

Studies on methanol extract of *Sargassum wightii* and its effect on anticancer activity against mammalian cell lines

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ABSTRACT: : Seaweeds have been screened extensively to isolate life saving drugs or biologically active substances all over the world. It is rich in secondary metabolites which include alkaloids, glycosides, flavonoids, saponins, tannins, steroids, related active metabolites which are of great medicinal value and have been extensively used in drug and pharmaceutical industry. In the present study investigated that the quantitative estimation was showed the presence of Flavanoids, Phenols and Alkaloids at Percentage of 20.92, 29.20 and 77.58 respectively in the methanolic extract of *S.wightii*. In MCF-7 cell line minimum cell viability (36.01%) and maximum cell inhibition (63.99%) were shown the methanolic extracts of *S. wightii*. The CTC_{50} values were found to be 138.89 $\mu\text{g/ml}$ in MCF-7, 186.65 $\mu\text{g/ml}$ in PC-3 and 91.33 $\mu\text{g/ml}$ in DLA cell lines. Among this, minimum cell viability was 32.31% noted in the DLA cell line at 200 $\mu\text{g/ml}$ extract concentrations.

Key Words: Seaweeds, medicinal value, methanol extract, MCF-7 cell line and cell viability.

Introduction

Cancer is a complex group of disease that have caused major global health problem, with significant association with death and disability. It arises from a series of mutations, as a result of genetic instability and environmental factors (Al-Hajj *et al.*, 2003). Cancer is caused by external factors, such as tobacco, infectious organisms, and an unhealthy diet, and internal factors, such as inherited genetic mutations, hormones, and immune conditions. These factors may act together or in sequence to cause cancer. Ten or more years often pass between exposure to external factors and detectable cancer. Cancer is one of the major concerns around the world (Parasramka and Gupta, 2012), as it is one of the leading causes of death worldwide. It is a group of disease where it affects all living cells, at all ages and in both genders (Nair, *et al.*, 2005). In 2008, about 12.7 million cases and 7.6 million deaths were caused due to cancer, this has dramatically increased recently. Several treatments to prevent and cure cancer have been done, such as radiotherapy, chemotherapy, or using synthetic medicine. In general, anticancer therapy has been felt to be sufficient to give good results, but it has side effects and the costs are quite (Siswandono *et al.*, 2000).

Therefore, many people have turned to traditional medicines that are considered more safe and economical, because it uses natural ingredients. Natural ingredients contain several active compounds which give pharmacological effects. In general, those active compounds are secondary metabolites (Liu *et al.*, 1998). Secondary metabolites have been known as sources of medical therapy, for example as antibacterial and anticancer medicines, Majority of secondary metabolites are synthesized by organisms to adapt to their environment, thus, the search of secondary metabolites that are able to act as anticancer bioactive mainly focused on organisms that live in extreme environmental condition. One of extreme environments is at tidal area. Organisms which live at tidal area and have been reported to contain anticancer bioactive compounds.

Marine algae are an important source of medicinal products in the marine ecosystem. Particularly seaweeds are directly exposed and are susceptible to ambient microorganisms such as bacteria and fungi. Seaweeds are able to produce a variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with cytostatic, antiviral, anthelmintic, antifungal and antibacterial activities have been detected in green, brown and red algae (Lindequist 2001). On account of this, the present study has been aimed to investigate the phytochemical and pharmacological activities of *Sargassum wightii*.

Materials and Methods

Collection of Algae materials

The fresh algae sample of *Sargassum wightii* was collected randomly from the Rameshwaram sea shore, Ramanathapuram district, Tamil Nadu, India. Algae materials were washed under running tap water and distilled water, air dried and then homogenized to fine powder and stored.

Preparation of extract

Crude sample extract was prepared by Soxhlet extraction method. About 20gm of powdered material was uniformly packed into a thimble and extracted with 250ml of methanol extract. The process of extraction has to be continued for 24 hours or till the solvent in siphon tube of extractor become colourless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40°C till all the solvent got evaporated. Dried extract was kept in refrigerator at 4°C till future use (Udhaya Prakash et al., 2012).

Quantitative phytochemical analysis

Estimation of Alkaloids

Alkaloid was determined by using Harborne (1973) method. 5g of the seaweed powder sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Conc ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Estimation of Flavonoids

Ten grams of seaweed powder was repeatedly extracted with 100ml of 80% aqueous methanol at room temperature. The mixture was then filtered through a filter paper into a pre-weighed 250ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed. The percentage flavonoid was calculated by difference (Dewanto et al., 2002).

