administered intraperitoneally (i.p.) and were dissolved in 1% DMSO and 99% saline water. The control group received the vehicle (1% DMSO, 99% saline water) (2.5 ml kg^{-1}, i.p.). The reference group received acetylsalicylic of lysine (ASL, 300 mg kg^{-1}, i.p). All drugs were administrated 30 min before the injection of carrageenan. Measurement of paw size was done by means of volume displacement technique using plethysmometer (Ugo Basile no.7140) immediately before carrageenan injection and 1, 2, 3, 4 and 5 h after carrageenan injection. Percentages of inhibition in our anti-inflammatory tests were obtained for each group using the following ratio:

$$[(V_t - V_o)_{control} - (V_t - V_o)_{treated}] \times 100 / (V_t - V_o)_{control},$$

Where, V_t is the average volume for each group and V_o is the average volume obtained for each group before any treatment. Lower and or higher doses were administered, in order to study doses dependency of the anti-inflammatory activity.

Anti-proliferative activity

Cell line and culture conditions

The human tumor cell lines A549 (lung cell carcinoma), HCT15 (colon cell carcinoma) and MCF7 (breast adenocarcinoma) were purchased from the American type culture collection (ATCC; Manassas, VA, USA). Freshly trypsinized cells were seeded and grown in DMEM supplemented with 10% (v/v) FBS, and 1% penicillin/streptomycin, all obtained from Biochrom AG (Berlin, Germany). They were grown on flasks (Nunc, Denmark) at 37°C in a humidified atmosphere containing 5% CO_2. Cells were replicated every 2 to 4 days and the medium changed once in-between.

Viability assay

Viability was determined using the MTT (Sigma Aldrich Chimie, Saint- Quentin- Fallavier, France) assay reported by Mosmann (1983). However, the development of this rapid colorimetric assay, which relies on the ability of mitochondrial dehydrogenase enzymes to convert 3, -4, 5 dimethyithiazol- 2, 5 diphenyl tetrazolium bromide (MTT) to a purple formazan precipitate, has simplified large scale screening of cells and drugs. The formazan crystals were dissolved and the optical density measured using a microplate reader. The use of MTT has thus become the method of choice because of its simplicity and adaptability to automation. Concentrations ranging from 25 to 500 (µg ml^{-1}) of the organic extracts of *C. crinita* were prepared from the stock solutions by serial dilution in DMEM to give a volume of 200 µl in each well of a microplate (96 wells) as described earlier. The final concentration of DMSO in the culture medium was maintained at 1% (v/v) to avoid toxicity of the solvent. Known number of A549, HCT15 or MCF7 cells (10^3) were transferred into 96- well plates (Nunc, Denmark) in a volume of 200 µl of culture medium and incubated for 24 h before addition of test compounds. After 24 h, cells were exposed at 37°C to known concentrations of the different organic extracts to be tested. After drug exposure, cells were washed with phosphate-buffered saline (PBS) and then reincubated in fresh culture medium for a further 48 h, then the culture medium was removed and 200 µl of MTT reagent (diluted in culture medium, 0.5 mg ml^{-1}) was added. Following incubation for 4 h, the MTT medium was removed and DMSO (200 µl) was added to dissolve the formazan crystals. Absorbance values were measured with a microplate reader (Bio Tek EL 340, USA) using a test wavelength of 570 nm and a reference wavelength of 630 nm.

The relative cell viability was expressed as the mean percentage of viable cells compared with DMSO-treated cells. Appropriate DMSO dilutions were used as controls. DMSO did not affect proliferation of any cell line. Cytotoxicity is expressed as the concentration of samples inhibiting cell growth by 50% (IC_{50}), compared with the control (using cells treated with 1.0% DMSO). All tests and analyses were run in triplicate and averaged.

