IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 8, Issue 3 (Nov. – Dec. 2013), PP 06-11
www.iosrjournals.org

In vitro cancer chemopreventive properties of polysaccharide extract from the brown alga, *sargassum wightii.*

Manojkumar.K[1] , seema [2]
1(*Department of Pharmacy, Baba Haridos College of pharmacy and technology / Board of Technical Education New delhi / India.*)
2 *(Department of Pharmacy, Baba Haridos College of pharmacy and technology / Board of Technical Education New delhi / India.*)

Abstract : *Polysaccharides of edible algae attracted extensive interest due to their numerous biological activities. Sargassum wightii C. Agardh, belongs to Sargassaceae, is a brown algae in sea shores in Mandapam Rameshwaram and in and aroud areas. The cancer chemopreventive activity of different fractionsof water-soluble polysaccharide extract derived from S.wightii. Estimation of cancer chemopreventive activity, specifically anti-initiation, including the modulation of carcinogen metabolism and the antioxidant capacity,.poly sacharides from Sargassum wightii have a broad spectrum of novel cancer chemopreventive agents. cell-and enzyme- based in vitro assays with markers relevant for measuring inhibition of carcinogenesis during the initiation, promotion, and progression stage. These bioassay systems offer effective identification and evaluation of lead compounds for the development of effective chemopreventive agents and the elucidation of their mechanism of action.*

Keywords: *Apoptosis, Anti-promoting, Cancer chemo prevention, MTT assay, Sargassum wightii*

I. Introduction

Brown algae contain large amounts of cell-wall polysaccharides,most of which are sulfated polysaccharide fucoidans (Asker et al.,2007[1]).Algal polysaccharides attracted extensive interest to study their numerous biological activities. Recently, several investigations have been focused on the isolation and function of the polysaccharides derived from different Sargassum species, which revealed multiple biological activities such as hepatic and renal protective activity (Josephine et al., 2007 [2]; Raghavendran et al.,2007[3]) and antioxidant (Jung et al., 2008[4]; Zhou et al., 2008[5]), anti-tumor,anti-angiogenic (Dias et al., 2005[6]), anti-inflammatory (Chang et al., 2008[7]), anti-coagulant (Athukorala et al., 2007[8]), anti-viral(Zhu et al., 2006[9]) and anti-vasculogenic (Dias et al., 2008 [10])activities.Generally, the biological activity of polysaccharides from marine algae is related to the molecular size, type of sugar, sulphate content, type of linkage and molecular geometry are also known to have a role in their activities (Zhu et al., 2004[11]). Edible seaweedshave high nutritional values as sources of vitamins, minerals, and non-caloric dietary fibers and as potential sources of biological active ingredients (Yan et al., 1999[12]). *Sargassum wightii.* (Turner) C. Agardh is one of the edible brown algae in red sea shores in Egypt and it also widely distributed in Japan and India . *S. wightii* belongs to class Phaeophyceae, order Fucales, family Sargassaceae. *S. wightii.* has Unambiguous Synonyms including Carpacanthus latifolius (Turner), and Fucus latifolius Turner. In a recent report, *S. wightii.* was found to have promising anti-viral activity (Asker et al., 2007[13]). During the cancer multi-stage cascade, normal cells undergo initiation, promotion, and progression processes. Extensive researches on the cellular and molecular basis of the carcinogenesis cascade provides a targeted approach for cancer chemoprevention, which aims to halt or reverse the development and progression of precancerous cells through use of non cytotoxic doses of nutrients and/or pharmacological agents (Theisen, 2001[14]). Identification of novel effective cancer chemopreventive agents has become an essential worldwide strategy in cancer prevention. Finding of cancer chemopreventive activity could elevate seaweed *(S. wightii.*) value as a food or additive as expanding its market and uses in food industries.The extracted different fractions of watersoluble polysaccharide from *S. Wightii* have cancer chemopreventive activity that prevent different stages of carcinogenesis process.

