Int J Pharm Bio Sci 2012 July; 3(3): (B) 333 - 345

Research Article Bio Chemistry

International Journal of Pharma and Bio Sciences ISSN 0975-6299

CHEMOPREVENTIVE PROPERTIES OF SULFATED POLYSACCHARIDE EXTRACTS FROM SARGASSUM SILIQUOSUM J. G. AGARDH (SARGASSACEAE)

ROSS D. VASQUEZ[1,*2], JOHN DONNIE A. RAMOS[2,3,4], SAMUEL D. BERNAL[5,6]

[1] Faculty of Pharmacy
[2] The Graduate School
[3]College of Science
[4]Research Center for Natural and Applied Sciences, University of Santo Tomas, Espaňa, Manila, Philippines 1008
[5]The Medical City, Makati City, Philippines
[6] Globetek Science Foundation, Makati Philippines

ABSTRACT

Sulfated polysaccharides from brown seaweeds attracted extensive interest due to their numerous biological activities and structural diversity. Sulfated polysaccharides isolated from *Sargassum siliquosum* J. G Agardh were investigated for its chemopreventive potential using *in vitro* assays. Estimation of chemopreventive activity included antioxidant (cancer anti-initiation); Nitrite Assay (cancer anti-promotion) and cytotoxicity (cancer anti-progression) assays representing the 3 stage paradigm of carcinogenesis. Extracted water soluble polysaccharides containing sulfate were identified by FT-IR and Ashing Acid Digestion Ion chromatography with IR spectra comparable to standard fucoidan and alginate. Sulfated polysaccharides showed significant radical scavenging activity in DPPH radicals, significantly suppressed production of nitric oxide in LPS-stimulated promyelocytic leukemic cells (PML) and displayed significant antiproliferative activity in both Hep G2 and renal carcinoma cells *in vitro*. Results herein indicated that sulfated polysaccharide of *S. siliquosum* possesses chemopreventive activities and their use as potential natural reagents might for cancer therapy should be given priority.

KEYWORDS; ***Sargassum siliquosum*, sulfated polysaccharide, *ashing-acid digestion, antioxidant, nitric oxide, antitumor activity***

ROSS D. VASQUEZ
Faculty of Pharmacy, The Graduate School

***Corresponding author**

INTRODUCTION

Polysaccharides obtained from algae represent structurally diverse class of macromolecules. In view of their potent application in medicine, more research have been focused on the isolation, purification and structure-bioactivity of these polysaccharides[1]. Sulfated polysaccharides are complex, heterogenous and bioactive macromolecules in which some of the hydroxyl groups in sugar residues are substituted with sulfate groups[2]. These compounds exhibit biological activities including vasodilation[3], anticoagulant[4], antivirus[5], antitumor[6], immunomodulatory[7], anti-inflammatory [8], antioxidant[9], antiherpetic[10], and hepatoprotective[11]. Generally, the biological activity of polysaccharides from marine algae is related to the molecular size, type of sugar, sulfate content, type of linkage and molecular geometry are also known to have a role in their activities[12,13]. The unique distributed sulfation pattern of high sulfated polysaccharides is believed to regulate its functional specificity[14].

Sargassum siliquosum J. G Agardh is widely distributed in the Philippines with various ethnobiological uses such as food source, fertilizer, insecticidal, sea-urchin and abalone culture and also in health drinks[15]. Despite the wide variety of application of *Sargassum*, chemopreventive potential has never been reported.

The search for novel effective but cheap cancer chemopreventive agents has become a worldwide strategy in cancer prevention. Understanding the cellular and molecular basis of carcinogenesis provide an approach as target for cancer chemoprevention. These approaches include the use of non cytotoxic doses of nutrients and pharmacological agents to stop or reverse the development and progression of precancerous cells. Thus, findings of effective and safe chemopreventive activity could elevate the value of *S. siliquosum* derived products and may expand its market in the food and pharmaceutical industries and may validate the potential use of *S. siliquosum* as an adjuvant supplement for cancer patients, whose immune system functions were suppressed during chemotherapies. This study examines the free radical scavenging activity, effects on NO production in LPS-stimulated PML cells and effects on cancer cell proliferation of sulfated polysaccharides from *S.* ***siliquosum*** J. G. Agardh.

MATERIALS AND METHODS

(i) Extraction of polysaccharides from S. siliquosum J.G Agardh

S. siliquosum was collected from Balibago, Calatagan Batangas, Philippines. The algal sample was authenticated by Professor Gavino A. Trono, Marine Science Specialist at the Marine Science Institute of the University the Philippines. About 1000g fresh weight biomass of *S. siliquosum* were cleaned and washed with distilled water. The samples were air-dried at room temperature, grounded into fine particles using Wiley mill, and sieved in a 2mm mesh. Polysaccharides were extracted from *S. siliquosum* according to Michailovna et al.[16] and Gamal-Eldeen et al.,[17] with some modifications *(Fig. 1).* The powdered algal sample was extracted repeatedly with 80% ethanol at 22°C for 22 h to remove the low molecular weight components. The resulting residual materials were extracted with boiling distilled water for 5 h repeatedly for three times. The filtrate of the water extract was concentrated by rotary evaporation to give **(AQ).** The dried residue was subjected to further extraction using 0.1 M HCl at 20-25°C for 12 h. The filtrate was adjusted to pH 6.0 by IM NaOH and was concentrated to 1/5 of its original volume, precipitated with 96% ethanol, and centrifuged for 20 min at 3000g to give **(F1).** The residue was boiled at 100°C for 3 h and 1.5 **h**, then filtrates were combined and concentrated to 1/5 of original volume. The pH of the concentrate was adjusted to 2.0-2-5 using 1M HCl and centrifuged for 15 min at 3000g to separate precipitate from the supernatant. The precipitate was alkalinized using 1M NaOH and precipitated with 96% ethanol to give **(L1).** The

supernatant was precipitated with 96% ethanol to give **(L2).** On the other hand, the residue after extraction with boiling water was treated with 1% $NaHCO_3$ at 65°C for **3 h**, added with 4M NaCl before reprecipitation with 96% Ethanol to give **(A1).** Finally AQ, F1, L1, L2, A1 were evaporated to dryness, lyophilized, used for chemical analysis and tested for cancer chemopreventive properties.

