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Research Article

Cytotoxic and Protective Effects of Sulfated Polysaccharide from *Codium edule* P. C. Silva against UVB-Induced Matrix Metalloproteinase-1 (MMP-1) Production and Skin Damage

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ABSTRACT

Objective: The study aimed to evaluate the chemical content, cytotoxic, and protective effects of polysaccharide fractions (CFs) from *Codium edule* against human breast cancer cells (MCF-7), UVB-induced MMP-1 production and damaged rats skin. Materials and methods: Ion exchange chromatography was used for the isolation of CFs from the crude polysaccharide (CP) of *C. edule*, which was subsequently analyzed for their chemical composition. Cytotoxic and proliferative activities of CFs were tested on MCF-7 and human dermal fibroblasts (HDFs) using MTT assay. Protective activity of polysaccharide fraction 1 (CF1) was evaluated on Sprague-Dawley rats exposed to UVB radiations. Animals were orally fed with 50, 150, 200 mg/kg BW CF1 before exposure to UVB radiation (1.14 µW/cm^{2,} 30 minutes) daily for 7 days. Rat's skin was evaluated by histological examination and measurement of severity index. The levels of matrix metalloproteinase-1 (MMP-1) in rats plasma were quantified by ELISA. Results and Conclusion: CFs afforded carbohydrates (1.93 - 14.9%), protein (1.3 - 4.1%) uronic acid (1.11 - 4.49%) and sulfate (2.48 - 6.3%). CF1 was cytotoxic to MCF-7 (≤ 80%, IC_{50} = 38.32 µg/mL), proliferative on normal HDFs, and inhibitory against UVB-induced MMP-1 production in fibroblast cells (≤97%) and rats skin (EC_{50} 85.12 mg/kg BW). Oral treatment with CF1 resulted to lower severity index, absence of hyperplastic response in dermal skin section, and decreased MMP-1 levels compared to negative (UVB exposed, no treatment) and positive (UVB exposed + piroxicam) groups ($p < 0.05$). Results strongly suggest the cytotoxic, protective and anti-metastasis potential of CF1 against MCF-7, UVB-induced skin damage and irregular MMP-1 production.

Keywords: cancer, cytotoxicity, fibroblast, matrix metalloproteinase-1, MCF-7, UVB, skin damage

INTRODUCTION

Bioactive compounds from marine green algae are interesting molecules due to their notable antitumor, anticoagulant, anti-inflammatory, hepatoprotective and immunomodulating properties[1-3]. The bioactivity of algal polysaccharides is associated to the degree of sulfation, uronic acid content, monosaccharide composition and glycosidic bonds[4]. Ulvan and galactan are polysaccharide present in the cell wall of green algae *Ulva* (Ulvaceae), *Enteromorpha* (Ulvaceae), *Monostroma* (Monostromataceae), *Caulerpa* (Caulerpaceae), and *Codium* (Codiaceae) species with repeating disaccharide moieties of sulfated rhamnose and uronic acid (glucuronic or iduronic)[5]. Ulvan and galactan are understudied compared to brown and red algal polysaccharides but are attracting the interest of medical researchers due to their reported effects on immune system, apoptosis and destructive proteases such as matrix metalloproteinases (MMPs)[6,1,2]. The expression and activity of MMPs increase significantly in various pathological conditions characterized by destructive oral disease, inflammation, tumor growth and metastasis[7]. Thus, it is valuable to discover a local source of an effective but non-toxic compound from marine product that has the potential to inhibit or regulate the excessive production of destructive MMPs. *C. edule*, commonly known as "puk-puklo" is an edible seaweed sold seasonally in the local market in the Northern Luzon, Philippines, and is being used in alternative medicine for its anthelmintic, antibacterial and antitumor properties[8]. At present, there are still no reports on the characterization and biological activities of sulfated polysaccharide of *C. edule* from the Philippines. Hence, this study was conducted to evaluate the chemical composition and activities of sulfated polysaccharides from *C. edule* collected at Cagayan Province, Philippines. The cytotoxic, proliferative and protective potential of the

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Table 1: Chemical composition (%) of polysaccharide fractions from *Codium edule* extracted with hot water and fractionation by anion exchange chromatography on DEAE Sepharose Column.

	Yield	Total Carbohydrates	Sulfate	Protein	Uronic Acid	Ash	Moisture	Fats
Crude	14.19[a]	14.9	6.3	4.1	4.49	77.2	3.0	1.0
CF1	32.[b]	6.08	3.08	1.3	1.49	nd	nd	nd
CF2	7.1 [b]	4.97	2.67	*tr*	1.11	nd	nd	nd
CF3	2.5 [b]	1.93	2.48	*tr*	*tr*	nd	nd	nd

a Yield (weight of crude/weight of seaweed powder) X 100
b Yield (weight of fraction/weight of crude) X 100
tr – Percentages lower than 1% are given as traces (*tr*)
nd – not done

polysaccharide fractions from this alga on MCF-7, irradiated fibroblast and UVB-induced skin damage in rats is first reported.

MATERIALS AND METHODS

Isolation of polysaccharide fractions

Fresh algal thalli (2 kg) was collected on April 2016 at low tide at a depth of 1−5 m along the coast of Santa Ana Cagayan (Crocodile Island), GPS 18° 31′ 11″N 122° 9′ 6″ E. Dr. Gavino C. Trono of Seaweed Taxonomy Laboratory, University of the Philippines Diliman Marine Science Institute identified the alga. A voucher specimen (No. USTH014316) was deposited at the University of Santo Tomas Herbarium. A collection certificate was secured from the Bureau of Fisheries and Aquatic Resources (BFAR) Region 2. Fresh algal materials were thoroughly washed with seawater followed by distilled water to remove extraneous matter and debris. Samples were air-dried under shade for two weeks. CFs were isolated according to reported methods[6,3,2,1]. The milled sample (200 g) was soaked in 85% ethanol (1:10 w/v) overnight at room temperature. The alcoholic portion was removed to retain the residue. The residue was then rinsed with acetone (1:1 w/v) and dried at room temperature. Two hundred grams of dried residue was extracted with distilled water (1:10 w/v) at 60 - 65 °C with occasional stirring for 4 h, cooled, and centrifuged at 10,000 rpm for 10 min at room temperature. The collected supernatant was concentrated by evaporation under reduced pressure at 60°C to approximately 500 mL, added with Ethyl alcohol (EtOH, 99%) (1:1 v/v) and kept at 4°C overnight. The precipitate was collected using Whatman filter paper (0.45 µm), washed with EtOH (99%) (1:10 w/v), followed by acetone (1:10 w/v), and allowed to dry to yield the crude polysaccharide (CP). CP was then fractionated by ion-exchange chromatography using DEAE Sepharose fast flow column (G.E. Healthcare Life Science, USA). CP (100 mg - 1 g) was dissolved in ultrapure water (35 - 50 mL) (Elix Millipore, Merck) at 60 - 65°C for 20 to 30 min and run down to the column previously stabilized in H_2O. Water was used as first elution solvent and followed with NaCl solutions from 0.5 M to 2 M. Three fractions of 2 to 9 mL were obtained and labelled as CF1, CF2 and CF3. All fractions were dialyzed, freeze-dried and stored at -20°C until use.