II. Materials and methods

2. 1. Preparation of Solvent extracts :

Collected the *sargsuum wihgtii* from sea shore. Collection of the *sargsuum wihgtii* from the marine environment of different sea shore in Tamil nadu.Thoroughly washed mature plant should shade dried and then powdered with the help of a blender. 25 g of the powder filled in the thimble and extracted successively with, chloroform, ethanol and aquase using a Soxhlet extractor for 48 h. All the extracts concentrated using rotary flash evaporator and preserved at 5^0C in air tight bottle until further use. Phytochemical analysis of all the evaporated solvent extracts would conducted the procedure of Indian Pharmacopoeia (1985).To exctract the

poly saccharides and study the anti oxidant activity from *sargsuum wihgtii.* The powdered algal sample was extracted repeatedly with 80% ethanol at 80^0C, to eliminate the low molecular weight ingredients. The resulted residual materials(E1) extracted, with boiling distilled water at 100^0C for 4 h, repeatedly for three times. The filtrate of the water extract discarded, and the dried residue submitted to another extraction by 0.1 M HCl at 95^0C for 4 h, repeatedly for three times. After filtration, the filtrate (A) and the precipitate (B) separated and then (A) was concentrated to 1/6 of its original volume, precipitated with 3% aqueous cetyl pyridinium chloride, and then centrifuged for 15 min at 3000g. The supernatant should precipitated with 99% EtOH to give (E2) and the pellet dissolved in 4 M NaCl at 40^0C for 20 h before re-precipitation with 99% EtOH, as previously mentioned with boiling water, to give (E3). The dried residue (B) was extracted again with 1.25 M NaOH at 28^0C for 24 h, the extraction was repeated two times then filtered. The filtrate was concentrated under reduced pressure, adjusted to pH 6 with 99% acetic acid, and precipitated with 99% ethanol to give (E4). Finally, E1, E2, E3, and E4 were lyophilized, and the powdered extracts were tested for their cancer chemopreventive activities.. The extracted polysaccharides solution should pretreated through a membrane with the pore size of 4.5×10^4 mm The ultrafiltration was performedwith a Millipore Ultrafiltration System equipped with different membranes with the area of 0.1 m^2 Crude polysaccharides obtained from ultrafiltration, were dissolved in 0.1 M NaCl (10 mg/ml) and 2 ml of solution were applied to a column of DEAE Cellulose-52 (2.6 x 30 cm), followed by stepwise elution with 0.1, 0.3, and 0.5 M sodium chloride solutions at a flow rate of 60 ml/h. Eluate (5 ml/tube) was collected automatically and the carbohydrates were determined by the phenol–sulfuric acid method, using glucose as standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956)

2.2. Invitro cancer activity study in different assay methods

Anti-proliferative activity against various tumor cell lines will estimated by the3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide by MTT assay. Cell cycle analysis by standard methods and by flow cytometry . the apoptosis study by using Apoptest Kit(DakoCytomation).

2.3.Collection of cell line

Malignant cancer cell lines including human gastric adenocarcinoma (AGS), human cervix carcinoma cell lines (HeLa), human breast cancer cell line (MCF-7), rat pheochromocytoma cell line (PC12) and non-malignant cells were collected from Amala Cancer Institute, Kerala, India.

AGS, HeLa, MCF-7, PC12 and NIH 3T3 were obtained from Amala Cancer Institute, Kerala, India and maintained at 37^0C in a humidified atmosphere (90%) containing 5% CO_2. Cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) with 5% (v/v) fetal bovine serum, 100 U/mL penicillin, and 100 lg/mL streptomycin. Cells were seeded overnight, and then incubated with various concentrations of different poly saccaride extracts for 24 h and 48 h.For MTT assay, cells were seeded at 5000 cell/well onto 96-well culture plates. For assay of apoptosis, cells were seeded at 100,000 cell/well onto a 24-well plate. For each concentration and time course study, there was a control sample which remained untreated and received the equal volume of medium.

2.4. Apoptosis

Apoptotic cells were detected using PI staining of treated cells followed by flow cytometry to detect the so-called sub- G1 peak (Zhang et al., 1999[15]). Briefly, HeLa cells were cultured overnight in a 24-well plate and treated with S. wightiifor 24 h. Floating and adherent cells were then harvested and incubated at 4 ^{0}C overnight in the dark with 750 lL of a hypotonic buffer (50 lg/mL PI in 0.1% sodium citrate plus 0.1% Triton X-100) before flow cytometric analysis using a FACScanflow cytometer (Becton Dickinson). About 10,000 events were acquired with FACS. The 10,000 events were acquired with FACS.