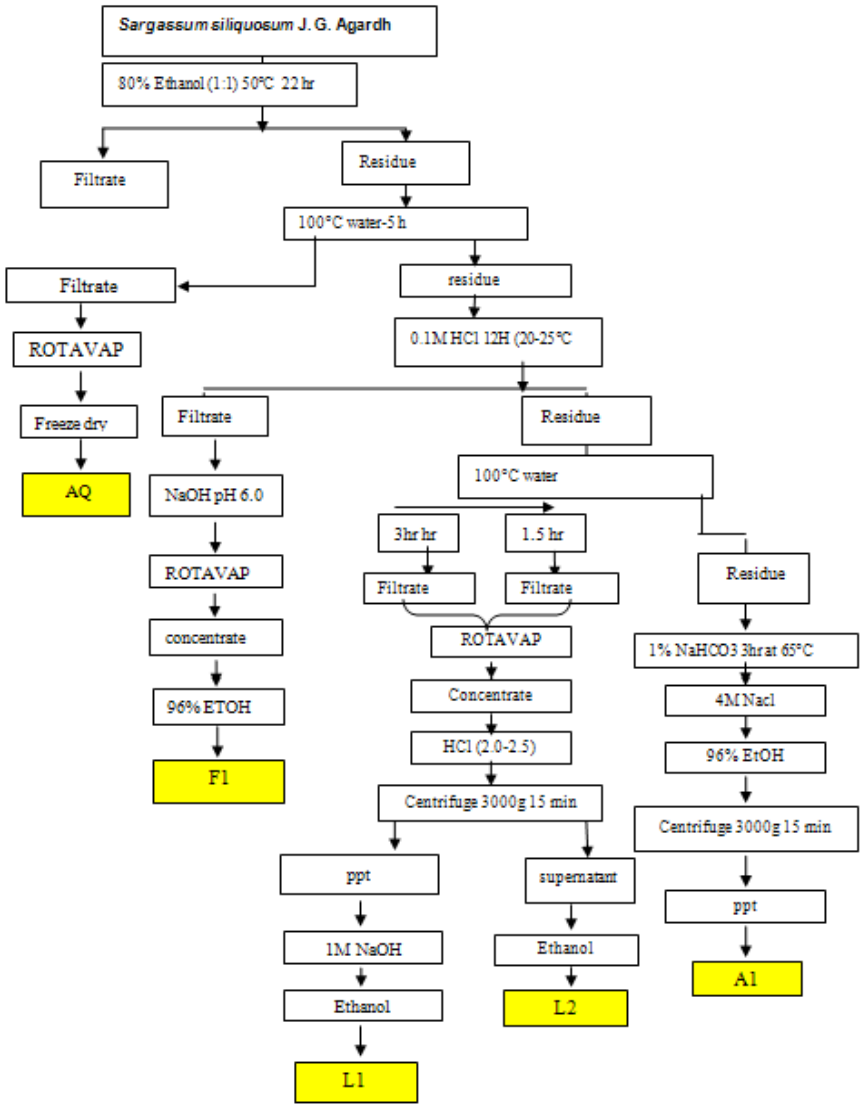


Figure 1.

***Scheme of the extraction of polysaccharides from Sargassum siliquosum J.G. Agardh. Lyophilized fractions (AQ, F1, L1, L2 & A1) were chemically analyzed and were used for the analysis of chemopreventive activities*.**

(ii) Ashing-Acid Digestion Ion Chromatography

The percentage of sulfate composition was quantified using Dionex DX-120 Ion Chromatography with IonPac AS4A-SC 2-mm column. Briefly, 5.0 g of lyophilized sample were ashed, digested with 1M HCl and filtered through 0.45µm membrane filter. Five microliters (5µl) were used as the injection volume. A solution of 1.8 m*M* $NaHCO_3$ + 1.7 m*M* Na_2CO_3 was used as the eluent, and electrical conductivity was used as the mode of detection. The degree of sulfation was computed from standard curve prepared from

the peak area readings of standard sulfates: 4.4189 ppm; 8.377ppm, 17.6755ppm and 35.3509 ppm respectively.

(iii) FT- Infrared spectroscopy

The Fourier transform IR spectra (FT-IR) were recorded with a Shimadzu IR spectrophotomer (model 8300) between 400 cm^{-1} and 4000 cm^{-1}. The samples were analyzed as KBr pellets. Extracts were compared with standard fucoidan (Sigma-Aldrich) and sodium alginate (Sigma-Aldrich).

(iv) Cell lines

Several cell lines used through this study including human hepatocellular carcinoma (Hep G2); human colon carcinoma cells (HCT); human renal cell carcinoma (RCC) and promyelocytic leukemic cells (PML) were provided by Dr. Sam Bernal of the Globetek Science Foundation, Philippines. Hep G2, HCT and RCC were routinely cultured in Dulbeco's Modified Eagle's Medium (DMEM). PML were grown in phenol free RPMI-1640. Media were supplemented with 10% fetal bovine serum (FBS), 2mM glutamine, containing 100U/ml penicillin G sodium, 100U/ml streptomycin **sulfate**. Cells were maintained in humidified air containing 5% $\mathbf{CO_2}$ at 37°C.

