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Research Article

FUCOSE-CONTAINING SULFATED POLYSACCHARIDES FROM *SARGASSUM WIGHT II:* EXTRACTION TECHNOLOGY AND ANTICANCER ACTIVITY ASSESSMENT

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ABSTRACT

Marine seaweed that is washed up on the coastline is a nuisance as its degradation produces a foul a smell and generates waste problems. Exploitation of coastline polluting seaweeds such as *Sargassum* sp., *Ulva* sp., and other beach cast seaweed species for various commercial applications will generate new valuable products that may help lessen coastal pollution by seaweeds and create new seaweed based resources. Thus, utilization of these natural resources is of great importance. The objectives of this study were to develop a technology to extract bioactive compounds from brown seaweeds, and investigate their bioactivity. The extraction of fucose containing sulfated polysaccharides (FCSPs) and/or crude fucoidan from *Sargassum wightii* was performed, and the bioactivity of the isolated FCSPs was investigated. Fucoidan is a term used to describe a class of sulfated polysaccharides extracted from brown seaweed, which contains substantial amounts of fucose; varying amounts of galactose, xylose, and glucuronic acid; and differing glycosidic linkages, and are variously substituted with sulfate and acetyl groups and side branches containing fucose or other glycosyl units. Thus, seaweed can be a potential source of biomass and bioactive compound notably FCSPs. This study proved the hypotheses that different extraction conditions have crucial influenced to the chemical nature of FCSPs. The study also demonstrated that unfractionated FCSPs are able to exert bioactive actions such as anticancer and immune modulating properties in *invitro* studies performed using HCT 116 (Human Colon Tumor cell line).

Keywords: *Sargassum wightii*, Anticancer, Fucoidan, HCT 116.

INTRODUCTION

Seaweed that washes up on the coastline often generates waste problems for populations residing seaside owing to microbial accumulation and unpleasant odors. Utilization of beach cast seaweeds such as *Ulva* sp., *Sargassum* sp., and other nuisance seaweed species for advantageous applications may alleviate these problems and create valuable seaweed based products. The brown seaweeds are usually found in tidal splash zones or rock pools, and certain species such as Sargassum are found floating on the shorelines. Brown algae are typically used for the production of alginate, which is commercially used as an ingredient for different industrial, biotechnology, and food applications.

Some seaweed species could be potential sources of functional dietary fiber and polysaccharides with bioactive properties (Lahaye 1991). Recent developments in seaweed utilization include applications involving naturally derived seaweed extracts and bioactive compounds such as fucose containing sulfated polysaccharides (FCSPs) and/or fucoidan in some cosmetic products and food supplements. Hiroomi Funahashi *et al.*, (1999) investigated the chemo preventive effects of Wakame seaweed on breast cancer and reported a suppressive effect on the proliferation of DMBA induced rat mammary tumors.

Fucoidan is a term used to described a class of sulfated polysaccharide extracted from the

seaweed class Phaeophyceae, which consist almost entirely of fucose and ester sulfate (Percival and McDowell 1950). In addition to fucose, different types of FCSPs may also contain galactose, mannose, xylose, glucose, and/or glucuronic acid, usually in minor amounts (Bilan and Usov, 2008).

Extraction using dilute acetic acid and subsequent purification was first performed by Kylin (1915) to isolate the substance from various species of Laminaria and Fucus. A parallel report was published that year by Hoagland and Lieb (1915), who isolated another water soluble polysaccharide that was closely related to, fucoidan from *Macrocystis pyrifera* and was shown to contain L-fucose and a high proportion of calcium and sulfate. Nelson and Cretcher (1931) extracted fucoidan from *Macrocystis pyrifera* by repeated extended (48 hours) extraction with dilute HCl followed by FCSP isolation by ethanol precipitation and revealed the presence of sulfate in the form of ester groupings in the precipitated product. Although differently extracted and purified FCSPs have been reported to exert bioactivity (Holtkamp *et al*., 2009), unfractionated FCSPs has also been found to reduce cell proliferation of lung carcinoma and melanoma cells, exert immunopotentiating effects in tumor bearing animals, and to activate natural killer (NK) cells in mice, leading to antitumor activity efficacy (Takahashi, 1983; Ale *et al*., 2011; Foley *et al*., 2011). Kim *et al.* (2010) applied a crude polysaccharide composed predominantly of sulfated fucose from *F. vesiculosus* to human colon cancer cells *in vitro* and concluded that this polysaccharide from brown seaweed induces apoptosis. Moreover, commercially available crude fucoidan was tested on human lymphoma HS Sultan cell lines and was found to inhibit proliferation and induce apoptosis by activating caspase -3 (Aisa et al., 2005). It was reported recently that FCSPs from *Sargassum sp.* and crude fucoidan from F. vesiculosus induced apoptosis in melanoma cells (Ale et al., 2011).

