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Golden Research Thoughts

STUDIES ON BIOLOGICAL COMPOUNDS AND THEIR ANTICANCEROUS ACTIVITY OF *SARGASSUM WIGHTII GREVILLE*

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ABSTRACT

The macroalgae *Sargassum wightii Greville* was collected from Mandabam - Rameswaram, Coastal area of Tamil Nadu, India. The biochemical parameter includes carbohydrate, protein and lipids were determined, followed by biopigment analysis for chl a and chl b was estimated. Fatty acid methyl esters were analyzed using gas chromatography mass spectrometry (GC-MS) and the compounds for FAME were resulted. Fourier transform infra-red spectroscopy (FT-IR) was studied and shows the presence of alcohols, Phenols, amines, amides, aromatics, esters, alkanes and alkenes confirms the presence of hydrocarbons from the FAME. The antiproliferative activity of the macroalgae *S.wightii* was analyzed by using the Methylthiazolyl diphenyl- Tetrazolium bromide (MTT) assay with ethanol and chloroform extracts.

KEYWORDS :*Sargassum wightii Greville*, Biochemical, Biopigment, Lung cancer, MTT assay.

INTRODUCTION

Algae are unicellular or multicellular organisms that photosynthesize, but lack the features of leaves, roots, seeds and flowers of the 'higher' vascular plants (mosses, flowering plants, liverwort etc). Macroalgae are commonly known as "seaweed" and are known as "phytoplanktons" which are macroscopic is a multicellular organism (Munro *et. al.*, 1999). The holdfast has an appearance similar to

the roots of plants but nutrients are absorbed by the entire macroalga from the source of water. Sea weeds or marine algae are potentially prolific sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents (Chanda et. al., 2010).

Sargassum (Family Sargassaceae, Order Fucales) represents the most common species of brown macroalgae in tropical to warm temperate waters (Guiry *et. al.*, 2013). It is the most diverse genus of marine macrophytes with more than 130 described species (Xie *et al*. 2013), with 28 species in Korea (Hwang *et al*. 2006). It provides food, alginates, feed, and bioactive compounds for people who harvest or culture *sargassum* (Belleme and Belleme 2007, Zhao et al. 2008, Xie *et al.* 2013). It is widely distributed on the southern coasts of Tamil Nadu, India and many parts of Asia. (subbarao et al 2006). The distribution of marine macroalgae from four southern districts Kanyakumari, Thiruneveli, Tuticorin and Ramanathapuram of Tamil Nadu (Indian Journal of Geo-Marine Sciences, 2014).

Gas Chromatography (GC) and Mass Spectrometry (MS) is a combination of two different analytical techniques, is used to analyze complex organic and biochemical mixtures (Skoog *et al.*, 2007). The gas chromatography portion separates volatile and semi-volatile compounds with great resolution, but it cannot identify them. Mass Spectrometry can provide detailed structural information on most compounds such that they can be exactly identified and quantified, but it cannot readily separate them. (Oregon State University, 2012).

Fourier Transform Infra-Red spectroscopy (FT-IR) has wide applicability in structure elucidation, which are either synthesized chemically or of natural origin (Sawant and Baravkar, 2011). Infrared absorption spectroscopy is the study of interaction of infrared radiation with matter as a function of photon frequency. Fourier Transform Infrared Spectroscopy (FTIR) provides specific information about the vibration and rotation of the chemical bonding and molecular structures, making it useful for analyzing organic materials and certain inorganic materials.

Cancer is the uncontrolled growth of abnormal cells in the body. Lung cancer tends to spread or metastasize very early after it forms, it is a very life-threatening cancer and one of the most difficult cancers to treat. The principal function of the lungs is to exchange gases between the air we breathe and the blood. Through the lung, carbon dioxide is removed from the bloodstream and oxygen from inspired air enters the bloodstream. Lung cancers can arise in any part of the lung, but 90%-95% of cancers of the lung are thought to arise from the epithelial cells, the cells lining the larger and smaller airways (bronchi and bronchioles); also called as bronchogenic cancers or bronchogenic carcinomas. The incidence of lung cancer is strongly correlated with cigarette smoking, with about 90% of lung cancers arising as a result of tobacco use. Radon gas is a known cause of lung cancer, with an estimated 12% of lung-cancer deaths attributable to radon gas.

MATERIALS AND METHODS

Collection and Authentication of Sample

The algal samples were collected from coastal region of Mandabam, Rameswaram - Tamil Nadu, and it was identified as *Sargassum wightii Greville*, with the help of Seaweed Manual – National Institute of Oceanography. The specimens were authenticated by Prof. S. Elumalai, Department of Biotechnology, University of Madras, Chennai, India, and Prof. M. Baluswami, Professor and Head, Department of Plant Biology and Plant Biotechnology, Madras Christian College, Tambaram, India.