Anti-oxidant activity (AOA)

Total phenolic content (TPC)

The total phenolic content of organic extracts of *C. crinita* was estimated by the method of Taga et al. (1984). Briefly, 100 µl aliquot of sample were mixed with 2.0 ml of 2% Na_2CO_3 and allowed to stand for 2 min at room temperature. After incubation, 100 µl of 50% Folin- Ciocalteu's phenol reagents were added, and the reaction mixture was mixed thoroughly and allowed to stand for 30 min at room temperature in the dark. Absorbance of all sample solutions was measured at 720 nm using spectrophotometer (Jenway 6505 UV/Vis). A calibration curve of gallic acid (ranging from 0.05 to 1 mg ml^{-1}) was prepared, and TPC was standardised against gallic acid and expressed as mg gallic acid equivalent per gram of sample on a dry weight basis (DW). All determinations were performed in triplicate.

DPPH radical scavenging activity

DPPH is a chromogen-radical-containing compound that can directly react with anti-oxidants. DPPH has been used extensively as a free radical to evaluate reducing substances and is a useful reagent for investigating the free radical scavenging. When the DPPH radical is scavenged by anti-oxidants through the donation of hydrogen to form a stable DPPH-H molecule, the colour is changed from purple to yellow. DPPH radical scavenging activity of the three organics extracts of *C. crinita* were determined according to the method of Kim et al. (2002). Each sample stock solution (1mg ml^{-1}) was diluted to final concentration of 500, 250, 100, 50 and 10 (µg ml^{-1}) in ethanol. A total of 0.5 ml of 30 mM DPPH ethanol solution was added to 0.5 ml of sample solution at different concentrations and allowed to react at room temperature. After 30 min, the absorbance (A) was measured at 520 nm. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{Radical scavenging capacity (RSC, \%)} = 1- [(A_{\text{sample}} - A_{\text{sample blank}})/A_{\text{control}}] \times 100.$$

Where the $A_{control}$ is the absorbance of the control (DPPH solution without sample), the A_{sample} is the absorbance of the test sample (DPPH solution plus test sample), and the $A_{sample\ blank}$ is the absorbance of the sample only (sample without DPPH solution). Synthetic anti-oxidant, Trolox was used as positive control. Concentration of extract required to reduce DPPH radicals by 50% (IC_{50}) was calculated by linear regression of plots, where the abscissa represented the concentration of tested marine algae extracts and the ordinate average percent of scavenging capacity from three replicates. DPPH was expressed in terms of Trolox equivalent anti-oxidant capacity (TEAC) which was calculated based on its concentration of extract required to reduce DPPH radicals by 50% (IC_{50}), as follows:

$$\text{TEAC (mg Trolox/100 g)} = IC_{50}\,(\text{Trolox})/IC_{50\ \text{sample}} \times 100.$$

Ferric - reducing antioxidant power (FRAP)

Reducing power of the three organic extracts of *C. crinita* was determined by the method of Oyaizu (1986). Briefly, 1.0 ml of each sample dissolved in distilled water was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1.0%). Reaction mixture was incubated for 20 min at 50°C. After incubation, 2.5 ml of trichloacetic acid (10%) was added, and the mixture was centrifuged for 10 min. Finally, 2.5 ml of the upper layer were mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1%). The solution was incubated at ambient temperature for 30 min for colour development. Absorbance of all the sample solutions was measured at 700 nm, and compared to a gallic acid calibration curve. The data were presented as gallic acid equivalent per gram of seaweed material (GAE/g). A greater value of GAE related to greater reducing power of the sample.

Data and statistical analysis

Data were expressed in mean ± S.E.M and statistical significance was evaluated using student's test for paired data and differences were considered significant when $p < 0.05$.

RESULTS

Evaluation of the anti-inflammatory activity

Organic extracts of *C. crinita* ($CHCl_3$, AcOEt and MeOH) were investigated for their anti-inflammatory activity using carrageenan induced hind paw oedema. The experimental results are listed in Table 1. There was a significant reduction ($p< 0.001$) in carrageenan-induced rat paw oedema at 50 mg kg^{-1} dose of organic extracts of *C. crinita* and at 300 mg kg^{-1} dose of ASL, over a period

Table 1. Effect of the administration of $CHCl_3$, AcOEt and MeOH extracts of *C. crinita* and reference drug (ASL) in carrageenan induced rat paw oedema.