(v) DPPH radical scavenging Assay

The antioxidant activity of the extracts was analyzed through their scavenging ability against 1,1 diphenyl-2picryl-hydrazyl (DPPH) radicals. The activity was compared with standard Ascorbic acid. The bleaching of DPPH was monitored at an absorbance of 517 nm. Percent inhibition was calculated using the equation used by Song et al.[18].

$$\text{Scavenging effect (\%)} = \left[1 - \frac{A\text{sample}517}{A\text{control}517}\right] \times 100$$

(vi) Nitrite Assay

Nitric Oxide (NO) is an essential key indicator in the inflammation process[19]. The accumulation of nitrite, an indicator of NO synthesis was measured by Griess reagent. Promyelocytic leukemic cells (PML) were grown in phenol red free RPMI containing 10% FBS. Cells were incubated for 24 h with bacterial lipopolysaccharide (LPS Sigma, 10 µg/ml) with or without extracts (25-175 µg/ml). Briefly, **50µl** of cell culture medium were mixed with 50µl of Griess reagent and incubated for 10 min. The absorbance was measured at 550 nm. Standard curve was **plotted** using serial concentrations of sodium nitrite.

(vii) Cytotoxicity assay

Anti-proliferative activity against various tumor cell lines were estimated by the 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H terazolium bromide (MTT) assay. Cells (5 X 10^5 cells/ml) of select cell lines were incubated with or without extracts (0-175µg/ml) at 37°C before submitted to MTT assay. The percentage cell growth inhibition was expressed as **percent inhibition** (%) = [(A0 −A1)/A0]×100. The treatment with Mitomycin, Doxorubicin and Paclitaxel was used as reference anti-cancer agents.

RESULTS AND DISCUSSION

Polysaccharides from S. siliquosum

Sulfate was successfully separated from polysaccharides after acid treatment and were [DELETE] eluded between 6.35-6.52 minutes. The average retention time, peak areas, amount of sulfate in extracts per 5 mg ashed sample are represented in ***Table 1*** and *Fig. 2*. The homogeneous and almost negligible amount of sulfate present in L1, L2 and A1 was in accordance with the result of extraction procedure by Mikhailovna et al. (2009). In Mikhailovna's extraction procedure, F1, L1, L2 and A1 corresponded to fractions containing fucoidan, laminaran, polymannuronate, sodium alginate respectively. Thus, only AQ and F1 were considered as fractions containing sulfated polysaccharide. The very small amount of sulfate present in L1, L2, A1 may be considered residual sulfate left in isolation procedure. Moreover, it is noted that sulfate content of polysaccharides from *S. siliquosum* were relatively lower than other sulfated polysaccharides from other brown algae. The

degree of sulfation of heparinoid active two sulfated polysaccharides from *Monostroma nitidum* was 34.4%/30mg [20]. Vischuk et al.[21] reported that sulfated polysaccharides from *Undaria finnatifida contained* 23% sulfate esters after anion-exchange chromatography in DEAE-cellulose. Sulfate contents of native and oversulfated fucoidan from *Cladosiphon okamuranus* were estimated to be 13.5% and 32.8% respectively[22].

A variety of procedures have been developed for determining the sulfate ester content of various biomolecules. Ion chromatography has been increasingly utilized for the determination of inorganic sulfate in clinical and environmental samples. In principle, determination of inorganic sulfate liberated after hydrolysis of sulfated polysaccharides and sulfolipids can be a method of choice when hydrolysis is complete. Compared with conventional methods, ion chromatography has demonstrated increased specificity and sensitivity as well as the inherent capacity to simultaneous determination of various anions, and it has been applied to samples from biological and environmental origin. The analyses of elements in the biomas and/or ash formed in the dry ashing process are important parameters in biomass investigation nowadays. In the present study, ashing acid digestion ion chromatography was employed to separate and determine percent sulfate of polysaccharides extracted from *S. siliquosum*.

Previous studies have shown that the composition and complexity of sulfated polysaccharides can vary considerably among algal species. These differences could be attributed to harvest season, age, extraction procedure, and separation technique[24]. Since, we wanted to relate chemopreventive activities of *S. siliquosum* polysaccharides to degree of sulfation, only AQ, F1 and A1 were used in the succeeding assays.

Table 1

***Retention time, peak areas and percent sulfate in the five extracts from S. siliquosum. Values are means of duplicate experiments*.**

Extracts	Retention time	Peak areas	Sulfate, %
AQ	6.40	940956	11
F1	6.52	834406	3.5
L1	6.47	836489	0.31
L2	6.42	1333737	0.48
A1	6.43	541591	0.21

For FT-IR analysis, the broad and intense band 3400 cm^{-1} common to all polysaccharides represents O-H stretching of hydroxyls and bound water which overlaps in part with the CH stretching between 2850-3000 cm-1 [24]. The bands found between 1000-1300 cm^{-1} in all samples represent C-O vibrations (2-bands) and band around 1630-1695 may be attributed to C=O strong stretching indicating the acidic nature of polysaccharides[25]. Moreover, the strong band between 1350-1450 **cm^{-1}** corresponds to sulfate groups while the band between 700-900 cm^{-1} in the samples and standard represents S-OR esters. The band between 1050-1200 seen in *S. siliquosum* polysaccharides but not in fucoidan and alginate may be attributed to the the C=S thiocarbonyl. The band between 950-1250 cm^{-1} represents weak P-H phosphine P-H bending, which may account for the characteristic odor of the samples. The band between 900-1050 cm^{-1} in A4 confirms presence of P-OR esters stretching similar to standard alginate band of 944.94 cm^{-1}.

Only AQ, F1 and A1 were subjected to FT-IR because only these fractions were

considered for chemopreventive assays. The chromatograms are shown here as acquired, without corrections or any further manipulation. The FT-IR spectra of these polysaccharides are represented along with the spectra of standard fucoidan (Sigma, Aldrich) and sodium alginate (Sigma, Aldrich) for comparison.