Preparation Of Crude Extract

The Sample was shade dried and was powdered with the help of mechanical pulverizer. The powdered material was then soaked in 1000 mL of Chloroform and ethanol. The extract was suction

filtered using Whatmann filter paper. This was repeated for 2 to 3 times and similar extracts were pooled together and concentrated at 40 to 45˚C under reduced pressure using vacuum rotary evaporator. The concentrated crude ethanolic extract was subjected to Gas chromatography and Mass spectrometry (GC-MS), Fourier Transform Infrared spectroscopy (FT-IR) and Chloroform and ethanolic extract was performed to carry out its biological activity (Anticancer). (Harborne, 1998; Raaman, 2006). Gas Chromatography and Mass Spectrometry (Gc- Ms) Analysis of Sargassum wightii Greville

About 1 mL of reagent 1 was poured to the fresh algal samples and vortexed for 5-10 sec. using a cyclomixer. The mixture was incubated in a water bath at 100°C for 5 min. and again vortexed for 5-10 sec. Then after incubated at 100°C for 25 min. reagent 2 was added and vortexed for 5-10 min. followed by thermal incubation at 80°C and rapidly cooled down to 4°C. Reagent 3 was added the mixture and mixed gently up to 10 min. Two phases were seen blatantly from which, the lower phase was conserved. To the content 3 mL of reagent 4 was added followed by mixing for 5 min. and obtained upper phase was removed. The lower phase rich in fatty acid methyl esters were stored in a vial at 4°C in a refrigerator.

The Fatty Acid Methyl Esters (FAME) was analyzed with the help of a gas chromatography equipped with flame ionization detector (FID) (Perkin Elmer, USA). A SP-2560 column (100 m × 0.25 mm I. D., 0.20 µm) (Sigma, Germany) along with standard fatty acid Supelco 37 Component FAME mix from Supelco (Bellefonte, PA, USA) was employed. About 5 µl of the sample was injected and the GC conditions were injector temperature: 260°C; Column temperature: 140oC and detector temperature: 260°C. Helium was used as a carrier gas with the flow rate of 1 mL/min. The unknown FAMEs were determined in comparison with the retention times of the standard FAMEs (Supelco) using a mass spectra from NIST library.

Fourier Transform Infrared Spectroscopy (Ft-Ir) Analysis of *Sargassum wightii Greville*

The FAME sample were analyzed under infrared (Perkin Elmer model spectrum – I PC). The FT-IR spectra with the resolution of 4 cm^{-1}, Scan Number: 3 were performed after the evaporation of the lipid fraction on Thalium bromide tablets. The FT-IR spectrums of all the FAME samples were obtained as a percentage of transmission ranged from 450 cm^{-1} to 4000 cm^{-1}.

BIOCHEMICAL ANALYSIS

Estimation of Protein

The protein was estimated by Biurette method (Raymont *et. al* ., 1964). To 5 mg of dried powdered sample, 1mL of distilled water followed by 4mL of biurette reagent were added and incubated for 30 minutes in the room temperature. Then the mixture was centrifuged for 10 minutes at 4000rpm. The supernatant solution was collected and the optical density was measured in a Spectrophotometer at 540 nm.

Estimation of Lipid

The lipid was estimated by using chloroform methanol mixture as described by Folch *et al.* (1957). 10 mg of dried powder sample taken in a test tube, 5 mL of chloroform- methanol (2:1) mixture was added. The mixture was incubated at room temperature for 48hrs after closing the mouth of the test tube with aluminium foil. After the incubation, the mixture was filtered using a filter paper. The filtrate was collected in a 10 mL preweighed beaker, which was kept on a hot plate. The chloroform methanol mixture was evaporated leaving a residue at the bottom of the beaker. The beaker with the residue and the weight of the empty beaker was calculated to know the weight of the lipid present in

the sample.

Estimation of Carbohydrates

The Carbohydrate content was estimated by Anthrone method (Roe, 1955). Seaweed sample was soaked in 80% ethanol and was centrifuged at 4000 rpm. 5mL of anthrone reagent was added to the 0.5 mL of supernatant. The tubes were kept in a boiling water bath for 15 minutes and kept in a dark room for 10 minutes. The developed colour intensity was read in a spectrophotometer at 650 nm. Carbohydrate content was calculated by referring to a standard D-Glucose and the results are expressed as mg/g sugar.

Estimation of Total Chl A and Chl B

The green photosynthetic pigment Chl a and Chl b were estimated by Jeffrey and Humphery (1975) method and its equation given below.

Chl a = 11.93E664 - 1.93E647 μgml^{-1}
Chl b = 20.36E647 - 5.50E664 μgml^{-1}

The absorbance values at 664 and 647 nm were recorded daily for about 16 days using a UV-Vis. Spectrophotometer.

Antiprolifeerative Activity of *Sargassum Wightii Greville* in A549 Cell Lines

Cell Line and Culture

A549 cell line was obtained from National center for cell sciences Pune (NCCS). The cells were maintained in Minimal Essential Medium supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 µg/mL) in a humidified atmosphere of 50 µg/mL CO2 at 37 ˚C.

Reagents

MEM was purchased from Hi Media Laboratories Fetal Bovine Serum (FBS) was purchased from Cistron laboratories Trypsin, methylthiazolyl diphenyl- tetrazolium bromide (MTT) and Dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai, India.