III. Results

3.1. Cytotoxicity of various fractions of S. wightii

Cytotoxicity of total ethanol extract of *S. Wightii* and its different fractions were examined on malignant cell lines. Firstly,malignant cells were incubated with various concentrations of total ethanol extract of poly saccaride E1 from *S. wightii* (5–1000 lg/mL) for 48 h. The result showed this extract decreased cell viability in a concentration- dependent manner and the toxicity started at a concentration as little as 80 lg/mL (Fig. 1). Our findings about the defatted fraction of *S. wightii*(6.25-1280 lg/mL) showed its anti-proliferative properties against Fig. 2. Dose-dependent growth inhibition of malignant cell lines by total ethanol extract (5–1000 lg/mL) after 48 h. Viability was quantitated by MTT assay. The toxicity started as little as 80 lg/mL. Results are mean ± (n = 3). *P < 0.05, _P < 0.01 and #P < 0.001 compared to control (Fig. 3). MCF-7 was greatest and decreased successively against AGS, HeLa and PC12 cells after 24 h (Fig. 2).In order to compare the cytotoxicity of solvent fractions of *S. wightii* against malignant cells, another MTT assay was carriedout for different concentrations (5–160 lg/mL). Among them, E1 was found to be more effective than the other

polysaccarides extract of the plant (Fig. 3), while other fractions showed no prominent cytotoxicity on the cell lines tested (Table 1). E1 showed inhibitory effects on the proliferation of malignant cells but not on non-malignant cells indicating a degree of specificity for malignant cell lines. The IC50 values of this fraction against AGS, HeLa, MCF-7 and PC12 cell lines after 24 h were determined, 43.61 ± 4.1, 46.92 ± 3.6, 99.38 ± 2.9, 158.8 ± 5 lg/mL respectively (Table. 1). The HeLa cells as the most sensitive cells were chosen for further comparative studies with baicalein as a positive control. E1 inhibited the proliferation of HeLa cells in a manner that is comparable with baicalein (Fig. 4).

3.2. Role of apoptosis in HeLa cells treated with S. Wighti

Apoptosis following treatment with the E1 of *S. Wighti* (50 lg/mL) was measured with PI staining and flow cytometry, aiming to detect the sub-G1 peak resulting from DNA fragmentation. Flow cytometry histogram of the positivecontrol, in which cells were cultured in serum free medium (Moon, 2008[16]), and the E1 treated cells exhibited a sub-G1 peak in HeLa cells which indicates the involvement ofan apoptotic process in E1 induced cell death (Fig. 5).

IV. Discussion

Plant derived poly saccaride compounds have multitask effects exerting influence on different levels and via different mechanisms. There is strong evidence supporting the positive role of medicinal plants in oncology, and that they affect all phases of the cancer process .Plants have played an important role as a source of effective anti-cancer agents and it is significant that over 60% of currently used anti-cancer agents are derived from natural sources including plants, marine organisms and micro-organisms (Cragg et al., 1997[17]; Valeriote et al., 2002[18]). In this study, the cytotoxic and proapoptotic effects of *S. wightii* on different cancer cell lines were investigated. To the authors knowledge this is the first report on *S. Wightii* induced toxicity in cancer cell lines. Our data confirmed that *S. wightii* extract has cytotoxic activity against AGS, HeLa, MCF-7 and PC12 cell lines which are consistent with previous studies conducted on other species .

In the next step, the purification by solvent extraction for the isolation of active components of *S. wightii* was used and the potential antitumor activity of the poly saccarides was compared. It was found that the polysaccaride E1 had the greatest anti-proliferative activity in vitro. The effects of the E1 on malignant and non-malignant cells demonstrated a degree of specificity for malignant cell lines.Among different fractions of the previously studied sargasum specious , *S .lactifolium* . A crude natural product extract is generally an extremely complicated mixture of several compounds that possess variable chemophysical properties often with opposing pharmacological activities (Sengupta et al., 2004[19]). Removing compounds which possess opposing effect ensure safe use of herbal medicine. The fundamental strategy for separating these compounds is based on their chemophysical properties that can be cleverly exploited to initially separate them into various chemical groups. However, from the literature search of the related genera and families, it is possible to predict the types of compounds that might be present in a particular extract. This tentative prediction on possible identity of the classes of compounds may help choose suitable extraction and partitioning methods, and solvents for extracting specific classes of compounds. Plant natural productsare usually extracted with solvents of increasing polarity (Otsuka, 2006[20]).