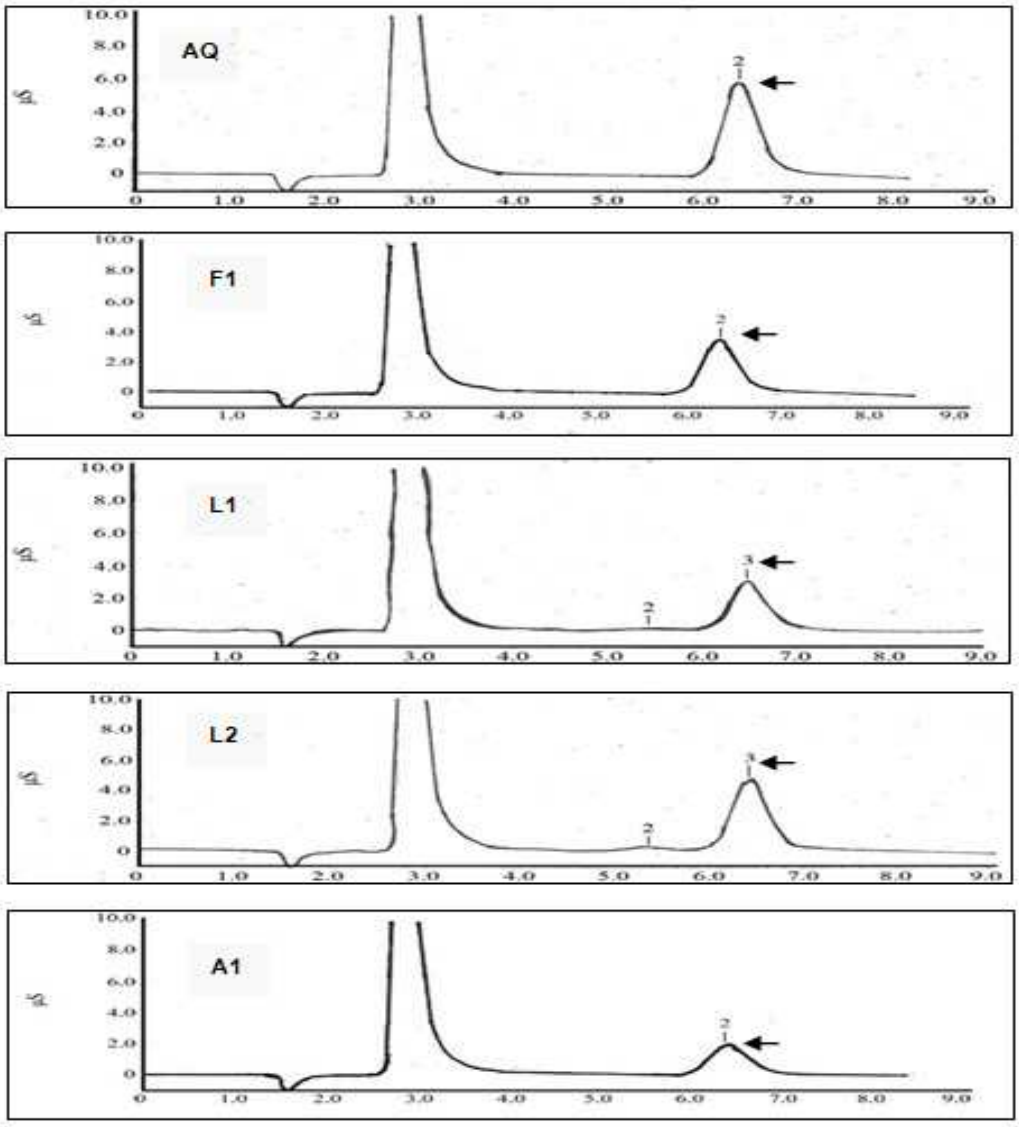


Figure 2.

The sample chromatograms of 5 polysaccharides from S. **siliquosum*****: fucoidan containing fraction (F1); laminaran containing fraction (L1); polymannuronate containing fraction (L2); alginate containing fraction (A1); Aqueous extract (AQ). The sulfate analysis was carried out as described in the experimental section. Sulfate was successfully eluted for all extracts (arrow).***