MATERIALS AND METHODS

Collection of Seaweeds

The seaweed *Sargassum wightii* was collected from Vedalai region near Rameswaram, Tamil Nadu and was isolated cleaned from epiphytes, extraneous matters. Sample was collected in sterilized container, and transported to the laboratory. Sample was washed thoroughly with sea water then sterile distilled water, air dried, cut into small pieces and then ground until a fine powder is obtained.

Extraction of FCSPs from *S.wightii*

The Sargassum FCSP (Fucose-containing sulfated polysaccharides) product used was extracted from *S. wightii* by single-step extraction. The dried seaweed was ground and filtered. 100 g of dried ground seaweed was extracted in 2 L of 0.03 M HCl with continuous stirring for 4 hours at 90˚C water bath. The suspended seaweed was filtered, and the extract was precipitated using 60% ethanol, then centrifuged at 10,600 rpm for 10 minutes, and the resulting pellet was dried. This dried pellet constituted the fucose-containing sulfated polysaccharides. The pulverized moisture free FCSP sample was extracted with 200 mL of ethanol and condensed in a rotary evaporator at 40 rpm to remove excess solvent and stored.

Cell Line Maintenance

The cell lines namely HCT 116. The cell was cultured in DMEM medium supplemented with 10% FBS with 100 U/mL streptomycin. Cells were maintained in a humidified atmosphere of 5% CO_2 incubator at 37˚C until the confluency stage was attained. The medium was replaced for every two days and maintained strictly. The cells were dissociated with trypsin phosphate versenal glucose in phosphate buffered saline. The stock cultures were grown in 25 cm^2 tissue culture flasks. The experiments were commenced after confluency was attained.

MTT Assay

Microculture Tetrazolium Assay - Cell viability was observed and cytotoxic index LC50 was calculated. NCIH 460 cells (2.5 × 104 cells/mL) were seeded in seven columns of 96 well microplates and incubated for 24 hours (37˚C, 5% CO2 air humidified). Then 20 µL of prepared concentrations (0.01, 0.10, 1.00, 10.00, 100.00 µg/mL) of extract was added to each column and incubated for next 48 hrs in the same condition. The untreated cells incubated for 48 hrs are specified for control. To evaluate cell survival, 20 µL of MTT solution was added to each well and incubated for four hours. After four hours, carefully the supernatant was removed leaving formazan crystals. 150 µL of DMSO was added to each well. The crystals were dissolved and the absorbance was read at 570 nm. The percentage of growth was calculated by the following formula:

Percentage growth % =

$$(T - T_0)/(C - T_0) \times 100 \quad \text{(when } T > T_0\text{)}$$

Where, T = cells treated with compounds for 48 hrs; T_0 = untreated cells assayed before

compound addition, C = untreated cells incubated for 48 hours (control).

RESULTS AND DISCUSSION

Extraction of FCSPs from *S.wightii*

Fucose – containing Sulphated polysaccharides (FCSPs) were extracted from *S.wightii* and assayed for its anticancer activity.

MTT Assay

The FCSP showed highest rate of cell death at 100 µg/mL and IC50 value was identified to be 80 µg/mL. Thus, confirmed the presence of anticancer activity of the FCSP extracted from *Sargassum wightii.*

Macrophage activation by polysaccharides is mediated through specific membrane receptors. The major receptors reported for polysaccharide recognition in macrophages are glycoproteins including Toll like receptor-4 (TLR-4), cluster of differentiation 14 (CD14), competent receptor-3 (CR-3), and scavenging receptor (Teruya *et al*., 2009). They are mediated by intracellular signaling pathways, and the family of mitogen-activated protein kinases (MAPKs) plays a critical role, notably in the production of nitric oxide, which can lyse tumors (Teruya *et al*., 2009). MAPK family members such as p38 MAPK, extracellularly regulated kinase, and stress activated protein kinase/c N-terminal kinase play an important role in the activation of macrophages by polysaccharides such as FCSPs (Teruya *et al*., 2009). Activated MAPKs lead to activation of transcription factors resulting in induction of various genes. Activation of macrophages induces the production of cytokines such as interleukin-12 which in turn stimulate the development of T-cells. T-cells produce IL-12 that in turn activates NK cells proliferation. The NK cells themselves produce immunologically important cytokines, notably IFN (gamma), which can further provoke the participation of macrophages in the stimulation of T-cell via induction of IL-12 (Maruyama *et al*., 2006). The antitumor mechanism of FCSPs appears to be associated with the significant enhancement of the cytolytic activity of NK cells augmented by increased production of the macrophage mediated immune response. The slated NK cell killing occurs via release of granules containing perforin, which opens up pores in target cell membranes through which the granzymes can enter and induce apoptosis (Kindt *et al*., 2007).