Determination of Total phenols

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5 ml of the extract was pipetted into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The plant samples were made up to mark and left to react for 30 min for colour development. This was measured at 505nm (Siddhuraju and Becker, 2003).

In vitro Cytotoxicity Activity

MTT assay

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, coloured formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the cells (Mossman, 1983).

Cell Lines and Culture Medium

MCF-7 (Human, Breast cancer), PC-3 (Human prostate cancer cell line) and DLA (Dalton lymphoma acetic tumour cell line) cell cultures were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in Dulbecco's modified Eagle's medium (DMEM). Medium was supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO₂ at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm² culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of Test Solutions

For cytotoxicity studies, each weighed test samples were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serially two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of Cell Viability by MTT Assays

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10⁵ cells/ml using medium containing 10% FBS and were used for the determination of cell viability by MTT assays. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth

inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC50) values is generated from the dose-response curves for each cell line.

Result

Quantitative analysis

The quantitative analyses of the phytoconstituents were also done in the *S.wightii*. The phytochemicals such as alkaloids, flavonoids and phenols were quantitatively estimated in methanolic extract of *S. wightii* (Fig.1). Methanol extract had 77.58% alkaloid when compared to flavonoids (29.2%) and phenol (20.9%) constituents. The present investigation showed significant variation in the contents like alkaloids, flavonoids and phenols.

In vitro Anti-cancer assay

The test for Cytotoxicity of the methanolic leaf extract of *Sargassum wightii* was conducted using the MCF-7, PC-3 and DLA cell line by using MTT assay. Growth of cells in the presence of the extracts were quantitated by the ability of living cells to reduce the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-terazolium bromide (MTT) to a blue formazan product. Different test concentrations ranging from 10 to 200 µg/ml of the methanol extract was taken to study the cytotoxicity. The anticancer activity of methanol extract was measured using MTT assay.

PC -3 Cell line

PC3 cell lines are used in prostate cancer research. These cells are useful in investigating the biochemical changes in advanced prostatic cancer cells and in assessing their response to chemotherapeutic agents. The present study revealed that the highest percentage of cytotoxic activity against PC-3 cell line was showed the 200 µg/ml concentration of methanolic extracts. The maximum cytotoxicity (52.87 %) and minimum cell viability (47.13 %) were obtained the 200 µg/ml concentration of methanolic extracts (Table.1). For the PC-3 cell line, the 50 % cytotoxicity concentration (CTC₅₀) was found to be 186.65 µg/ml in methanol extract was required to inhibit the cell growth by 50%.

MCF-7 Cell Line

The MCF-7 cell line was derived from the human breast adenocarcinoma cells. In the presence study revealed that, the highest percentage of cytotoxic activity (63.99 %) against in MCF-7 cell line was seen with methanolic extract of *S. wightii* at the concentration of 200 µg/ml (Table.2). Results showed that the reduced cell viability of cancer cell lines with increasing concentrations (10–200 µg/ml) of methanol extract. For the MCF-7 cell line, the CTC₅₀ value was found to be 138.89 µg/ml in methanol extract was required to 50% inhibit the cell growth.

DLA Cell Line

S. wightii was found to be cytotoxic towards DLA cells only at higher concentration (200 µg/ml). MTT assay was based on the reduction of MTT by mitochondrial dehydrogenase to purple formazan product. The maximum cytotoxicity was showed the 67.69 % in the methanolic extract at the concentration of 200µg/ml (Table.3). Results showed that at higher concentrations there is significant cell mortality. The IC₅₀ value of methanolic extract showed the 91.33 µg/ml was required to inhibit the cell growth by 50%. The cytomorphological changes of DLA cell lines at different concentrations involve intracellular suicide program possessing morphological changes like cell shrinkage, oxidative stress, coiling and biochemical response leading to apoptosis. It is quite obvious from the results that the apoptosis rate of DLA cell lines increases with increase in concentration of extracts.