Determination of organic composition

The organic composition of the polysaccharide was determined by phenol-sulfuric acid[9-10], Bradford[11], sulfamate/m-hydroxydiphenyl[11], Solvent Extraction/Gravimetry[12-13] methods for carbohydrate, protein, uronic acid, fat and ash contents, respectively. The results were expressed as percent composition (w/w) in comparison with known standards.

Determination of percent sulfation

The percent sulfate content in CP was determined by Ashing-acid ion chromatography (Dionex DX-120 Ion Chromatography, IonPac AS4A-SC 2-mm column). Five hundred milligrams of CP was ashed, digested with 1M HCl, and filtered through 0.45 µm membrane filter. Five microliter of filtrate was used as the injection volume. A solution 1.8 mM $NaHCO_3$ + 1.7 mM Na_2CO_3 was used as the eluent, and electrical conductivity was used as the mode of detection. Percent sulfate was determined by regression analysis in comparison with standard sulfates (0.5 M – 2.0 M). The sulfate content of hydrolyzed CFs was estimated by $BaCl_2$ method and the absorbance was recorded at 360 nm using K_2SO_4 as a standard[10,2].

FT-IR analysis

The polysaccharide was relied on an IR Affinity-1S Fourier Transform Infrared (Shimadzu). A salt disc of 10 mm diameter was prepared by mixing and compressing 10 mg of polysaccharide with 100 mg of dried potassium bromide (KBr). FT-IR bands were recorded between wave numbers of 4000 and 400 cm^{-1}.

2.5. NMR Spectroscopy

Samples (10 - 20 mg) were dissolved in 0.5 mL deuterium oxide using 5 mm tubes. Signals were recorded at room temperature on a Bruker 300 MHz spectrometer (Billerica, MA, USA) following the parameters of spectral width of 4.5 KHz, an acquisition time of 1.82 s, a relaxation delay of 1.82 s for 100 scans. The chemical shift was expressed in parts per million (ppm)[14].

Cell Culture Conditions

HDFs (PCS-201-010) and MCF-7 (ATCC HTB-22) were routinely cultured in Dulbeco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Sigma), 2 mM glutamine, containing 100U/mL penicillin, 100 U/mL streptomycin and 100 U/mL mycostatin. Cells were maintained in a humidified chamber with 5% CO_2 at 37°C. Cells were detached from the flask upon reaching 80% confluence with trypsin-

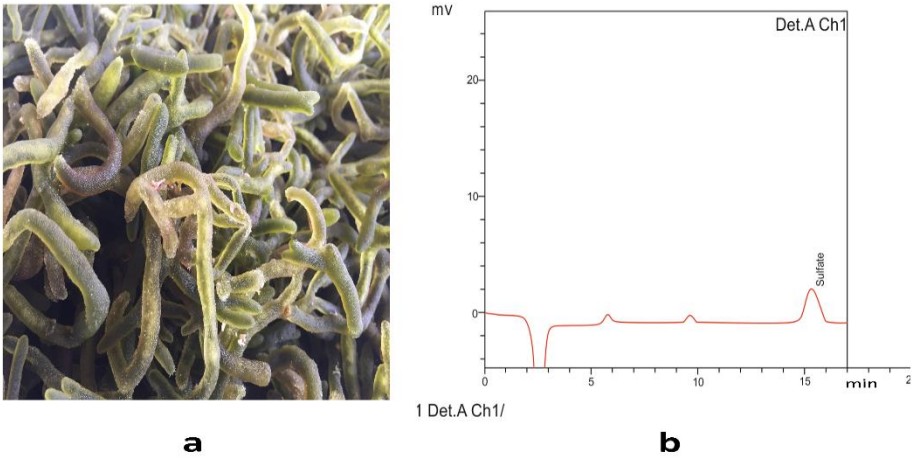

Figure 1: Fresh thalli of *C. edule (a);* and sulfate ion chromatogram of CP (*c*).

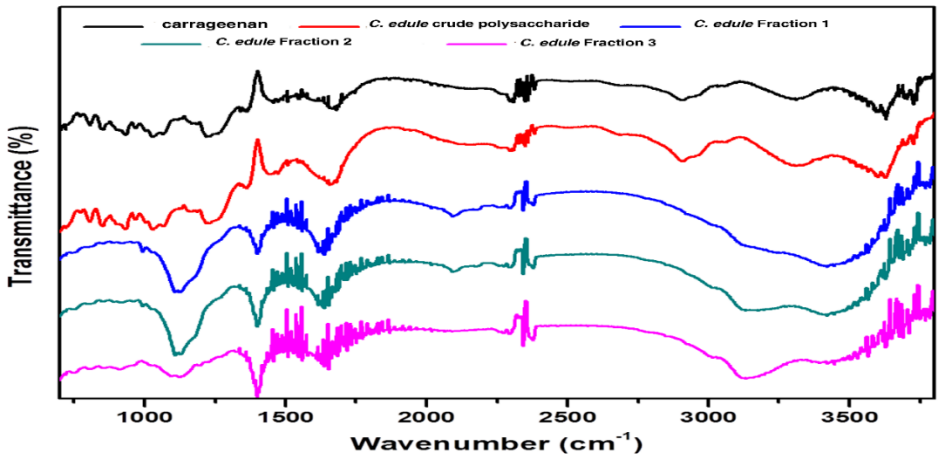

Figure 2: FT-IR spectra of CP and CFs in comparison with carrageenan.