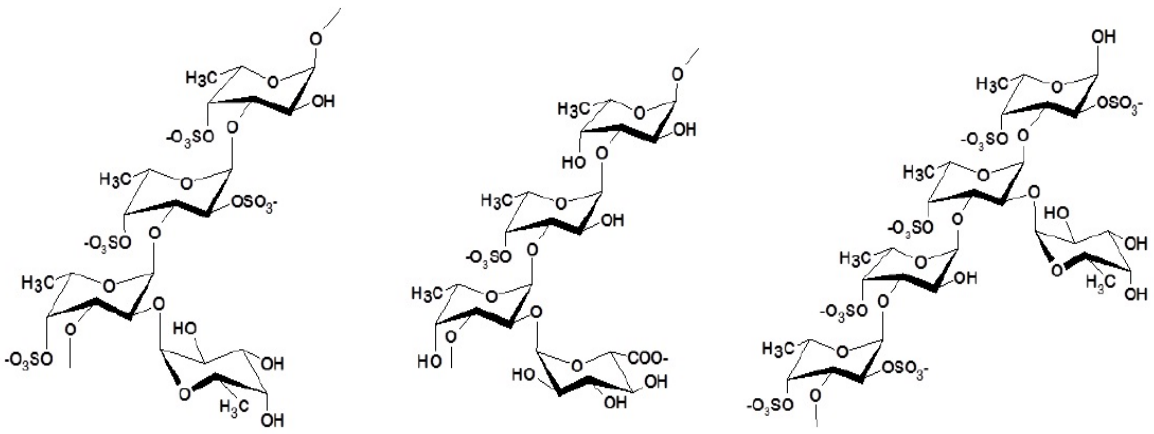
CONCLUSION

Fucoidan—(FCSPs)—are an important group of polysaccharides that show remarkable biological activities. The preservation of the structural integrity of the FCSPs molecules nevertheless appears crucial for maintaining the biological properties and it has been clearly shown that the extraction treatment employed affects the composition and thus the structural features of the FCSPs substances. The diverse structures and varied chemical composition of FCSPs may have hindered the development of an in-depth understanding of the precise properties of significance for specific bioactivity effects. The anticancer bioactivity of fucose-containing sulfated polysaccharides (FCSPs) from *Sargassum wightii* was demonstrated through evaluation of inhibition of HCT116 cells *in vitro*. The structural traits of the FCSPs products were predicted to be complex and to differ among the different FCSPs making it delicate to draw definite conclusions about structural effects and mechanisms.

Table 1: Anti–Cancer activity

Concentration (µg/mL)	Cell Viability (%)
20	83.29
40	72.35
60	60.90
80	47.43
100	28.36

Fig. 1: Brown Seaweeds - *Sargassum wightii*



(a)

