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## Antitumour Activity of *Sargassum wightii* (Greville) Extracts against Dalton's Ascites Lymphoma

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**Abstract:** Seaweeds have been used by mankind as medicine and food for more than 13,000 years. Marine algae are considered to produce a valuable phytoconstituents characterized by a broad spectrum of antitumor activities. The aim of the present study was to explore the effect of different solvent extracts of *Sargassum wightii*, Greville against Dalton's Ascitic Lymphoma (DAL) in Swiss male albino mice. DAL cells were injected intraperitoneally  $1 \times 10^6$  cell to the mice. Two days after cells injection the animals were treated with different solvent extracts of *Sargassum wightii* at dose of  $200 \text{ mg kg}^{-1}$  for 14 days. 5-fluorouracil ( $20 \text{ mg kg}^{-1}$ ) was used as reference drug. On day 11, cancer cell number, packed cell volume, decrease in tumour weight of the mice, increase in life span and hematological parameters were evaluated and compared with the same parameters in control. A significant increase in the life span and a decrease in the cancer cell number and tumour weight were noted in the tumour-induced mice after treatment with the extract. The haematological parameters were also normalized by the ethanolic and chloroform extracts in tumour-induced mice. These observations are suggestive of the protective effect of ethanolic extract of *Sargassum wightii* is comparatively better than other two tested extracts against Dalton's Ascitic Lymphoma (DAL).

**Key words:** *Sargassum wightii*, Dalton's ascitic lymphoma, fucoidan

### INTRODUCTION

Over the past several decades, seaweeds and their extracts have generated an enormous amount of interest in the drug development industry as the fresh source of bioactive compounds with immense therapeutic potential. The exploration of seaweeds for commercial utilization is under extensive investigations since the world is facing the damage of the shortage of conventional herbal medicine. The phytochemicals from marine algae are extensively used in various industries such as food, gelling, stabilizing, confectionary, pharmaceutical, dairy and textile.

Seaweeds are considered to produce a pronounced variety of secondary metabolites characterized by a broad spectrum of pharmacological activities. Compounds with antitumor activity (Gothoskar and Ranadive, 1971), antiviral (Serkedjieva, 2004), antifungal (Tang *et al.*, 2002), antioxidant effects (Xu *et al.*, 2004), antibacterial properties (Bansemir *et al.*, 2006), cytotoxic (Tang *et al.*, 2002), antiradical activity Chakkaravarthy and Kumar

(2009), larvicidal effect (Thangam and Kathiresan, 1991) and inhibit human immunodeficiency virus activity (Sugawara *et al.*, 1989).

The antitumor activity was one of the most important activities in marine drugs and many marine algae and their metabolites have been showed potent cytotoxicity. These metabolites have played an significant role in leading to new phytochemical from algae for antitumor drugs. Xu *et al.* (2004) has reported four species of *Rhodophyta* algae and three species of *Phaeophyta* exhibited cytotoxic effects against KB and HT-29 cancer cell lines in the east of Asia.

Seaweeds belong to a group of plants known as algae. Seaweeds are classified as *Rhodophyta* (red algae), *Phaeophyta* (brown algae) and *Chlorophyta* (green algae) depending on their nutrient and chemical composition (Kuda *et al.*, 2002).

*Sargassum wightii*, Greville (*Sargassaceae*) belonging to the family *Sargassaceae* is an abundant marine brown alga commonly found in the shorelines of India, coast of Taiwan and shore waters of northern

Arabian Sea. It is a macroscopic, multicellular, photosynthetic, non-vascular, pelagic marine species rich (Sumich and Morrissey, 2004) in sulphated polysaccharides that manifest potent free radical scavenging (Park *et al.*, 2005) and antioxidant effects (Xue *et al.*, 2001).

Carbohydrate steroid, flavonoids, phenols, terpenoids and sulphated polysaccharide, a potential natural antioxidant which are not found in land plants property (Chakkaravarthy and Kumar, 2009). In addition to vitamins and minerals, Highest amount of protein and lipid in seaweeds are most adequate for consumption compared to other land vegetables mainly due to their high content in essential amino acid and relatively high level of unsaturated fatty acid (Peter *et al.*, 2005; Lahaye, 1991; Vrillon, 1993). Arunkumar *et al.* (2005) has reported the presence of Sulphoglycerolipid 1-0-palmitoyl-3-0-(6'-sulpho- $\alpha$ -quinovopyranosyl)-glycerol isolated from the methanolic extract of the brown seaweed *Sargassum wightii* inhibited the growth of *Xanthomonas oryzae* pv. *oryzae* which causes bacterial blight of rice.