The larger the variety of compounds that are extracted by the extractant, the better the chance that biologically active components will also be extracted if a specific class of chemical component is not targeted (Eloff, 1998[21]). Similarly, in our study the cytotoxic and apoptogenic properties of *S. Wightii* poly saccaride extract could also be attributed to these flavonoids. It is considered important to screen apoptotic inducers from plants, either in the form of crude extracts or as components isolated from them. In the present study *S. wightii* - induced apoptosis was involved in the induction of cell death. Apoptosis is characterized by distinct morphological features including; chromatincondensation, cell and nuclear shrinkage, membrane blebbing and oligonucleosomal DNA fragmentation (Wyllie, 1997[22]; Broker et al., 2005[23]; Mousavi et al., 2008[24]). As shown in Table 2, while *S. wightii* (50 lg/mL) induced remarkable cell toxicity in the HeLa cells, apoptosis only partially contributed to this toxicity. It has been reported that DNA fragmentation creates small fragments of DNA that can be eluted following incubation in a hypotonic phosphate–citrate buffer. When cells are stained with a quantitative DNA-binding dye such as PI, aiming to detect the sub-G1 peak resulting from DNA fragmentation, cells that have lost DNA will take up less stain and will appear to the left of the G1 peak. (Brohem et al., 2009[25]). Overall, this study showed that *S. wightii* inhibits the proliferation of a variety of malignant cell lines with the involvement of apoptosis or programmed cell death. Further studies are needed to fully recognize the mechanisms involved in cell death. *S. wightii* could be considered as a promising chemotherapeutic agent in cancer treatment.

TABLE 1

Doses of polysaccarides of *S. wightii* inducing 50% cell growth inhibition (IC50) against malignant cell lines. Cells incubated with different concentration of extracts for 24 h. IC50 values were expressed as the mean ± SEM (n = 3).

Cell lines	*E1*	*E2*	*E3*	*E4*
AGS	*43.61 ± 4.1*	*> 300*	*> 300*	*> 300*
HeLa	46.92 ± 3.6	*> 300*	*> 300*	*> 300*
MCF-7	*99.38 ± 2.9*	*> 300*	*> 300*	*> 300*
PC12	*158.8 ± 5*	*> 300*	*> 300*	*> 300*

TABLE 2

The proportion of apoptosis in *S. wightii* -induced cytotoxicity in HeLa cells. Cells were treated with 50 lg/mL of E1polysaccaride for 24 h.

HeLa	
Apoptosis(%)	*22.7 ± 4.1*
Cell toxicity(%)	*58.3 ± 0.7*

Cell toxicity was quantitated by MTT assay. Apoptotic cells were determined using PI staining of DNA fragmentation by flow cytometry (sub-G1 peak). Results are mean ± SEM (n = 3).

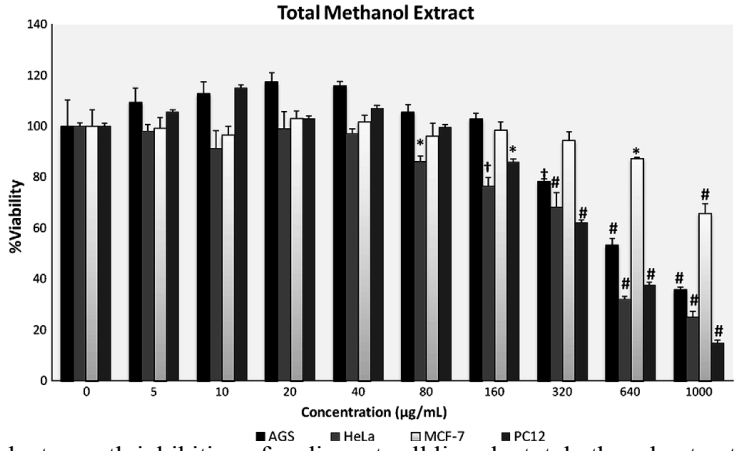

Fig. 1. Dose-dependent growth inhibition of malignant cell lines by total ethanol extract (5–1000 lg/mL) after 48 h. Viability was quantitated by MTT assay. The toxicity started as little as 80 lg/mL. Results are mean ± (n = 3). *P < 0.05, _P < 0.01 and #P < 0.001 compared to control.