Sample	Dose (mg/kg)	Oedema (10^{-2} ml) (mean ± S.E.M) (h)			Oedema inhibition (%) (h)		
		1	3	5	1	3	5
Control	-	27.20 ± 1.10	69.83 ± 1.47	74.17 ± 1.72	-	-	-
Acetylsalicylate of lysine	300	14.33 ± 0.52***	18.17 ± 1.33***	21.33 ± 1.21***	47.31	73.97	71.43
***C. crinita* extracts**							
$CHCl_3$	25	13.50 ± 1.38***	17.85 ± 2.67**	19.33 ± 0.82***	49.81	74.43	73.93
	50	10.17 ± 0.95***	12.75 ± 1.85***	14.17 ±1.17***	62.61	81.74	80.89
AcOEt	25	14.67 ± 1.21**	16.83 ± 0.75***	19.14 ± 1.68**	46.06	75.89	74.19
	50	08.33 ± 0.75***	10.50 ± 1.47***	12.70 ± 0.55***	69.37	84.96	82.87
MeOH	25	19.67 ± 1.63**	22.33 ± 2.66**	23.57 ± 0.72***	27.68	68.02	68.22
	50	10.75 ± 0.75***	15.67 ± 0.82***	17.50 ± 1.05***	60.47	77.55	76.40

Chloroform ($CHCl_3$), ethyl acetate (AcOEt), methanol (MeOH), acetylsalicylate of lysine (ASL): Reference drug. Control: 1% DMSO; The values represents the means difference of volume of paw ± S.E.M; n = 6. **p < 0.01 and ***p < 0.001 significant from the control.

Table 2. *In vitro* growth inhibitory activity of organic extracts from *C. crinita* on three human tumor cell lines A549 (lung cell carcinoma), HCT15 (colon cell carcinoma) and MCF7 (breast adenocarcinoma).

Samples	IC_{50} (µg ml^{-1})		
	A549	HCT15	MCF7
$CHCl_3$	367 ± 0.001	41 ± 0.003	37 ± 0.001
AcOEt	131 ± 0.014	58 ± 0.003	65 ± 0.009
MeOH	276 ± 0.025	446 ± 0.015	80 ± 0.010

IC_{50}, 50% inhibition of cell growth.

of 3 h. Treatment with $CHCl_3$ and AcOEt extracts of *C. crinite* (at the dose of 50 mg kg^{-1}, i.p.) inhibited the formation of the oedema by 81.74 and 84.96%, respectively in the third hour of experimentation (Peak of oedema formation). Both results were statistically significant compared to the control. In addition, the MeOH extract of *C. crinita* also produced inhibitory effect at 50 mg kg^{-1} dose, on carrageenan oedema in the third hour, by 77.55% when compared to the control ($p < 0.001$). This results is quite similar to the one observed for the group treated with ASL (300 mg kg^{-1}), which inhibited oedema formation by 73.97%.

Evaluation of the anti-proliferative activity

The inhibitory effect of the chloroformic, the ethyl acetate and the methanolic extracts of *C. crinita* were determined by exposure of A549, HCT15 and MCF7 cells, to increasing concentrations of all extracts in a stepwise for 24 h. The concentration of each extract which reduced cell survival by 50% (IC_{50}) was determined from cell survival curves and the results are presented in Table 3. As indicated in Table 2, treatment of HCT15 and MCF7 cells with chloroformic extract resulted in loss of cell viability with IC_{50} values respectively of 41 and 37 µg ml^{-1}. Moreover, treatment of HCT15 and MCF7 cells with ethyl acetate extract resulted in loss of cell viability, too, with an IC_{50} values of 58 and 65 µg ml^{-1}, respectively. Furthermore, treatment with the methanolic extract inhibited the proliferation only of MCF7 cell, with an IC_{50} value of 80 µg ml^{-1} whereas, A549 cell line was more resistant to all extracts, as shown by the respective IC_{50} of these cancer cell lines. The effect of all extracts on different cell lines was studied by measuring cell numbers by the MTT assay after the treatment of the cultures with each extract for 24 h. The treatment of HCT15 and MCF7 cell lines with the chloroformic and the ethyl acetate extracts clearly reduced cell numbers (Figures 1 and 2); whereas the methanolic extract did not have an evident inhibitory effect on cell proliferation, only on MCF7 cell lines (Figure 3). The effect of the organic extracts on cell viability was