There are no investigation on the different solvent extracts of *S. wightii* are exposed for its activity against treatment for cancer and the present study was carried out to evaluate the antitumor activity of *S. wightii* against Dalton's Ascitic Lymphoma (DAL) in Swiss mice.

## MATERIALS AND METHODS

**Collection of plant material:** *Sargassum wightii* (Greville) was collected from the gulf of manner, mandapam, Ramanapuram, tamilnadu, India. The plant was identified and authenticated by Dr. K. Eswaran, scientist incharge, Central salt and marine chemicals research chemicals research institute (CSIR), marine algal research station, mandapam, ramanapuram, tamilnadu, india.

The collected seaweeds were then thoroughly washed with sea water followed by distilled water to remove the epiphytic growth and foreign matter. For drying, washed seaweeds were blotted on the blotting paper and spread out at room temperature in shade. The shade dried seaweeds were grounded to coarse powder using tissue blender. The powdered samples were then stored in refrigerator before use.

**Preparation of aqueous extract:** Dried coarse powdered material (250 g) of air dried powder of *S. wightii* Greville was extracted with solvents viz., ethyl acetate, chloroform and ethanol. The extraction was carried out with 2.5 l of each solvent in rotary evaporator at 40°C under reduced pressure for 72 h. After cooling, the resulting materials

were transferred to a watch glass and placed in a desiccators containing calcium chloride. The crude extracts were dissolved in the respective solvents and were subjected for preliminary phytochemical analysis.

**Preliminary phytochemical screening:** Phytochemical screening of the different solvent extracts of *S. wightii* Greville were tested for the identification of phytochemical constituents according to standard procedures by Harborne (1998).

**Experimental animals:** Swiss male albino mice of weighing between 20±5 g were used. The animals were fed with sterile animal chow and water *ad libitum*. The mice were used after acclimatization under controlled conditions of temperature of 24±2°C, humidity of 50±5% and 10-12 h of light and dark cycles, respectively for one week. The toxicity and anti-tumour experimental study were conducted after obtaining the approval of Institutional Animal Ethical Committee, Padmavathi College of Pharmacy. Animal experiments were performed in accordance with the principles of good laboratory practices and CPCSEA guidelines of the Government of India.

**Acute oral toxicity study:** Acute oral toxicity was performed by following OECD guideline-420 fixed dose procedure for ethyl acetate, chloroform and ethanol extract of *S. wightii* Greville and it was found that dose increasing up to 2000 mg kg<sup>-1</sup> body weight, shown no toxicity or mortality in swiss male albino mice. The LD<sub>50</sub> of each solvent extract of *S. wightii* as per OECD guidelines 420 is greater than 2000 mg kg<sup>-1</sup>.

## Animal experimental design

**Tumour cell lines:** Dalton's Ascitic lymphoma (DAL) was obtained through the courtesy of the Amala cancer research centre, amala nagar, Trissur, kerala. DAL cells were maintained by weekly intraperitoneal (i.p.) inoculation of 1×10<sup>6</sup> cells/mL/mouse.

**Determination of antitumour activity:** Swiss male albino mice (20±5 g) were randomly placed into six groups each of six animals and housed in separate cages. All the groups except group I were injected with DAL Cells (1×10<sup>6</sup> cells/mL/mouse.i.p.). This was taken as day 0. Group I served as normal saline control (5 mL kg<sup>-1</sup>, p.o.) and Group II served as DAL control. On day 1, the Ethyl Acetate (EA), Chloroform (CF) and Ethanol (EL) extract of *S. wightii* at a dose of 200 mg kg<sup>-1</sup> (Gp-III to V) were administered orally and 20 mg kg<sup>-1</sup> of 5-Fluorouracil (5-FU) treated group (Gp VI) of six each and continued for

14 consecutive days. Group I was reserved as solvent control, it was not treated with any extract but only with saline. On day 15, mice were sacrificed 24 h after the last dose (Gothoskar and Ranadive, 1971).