Fig. 2: Typical structure of fucoidan (FCSPs) obtained from *S.wightii*

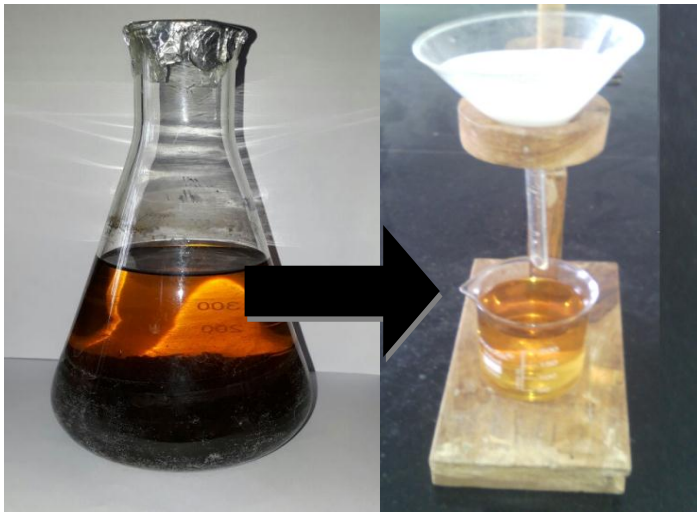


Fig. 3: Stages in FCSP Extraction

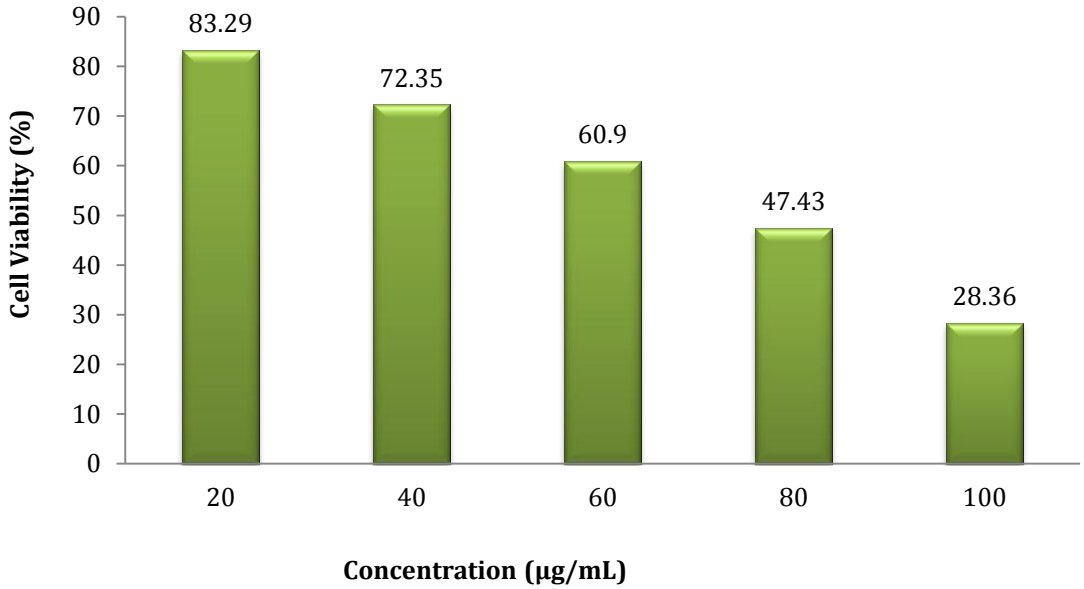


Fig. 4: Anti-cancer activity of FCSP at different concentrations

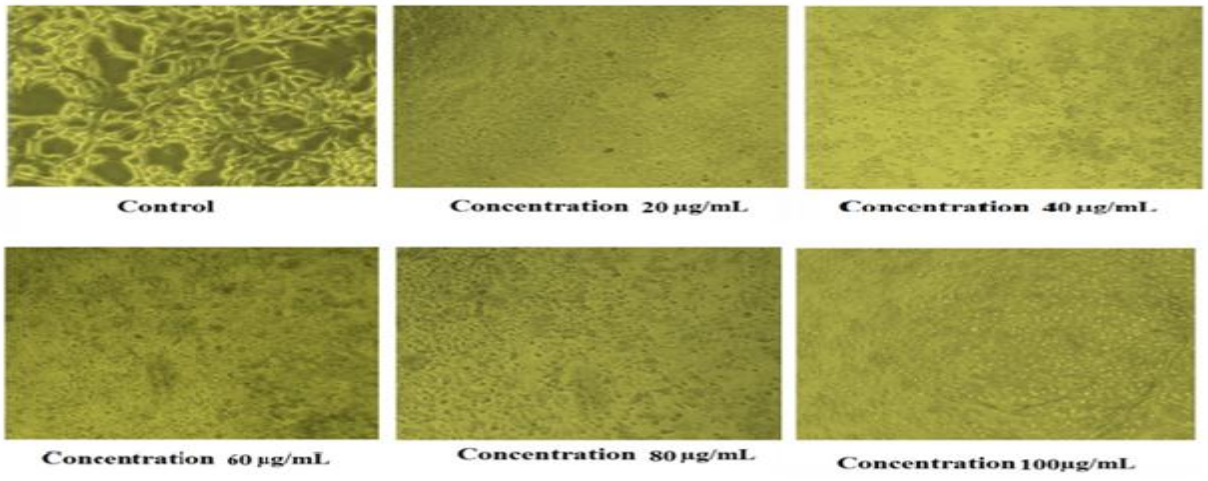


Fig. 5: Photographs of HCT 116 cell line treated with different concentrations of FCSP (*S.wightii*) under inverted microscope (magnification 10X)

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