Discussion

Seaweeds also contain a range of unique phytochemicals not present in terrestrial plants. As such, edible seaweeds may be the only relevant dietary source of some of these factors. Seaweeds known as medicinal are rich in secondary metabolites which include alkaloids, phenols, flavonoids, saponins, steroids and related active metabolites are of great medicinal value and have been extensively used in the drug and pharmaceutical industry. Recently, a number of studies have been reported on the phytochemistry of seaweeds across the world (Selvin and Lipton, 2004). Prostate cancer is a leading cause of cancer death, in humans that was related to the metastatic disease caused due to mutations and different gene expressions. PC3 are prostate cancer cell lines that are used extensively in prostate cancer research (Herman *et al.*, 1995). The present study revealed that the highest percentage of cytotoxic activity against PC-3 cell line was showed the 200 µg/ml concentration of methanol extracts (Table.1). Some studies recently evaluated the effect of a brown seaweed (*Sargassum muticum*) methanol extract (SMME) on the proliferation of MCF-7 and MDA-MB-231 breast cancer cell lines (Namvar *et al.*, 2013) by conducting morphological assessments of

apoptosis, caspase assays and chick chorioallantoic membrane (CAM) assays. In the present study, the CTC₅₀ value was found to be 138.89 µg/ml in methanol extract in MCF-7 cell line (Table.2). Anjana *et al.*, (2014) reported that the decrease in the cancer cell number observed in the ethanolic extract of *Sargassum wightii* - treated group cancer animals indicates that the test drug has a significant inhibitory effect on the tumor cell proliferation. Whereas the rest results using methanol extract found to be cytotoxic towards DLA cells only at higher concentration. Results showed that at higher concentrations there is significant cell mortality (Table.3).

Table. 1 In vitro anticancer activity of methanolic extracts of *S. wightii* in PC -3 Cell line

Extract concentration (µg/ml)	OD Value	%CTC	CTC ₅₀ (µg/ml)	Cell Viability (%)
10	0.432	24.60	186.65	75.40
50	0.407	28.97		71.03
100	0.361	36.99		63.01
150	0.323	43.63		56.37
200	0.270	52.87		47.13

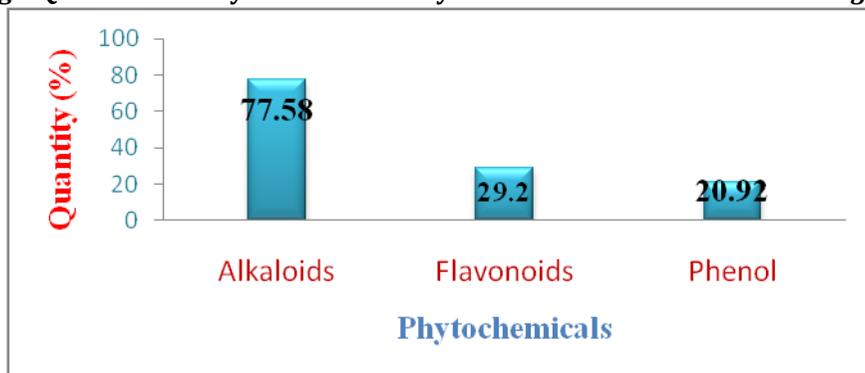
Table. 2 In vitro anticancer activity of methanolic extracts of *S. wightii* in MCF-7 Cell line

Extract concentration (µg/ml)	OD Value	%CTC	CTC ₅₀ (µg/ml)	Cell Viability (%)
10	0.404	16.87	138.89	83.13
50	0.341	29.83		70.17
100	0.283	41.76		58.24
150	0.320	52.67		47.33
200	0.175	63.99		36.01

Table. 3 In vitro anticancer activity of methanolic extracts of *S. wightii* in DLA Cell line

Extract concentration (µg/ml)	OD Value	%CTC	CTC ₅₀ (µg/ml)	Cell Viability (%)
10	0.340	30.04	91.33	69.96
50	0.287	40.94		59.06
100	0.213	56.17		43.83
150	0.164	66.25		33.75
200	0.157	67.69		32.31

Fig.1 Quantitative Phytochemical analysis of methanolic extracts of *S. wightii*



Conclusion

In this research work, *S.wightii* was investigated for anticancer properties. Preliminary analysis showed the presence of phytochemical, antioxidant and anti-microbial properties. Anti-cancer properties were verified using Human Breast Adeno carcinoma, Prostate Cancer cell line and Daltons Lymphoma Ascites cell line. From the above findings it could be concluded that the leaves of methanol extract of *S.wightii* exhibited potent anticancer activity against mammalian cell lines. Further isolation and

purification of bioactive compound from *S.wightii* may reveal the presence of potent novel anticancer agent and also to explore the exact mechanism of action of the anticancer activity.

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