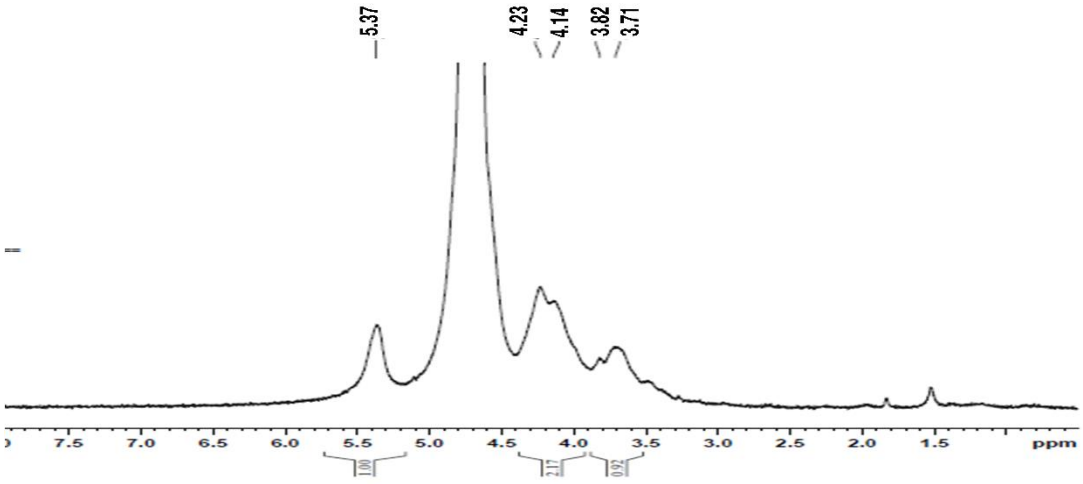

Figure 3: ^{1}H NMR spectrum at 500 MHz of CF1 from *C. edule.*

EDTA and splitted at 1:5 ratio until assays.

MTT assay

Cytotoxicity assay was performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT) kit (Sigma Aldrich). MCF-7 and HDFs cells were counted under a microscope using a hemacytometer (Hausser Scientific) and plated (1 X 10^5 cells/well) into the 96-well microtiter plates. After 24 h,

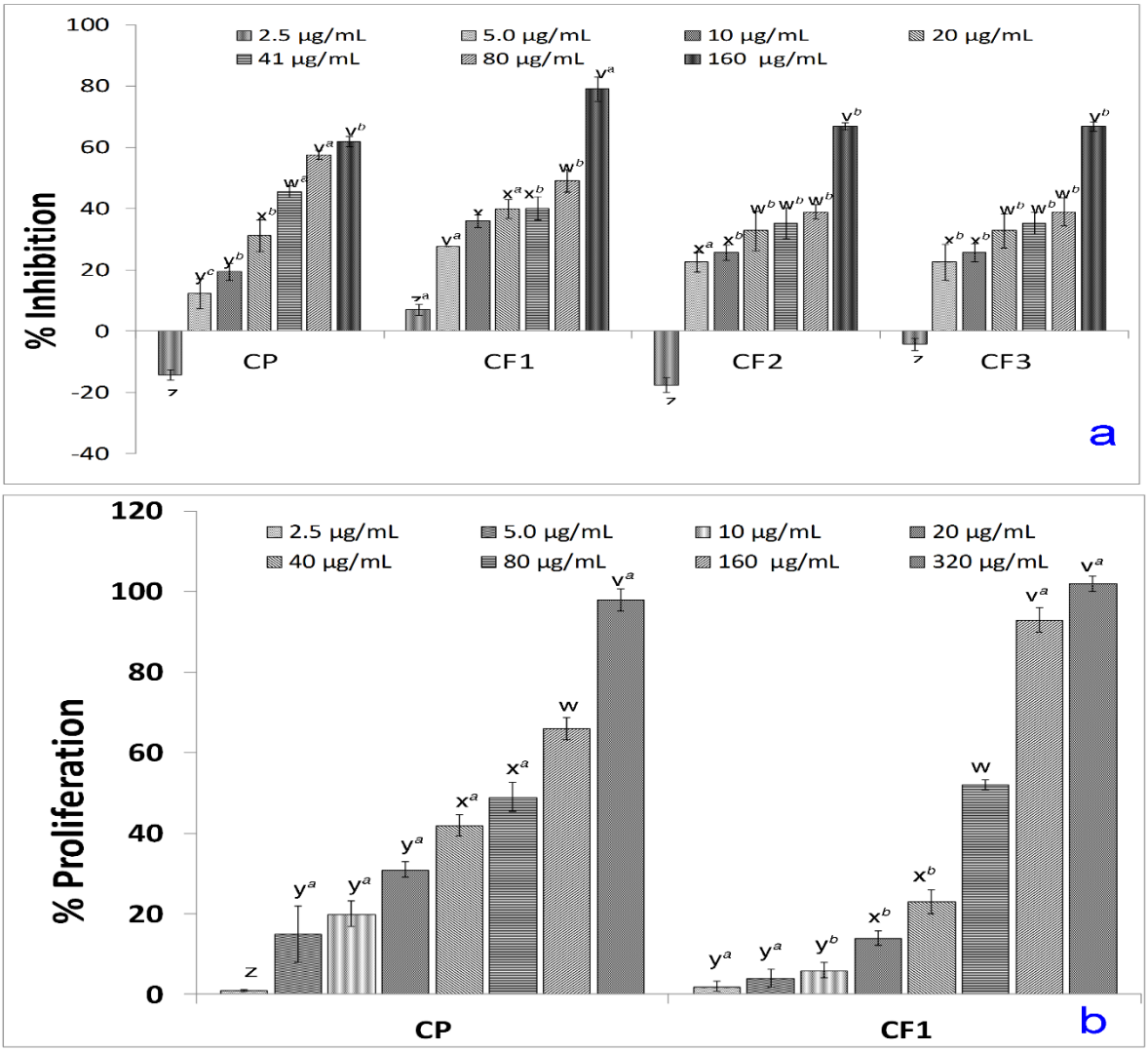

Figure 4: Effects of C. edule on MCF-7 and HDFs: Cytotoxic activity of *C. edule* against MCF-7 breast cancer cells (*a*); proliferative effect on HDFs (*b*). Cells (1 x 10^5 cells/mL) were incubated for 48 h with different concentrations of polysaccharides (0 to 160 µg/mL) with Doxorubicin as positive control. The data are presented as means ± standard deviation (n=3). The letters $^{v, w, x, y, z}$ indicate significant difference ($p < 0.05$) between the concentrations of CP and CFs with $^{a, b, c}$ indicating significant difference ($p<0.01$) between the CP and CFS at each concentration.

the culture medium was removed and replaced by 100 µL serial dilutions (0, 10, 20, 40, 80, 160 µg/mL) of polysaccharide extracts pre-filtered with 0.2 µM Nylon membrane filter (Sigma Aldrich, USA) and incubated for 48 h. The formazan salt was dissolved by DMSO and the absorbance was read at 570 nm (Thermoscientific Multiskan Go Reader). The cell inhibition ratio (%) was computed using the equation= $(A_c\text{-}A_e)/A_u \times 100$, where A_c and A_e are the absorbance of untreated group and treated group, respectively. For HDFs, the proliferation ratio (%) was computed using the equation= $A_c/A_e \times 100$ where A_c and A_e are the absorbance of the test group and control group, respectively. Assays were done in triplicate.