In Vitro assay for Anticancer Activity (MTT Assay) (Mosmann, 1983)

Cells (1×10^5/well) were plated in 24-well plates and incubated in 37^0C with 5% CO_2 condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or MEM without serum. 100µl/well (5mg/mL) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1mL of DMSO was added in all the wells. The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC50) was determined graphically. The % cell viability was calculated using the following formula:

% Cell viability = A570 of treated cells / A570 of control cells × 100

Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

RESULTS

Sampling Sites

The abiotic factors variations in the major and minor constituents of various areas of, Mandabam, Rameshwaram district, Tamil Nadu, India during February. The collected Macro algae are *Sargassum wightii Greville* was collected from Rameswaram where the pH ranges from 7.6 – 8.1(Fig. 1).

Fig 1. *Sargassum wightii Greville*

Identification

The Macroalgae was identified through the manual, **"Seaweed Manual National Institute of Oceanography"** by V. K. Bhargalkar, were the section of specimen was taken and with the help of the above mentioned book, the specimen was identified as *Sargassum wightii Greville* and was authenticated by Dr. S. Elumalai and Dr. M. Baluswami.

Sample Preparation

The sample *Sargassum wightii Greville* was kept in hot air oven for a period of two weeks and was allowed to dry. The dried samples were weighed initially and then it was coarsely powdered with the help of mortar and pestle (Fig. 2).

Fig 2. Sample preparation of *Sargassum wightii Greville.*

Biochemical Analysis of *Sargassum wightii Greville*

In the present study, the protein, carbohydrate and lipid content was observed in seaweed *Sargassum wightii Greville*. Of which the protein content by biurette method was estimated as 16.34±.04% followed by Carbohydrate content of *Sargassum wightii Greville* by Anthrone method was estimated as 54.09±0.66% and finally the lipid content of *Sargassum wightii Greville* by Folch's method estimated as 0.51±.002% of which carbohydrate content was more compared to protein and lipid (Fig. 3).

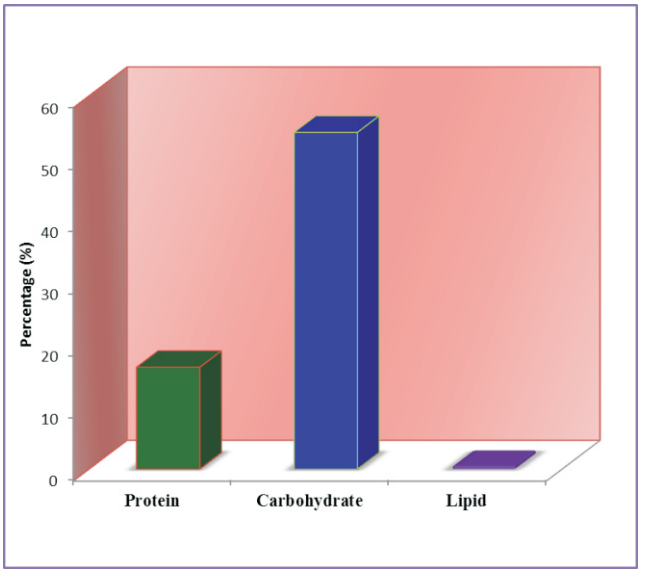


Fig. 3. Biochemical composition of Sargassum wightii Greville

BIOPIGMENT ANALYSIS OF *SARGASSUM WIGHTII GREVILLE*

The chl a and b content was observed in seaweed *Sargassum wightii Greville*. Of which the Chl a was estimated as 33.80µg and Chl b content was estimated as 34.90 µg. The Chl b content was more compared to Chl a (Fig. 4).

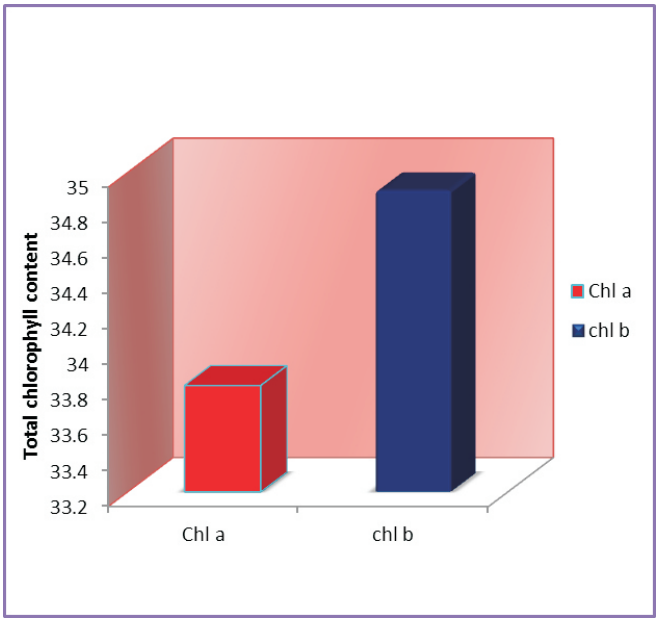


Fig. 4. Biopigment analysis of *Sargassum wightii Greville*

Crude Extraction of *Sargassum Wightii Greville.*

The powdered sample was soaked in 100mL of chloroform and Ethanol. The extract was suction filtered using Whatmann filter paper. This was repeated for 2 to 3 times and similar extracts were pooled together and concentrated at 40 to 45˚ C under reduced pressure. The concentrated crude extract was subjected to identify the compounds and their functional groups using GC-MS and FTIR techniques and followed by it the sample was analysed for Antiproliferative activity against lung cancer A549 cell line.