Table 3. Amount of phenolic compounds and anti-oxidant activities of organic extracts of *Cystoseira crinita.*

Samples	TPC (mg GAE/g dried sample)	DPPH		FRAP (mg GAE/g dried sample)
		IC_{50} (µg ml^{-1})	TEAC (mg Trolox/100 g dried sample)	
$CHCl_3$	402.44 ± 0.004	103 ± 0.004	87.37 ± 0.004	3.58 ± 0.005
AcOEt	406.22 ± 0.001	96 ± 0.002	93.75 ± 0.003	4.62 ± 0.004
MeOH	261.53 ± 0.006	107 ± 0.001	84.11 ± 0.005	1.26 ± 0.001
Trolox		90 ± 0.002		

Results are expressed as means ±SD (n = 3). DPPH was expressed in terms of Trolox Equivalent. TEAC: Trolox equivalent anti-oxidant capacity was calculated based on its concentration of extract required to reduce DPPH radicals by 50% (IC_{50}) as follows: TEAC (mg Trolox/100 g) = IC_{50} (Trolox)/IC_{50} sample x 100.

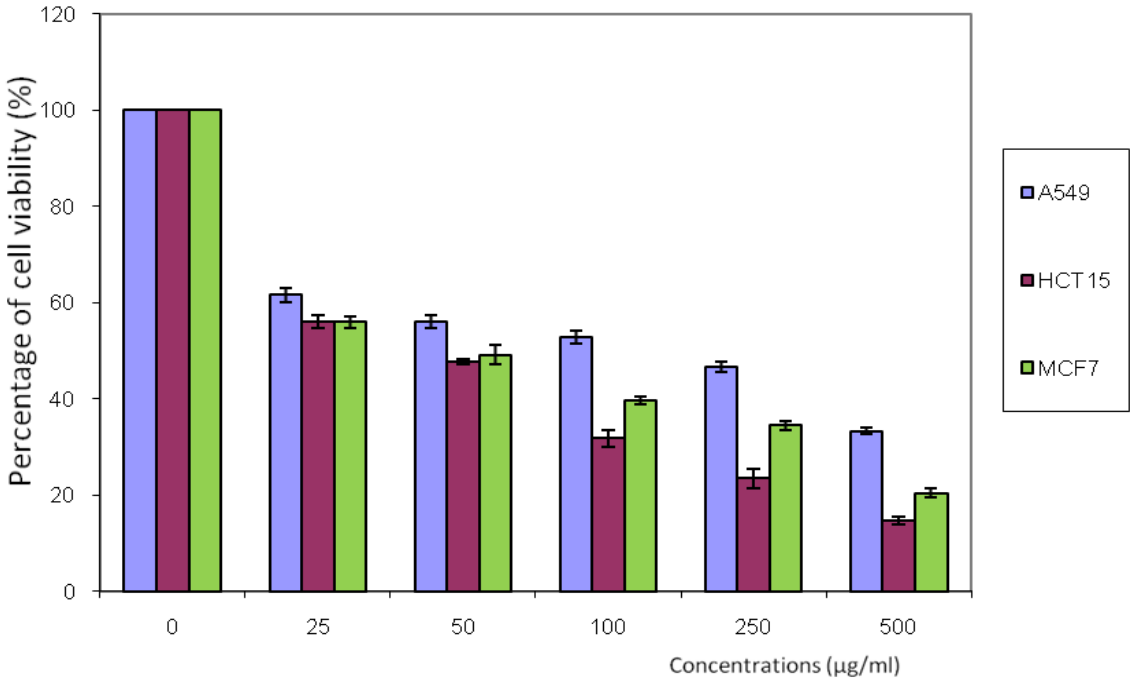

Figure 1. Effect of the chloroformic extract of *Cystoseira crinita* ($CHCl_3$) on the viability of three human tumor cells lines (A549: lung cell carcinoma; HCT15: colon cell carcinoma and MCF7: breast adenocarcinoma) expressed as (%) of cell viability to the control. Statistical significance is based on the difference when compared with the cells without extracts (*p < 0.05, **p < 0.01 and ***p < 0.001).