Induction of MMP-1 production in HDFs and Treatment

HDFs (5×10^5 cells/mL) were plated into a 12-well plate containing phenol red-free DMEM supplemented with 10% FBS, 2 mM glutamine, 100U/mL penicillin, 100 U/mL streptomycin and 100 U/mL mycostatin to 80% confluence. Cells were then starved in serum free DMEM for 24 h, washed with phosphate-buffered saline (PBS) twice, and exposed to UV-B irradiation (UV-B lamp (Reptisun, 290-320 nm, 25 mjoules/cm^2) as measured with a VLX-3W research radiometer (UVItec, USA). The culture medium was then removed and replaced by 500 µL serial dilutions of CFs (0 - 250 µg/mL) dissolved in phenol-red free DMEM. After 48 h, the supernatant was collected in 2 mL centrifuge tube and stored at -80°C until assay. The level of secreted MMP-1 was measured with ELISA kit (Abcam). Quantity of MMP-1 was calculated from standard curves of recombinant MMP-1 standard by a linear regression method. Percent inhibition was calculated using the equation (%) = $(A_u\text{-}A_t)/A_t \times 100$, where A_u and A_t are the secreted MMP-1 (pg/mL) of untreated group and treated groups, respectively.

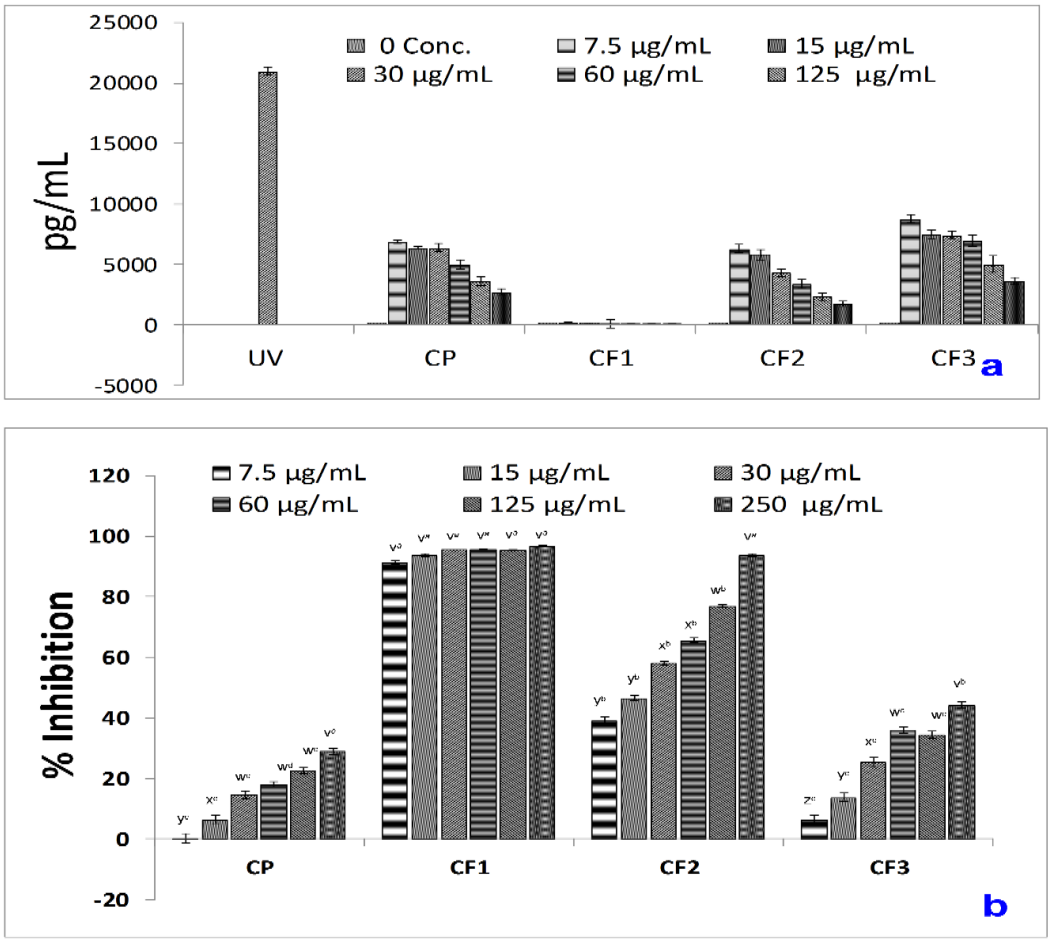

Figure 5: Inhibitory effect of *C. edule* on the production of MMP-1 in UVB-induced HDFs: concentration of MMP-1 in pg/mL *(a);* 5 inhibition *(b).* HDFs (5 x 10^5 cells/mL) were exposed to 25 mjoules/cm^2 of UVB for 30 seconds and incubated for 48 h with different concentrations of polysaccharides (0 to 250 µg/mL) in phenol red- free DMEM. The data are presented as means ± standard deviation (n=3). The letters $^{w, x, y, z}$ indicate significant difference ($p < 0.05$) between the concentrations of the crude and the fractions, with $^{a, b, c}$ indicating significant difference ($p<0.01$) between CP and CFS at each concentration.

Acute oral toxicity

Acute oral toxicity was performed following OECD 425 guidelines in eight-week old Sprague-Dawley rats (200 ± 20 g). Nine rats were divided in three groups with 3 animals each. Three rats were orally dosed with 2,000 mg/kg CF1 using oral canula. CF1 was tested because it displayed the highest percent inhibition against MMP-1 production in irradiated HDFs. CF1 was also used in MMP-1 assay because it did not cause any mortality and abnormalities in rats throughout the experiment.