Gas Chromatography and Mass Spectrometry (Gc – Ms) Analysis of *Sargassum wightii Greville.*

The Gas Chromatography and Mass Spectrometry (GC- MS) analysis of crude ethanolic extract of *Sargassum wightii Greville* showed the presence of 5,8,11 – heptadecatrienoic acid, Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis, (E)-10-Heptadecen-8-ynoic acid methyl ester, 6-Octadecenoic acid, (Z), Heneicosane, ethanol 2-(9-octadecenyloxy)- (z), n-hexadecanoic acid and oxiraine,dodecyl are the compounds present in the sample (Table 1 and Fig 5).

Table 1. Analysis of GC-MS from crude ethanolic extract of *Sargassum wightii Greville*

Retention Time	Compound Name	Molecular formula	Molecular weight (g/mol)
19.78	5,8,11 – heptadecatrienoic acid	$C_{17}H_{28}O_3$	280.41
16.06	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis-	$C_{19}H_{36}O_3$	312.4873
17.16	(E)-10-Heptadecen-8-ynoic acid methyl ester	$C_{18}H_{30}O_2$	278.429600
18.37	6-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$	282.4614
20.42	Heneicosane	$C_{21}H_{44}$	296.57406
15.11	ethanol 2-(9-octadecenyloxy)- (z)-	$C_{20}H_{40}O_2$	312.5304
16.66	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241
17.89	oxirane,dodecyl-	$C_{14}H_{28}O$	212.3715

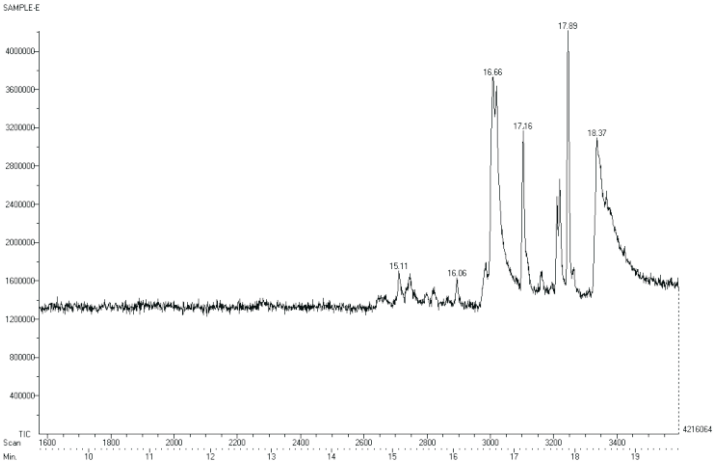


Fig 5. GC chromatogram of *Sargassam wightii*

Fourier Transform Infrared Spectroscopy (FT-IR) of *Sargassum wightii Greville.*

The volatile products can be found in the spectrum for *Sargassum wightii Greville*. The collected extractions give bands at 604 and 3395 cm^{-1}, so all are cis-isomer, as expected from alga lipid because trans isomers produce a strong band at 970 cm-1 and a weak band at 3012 cm^{-1} while cis-isomers gave medium nearby 720 and 3012 cm^{-1} bands (Fig 6). An analysis of the IR spectrum showed the main composition stage, reveals the existence of the absorption bands characteristic of these five different bonds:

- C═O: Carbonylic compounds (aldehydes, acids, etc.) are the strong C O stretching absorption band in the region of 1736–1461 cm^{-1}. If esters, this band appears in the 1750–1735 cm^{-1}.
- C—O—C (Ethers): These stretching vibrations produce a strong band in the 1200–900 cm^{-1} region.
- C—H: absorption bands characteristic of the vibrations of C—H bonds, as an example, 2960 and 2875 cm^{-1} correspond to the asymmetric and symmetric vibrational modes of methyl groups, respectively, and 2924 and 2853 cm^{-1} correspond to the asymmetric and symmetric vibrational modes of methylene groups, respectively.
- CO_2: they produce strong bands in between 2800-2000 cm-1 as well as in 700 cm^{-1} region.
- H_2O: the adsorption bands of water can be observed in the range of 1800-1200 cm^{-1}.

As many algal species have been found to grow rapidly and produce substantial amounts of TAG referred to as oleaginous algae. It has long been postulated that algae could be employed as a cell factories to produce lipids for bioprospecting (Benemann 1982). There is an increase in total lipids stationary phase of macroalgal cells. De novo biosynthesis and conversion of certain existing membrane polar lipids into triacylglycerols may contribute to the overall increase in TAG (Table 2).