evaluated by determining the percentage of MTT reduction upon incubation of A549, HCT15 and MCF7 cells with increasing extract concentrations in the range of 25 to 500 µg ml^{-1}. As shown in Figures 1 and 2, the chloroformic and the ethyl acetate extracts produced a dose-dependent reduction on cell viability. Indeed, at lower concentrations of the chloroformic and the ethyl acetate extracts of *C. crinita* (25 and 50 µg ml^{-1}), the number of HCT15 and MCF7 cells started to decrease markedly.

Figure 3 shows that higher concentrations (250 µg ml^{-1}) rapidly reduced the number of HCT15 and A549 cells. These results suggest that the chloroformic and the ethyl acetate extracts of *C. crinita* had an obvious toxic effect on HCT15 and MCF7 cells at low concentrations but the methanolic extract inhibited HCT15 and A549 cells at higher concentration. All organic extracts of *C. crinita* had smaller effect on A549 cell line (IC_{50} values ranged from 131 to 367 µg ml^{-1}).

Evaluation of antioxidant activity

Total phenolic content

The total phenolic of *C. crinita* extracts was measured according to Folin- Ciocalteu method. The Folin–Ciocalteu regent determines total phenols, producing

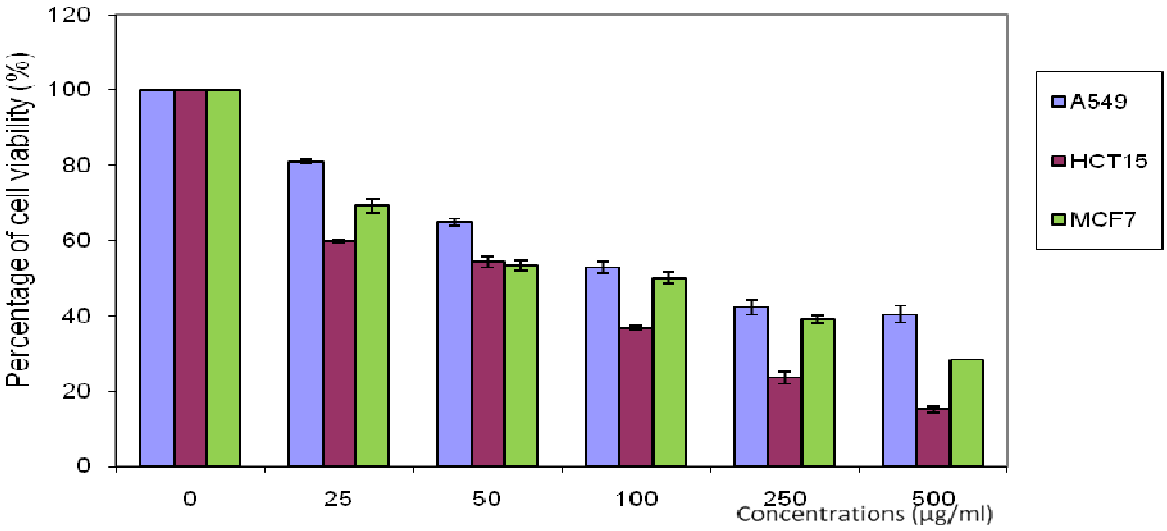

Figure 2. Effect of the ethyl acetate extract of *Cystoseira crinita* (AcOEt) on the viability of the three human tumors cells lines (A549: lung cell carcinoma; HCT15: olon cell carcinoma and MCF7: breast adenocarcinoma). Statistical significance was based on the difference when compared with the cells without extracts (*p < 0.05, **p < 0.01 and ***p < 0.001).