Induction of MMP-1 production in rats and treatment

Eight-week old female Sprague-Dawley rats (200 ± 20 g) were purchased from Plegaria, Inc., (Philippines' Bureau of Animal Industry Certification No. LAF0015). The animals were acclimatized for 7 days in a well-ventilated animal house at controlled temperature (25 ± 2°C), 12-h light/dark cycle with laboratory diet and drinking water *ad libitum*. After acclimitization, rats were divided into 6 groups with 5 rats each: Normal Control (control : without UV and treatment); I (negative control : UVB − extract); II (positive control : UVB + piroxicam 10 mg/kg BW); III (UV + 50mg/kg CF1 BW); IV (UVB, + 100mg/kg CF1 BW) and V (UVB + 200mg/kg CF1). The pre-shaved dorsal side of the rats (1.5 cm x 2.5 cm) was irradiated using ReptiSun UVB light (1.14 µW/cm^2) for 30 min daily for one week. The severity of skin damage was evaluated 24 h post exposure for seven days. Severity scores were based on gradiations of scarring, redness, rashes, scaly texture of the skin, loss of elasticity and wrinkling[15]. On the 8th day, blood sample was collected in Ethylenediaminetetraacetic acid (EDTA) tubes and the level of secreted MMP-1 in rats' plasma was quantified by ELISA. Baseline level of MMP-1 in rats was obtained before the treatment. A permit was secured from the University of Santo Tomas Institutional Animal Care and Use Committee (UST-IACUC) before the assay was conducted.

Statistical Analysis

Data were presented as the mean ± SEM of three independent measurements. Data were analyzed by SPSS 20.0 and Prism ver 7.0. Statistical differences between groups were tested by one-way ANOVA and Tukey's multiple comparison test. P values of less than 0.05 were considered significant. Absolute IC_{50} and EC_{50} values

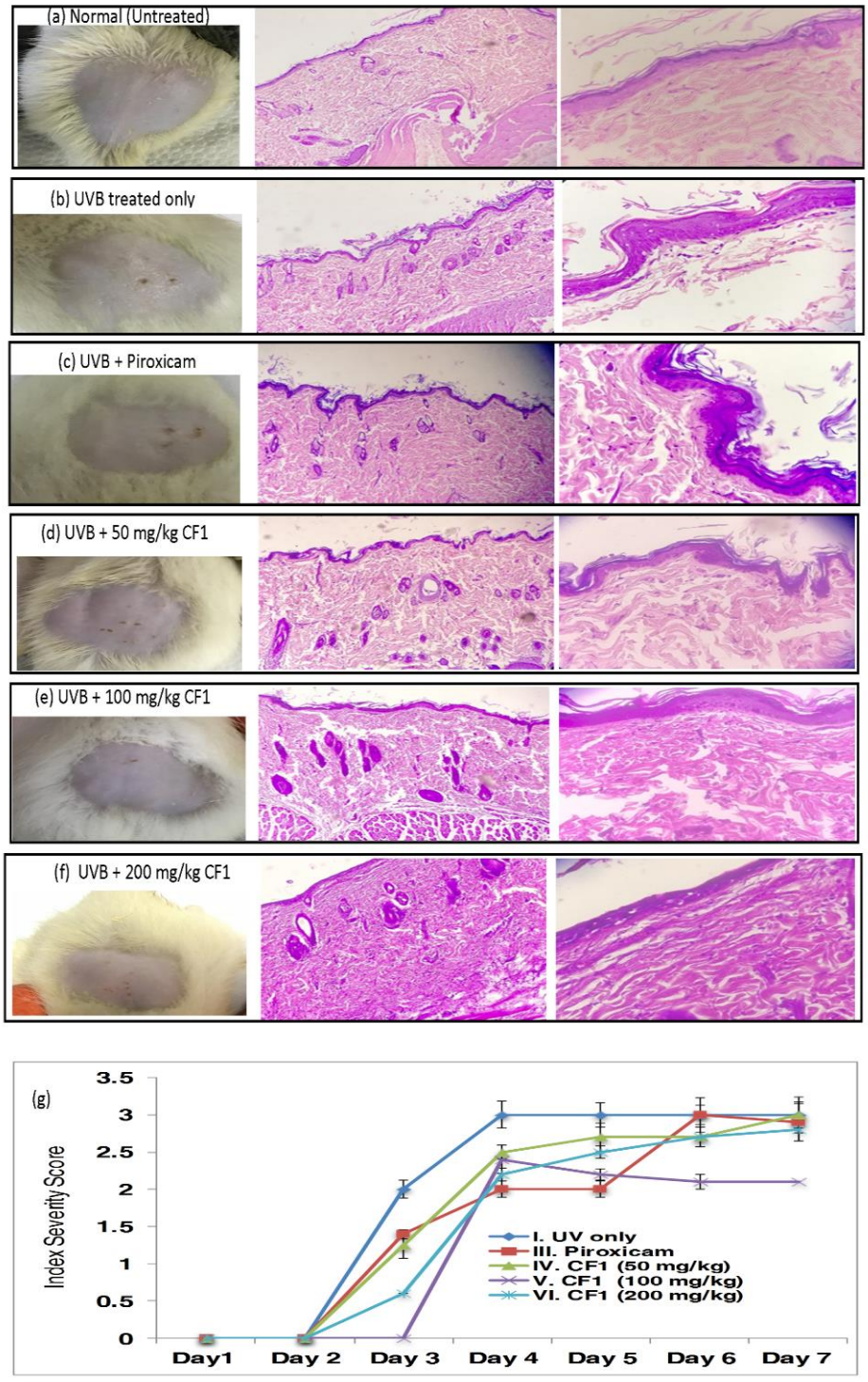

Figure 6: Effect of C. edule on UVB-induced damage on rat's skin: Histopathology of rats skin exposed to UVB radiation: normal non-UVB exposed (*a*); UVB exposed without any treatment (*b*); UVB exposed + piroxicam (*c*); UVB exposed + 50 mg/kg CF1 (*d*); UVB exposed + 100 mg/kg CF1 (*e*) and UVB exposed + 200 mg/kg CF1(*f*); mean severity index score (*g*). Left to Right image (rats skin, LPO (X150), HPO (X400).

were calculated by non-linear regression curve-fit function of the Graph Pad Prism 6 Software at 95% confidence interval.