Table 2. Functional group analysi by FT – IR from *Sargassum wightii Greville*

Frequency	Bond	Functional group
3395 cm^{-1}	O-H stretch, H- bonded, N-H Stretch	alcohols, Phenols, 1^{O}, 2^{o} amines, amides
3011 cm^{-1}	=C-H, =C-H stretch	aromatics, alkenes
2924 cm^{-1}	C-H Stretch	Alkanes
2853 cm^{-1}	C-H Stretch	Alkanes
1736 cm^{-1}	C=O Stretch	esters, Saturated aliphatic, aldehydes, saturated aliphatic
1645 cm^{-1}	-C=C – Stretch, N-H bend	alkenes, 1^{o} amines
1461 cm^{-1}	C-H bend, C-C stretch (in ring)	alkanes, aromatics
1408 cm^{-1}	C-C Stretch (in – ring)	Aromatics
1167 cm^{-1}	C-H wag t- CH_2X_1, C-N Stretch	alkyl halides, aliphatic amines
1075 cm^{-1}	C-N Stretch	aliphatic amines
887 cm^{-1}	N-H wag, C-H "oop"	1^{O}, 2^{o} amines, amides, aromatics
721 cm^{-1}	C-CL stretch, C-H rock	alkyl halides, alkanes
604 cm^{-1}	C-CL stretch, C-Br Stretch	alkyl halides

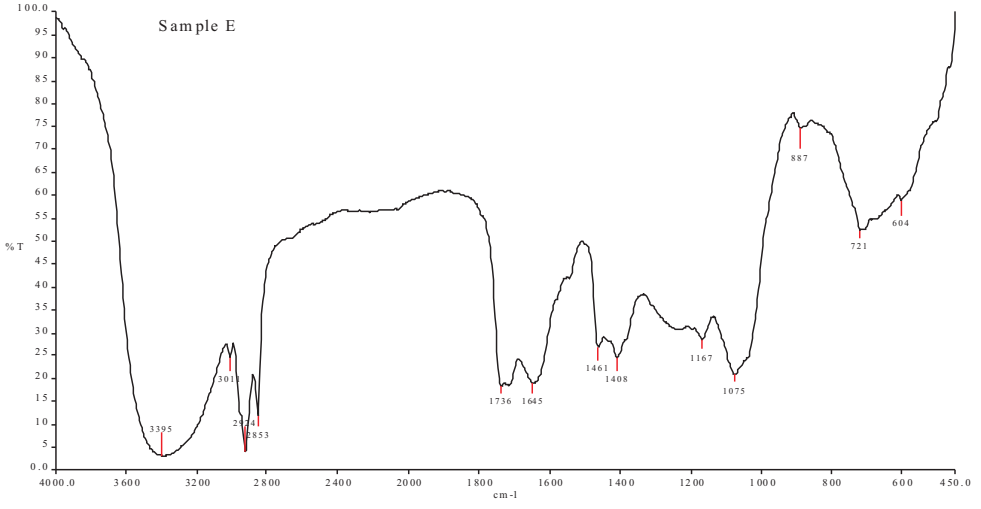


Fig 6. FT-IR spectrum of *Sargassum wightii Greville*

Antiproliferative activity of A549 lung cancer cell line

Chloroform and Ethanol extract of *Sargassum wightii Greville* were tested the cytotoxicity on lung cancer (A549) cancer cell lines with the concentration from 7.8 µg/mL to 1000µg/mL. *Sargassum wightii Greville* showed various ranges of viability with dose dependent. At the concentration of 1000 µg/mL, chloroform extract showed the cell viability of 34.07% and Ethanol extract showed the cell viability of 32.96 % against lung cancer cell line (A549), The normal structural morphology were altered the dose dependent manner when compared with the normal cell. The IC_{50} value for lung cancer (A549) of chloroform extract showed 125 µg/mL, where ethanol extract showed 62.5. Thus ethanolic extract of *Sargassum wightii Greville* showed apoptopsis at a low rate compared to the chloroform extract. (Table 3; Table 4; Fig. 7, Fig. 8, Fig. 9 and Fig. 10).

Table 3. Anticancerous activity of chloroform extract on A549 lung cancer cell line in *Sargassum wightii Greville*

S.No.	Concentration (µg/mL)	Dilutions	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.184	34.07
2	500	1:1	0.207	38.33
3	250	1:2	0.235	43.51
4	125	1:4	0.264	48.88
5	62.5	1:8	0.289	53.51
6	31.2	1:16	0.314	58.14
7	15.6	1:32	0.347	64.25
8	7.8	1:64	0.368	68.14
9	Cell control	-	0.540	100