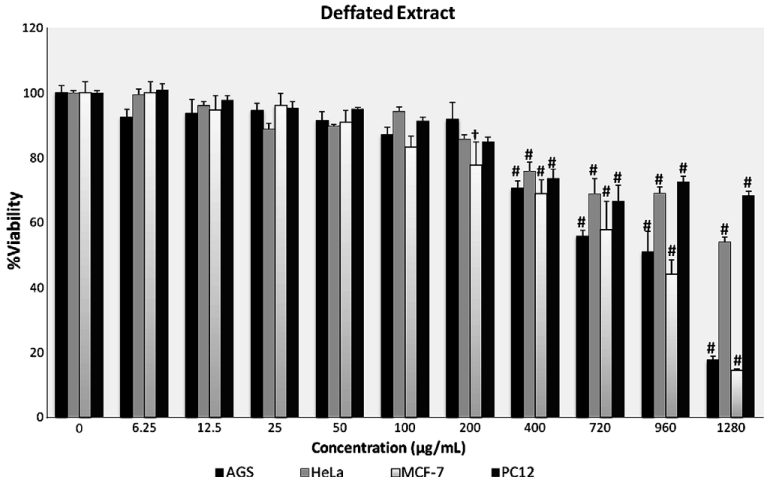

Fig. 2. Dose-dependent growth inhibition of malignant cell lines by defatted (6.25–1280 lg/mL) after 24 h. Viability was quantitated by MTT assay. Results are mean ± SD (n = 3). *P < 0.05, _P < 0.01 and #P < 0.001 compared to control.

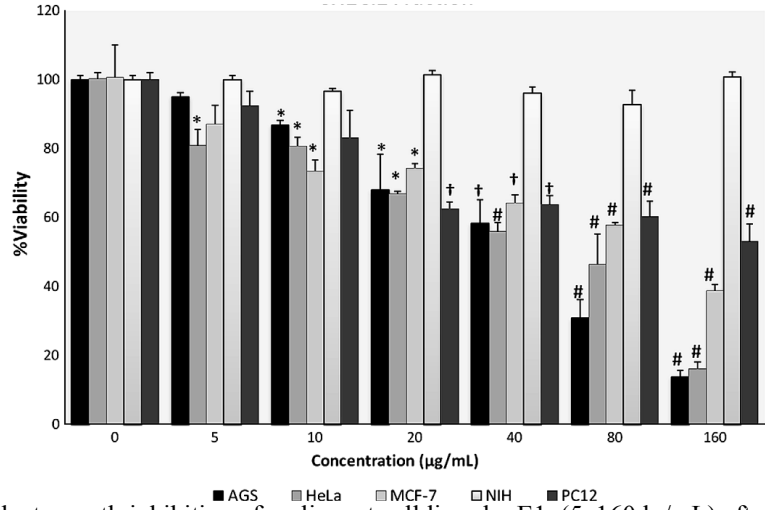

Fig. 3. Dose-dependent growth inhibition of malignant cell lines by E1 (5–160 lg/mL) after 24 h. Viability was quantitated by MTT assay. The IC50 values of this fraction against AGS, HeLa, MCF-7 and PC12 cell lines after 24 h were determined, 43.61 ± 4.1, 46.92 ± 3.6, 99.38 ± 2.9, 158.8 ± 5 lg/Ml respectively. Results are mean ± SD (n = 3). *P < 0.05, _P < 0.01 and #P < 0.001 compared to control.

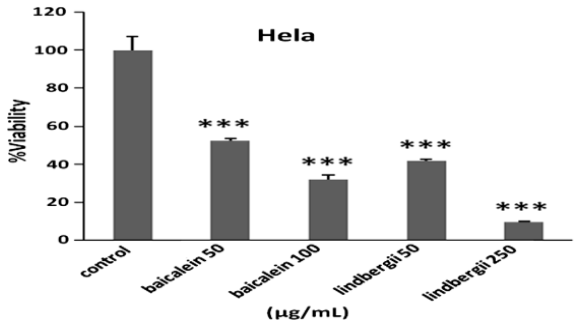

Fig. 4. Growth inhibition of HeLa cells by E1 (50 and 250 lg/mL) compare with baicalein (50 and 100 lM) after 24 h. Viability was quantitated byMTT assay. E1 could inhibit the proliferation of cells that is comparable with baicalein. Results are mean ± SD (n = 3). ***P < 0.001 compared to control.