**Determination of tumor cell count:** The ascitic fluid was taken in a RBC pipette and diluted 1000 times. Then a drop of the diluted cell suspension was placed on the Neubauer counting chamber and the number of cells in 64 small squares was counted.

**Estimation of viable tumor cell count:** The cells were then stained with Trypan blue (0.4% in normal saline) dye. The cells that did not take up the dye were viable and those that took the stain were nonviable. These viable and nonviable cells were counted:

$$\text{Cell count} = \frac{(\text{No. of cells} \times \text{dilution})}{(\text{Area} \times \text{Thickness of liquid film})}$$

**Percentage increase life span:** The mortality was monitored by recording the effect of different extracts of *S. wightii* on tumor growth and percentage increase in life span were calculated (Sur and Ganguly, 1994).

**Hematological studies:** The effect of different solvent extract of *S. wightii* on RBC, WBC counts and hemoglobin were done by standard procedures from freely flowing tail vein blood (Price and Greenfield, 1958).

**Statistical analysis:** Results were subjected to one-way ANOVA.  $p < 0.05$  was considered significant. The post hoc analysis was carried out by Dunnet's multiple comparison test.

## RESULTS

**Acute oral toxicity study:** Different solvent extract of *S. wightii* were studied for oral acute toxicity at dose of 2000 mg kg<sup>-1</sup>. The extract was found devoid of neither mortality of the animals nor any visible clinical signs of general weakness in the animals. Hence, 2000 mg kg<sup>-1</sup> was considered as LD<sub>50</sub> cut-off value. So the dose of 200 mg kg<sup>-1</sup> (1/10th of 2000 mg kg<sup>-1</sup>) was selected for ethyl acetate, chloroform and ethanol extract of *S. wightii* based on fixed dose method of OECD guidelines.

**Phytochemical screening:** The results from the preliminary qualitative phytochemical analysis of the *S. wightii* extracts revealed the presence of protein, aminoacids, phenolic compounds, flavonoids in three solvent extracts.

Table 1: Preliminary phytochemical analysis of crude extracts of *S. wightii*

Test	Ethyl acetate Extract	Chloroform extract	Ethanol extract
Carbohydrates	-	+	+
Proteins and Amino acids	+	+	+
Steroids	+	-	+
Glycosides	+	-	+
Alkaloids	+	+	+
Phenolic compounds	+	+	+
Flavonoids	+	+	+
Tannins	-	-	+
Saponins	-	-	+
Terpenoids	-	-	-
Mucilage	-	-	-

+: Present, -: Absent

Carbohydrates, alkaloids, tannins and saponins are present only in ethanol and chloroform extract of *S. wightii*. Steroids and glycosides present only in ethanol and ethyl acetate extract of *S. wightii*. Tannins and saponins are present only in ethanol extract (Table 1).

**Antitumor activity:** The intraperitoneal inoculation of DAL cells in the mice produces increased proliferation of cells in group II. In the case of tumor growth response study, treatment with 200 mg kg<sup>-1</sup> of ethanol and chloroform extract of *S. wightii* showed significant ( $p < 0.001$ ) reduction in tumor volume (Table 2).

The effect of different extracts of *S. wightii* on life span, viable cell count and nonviable cell count were depicted in Table 2. It revealed that there was increase in mean survival time. Administration of different extracts appreciably decreases the viable cell count compared to DAL bearing mice.

Ethanol and chloroform extract of *S. wightii* treated mice survived upto 35.67±2.4 and 27.89±0.85 days, respectively whereas the DAL control mice survived upto 20.74±1.23 only. The percentage increase in lifespan of ethanol and chloroform extract treated mice increased by 71.98 and 34.47%, respectively (Table 2). Ethanol extract of *S. wightii* treatment reduces the tumour volume to greater extent compared with other two extracts treatment.

In case of the haematological parameters, DAL control mice showed reduced RBC count but increase in WBC count than normal group. The treatment with ethanol and chloroform extract also reduced the RBC count significantly to 12.83±1.09 10<sup>6</sup> mm<sup>-3</sup> and 12.27±0.76 10<sup>6</sup> mm<sup>-3</sup>, respectively. Similarly both extracts restored the WBC value to 16.57±1.07 10<sup>6</sup> mm<sup>-3</sup> and 18.27±0.92 10<sup>6</sup> mm<sup>-3</sup>, respectively. Haemoglobin content in DAL control mice decreased significantly when compared with normal group. But the ethanol and chloroform extract of *S. wightii* treated mice increased haemoglobin content to 18.42±1.05 g% and 19.89±0.76 10.63±0.55 g %, respectively.