RESULTS AND DISCUSSION

Chemical Composition of CP and CFs

Three fractions were obtained from the elution of CP to DEAE Sepharose column. The proximate composition of

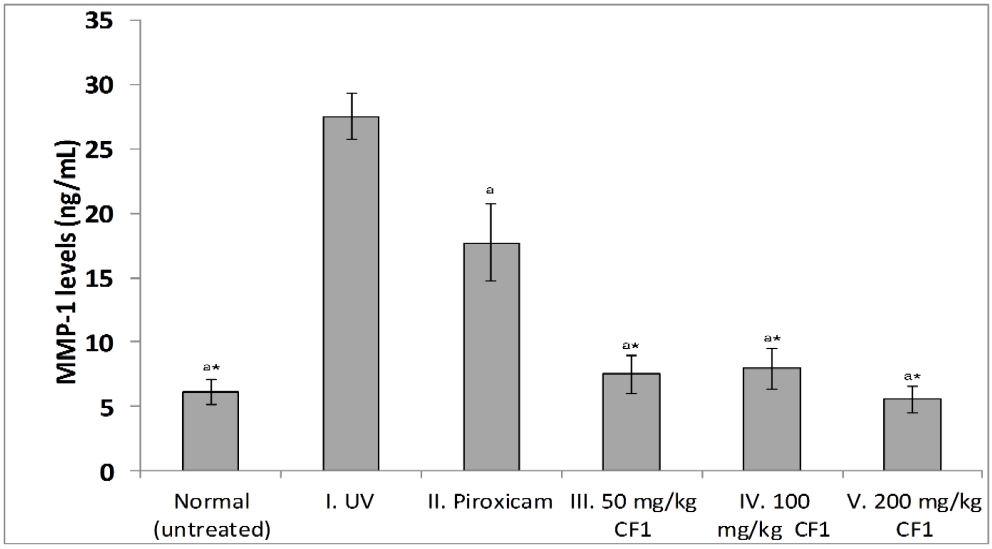

Figure 7: Inhibition of MMP-1 production in UVB - exposed rats. All values are mean ± SEM, n = 5, where ^{a} $p < 0.05$ (compared to UV), $^{a*}p < 0.01$ (compared to UV and Piroxicam).

C. edule is presented in Table 1. Sulfate ion was successfully eluted between 14 to 16 min (Fig. 1B). CFs afforded sulfate content of 2.48 - 6.3% (w/w) which was lower than 12 - 38%, 10.1 - 22.5%, 13% and 5.3 - 12.8% reported from *C. vermilaria* (Olivi) Delle Chiaje, *C. decorticatum* (Woodw.) M. Howe and *C. fragile* (Suringar) Hariot, respectively[16-17,13,2].

CP afforded 4.10% protein which was lower than the 10.4 - 20.3% reported in *Codium fragile*[18]. The protein content of CFs is comparable with 0.1- 1.9% reported in *Codium decorticatum*[10,3]. The protein content in marine algae ranges from 10 - 40% per dry weight and varies according to the species and seasons[19]. Generally, green seaweeds possess lower amount of protein than red seaweeds. CFs afforded 1.93 - 14.93% carbohydrate content which is lower compared to the values reported in brown and red counterparts. The uronic acid content of *C. edule* was lower than 1.4 - 2.0% reported in *C. fragile*[16,2].

Cell wall matrices of algae are composed of highly sulfated heteropolysaccharide with associated galacturonic acid, glucuronic acid, rhamnose, arabinose and galactose[17,2]. Determination of total protein in algae is usually done to find new sources of protein supplements[20]. The fat content of *C. edule* is comparable to values reported in most brown marine algae. *Hizikia* sp. (Harvey) Okamura (Sargassaceae) and *Eisenia bicyclis* (Kjellman) Setchell (Lessoniaceae) showed minimal content of fat from 0.7 to 0.9 g/100 d.w[21]. CP afforded 4.49% uronic acid content, which is higher than 2.0%, and 1.4% of *C. fragile*[16,3]. The major sulfated polysaccharides of green algae are sulfated heteropolysaccharides with galactose, xylose, arabinose, and uronic acid contents. The 77.2% ash content of CP was higher than 15.9% of *C. tomentosum* Stackh.[14,3]. The ash content of algae is usually determined to assess the value of algae as food, feed, and feedstock for biofuels. CP contained higher moisture than *C. tomentosum*[22].

FT-IR Spectra

FT-IR spectra of *C. edule* are shown in Fig. 2. The broad band around 3400 cm-^{1} was assigned to hydroxyl stretching vibration of polysaccharide (-OH) and a band at 2922 cm^{-1} corresponded to C-H stretch (alkane). Band at 1658 cm^{-1} was attributed to C=O stretches of amide C=N group while the band at 1259 cm^{-1} was assigned to S=O sulfate ester. The signals at 843 cm^{-1} and 1253 cm^{-1} were attributed to the bending vibration of C-O-S and the stretching vibration of S–O of sulfate group[12,3]. The regions at 1072 cm^{-1} and 890 cm^{-1} were assigned to the skeleton of galactans and agar specific band[23]. The bands at 1022 cm^{-1} and 1259 cm^{-1} corresponded to D-glucose and ester sulfate groups[14,4]. Thus, CP and CFs possess ester sulfate group naturally present in the galactan and ulvan polysaccharides of green algae.

^{1}H NMR Analysis

The ^{1}H NMR spectrum of CF1 (Fig. 3) clearly shows the presence of five anomeric proton signals at 5.37, 4.23. 4.14, 3.82, and 3.71 ppm and had relative integrals of 1.0, 2.17, 0.92 respectively. The ^{1}H-signals at 5.37 was assigned to the disulfated β-L-arabinopyranose units while the presence of small signals at 3.82 and 3.71 correspond to C5/H5,5'of α-L-arabinopyranose with low molecular weight[10,3]. Signal at 4.20 confirmed sulfation of C-2. Signals between 4.20 and 4.14 suggested the presence of α- (1,4) and β-(1,3) linked galactopyranosyl units[12,3, 24]. These signals are attributed to the galactan moiety in the polysaccharides that are seen in sulfated polysaccharides from other *Codium* species. For instance, *C. divarticum* (C.Agardh) Baisoletto and *C. vermilaria* were mainly composed of β-D-mannan and pyruvylated sulfated 3- and 6-linked β -D- galactans respectively[25]. Sulfated galactan from *C. cylindricum* Holmes contained glucose residues responsible for the formation of sulfated gluco-galactan. A mixed linkages of (1-3)-α and β-D- mannan was seen as the main backbone in the sulfated polysaccharides from *C. fragile*[17,3.] In this study, FTIR and NMR analyses strongly suggest that CP and CFs are polysaccharide-

protein complex with mixed α-(1,4) and β-(1,3) linked galactopyranosyl units typically present in sulfated galactan and ulvan polysaccharides. However, there is a need to purify them to know the exact identity, molecular weight and distribution of sulfate along the polymer chain. The purification of biologically active compound is hindered by some difficulties or limitations. Some of the limiting factors include the low concentration, difficulty in separation and instability of these bioactive compounds[26].