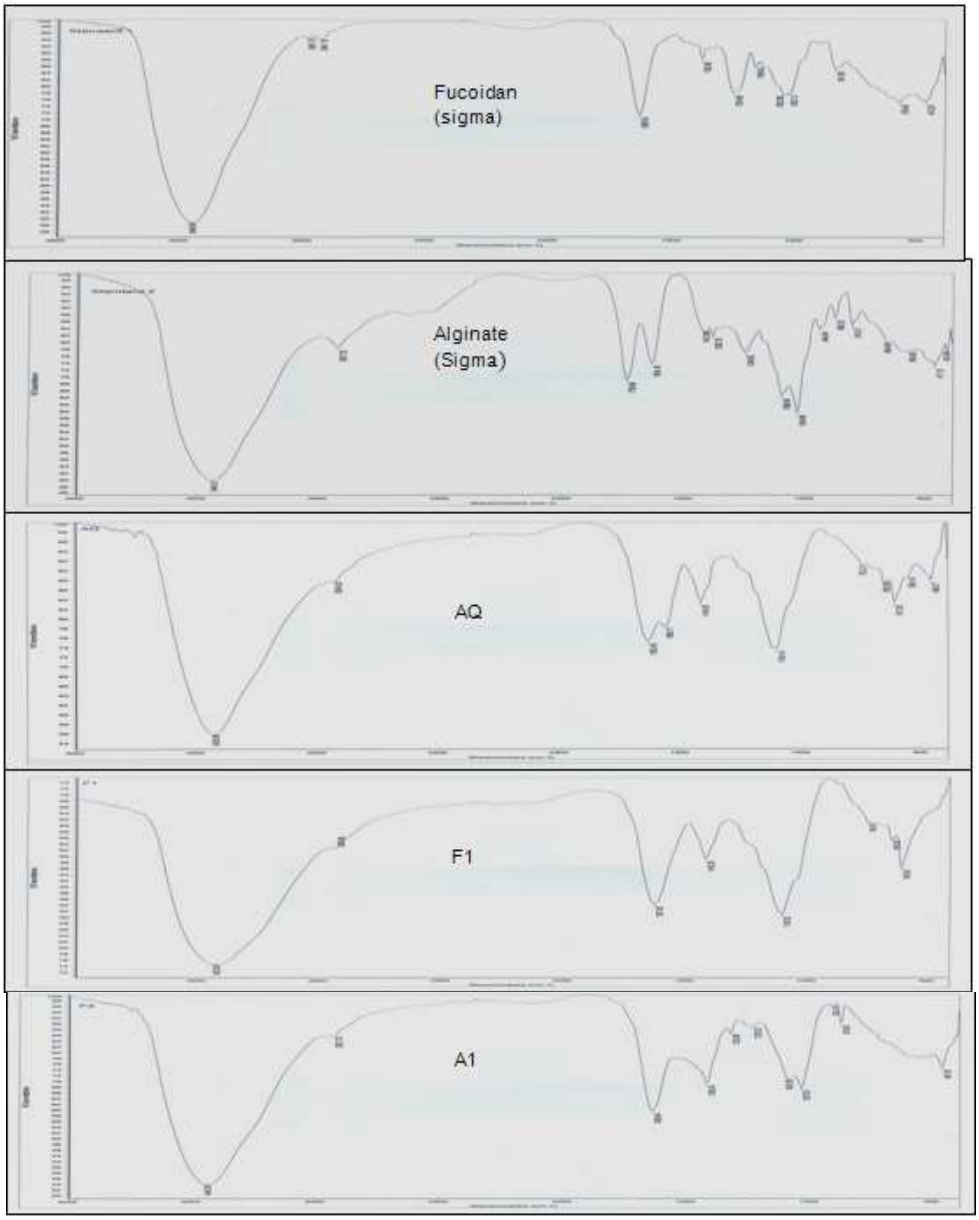


Figure 3.
Comparative FT-IR spectra of polysaccharides: lyophilized aqueous extract (AQ); alginate containing fraction (A1); fucoidan containing fraction (F1).

DPPH radical scavenging assay

Total DPPH scavenging of AQ, F1 and A1 at varying concentrations were measured and the results were depicted in *Fig.4.* A direct relationship between activity and increasing concentrations of the extracts were observed. The marked inhibitory effect of these polysaccharides on DPPH radicals was found to be dependent on sulfate content. However, scavenging activity of ***S. siliquosum*** polysaccharides **was** not comparable with Ascorbic acid. Our finding is in agreement with Ruperez et al.[26] where fucoidan containing fraction from *Fucus vesiculosus* exhibited higher antioxidant potency than that of laminaran and alginic acid containing fractions.

Sulfated polysaccharides were considered to play an important role as free radical scavengers in vitro and antioxidants for prevention of oxidative damage in living

organisms. The antioxidant capacity of polysaccharides extracted from *Sargassum* species has been documented in recent years. Sulfated polysaccharides from *S. thunbergii* significantly upregulated the expression levels of antioxidative enzymes superoxide dismutase (SOD-1), catalase, glutathione peroxidase , glutathione-S- transferases, glutathione reductase[27]. Although the antioxidant capacity of polysaccharide has been proven, the relationship between the structure of polysaccharides and the antioxidative mechanisms has not yet been elucidated. The activity of sulfated polysaccharides depends on several structural parameters such as amount of sulfate, degree of sulfation, the molecular weight, protein content, type of sugar and glycosidic branching. The huge structural diversity of these polysaccharides has given a major hindrance in the structure-activity relationship[28].

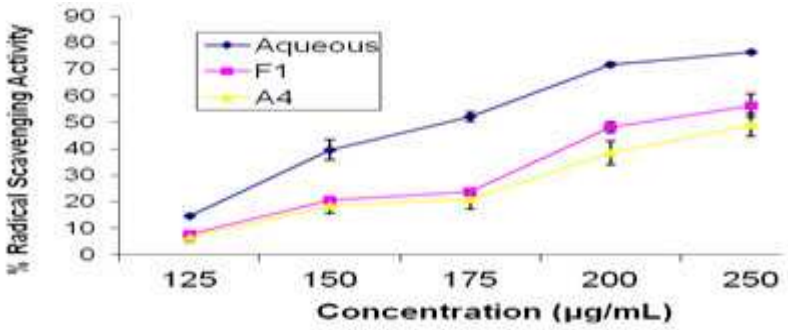


Figure 4.
Scavenging effects of the sample, AQ, F1, and A1 on DPPH radicals. Values are means ± SD (n=3).

Nitrite Assay

Treatment with AQ, F1 and A1 resulted in a dramatic inhibition of LPS-stimulated NO generation in dose dependent manner. A1 displayed 80% at 125µg/ml. AQ, Fuc and F1 displayed 60%, 62% and 46% inhibitory rate at 150µg/ml respectively. It was also observed that AQ, F1 and A4 stimulated production of NO at 175µg/ml which resulted to a decrease in inhibition of NO production *(Fig. 5)*. Thus, results presented interesting investigation on the dichotomy of effects of sulfated polysaccharides on NO production in PML cells. The dichotomy of effects of NO within the multistage model of cancer has generated considerable discussion over the past several years.

We investigated the possible effects of extract on production of NO in stimulated polymorphonuclear cells (PML) to assess their cancer-antipromoting activity. NO plays a variety of regulatory functions in vivo. This molecule is produced by three isoforms of the enzymatic nitric oxide synthase (NOS) which can regulate numerous physiological functions. Despite these beneficial effects, NO has been implicated as a deleterious agent in various pathophysiological conditions including cancer. NO has been proposed to be an important mediator of tumor growth. Within the paradigm of the multistage carcinogenesis model, NO has been reported to act in other stages of cancer growth in addition to initiation. For example, endogenously formed NO appeared to cause the neoplastic transformation of C3H 10T1/2 mouse fibroblast[29]. Different studies have shown that increased and continuous production plays a pivotal role in the regulation of carcinogenic process. NO and NO-derived reactive nitrogen species induce oxidative and nitrosative stress which results in DNA damage and inhibition of DNA repair enzymes[30]

.