Table 2: Effect of different extract of *S. wightii* on tumour volume, viable and non-Viable Cell Count in mice

Treatment group	% decrease in body weight (g)	Tumour volume (mL)	Mean survival time (days)	Viable cell count	Non-viable cell count
Group II: DAL control	8.13±0.35	4.89±0.18	20.74±1.23	9.57±0.48	0.81±0.32
Group III: DAL+EA (200 mg kg <sup>-1</sup> p.o)	7.97±0.24 <sup>ns</sup>	3.79±0.46 <sup>b</sup>	24.29±1.51 <sup>b</sup>	4.78±0.29 <sup>a</sup>	1.88±0.45 <sup>c</sup>
Group IV: DAL+CF (200 mg kg <sup>-1</sup> p.o)	7.22±0.48 <sup>c</sup>	2.86±0.83 <sup>a</sup>	27.89±0.85 <sup>a</sup>	3.16±0.79 <sup>a</sup>	2.16±0.41 <sup>b</sup>
Group V: DAL+EL (400 mg kg <sup>-1</sup> p.o)	6.34±0.71 <sup>a</sup>	1.82±0.27 <sup>a</sup>	35.67±2.4 <sup>a</sup>	2.89±0.44 <sup>a</sup>	4.38±0.68 <sup>a</sup>
Group VI: DAL+5-FU	5.82±0.63 <sup>a</sup>	1.23±0.58 <sup>a</sup>	41.78±1.69 <sup>a</sup>	2.46±0.65 <sup>a</sup>	5.49±0.82 <sup>a</sup>
Standard drug					

Values are expressed as the Mean±SEM, (n = 6) on day 14 of the experiment; Statistical significance (p) calculate by one way ANOVA followed by Dunnett's <sup>a</sup>p<0.001, <sup>b</sup>p<0.01, <sup>c</sup>p<0.05, NS: Non significant calculated by comparing treated group with DAL control

Table 3: Effect of different extract of *S. wightii* on haematological parameter in mice

Treatment group	Haemoglobin Hb (g %)	Red blood cells (10 <sup>6</sup> mm <sup>-3</sup> )	WBC Count (10 <sup>3</sup> mm <sup>-3</sup> )	Lymphocytes (%)
Group I: Normal control	16.22±0.34	10.26±0.34	11.23±0.73	71.84±2.34
Group II: DAL control	22.67±0.87	14.66±0.86	15.44±0.67	48.12±1.88
Group III: DAL+EA	19.07±1.12 <sup>a</sup>	13.08±0.46 <sup>b</sup>	17.83±1.21 <sup>a</sup>	52.73±3.67 <sup>ns</sup>
Group IV: DAL+CF	19.89±0.76 <sup>a</sup>	12.27±0.76 <sup>c</sup>	18.27±0.92 <sup>a</sup>	58.97±1.68 <sup>a</sup>
Group V: DAL+EL	18.42±1.05 <sup>a</sup>	12.83±1.09 <sup>b</sup>	16.57±1.07 <sup>a</sup>	62.31±2.72 <sup>a</sup>
Group VI: DAL+5-FU standard drug	17.48±0.58 <sup>a</sup>	11.48±0.58 <sup>a</sup>	11.23±0.73 <sup>a</sup>	68.75±1.79 <sup>a</sup>

Values are expressed as the mean±SEM, (n = 6) on day 14 of the experiment; Statistical significance (P) calculated by one way ANOVA followed by Dunnett's <sup>a</sup>p<0.001, <sup>b</sup>p<0.01, <sup>c</sup>p<0.05, NS: Non significant calculated by comparing treated group with DAL control

Table 3 showed that haematological parameters of tumour bearing mice on day 15 were found to be significantly different as compared to the different extracts of *S. wightii* treated groups. In tumour bearing mice, it was found that there was increase in WBC count and decrease in Haemoglobin content and RBC count.

In the current investigation, intraperitoneal inoculation of DAL cells in the mice produced an enormous increase in the cancer cell count, which indicated that there is progression of cancer in the animals. The decrease in the cancer cell number observed in the ethanolic extract treated (i.e., Group III and IV) animals proves that the tested extract is having significant inhibitory effect on the tumour cell proliferation.