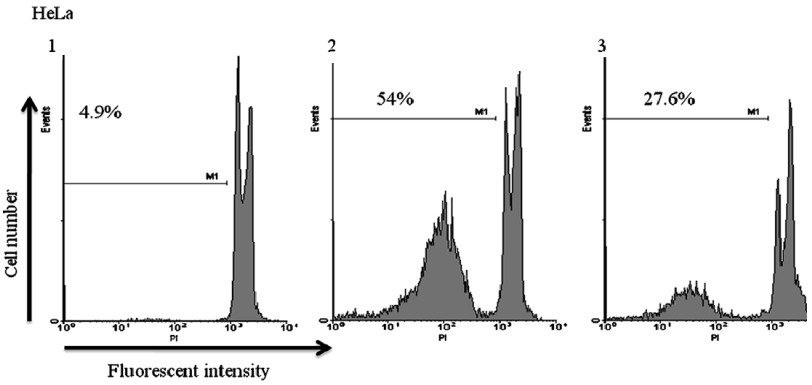

Fig. 5. Flow cytometry histograms of apoptosis assays by PI method in HeLa cells. Cells were treated with 50 lg/mL E1 poly saccaride for 24 h. Sub-G1 peak as an indicative of apoptotic cells, was induced in E1 poly saccaride -treated but not in control cells. 1: control, 2: serum free (positive control), 3: E1 poly saccaride Flow cytometry histogram of positive control in which cells cultured in serum free medium and CH2Cl2 fraction-treated cells, exhibited a sub-G1 peak in HeLa cells. It is indicating involvement of an apoptotic process in E1 poly saccaride -induced cell death.

References

[1] Asker, M.S., Mohamed, S.M., Ali, F.M., El-Sayed, O.H., 2007. Chemical structure and antiviral activity of water-soluble sulfated polysaccharides from Surgassum latifolium. J. Appl. Sci. Res. 3, 1178–1185.[1].

[2] Josephine, A., Veena, C.K., Amudha, G., Preetha, S.P., Sundarapandian, R.,Varalakshmi, P., 2007. Sulphated polysaccharides: new insight in theprevention of cyclosporine A-induced glomerular injury. Basic Clin. Pharmacol. Toxicol. 101, 9–15.[2]