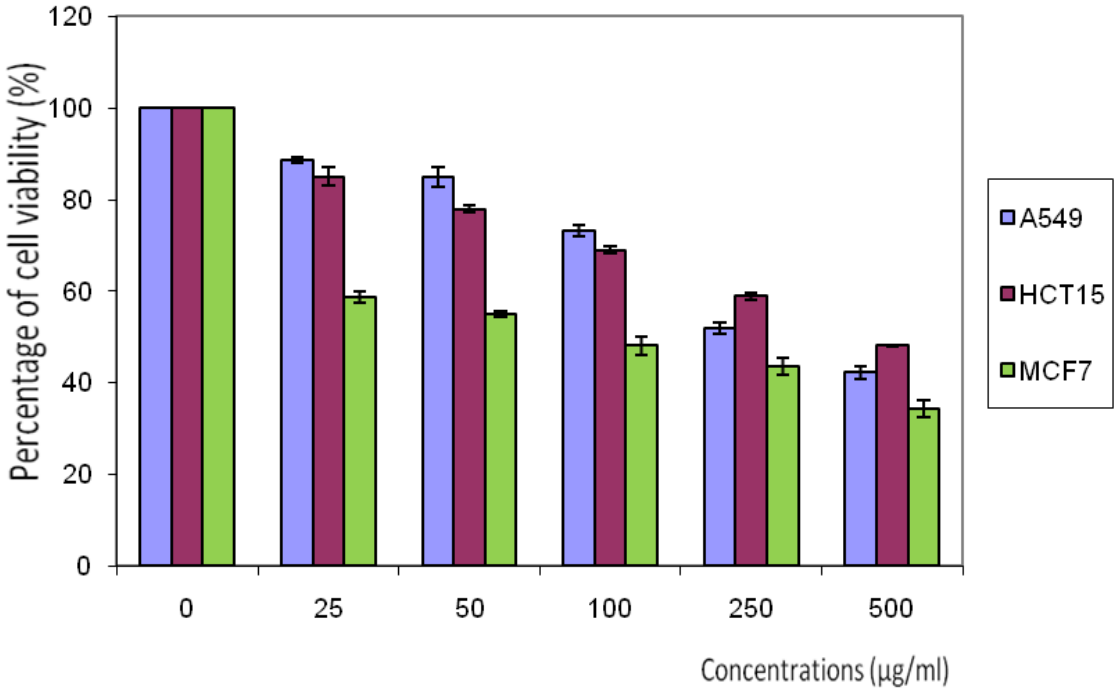

Figure 3. Effect of the methanolic extract of *Cystoseira crinita (*MeOH) on the viability of the three human tumor cells lines (A549: lung cell carcinoma; HCT15: colon cell carcinoma and MCF7: breast adenocarcinoma). Statistical significance is based on the difference when compared with the cells without treating extracts (*p < 0.05, **p < 0.01 and ***p < 0.001).

blue colour by reducing yellow hetero polyphosphate molybdate tungstate anions. The phenolic contents varied widely in the organic extracts of *C. crinita* and ranged from 261.53 to 406 mg GAE/g dried sample (Table 3). The highest level of phenolic was found in both ethyl acetate and chloroformic extracts, with values towards 406.22 and 402.44 mg GAE/g dried sample, respectively whereas, in the methanolic extract, TPC was

only 261.53 mg GAE/g dried sample. In fact, the phenolic content of this study extracts decreased in order of AcOEt> $CHCl_3$> MeOH.