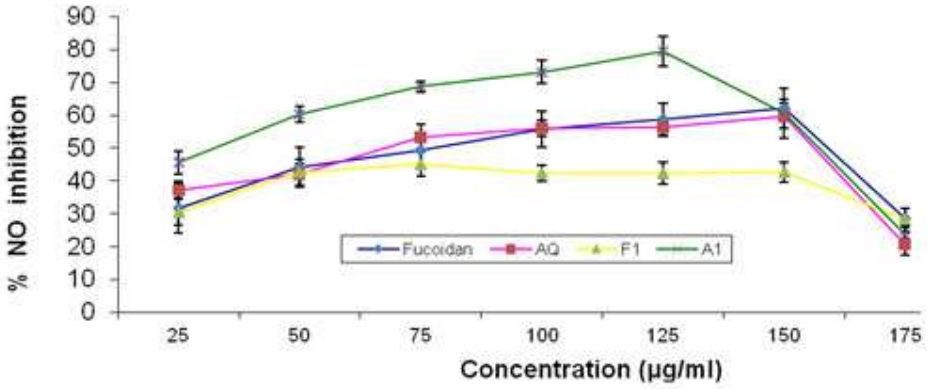
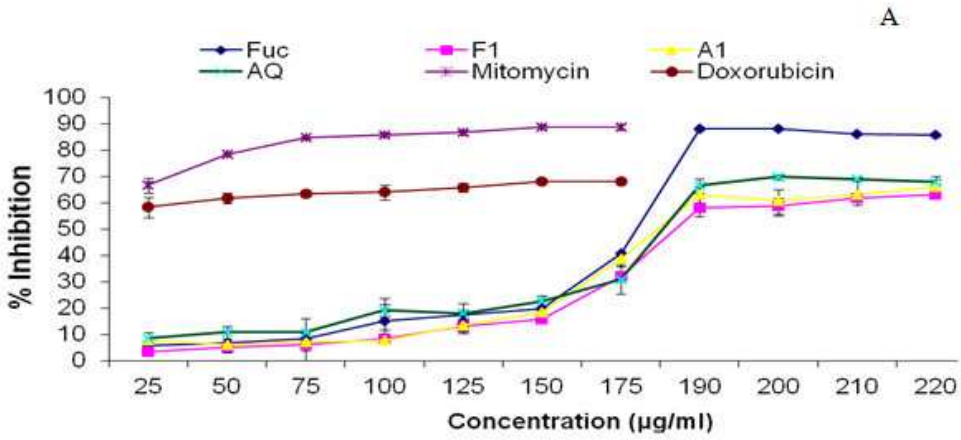


Figure 5.

Effect of the sulfated polysaccharides AQ. F1 and A1 on production of NO in LPS-stimulated promyelocytic leukemic cells (PML). Cells were seeded (2.5 x 105 cells/ml) in 96-well plates in 200 µl of 10% FBS-DMEM medium and then treated with extracts (25-175 µg/ml) and commercial fucoidan as positive standard for 24 h. Supernatants were collected and assayed by Griess reagent. Data were expressed as means ± SD. (n=3).

Cytotoxicity Assay

The sulfated polysaccharides AQ, F1 and A1 significantly suppressed the proliferation of Hep G2 in vitro after 48 h of treatment in dose and sulfate content dependent manner. The growth inhibition rate increased consistently with the sample concentration comparable with the commercial fucoidan and the differences was not significant among the *S. siliquosum extracts* ($p<0.05$). A plateu in growth was observed at 190-220µg/ml for all extracts. $IC_{50\ values}$ were 177.888; 185.305; 181.931; 183.038 µg/ml for fucoidan, F1, AQ and E4 respectively. Antitumor activity at 190µg/ml was highest in fucoidan (88%), followed by AQ (67%), A1(64%) and F1(58%) *(Fig.6a).* In the present study, the growth inhibitory effects of the sulfated polysaccharides from *Sargassum siliquosum* J.G Agardh was investigated and compared with Mitomycin, Doxorubicin, Paclitaxel and commercial Fucoidan. MTT assay, a well-established in vitro model for ctyotoxicity against cancer cell lines was used as one of the conventional methods for the screening of compounds with potential antitumor properties



The formation of cancer cells in human body can be directly induced by free radicals and natural anticancer drugs as chemopreventive agents have gained positive popularity in treatment of cancer. Anticancer activity is closely related to several structural parameters such as degree of sulfation, molecular weight, position of sulfation, monosaccharide composition and glycosidic linkage[31].The antiproliferative activity of many sulfated polysaccharides from algal species has been reported in recent years. Sulphated polysaccharides from *Grifolia frondosa* inhibited the SGC-7901 cell growth in a dose-dependent manner [32], while sulfated polysaccharide from *S. pallidum* presented promising antiproliferative effect in Hep G2 cells, A549 cells and MGC-803 cells respectively[33]. In the present study, the antiproliferative effect AQ, F1 and A1 activity in human renal cancer cells was dose-dependent and was related to sulphate content. There was significant percent inhibition comparable with commercial fucoidan *(Fig.6b),* however $IC_{50\ values}$ were not observed up to 600µg/ml. The extracts and paclitaxel did not present cytotoxic activity against human colon carcinoma cells *(Fig. 6c).* The differences on antitumor activities of *S. siliquosum* polysaccharides may be attributed to different structural parameters other than sulphate content such as different molecular weights, charge characteristics, monosaccharide distribution and glycosidic linkage.