[3] Raghavendran, H.B., Sathivel, A., Yogeeta, R.S., Devaki, T., 2007. Efficacy of Sargassumpolycystum Phaeophyceae sulphated polysaccharide against paracetamolinduced DNA fragmentation and modulation of membrane-bound phosphatases during toxic hepatitis. Clin. Exp. Pharmacol. Physiol. 34, 142–147.
[4] Jung, M., Jang, K.H., Kim, B., Lee, B.H., Choi, B.W., Oh, K.B., Shin, J., 2008.Meroditerpenoids from the brown alga Sargassum siliquastrum. J. Nat. Prod. 71, 1714–1719.
[5] Zhu, W., Chiu, L.C., Ooi, V.E., Chan, P.K., Ang Jr., P.O., 2006. Antiviral property and mechanisms of a sulphated polysaccharide from the brown alga Sargassum patens against Herpes simplex virus type 1. Phytomedicine 13, 695–701.
[6] Dias, P.F., Siqueira Jr., J.M., Maraschin, M., Ferreira, A.G., Gagliardi, A.R., Ribeiro-do- Valle, R.M., 2008. A polysaccharide isolated from the brown seaweed Sargassum stenophyllum exerts antivasculogenic effects evidenced by modified morphogenesis. Microvasc. Res. 751 (January), 34–44.
[7] Chang, H.W., Jang, K.H., Lee, D., Kang, H.R., Kim, T.Y., Lee, B.H., Choi, B.W., Kim, S., Shin, J., 2008. Monoglycerides from the brown alga Sargassum sagamianum: isolation, synthesis, and biological activity. Bioorg. Med. Chem. Lett. 1812 (June), 3589–3592.
[8] Athukorala, Y., Lee, K.W., Kim, S.K., Jeon, Y.J., 2007. Anticoagulant activity of marine green and brown algae collected from Jeju Island in Korea. Bioresour. Technol. 98, 1711–1716.
[9] Zhu, W., Chiu, M.C.L., Ooi, V.E.C., Chan, P.K.S., Angjr, O.P., 2004. Antiviral property and mode of action of a sulfated polysaccharide from Sargassum platens against herpes simplex virus type 2. Int. J. Antimicrob. Agents 24, 18–25.
[10] Dias, P.F., Siqueira Jr., J.M., Maraschin, M., Ferreira, A.G., Gagliardi, A.R., Ribeiro-do- Valle, R.M., 2008. A polysaccharide isolated from the brown seaweed Sargassum stenophyllum exerts antivasculogenic effects evidenced by modified morphogenesis. Microvasc. Res. 751 (January), 34–44.
[11] Zhu, W., Chiu, M.C.L., Ooi, V.E.C., Chan, P.K.S., Angjr, O.P., 2004. Antiviral property and mode of action of a sulfated polysaccharide from Sargassum platens against herpes simplex virus type 2. Int. J. Antimicrob. Agents 24, 18–25.
[12] Yan, X., Chuda, Y., Suzuki, M., Nagata, T., 1999. Fucoxanthin as the major antioxidant in Hijikia fusiformis, a common edible seaweed. Biosci. Biotechnol. Biochem. 63, 605–607.
[13] Asker, M.S., Mohamed, S.M., Ali, F.M., El-Sayed, O.H., 2007. Chemical structure and antiviral activity of water-soluble sulfated polysaccharides from Surgassum latifolium. J. Appl. Sci. Res. 3, 1178–1185. [13].
[14] Theisen, C., 2001. Chemoprevention: what's in a name? J. Natl. Cancer Inst. 93, 743– 774.
[15] Zhuang, C., Itoh, H., Mizuno, T., Ito, H., 2006. Antitumor active fucoidan from the brown seaweed, umitoranoo (Sargassum thunbergii). Biosci. Biotechnol. Biochem. 59, 563–567.
[16] Moon, E.Y., 2008. Serum deprivation enhances apoptotic cell death by increasing mitochondrial enzyme activity. Biomol. Ther. 16, 1–8.
[17] Cragg, G.M., Newmann, D.J., Snader, K.M., 1997. Natural products in drug discovery and development. J. Nat. Prod. 60, 52–60.
[18] Valeriote, F., Grieshaber, C.K., Media, J., Pietraszkewlcz, H., Hoffmann, J., Pan, M., McLaughlin, S., 2002. Discovery and development of anticancer agents from plants. J. Exp. Ther. Oncol. 2, 228–236.
[19] Sengupta, S., Toh, S.A., Sellers, L.A., Skepper, J.N., Koolwijk, P., Leung, H.W., Yeung, H.W., Wong, R.N., Sasisekharan, R., Fan, T.P., 2004. Modulating angiogenesis, the yin and the yang in ginseng. Circulation 110, 1219–1225.
[20] Otsuka, H., 2006. Purification by solvent extraction using partition coefficient. In: Sarker, S.D., Latif, Z., Gray, A.L. (Eds.), Natural Products Isolation, second ed. Humana Press, Totowa, New Jersey, pp. 269–273.[20]
[21] Eloff, J.N., 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants? J. Ethnopharmacol. 60, 1–8.
[22] Wyllie, A.H., 1997. Apoptosis, an overview. Br. Med. Bull. 53, 451–465. Yu, J., Liu, H., Lei, J., Tan, W., Hu, X., Zou, G., 2007. Antitumor activity of chloroform fraction of Scutellaria barbata and its active constituents. Phytother. Res. 21,
[23] 817–822.
[24] Broker, L.E., Kruyt, F.A., Giaccone, G., 2005. Cell death independent of caspases, a review. Clin. Cancer Res. 11, 3155–3162]
[25] Mousavi, S.H., Tavakkol-Afshari, J., Brook, A., Jafari-Anarkooli, I., 2009. Role of caspases and Bax protein in saffron-induced apoptosis in MCF-7 cells. Food Chem. Toxicol. 47 (8), 1909–1913.
[26] Brohem, C.A., Sawada, T.C.H., Massaro, R.R., Almeida, R.L., Rivelli, D.P., Ropke, C.D., da Silva, V.V., de Lima, T.M., Curi, R., Barros, S.B.M., Maria-Engler, S.S., 2009. Apoptosis induction by 4-nerolidylcatechol in melanoma cell lines. Toxicol. In Vitro 23, 111–119.