Effect of CP and CFs on viability of MCF-7 and HDFs

Both CP and CFs exerted notable cytotoxic effect against MCF-7 (Fig. 4). At 160 µg/mL, CF1 displayed the highest inhibitory effect (80%) against MCF-7 ($p = 0.002$). IC_{50} values of 40.46 µg/mL, 38.32 µg/mL, 96.83 µg/mL, and 53.70 µg/mL were determined for CP, CF1, CF2, and CF3, respectively. Cytotoxic effect of CFs was significantly lower than Doxorubicin (2.4 µg/mL) ($p < 0.05$). Interestingly, CF1 promoted the proliferation of HDFs as high as 104 ± 2.0% (Fig. 5). These findings are very important and may offer a strategy for cancer treatment. Drug developers are aiming to develop new drugs that are non-genotoxic, can kill cancer cells but with minimal toxic effect to healthy cells or few organs[22,2]. Marine algal polysaccharides are generally reported as safe and non-toxic to normal cells. For instance, ulvan, a biopolymeric macromolecule in green algal cell wall has been reported to maintain osmolar stability and provide protection to the cell[27]. A low molecular weight ulvan (MW < 5,000) isolated from *Ulva lactuca* inhibited the proliferation of Caco-2 by induction of low cell reactivity to *Ulex europeaus*-1 lectins, but did not exert any cytotoxic effect on normal colonocytes[28]. In this study, the observed proliferative effect of CFs on HDFs is possibly due to the interaction of organic contents of the polysaccharide[14,4].

Effect of CFs on UVB-induced production of MMP-1 in HDFs and rats

HDFs were exposed to 25 mJ/cm^2 dose of UVB irradiation to induce MMP-1 production. UBV irradiation usually leads to elevation of interstitial collagenase mRNA levels (MMP-1 and MMP-3) in cultured human dermal fibroblast[29]. Previous studies showed that irradiation of fibroblast cells from 25 - 80 mJ/cm^2 induced the expression of MMPs without being cytotoxic[30-32]. After 48 h, level of MMP-1 in cell supernatant of irradiated HDFs was highly elevated and treatment with CF1 significantly inhibited the MMP-1 production at a remarkable rate of ≤97% (Fig. 6 A-B) ($p<0.05$). The capacity of CF1 to inhibit MMP-1 production was not compared to any standard drug because of the absence of standard MMP-1 inhibitor used for research assays. At present, batimastat and marimastat are considered the most advanced anti-MMP1 in terms of preclinical and clinical development[33-34].

In rats, manifestations of UVB-induced damage which include redness, swelling and irritation were apparently visible three days after UVB exposure (Fig.6). These damages were not seen on skin of rats treated with CF1. The histological changes in rats skin were analyzed by H & E staining. The epidermis of normal non-UV exposed skin was observed to be thin with two to three layers of normal cells (Fig. 6*a*). Formation of sunburn cells and skin hyperplasia with five to nine cell layers thick were evident in rats after 5 consecutive days UVB irradiation. Irradiated dermis showed enlarged sebaceous glands, more vacuoles, and few dermal cysts in comparison with normal skin without UV exposure (Fig. 6*b*). Rats treated with CF1 showed better and intact skin histology than UVB exposed and UVB + piroxicam treated group (Fig. 6*c-f*). Oral feeding of 100 mg/kg CF1 in rats offered the best protection against UVB-induced damage as epidermis and dermal sections showed intact epidermis with very minimal sign of hyperplasia and absence of dermal cysts (Fig. 6*e*). A lower severity index score was also observed in CF1 treated groups compared to negative control UVB-treated group (Fig.6*g*). The observed protective effect of CF1 was supported by decreased MMP-1 production. UVB exposure induced three-to-four fold increase in the baseline plasma level of MMP-1 from 6.14 to 22.52 ng/mL. Oral treatment with CF1 (50, 100, 200 mg/kg BW) after UVB exposure significantly inhibited MMP-1 production by 75 - 81.48% when compared to both positive (UVB + piroxicam) and negative (UVB, no treatment) groups ($p < 0.05$) (Fig. 7). EC_{50} of CF1 was 85.12 mg/kg BW. These results clearly indicate the potential of CF1 in the field of drug development against tumor invasion and metastasis. MMPs are potential focus for medical intervention in conjunction with other cytotoxic treatment that intends to stop or kill tumor growth and metastasis[35]. The last few years of drug development against destructive proteases focused on the production of new generation of MMP-1 inhibitors that selectively target high expression of enzymes in diseased tissues. Studies were directed to improve selectivity profile, reduce drug toxicity, increase availability and binding efficacy[32,2]. Most studies on marine polysaccharides as anti-metastasis agent are focused on fucoidan and carrageenan[36]. The present findings strongly suggest the bioactive potential of sulfated polysaccharide from *C. edule* against MCF-7 and MMPs associated diseases such as skin disease, aging and cancer metastasis.

CONCLUSION

C. edule is a potential source of bioactive polysaccharide with significant sulfate, protein, and uronic acid content. CF1 notably inhibited the growth of MCF-7, promoted the proliferation of HDFs, regulated the MMP-1 production, and provided protection to rats skin against UVB radiations. Results strongly suggest that CF1 from *C. edule* should be given attention for the development of drug or nutraceutical against cancer and destructive proteases such as MMP-1. However, there is a need to determine the molecular structure, weight and distribution of sulfate group along the polysaccharide chain before an exact mechanism of action as anti- MMP-1 can be proposed.

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CONFLICT OF INTEREST

The authors report no financial or other conflicts of interest.

AUTHORS CONTRIBUTION

RDV was responsible for overall planning, implementation, experimental design and analysis of data. RBC, JGA and LS contributed to experimentation and analysis and/or interpolation of data. PDFA, DPJ, and JDP performed the animal model for MMP-1 assay. LS contributed to a critical reading of the manuscript and NMR analysis. RDV drafted the manuscript and all the authors have read the final manuscript and approved the submission.