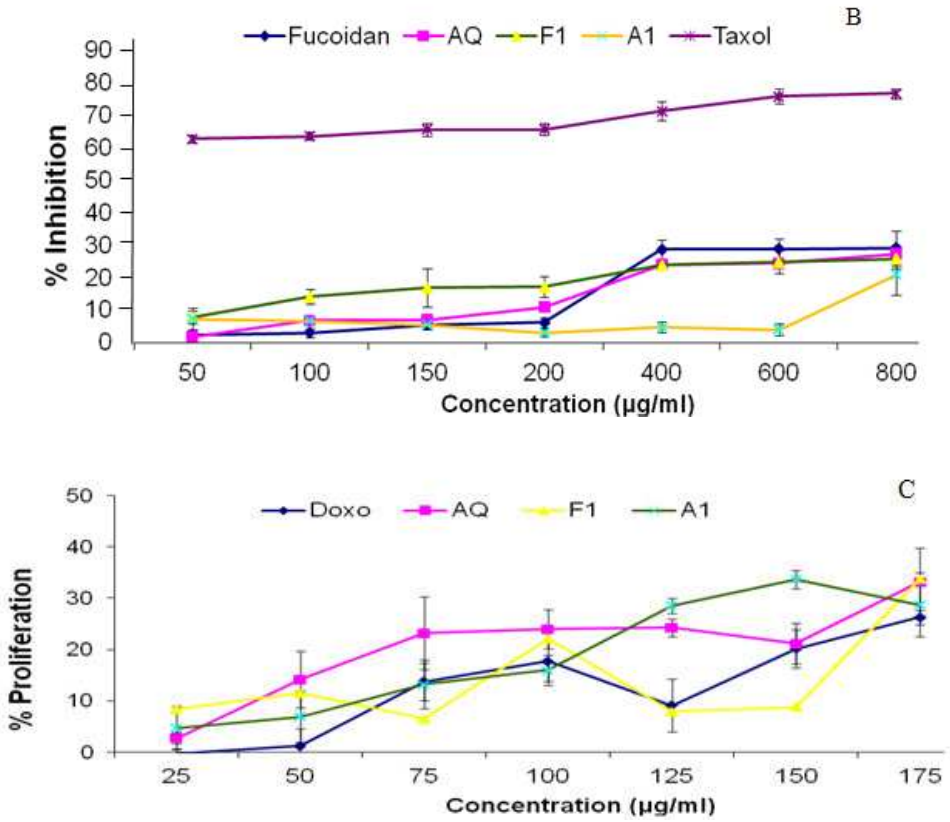


Figure 6.

Effect of the sulfated polysaccharides AQ, F1and A1 on cell proliferation of Hep G2 (A), human colon cancer cells (B) and human colon cancer cells (C). Cells were seeded (2.5 x 10^5 cells/ml in 96-well plates in 200µl of 10% FBS-DMEM medium, and then treated with extracts (25-220 µg/ml), Mitomycin,Doxorubicin and Taxol as standard anticancer drugs. The cell viability was assessed using the MTT assay. Data are represented as the means±SD as determined from triplicate experiments

CONCLUSION

In the present study, fractions containing fucoidan and alginate were isolated from *Sargassum* ***siliquosum*** J.G Agardh and their chemopreventive activities were evaluated. The results clearly demonstrated that fucoidan and alginate containing fractions from *S.* ***siliquosum*** displayed significant free radical scavenging activity on DPPH radical (anti-initiation), inhibited NO production in LPS-stimulated PML cells (anti-promotion) and specifically inhibited proliferation of cancer cells in vitro (anti-progression) in dose dependent manner. Our findings also indicated that sulfate content made great role in the observed chemopreventive activities. However, the relationship between the structure of polysaccharides and chemopreventive mechanisms is yet to be elucidated. It is of great importance to define the complete structure of *S. siliquosum* polysaccharide to elucidate the biological roles of polysaccharides and develop potential antitumor drugs.

.

ACKNOWLEDGEMENT

The research was funded by financial grant from the Department of Science and Technology-Philippine Council for Health and Research Development (Philippines). This research was supported **also** by the Globetek Science Foundation, Philippines.

REFERENCES

[1] Wijesinghe WAJP, Jeon YJ, Biological activities and potential industrial applications of fucose rich sulfated polysaccharides and fucoidans isolated from brown seaweeds: A review. Carbohyd Polym, doi:10.1016/j. carbpol. 2011. 12. 029. (2012).

[2] Li B, Zinjun FL, Zhao R, Fucoidan: Structure and Bioactivity. Molecules, 13: 1671-1695, (2008).

[3] Park BY, Shin WS, Um Y, Cho S, Park GM, Yeon DS, Kwon SC, Ham J, Choi B W, Lee S, Selective basodilation effect of sargahydroquinoic acid, an active constituent of *Sargassum micracanthum* on the basilar arteries of rabbits. Bioorg Med Chem Lett, 18: 2624 2627, (2008).

[4] Shanmugam M, Mody KHHeparinoid-active sulfated polysaccharides from marine algae as potential blood anticoagulant agents. Curr Sci India, 79(12): 1672-1681, (2010).

[5] Paskaleva EE, Lin X, Duus K, McSharry JJ, Veille CL, Thornber C, Liu Y, Lee D Y, Canki M *Sargassum fusiforme* fraction is a potent and specific inhibitor of HIV- 1 fusion and reverse transcriptase. Virol J, 5(8): 1-9, (2008).

[6] de Sousa AA, Torres MR, Pessoa C, de Moraes MO, Filho FDR, Alves ANN, Costa-Lotulfo LV In vivo growth-inhibition of Sarcoma 180 tumor by alginates from brown seaweed *Sargassum vulgare*. Carbohyd Polym, 69: 7-13, (2006).

[7] Huang X, Zhou H, Shang H, The effect of *Sargassum fusiforme* polysaccharide extracts on vibriosis resistance and immune activity of the shrimp, *Fenneropenaeus chinensis*. Fish Shellfish Immun, 20: 750-757, (2005).

[8] Yoon WJ, Ham YM, Kim SS, Yoo BS, Moon JY, Baik JS, Lee NH, Hyun C, Suppression of pro-inflammatory cytokines, iNOS and COX-2 expression by brown algae *Sargassum micracanthum* in RAW 264.7 macrophages. Eurasia J Biol Sci, 3: 130-134, (2009).

[9] Garcia-casal MN, Ramirez J, Leets I, Pereira AC, Uiroga MF, Antioxidant capacity, polyphenol content and iron bioavailability from algae (*Ulva* sp., *Sargassum* sp., and *Porphyra* sp.) in human subjects. Brit J of Nutr, 101: 79-85, (2009).