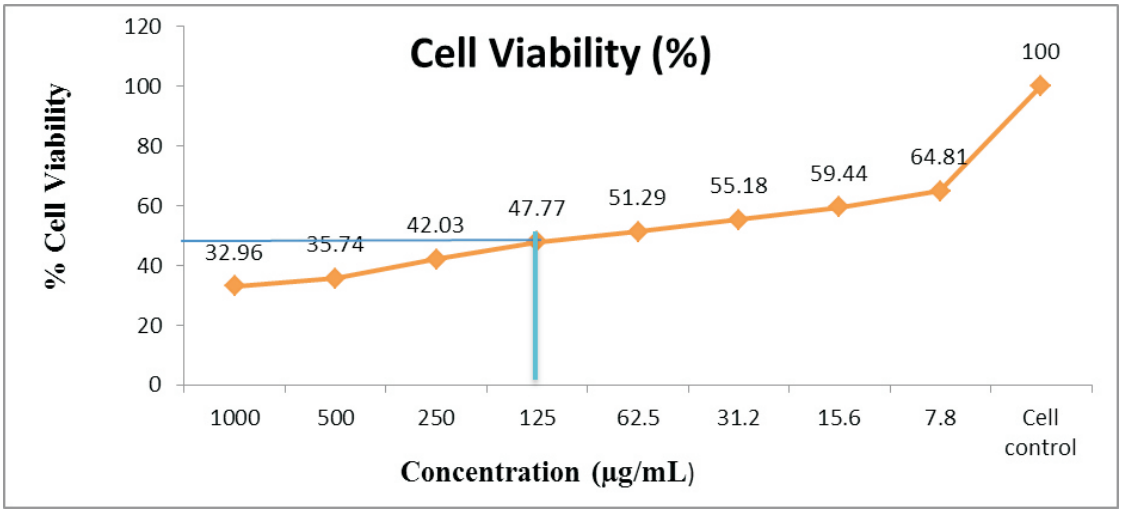


Fig 7. Anticancerous activity of chloroform extract of A549 lung cancer cell line

Table 4. Anticancerous activity of Ethanolic extract of A549 lung cancer cell line in *Sargassum wightii Greville*

S.No.	Concentration (µg/mL)	Dilutions	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.178	32.96
2	500	1:1	0.193	35.74
3	250	1:2	0.227	42.03
4	125	1:4	0.258	47.77
5	62.5	1:8	0.277	51.29
6	31.2	1:16	0.298	55.18
7	15.6	1:32	0.321	59.44
8	7.8	1:64	0.350	64.81
9	Cell control	-	0.540	100

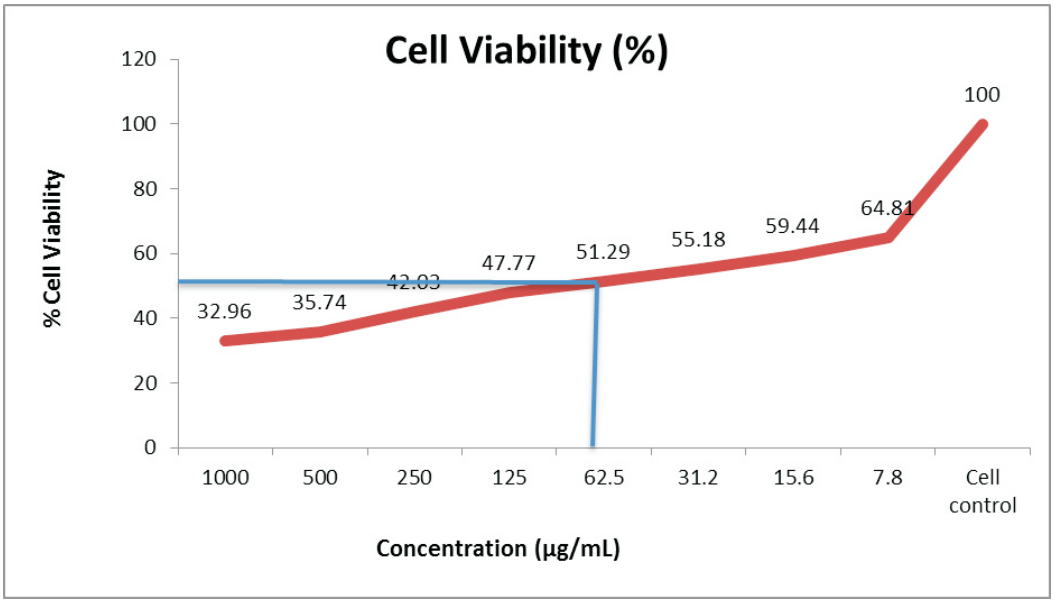
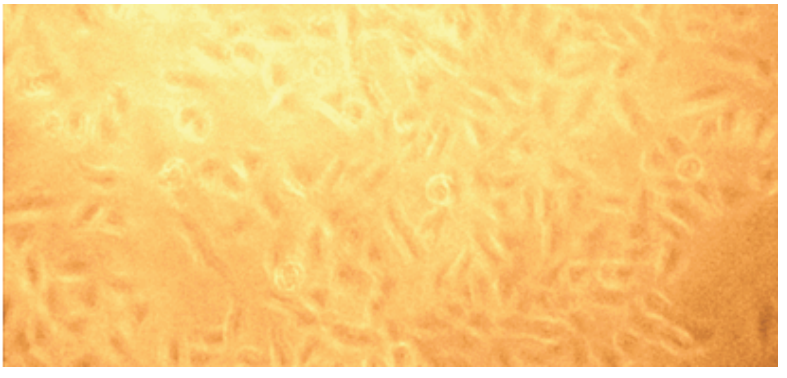
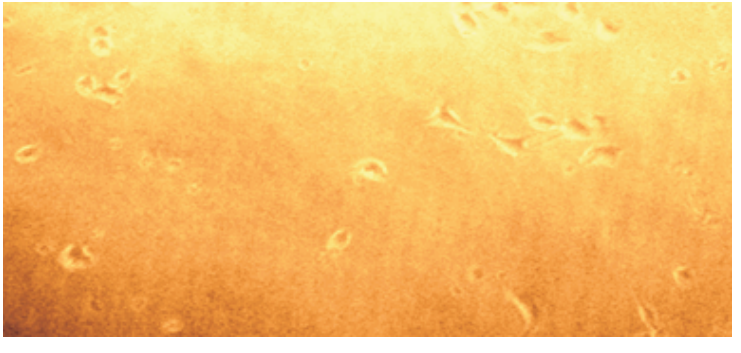