DPPH radical scavenging activity

Free radical scavenging activity was measured using DPPH, which is a stable free radical and in the presence of the total extract, it was scavenged; the anti-oxidant activity was defined as the mean of free radical scavenging capacity. So we examined the anti-oxidant effect of organic extracts of *C. crinita* by DPPH radical scavenging activity. These extracts were able to reduce the stable radical DPPH to the yellow coloured diphenyl prilhydrazine and the IC_{50} values were calculated and are presented in Table 3. The ethyl acetate extract of *C. crinita* exhibited excellent DPPH radical scavenging activity, with an IC_{50} value of 96 µg ml^{-1} whereas, the chloroformic and the methanolic extracts belonging to *C. crinita* exhibited less DPPH radical scavenging activity with an IC_{50} value of 103 and 107 µg ml^{-1}, respectively. However, the scavenging effects of these extracts decreased in the order of AcOEt > $CHCl_3$ > MeOH. These scavenging activities were found significantly similar to Trolox (90 ± 0.002 µg ml^{-1}), under the same experimental conditions. In the other hand, a correlation was found between the TPC and IC_{50}; when the TPC was high, the IC_{50} was low and results in high level of TEAC (Trolox equivalent anti-oxidant capacity).

This was due to the high level amount of polyphenolic constituents present in *C. crinita* which were capable of functioning as free radical scavengers.

Ferric- reducing anti-oxidant power (FRAP)

Table 3 shows greater differences in total anti-oxidant capacity measured by FRAP method, by comparison of the three organic extracts. FRAP values were found within the range of 1.267 and 4.624 mg GAE/g dried sample. The ethyl acetate extract of *C. crinita* had major ability to reduce Fe^{3+} following by the chloroformic extract, with the respective value of 4.624 and 3.586 mg GAE/g dried sample. The FRAP value of the MeOH extract of *C. crinita* had a lower FRAP value 1.26 mg GAE/g dried sample.

DISCUSSION

This study is the first report on the anti-inflammatory, anti-proliferative and anti-oxidant activities of the chloroformic, ethyl acetate and the methanolic extracts from *C. crinita.* Carrageenan has been widely used as a noxious agent able to induce experimental inflammation for the screening of compounds possessing anti-inflammatory activity. This phlogistic agent, when injected locally into the rat paw, produced a severe inflammatory reaction, which was discernible within 30 min (John and Nodine, 1999). The development of oedema induced by carrageenan is a biphasic event: the early phase (90 to 180 min) of the inflammation is due to the release of histamine, serotonin and similar substances. The later phase (270 to 360 min) is associated with the activation of kinin-like substances and the release of prosta-glandins, proteases and lysosome (Olajide et al., 1999). All organic extracts inhibited hind paw oedema and showed a dose-dependent anti-inflammatory activity but the results were different for each extract depending on the early/later phases. $CHCl_3$ and AcOEt extracts from *C. crinita* inhibited both phases of the carrageenan-induced oedema by reducing the release of histamine and serotonin and also the kinin-like substances and prostaglandins; while the MeOH extract from *C. crinita* inhibited particularly the later phases by restraining the kinin-like substances and prostaglandins productions. This pharmacological property may be attributed to the seaweed composition and to a possible molecular mechanism by effectively decreasing the production of the pro-inflammatory cytokines of IL-6 and IL-β and the expression of COX-2 and simultaneously elevating the level of anti-inflammatory cytokine IL-4 in the carrageenan-injected rat paw tissues (Moulin and Coquerel, 2002).

Brown algae have been shown to possess the ability to produce a great variety of secondary metabolites with very different skeleton types and functionalities (Blunt et al., 2003). Secondary metabolites from the genus *Cystoseira* have been widely studied. Polysaccharides (Cardozo et al. 2007), sterols and lipids (Heiba et al., 1997; Moreno et al., 1998) terpenes (Amico et al., 1990; Valls, 1993) and many diterpenoids have been isolated as the linear diterpenes or the acyclic and cyclic meroditerpenoids (Culioli et al., 2004; Valls et al., 1995) and they have been extensively investigated. These metabolites are suggested to act synergistically to exert the observed pharmacological activity and could possibly lead to the observed activities. It has been reported that these classes of compounds possess various important pharmacological activities including anti-inflammatory activity (Geetha and Varalakshmi, 2001). $CHCl_3$ and AcOEt extracts had an obvious anti-proliferative effect on HCT15 and MCF7 cancer cells. Moreover the methanolic extract exhibited a strong against the proliferation of MCF7 cell lines. The inhibition of proliferation and induction of cell death observed occurred in a concentration- and dose-dependent manner. It seemed that variation of cytotoxicity of organic extracts of *C. crinita* in different cells may be dependent upon the mechanisms affecting its internalization. Both $CHCl_3$ and AcOEt extracts of *C. crinita* by comparison to HCT15 and MC7 cells may have particular molecular receptor sites with which $CHCl_3$ and AcOEt extracts of *C. crinita* react