[10] Lee JB, Koizume S, Hayashi K, Hayashi T, Structure of rhamnan sulfate from the

green alga *Monostroma nitidum* and its anti-herpetic effect. Carbohyd Poly, 87: 572-577, (2010).

[11] Josephine A, Nithya K, Amudha G, Veena CK, Preetha SP, Varalakshmi P, Role of sulphated polysaccharides from *Sargassum wrightii* in Cyclosporine A-induced oxidative liver injury in rats. BMC Pharmacology, 8 (4): 64-68, (2008).

[12] Mandal P, Pujol CP, Damonte EB, Ghosh T, Ray B, Xylans from *Scinaia hatei*: structural features, sulfation and anti-HSV activity, Int J Biol Macromol., 46: 173-178, (2009).

[13] Mao W, Li H, Li Y, Zhang H, Qi X, Sun H, Chen Y, Guo S, Chemical characteristics and anticoagulant activity of the sulfated polysaccharide isolated from *Monostroma latissimum* (Chlorophyta), Int J of Biol Macromol, 44: 70-74, (2008).

[14] Karmakar P, Ghosh T, Sinha H, Saha S, Mandal P, Gosal PK, Ray B, Polysaccharides from the brown seaweed *Padina tetrastomatica*: characterization of sulfated fucans, Carbohyd Polym, 78: 416-421, (2009).

[15] Tupas LM, Montaňo NE, Effects of aqueous alkaline extracts from Philippine seaweeds as foliar spray on crops, Philipp J Sci, 17: 29-36, (1987).

[16] Michailovna SN, Igoverna IT, Mikhailovna UA, Igoverich MK, Gennadievich KV, Nikolevna ZT, Alexeevna E, Method of processing seaweeds, US 7, 611, 716B2, www. freepatentsonline.com. (2009).

[17] Gamal-Eldeen AM, Ahmed EF, Abo-Zeid MA, *In vitro* Cancer chemopreventive properties of polysaccharide extract from the brown alga, *Sargassum latifolium*, Food Chem Toxicol, 47: 1378-1384, (2009).

[18] Song H, Zhang Q, Zhang Z, Wang J, In vitro antioxidant activity of polysaccharides extracted from *Bryopsis plumose*, Carbohyd Polym, 80: 1057-1061, (2010).

[19] Muntane J, De la Mata M, Nitric oxide and cancer. World J Hepato, 2(9): 337- 344, (2010).

[20] Wu XZ, Chen D, Effects of sulfated polysaccharides on tumour biology, W Indian Med J, 55 (4): 270-272, (2006).

[21] Vischuk SO, Ermakova PS, Zvyagintseva TN, Sulfated polysaccharides from brown seaweeds *Saccharina japonica* and *Undaria pinnatifida*: Isolation, structural characteristics, and antitumor activity, Carbohyd Res, 346: 2769-2776, (2011).

[22] Teruya T, Konishi T, Uechi S, Tamaki H, Tako M, Anti-proliferative activity of oversulfated fucoidan from commercially cultured *Cladosiphon okamuranus* TOKIDA in U937 cells, Int J Biol Macromol, 41**:** 221-226, (2007).

[23] Llorente MJF, Garcia JEC, Concentration of elements in woody and herbaceous biomass as a function of dry ashing temperature, Fuel, 1273-1279, (2006).

[24] Freitas F, Alves VD, Torres CAV, Cruz M, Sousa I, Melo MJ, Ramos AM, Reis MAM, Fucose-containing exopolysaccharide produced by the newly isolated *Enterobacter* strain A47 DSM 21139, Carbohyd Polym, 83, 159-165, (2010).

[25] Mao WJ, Fang F, Li HY, Qi XH, Sun HH, Chen Y, Guo SD, Heparinoid-active two sulfated polysaccharides isolated from marine green algae *Monostroma nitidum*, Carbohyd Polym, 74: 834-839, (2008).

[26] Ruperez P, Ahrazem O, Leal JA**,** Potential antioxidant capacity of sulfated polysaccharides from the edible marine brown seaweeds *Fucus vesiculosus*, J Agr Food Chem, 50 (4): 840-845, (2002).

[27] Patra JK, Rath SK, Jena K, Rathod VK, Thatoi H, Evaluation of antioxidant and antimicrobial activity of seaweed (*Sargassum* sp.) Extract: A study on inhibition of glutathione-S-transferase activity, Turk J Biol, 32: 119-125, (2007).

[28] Chattopadhyay N, Ghosh T, Sinha S, Chattopadhyay K, Karmakar P, Ray B, Polysaccharides from *Turbinaria conides*: Structural features and antioxidant capacity**,** Food Chem, 118: 823-829, (2010).

[29] Mordan LJ, Burnett TS, Zhang LX, Tom J, Cooney RV, Inhibitors of endogenous nitrogen oxide formation block the promotion of neoplastic transformation in C3H 10T1/2 fibroblast, Carcinogenesis, 14: 1555-1559, (1993).

[30] Wink DA, Vodovotz Y, Laval J, Laval F, Dewhirst MW, Mitchell JB, The multifaceted roles of nitric oxide in cancer, Carcinogenesis, 19 (5): 711-721, (1998).

[31] Jiang Z, Okimura T, Yokose T, Yamasaki Y, Yamaguchi K, Oda T, Effects of sulfated fucan, ascophyllan, from the brown alga *Ascophyllum nodosum* on various cell lines: a comparative study on ascophyllan and fucoidan, J Biol Sci Bioeng, 110 (1): 113-117, (2010).

[32] Shi BJ, Nie XH, Chen LZ, Liu YL, Tao WY, Anticancer activities of a chemically sulfated polysaccharide obtained from *Grifolia frondosa* and its combination with 5-Fluorouracil against human gastric carcinoma cells, Carbohyd Polym, 68: 687-692, (2006).

[33] Ye H, Wang K, Zhou C, Liu J, Zeng X, Purification, antitumor and antioxidant activities in vitro of polysaccharides from the brown seaweed *Sargassum pallidum,* Food Chem, 111: 428-432, (2008).