Fig 8. Anticancerous activity of ethanolic extract of A549 lung cancer cell line

Normal A549 Cell line



Toxicity- 500 µg/mL



Toxicity- 125 µg/mL **Toxicity- 15.6 µg/mL**

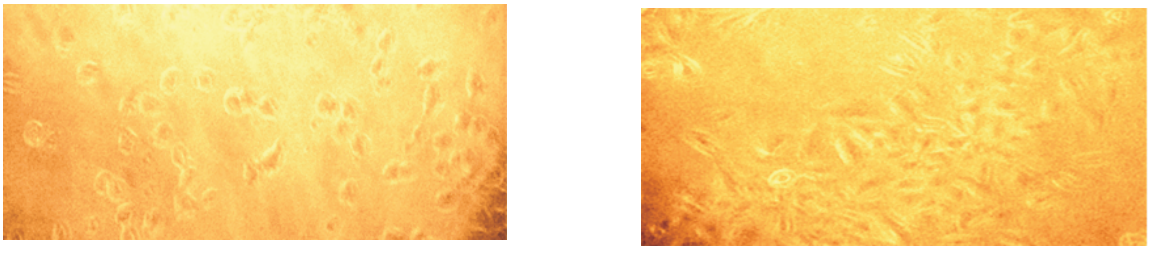
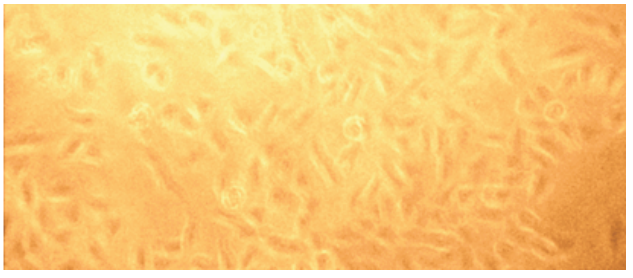
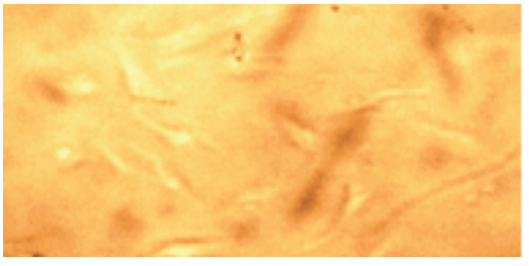


Fig 9. Anticancerous effect of chloroform extract of *S.wightii* on A549 lung cancer cell line

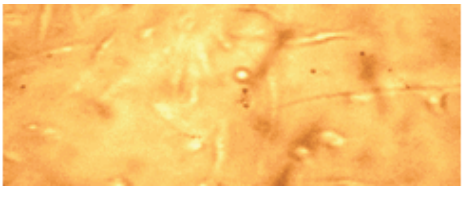
Normal A549 Cell line



Toxicity- 500 µg/mL



Toxicity- 62.5 µg/mL **Toxicity- 15.6 µg/mL**



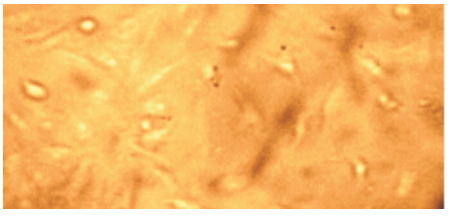


Fig 10. Anticancerous effect of Ethanolic extract of *S.wightii* on A549 lung cancer cell line

DISCUSSION

Eight different species of *Sargassum sp*. grow in the Ryukyu Islands and four of them, in the coastal areas of Kochi. In Tamil Nadu, the sample *Sargassum wightii* was present in Kanyakumari, Tirunelveli, Tuticorin, Ramanathapuram and mandabam coastal area of Tamil Nadu (Lima-Filho *et al.*, 2002). Since the sample shows predominant growth in mandabam coastal area compared to other places, and cited in many literatures the sample Sargassum wightii was collected from the same area

and was authenticated by Dr.S. Elumalai and Dr. M. Baluswami.

Macroalgae has been identified by various phycologist across the world. Of which "Salt water algae control: the ultimate guide" and "Macroalgae identification" by RothChyld and many other books have been carried out both in India and abroad to identify the macro algae. With these references, we have chosen seaweed manual from National Institute of Oceanography by V. K. Bhargalkar by taking specimen section for identifying the macroalgae Sargassum at species level.