REFERENCES

1. Lee JB, Ohta Y, Hayashi K, et al. Immunostimulating effects of a sulfated galactan from *Codium fragile*. Carbohydr Res 2010; 345 (10): 1452-1454.
2. Chale-Dzul J, Moo-Puc R, Robledo D. Hepatoprotective effect of the fucoidan from the brown seaweed *Turbinaria tricostata.* J Appl Phycol 2015*;* 27 (5): 2123-2125.
3. Peasura N, Laohakunjit N, Kerdchoechuen O, et al. Assessment of biochemical and immunomodulatory activity of sulphated polysaccharides from Ulva intestinalis. Int J Biol Macromol 2016; 91: 269-277.
4. Ngo DH, Kim SK. Sulfated polysaccharides as bioactive agents from marine algae. Int J Biol Macromol 2013; 62: 70-75.
5. Jiao G, Yu G, Zhang, J. et al. Chemical structures and bioactivities of sulfated polysaccharides from marine algae. Mar Drugs 2011: 9 (2): 196 - 223.
6. Karnjanapratum S, Tabarsa M, Lae CM, et al. Characterization and immunomodulatory activities of sulfated polysaccharides from *Capsosiphon fulvescens*. Int J Biol.Macromol 2012; 51 (5): 720–729.
7. Sorsa T, Tjaderhane L, Salo T. Matrix metalloproteinases (MMPs) in oral diseases. Oral Diseases 2004; 10 (6): 311-318.
8. Trono GC. Field Guide and Atlas of the Seaweed Resources of the Philippines. Bookmark, Inc. Philippines, 1997, 45-49.
9. Dubois M, Gilles KA, Hamilton JK, et al. Colorimetric method for determination of sugars and related substances. Analytical Chem 1956; 28:350-356.
10. Fernandez PV, Raffoc MP, Albhergina J, et al. Polysaccharides from the green seaweed *Codium decorticatum*: Structure and cell wall distribution. Carbohydr Polym 2015; 117: 836–844.
11. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72 (1-2): 248-254.
12. Li N, Mao W, Yan M. et al. Structural characterization and anticoagulant activity of a sulfated polysaccharide from the green alga *Codium divaricatum*. Carbohydr Polym 2015; 121: 175-182.
13. You SG, Yang C, Lee HY, et al. Molecular characteristics of partially hydrolyzed fucoidans from sporophyll of *Undaria pinnatifida* and their in vitro anticancer activity. Food Chem 2010; 119 (2): 554-559.
14. Seedevi P, Sudharsan S, Kumar SV, et al. Isolation and characterization of sulphated polysaccharides from *Codium tomentosum* (J. Stackhouse, 1797) collected from southeast coast of India. Adv Appl Sci Res 2013*;* 4 (5): 72-77.
15. Nagar KH, Srivasta AK, Srivasta R. et al. Evaluation of potent phytomedicine for treatment of psoriasis using UV radiation induced psoriasis in rats. Biomed Pharmacother 2016*;* 84: 1156-1162.
16. Surayot S, You SG. Structural effects of sulfated polysaccharides from *Codium* fragile on NK cell activation and cytotoxicity. Int J Biol Macromol 2017; 98: 117–124.
17. Tabarsa M, Karjanapatrum S, Cho ML, et al. Molecular characteristics and biological activities of anionic macromolecules from *Codium fragile*. Int J Biol Macromol 2013*;* 59: 1-12.
18. Ciancia M, Quintana I, Vizcarguenaga MI, et al. Polysaccharide from green seaweeds *C. fragile* and *C. vermilaria* with controversial effects on hemostasis. Int J Biol Macrmol 2007; 41 (5): 641-649.
19. Renaud SM, Luong-Van JT. Seasonal variation in the chemical composition of tropical Australian marine macroalgae. J Appl Phycol 2006; 18: 381-387.
20. Fan X, Bai L, Zhu L, et al. Marine Algae-Derived Bioactive Peptides for Human Nutrition and Health, J. Agric. Food. Chem 2014; 62 (38): 9211–9222.
21. Dawczynski C, Schubert R, Jahreis G. Amino acids, fatty acids, and dietary fiber in edible seaweed products. Food Chem 2006; 103 (3): 891-899.
22. Liu K. Characterization of ash in algae and other materials by determination of wet acid indigestible ash and microscopic examination. *Algal Res 2017;* 25: 307-321.
23. Souza WSB, Cerqueira MA, Bourbon AI, et al. Chemical characterization and antioxidant activity of sulfated polysaccharide from the red seaweed *Gracilaria birdiae*. Food Hydrocoll 2012*;* 27 (2): 287-292.
24. Senthikulmar D, Jayanthi S. Partial characterization and anticancer activities of purified glycoprotein extracted from green seaweed *Codium decorticatum*. J Funct Foods 2016; 25: 323-332.
25. Fernandez PV, Raffoc MP, Albhergina J, et al. Sulfated β-D mannan from green seaweed *Codium vermilara*. Carbohydr Polym 2012; 87: 916-919.
26. Athukorala Y, Kim KN, Jeo YJ. Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, *Ecklonia cava*. *Food Chem Toxicol* 2006; *44*(7)*:* 1065-1074.
27. Paradossi G, Cavalieri, Chiessi E. A conformational study on the algal polysaccharide ulvan. *Macromol 2002;* 35 (16): 6404-6411.
28. Kaeffer B, Bénard C, Marc LM, et al. Biological properties of ulvan, a new source of green seaweed

sulfated polysaccharides, on cultured normal and cancerous colonic epithelial cells. Planta Med1999; 65 (6): 527-531.
29. Brenneisen PJ, Wenk LO, Klotz M, Briviba T, Krieg H, Sies K, Scharffetter K. Central role of ferrous/ferric iron in the ultraviolet B irradiation-mediated signaling pathway leading to increased interstitial collagenase (matrix-degrading metalloprotease (MMP)-1) and stromelysin-1 (MMP-3) mRNA levels in cultured human dermal fibroblasts. J Biol Chem 1998; 273 (9): 5279-5287.
30. Kim J, Lee CW, Kim, EK, et al. Inhibition effect of *Gynura procumbens* extract on UV-B-induced matrix-metalloproteinase expression in human dermal fibroblasts. J Ethnopharmacol 2011*;* 137 (1): 427-433.
31. Chiang HM, Chen HC, Lin TJ, et al. *Michelia alba* extract attenuates UVB-induced expression of matrix metalloproteinases via MAP kinase pathway in human dermal fibroblasts. Food Chem Toxicol 2012; 50 (12): 4260-4269.
32. Choi HK, Kim DH, Kim JW, et al. *Labisia pumila* extract protects skin cells from photoaging caused by UVB irradiation. J Biosci Bioeng 2010; 109 (3): 291-296.
33. Rasmussen HS, Mc Cann PP. Matrix metalloproteinase inhibition as a novel anticancer strategy: A review with special focus on batimastat and marimastat. Pharmacol Therapeutics 1997; *75*(1): 69-75.
34. Mohan V, Talmi-Frank D, Arkadash V, et al. Matrix metalloproteinase protein inhibitors: highlighting a new beginning for metalloproteinases in medicine. Metalloproteinase in Medicine 2016; 3: 31-47.
35. Stetler-Stevenson WG. The role of matrix metalloproteinases in tumor invasion, metastasis, and angiogenesis. Surg Oncol Clin N Am 2001; 10 (2): 383-92.
36. Moon HJ, Lee SR, Shim SN, et al. Fucoidan inhibits UVB-induced MMP-1 expression in human skin fibroblasts. Biol and Pharm Bull 2008*;* 31 (2): 284-289.