The increase in tumour weight of Group II animals may be due to accumulation of peritoneal fluid as an abnormal enlargement of peritoneal cavity was observed in tumour-induced mice. Treatment with ethanolic extract of *S. wightii* treated mice reduced the tumour weight and hence increased the lifespan.

## DISCUSSION

When DAL is induced in animals, the cancer cell count in the peritoneal fluid has been used as the standard marker to confirm the proliferation of cells. In this study, increased cell count after 10 days confirmed the proliferation of cells in the group II control group animals.

The ethyl acetate, chloroform and ethanol extracts of *S. wightii* treated animals at the doses of 200 mg kg<sup>-1</sup> (group III, IV, VI) and 5-Fluorouracil (5-FU) at the dose of 20 mg kg<sup>-1</sup> (group VII) significantly inhibited the tumor

volume, tumour cell count and restore back the haematological parameters to more or less normal levels.

Myelosuppression and anaemia is the foremost difficulties encountered in the cancer chemotherapy (Price and Greenfield, 1958; Hoagland, 1982). But the results have evidently shown that all three extracts of *S. wightii* have restored back haemoglobin content and RBC count to normal. After 14 days of transplantation, ethanolic and chloroform extracts treated groups III and IV were able to reverse the changes in the haematological parameters to a greater extent consequential to tumour inoculation. An increase in RBC count and a decrease in elevated WBC count were reported as confirmatory indicators for the protection against DAL (Hoagland, 1982). However from the above observations on other hematological parameters it can be concluded that the *S. wightii* extracts possesses activity against DAL.

Different *S. wightii* extracts treatment was found to enhance nonviable cell counts in peritoneal exudates and decrease the viable cell count. It might be due to the absorption of ethanolic extract by viable cells which leads to lysis of cell through to the activation of macrophages or some cytokine production in peritoneal cavity. Viable cell count of the tumour bearing mice was significantly decreased while non-viable cell count were increased in ethanolic and chloroform extract treated groups when compared with DAL treated group.

Ascetic fluid is the direct nutritional source for tumour cells and a quick increase in ascetic fluid with tumour development would be a means to meet the nutritional requirement of tumour cells (Prasad and Giri, 1974). Prolongation of life span of animals is the trustworthy standard criteria for judging the potency of

anticancer drug (Clarkson and Burchenal, 1965). It can be inferred that extracts of *S. wightii* increased the life span of DAL bearing mice may be due to decrease the ascetic fluid volume and delay the cell division (Sur and Ganguly, 1994).

The phytochemical study indicated the presence of flavonoids, alkaloids and terpenoids in ethanol extract. Furthermore, flavonoids have a chemopreventive role in cancer through their effects on signal transduction in cell proliferation (Weber *et al.*, 1996) and angiogenesis (Fotsis *et al.*, 1997).

Thus, improved antitumour effect produced by the ethanol extract of *S. wightii* may be due to presence of Fucoidan, sulfated polysaccharides and flavonoids as well as its antioxidant potential (Zhuang *et al.*, 1995). Water-soluble form of Fucoidan has been reported in treatment of cholangio carcinoma and gallbladder carcinoma cell lines and findings indicate that the mechanisms of fucoidan action include the induction of apoptosis and the inhibition of the cell cycle (Fukahori *et al.*, 2008).

## CONCLUSION

Thus, anti-tumour effect produced by the both the extracts of *S. wightii* may be due to its flavonoids as well as its antioxidant potential. The ethanolic extract of *S. wightii* restore the mean survival time, decrease tumor volume count in treated mice. Thus present investigation suggests that ethanol and chloroform extracts of *S. wightii* possess potent anticancer activity against Dalton's Ascitic Lymphoma.

Therefore in conclusion, the present investigation showed a decrease in cancer cell count, tumour volume, RBC count as a confirmatory evidence for protection against DAL. Consequently increased WBC count, life span, haemoglobin content were observed with all three extracts of *S. wightii* treated mice.

Further studies to characterize the active principle and elucidate the mechanism of action of ethanolic extract of *S. wightii* are in progress using different animal model and cell lines. All these data point to the possibility of developing an ethanolic extract of *S. wightii* as a novel, potential phytochemical in the field of cancer management.

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