effectively to produce important cytotoxic responses. This finding provides the impetus for further studies to delineate the mechanisms of selective cytotoxicity. Further studies about cytotoxic activity on *Cystoseira* genus have showed various diterpenes which were identified as the bioactive compounds in *C. crinita* (Fisch et al., 2003), *C. myrica* (Blunt et al., 2005) and *C. usneoides* (Kukovinets and Kislitsyn, 2006). On the other hand, brown algae *C. usneoides* which contained usneoidone E, exhibited anti-viral and anti-tumor activity (Kukovinets and Kislitsyn, 2006).

Blunt et al. (2005) reported that *C. myrica*, collected in the Gulf of Suez, yielded four hydroazulene diterpenes, dictyone acetate, dictyol F monoacetate, isodictytriol monoacetate and cystoseirol monoacetate. These reports suggest that diterpenes compounds could be responsible for the anti-tumoral activities measured in *C. crinita* collected in our study. Organic extracts of *C. crinita* had significant anti-oxidant activity especially for both $CHCl_3$ and AcOEt extracts toward the DPPH free radical and FRAP assay. Many researchers have investigated the anti-oxidant factors of seaweeds. Several compounds were identified as anti-oxidants, including protective enzymes (Nakano et al., 1995), ascorbic acid (Morgan et al., 1980), lipophilic anti-oxidants (Takamatsu et al., 2003), phlorotannins (Jimenez-Escrig et al., 2001) and catechins (Yoshie et al., 2000). We hypothesize that these compounds might contribute to the activity. The observed anti-inflammatory and the anti-proliferative activity could be attributed to the total polyphenols compounds estimated in the organic extracts of *C. crinita*. In fact, polyphenols has emerged as one major category of natural products that is important to human health (Shahidi et al., 1992). Increasing scientific evidence shows that polyphenols are good anti-oxidants and are effective in preventing cardiovascular and inflammatory diseases, and can also be used as chemopreventing agents for cancer. These molecules might act as cancer-blocking agents, preventing initiation of the carcinogenic process and as cancer-suppressing agents, inhibiting cancer promotion and progression (Russo, 2007). Ragan and Glombitza (1986) reported the radical-scavenging activity of seaweeds to be mostly related to their phenolic contents. Anti-oxidant activity had also been reported to be concomitant with the development of reducing power (Wong et al., 2006). Some reports supported the relationship of cytotoxicity with anti-oxidant activity (Dwivedi et al., 2003). Yuan and Walsh (2006) proved anti-oxidant and anti-proliferative activities of extracts from a variety of marine algae on human cervical adenocarcinoma cells.

Thum et al. (1991) and Dubois (1996) reported that there is a close relationship between inflammation and cancer; in which tumor promoters recruit inflammatory cells to the application site and cancer development may also act by aggravating inflammation in the tissue and vice versa and that inflammatory cells are capable of inducing genotoxic effects (Rosin et al., 1994). The results of these screening investigations confirm the greater potential of the brown seaweed *C. crinita.*

Conclusion

This study demonstrated that $CHCl_3$ and AcOEt extracts of *C. crinita* exhibited a high anti-inflammatory activity. Moreover, these extracts have an anti-proliferative effect by reducing cell viability and excellent anti-oxidant activity.

All these findings support the need for further investigations to clarify the features underlying the anti-inflammatory and anti-tumor potencies of these extracts. Additional studies should be tested for the potent organic extracts to assess the *in vivo* biological activities and to identify specific phytochemicals responsible for their activities and to characterize the efficacious phyto-therapeutic and/or bioactive compound(s).

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