The seaweeds are shade dried for 10 days in a clean environment to avoid the contamination and they were dried in the oven at 60 ˚C for four hours to remove the moisture content. The oven-dried seaweeds were ground into a fine powder by using an agate mortar (Kannan, 2014). The dried samples were weighed and after adding of distilled water (100 ml), the samples were autoclaved at 121˚C for 15 min. (Kudo et al., 2005a,b). For preparation of the ethanol extract (EE) the dried samples were weighed (4 g), then 50 ml ethanol added to each sample, and the samples were shaken at 37˚C for 4 1/2 h. This was followed by centrifugation (2220 g for 10 min), the supernatant as the ethanol extract solution was used. In this case, the sample was then washed thoroughly with tap water followed by distilled water and then shade dried for a period of 2 weeks and then kept in hot air oven at 60 ˚C for overnight and sample was weighed and coarsely powdered with the help of mortar and pestle. Finally few mg of sample was soaked in ethanol and chloroform in twice the volume of the sample.

The biochemical contents of *Sargassum wightii Greville* from Port Okha were studied in relation to ecological factors (Murthy and Radia, 1978). They presented the month-wise proteins, carbohydrates, fat, crude-fibre, sodium, potassium, calcium and phosphorus contents of these algal species. Dhargalkar, 1966 estimating the major metabolites such as proteins, carbohydrates, lipids, of which carbohydrate, protein values also followed the same trend while lipids did not show any significant seasonal variation. Marked changes in the chemical constituents were found to occur with change of seasons, environmental conditions as well as in the various phases of plants growth and fruiting cycle. The seasonal variation in the major and minor constituents of green, brown and red algae (Pillai, 1957). In *Sargassum wightii* the maximum amount is present in carbohydrate and least amount in lipids and other phycologist from Chidambaram and other areas showed a significant result which confirms the result obtained.

The chl b content was comparatively high compared to that of chl a in *sargassum wightii* and this similar report was confirmed by other phycologists. The earlier investigation was in contrast to the present study, as the concentrations varied from 0.1 to 1.5% and the maximum growth rate, biochemical and pigment concentration was observed at 0.50% concentration.

Gas chromatography coupled to mass spectrometry is a versatile tool to separate, quantify and identify unknown (volatile) organic compounds and permanent gases. A high resolution mass spectrum equipped with a data system in combination with Gas Chromatography was used in the present study for the chemical analysis of active solvent. On comparison of the mass spectra of the constituents with the NIST library, seventeen peaks were obtained and the phytoconstituents were characterized and identified with retention times and relative percentages. Biological activity of *Sargassum wightii* might be due to the presence of fatty acid, Bromoacetic acid, hexadecyl ester (94.98%) in higher percentage. In our study, the GC-MS shows various compounds at different retention time with molecular formula and molecular weight. Among that the retention time was maximum at 20.42 showing the presence of Heneicosane compound and minimum retention time at 16.06 showing the presence of Oxiraneundecanoic acid.

In FTIR spectrum of both the samples were compared with that of the standard gallic acid. The FTIR spectrum of standard gallic acid showed thirteen major peaks at the range of 3395, 3011, 2924,

2853, 1736, 1645, 1461, 1408, 1167, 1075, 887, 721, 604 cm-1 , whereas the FTIR spectrum of the S. wightii samples also showed the same number of peaks lying between 3395 cm^{-1} and 604cm^{-1}, respectively. FT-IR spectrum of *Sargassum wightii* respectively exhibits the characteristic finger print band features. The strong absorption bands at 3395 cm^{-1} are representing the O-H stretch, H bonded, N-H stretch. The Weak band at 604 cm-1 are due to the stretching vibration of C-Cl stretch, C-Br stretch belonging to alkyl halides. The Seaweed Sargassum wightii contain a strong absorption band at 3371cm^{-1}.

Cancer is a formidable problem for people with the great majority of chemical compounds, which have been identified as cytotoxic to cancer cells, are also toxic to normal cells (Borchers *et al*., 1999). Therefore, it is very important to investigate novel antitumor substances with little toxicity to host. (Ananthi *et al.*, 2010; Cheng *et al*., 2010).

In this investigation, the antiproliferative study from *Sargassum wightii Greville*, in invitro condition and the anti-tumor activity of the polysaccharide was evaluated. SFP's could effectively prevent the formation of A549 lung adenocarcinoma, and extensively disrupted the architecture of tumor tissue. The Standing Field Potentials (SFP's) may indirectly play the role of antitumors activity through the releases of effective molecules TNF-α. Also, Standing Field Potentials (SFP's) exhibited significantly cytotoxicity to A549 lung adenocarcinoma cells. These results indicated that SFP's had anti-tumor activity in *invitro*. The immune system plays an important role in antitumor defense. Many reports suggested that anti-tumor activity of the polysaccharides were also mediated through enhancing the immune response (Cho and Leung, 2007; Schepetkin and Quinn, 2006). As per these investigation *Sargassum wightii* anti proliferative activity was carried out for lung cancer A549 which shows ethanol extract shows good activity compared to chloroform extract where chloroform shows IC50 value at 125µg/mL and ethanol shows IC_{50} value at 62.5 µg/mL.

CONCLUSION

The macroalgae *Sargassum wightii Greville* are commonly available species in the coastal region of Tamil Nadu. It consists of much bioactive compounds which are useful as pharmaceutical fields. In this study conclude that the Sargassum wightii Greville are showed very good anticancer activity in the Ethanol extraction when compared to the chloroform extraction. These are low cost and eco-friendly.

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