Marmara Pharmaceutical Journal 21/2: 269-277, 2017
DOI: 10.12991/marupj.300349

RESEARCH PAPER

Spectral Analysis of *Sargassum ilicifolium* (Turner) C.Agardh and *in vitro* Anti-proliferative Study of its Ethanolic Extract and Chloroform Fraction Against Colon Cancer (HT-29) and Lung Cancer (A549) Cell Lines.

M. Sumithra, V. Chitra, Radika Ramasamy

ABSTRACT

Spectral Analysis of *Sargassum ilicifolium* (Turner) C. Agardh and *in vitro* Anti-proliferative Study of its Ethanolic Extract and Chloroform Fraction Against Colon Cancer (HT-29) and Lung Cancer (A549) Cell Lines.

Current research in drug discovery from medicinal plants involves the identification of safe and inexpensive lead molecule towards diseases. Cancer is a second major national burden leading to cause death. Reducing the nation's cancer burden is a great challenge to win the "War of cancer". During the last decades, numerous novel compounds have been found from marine organisms with interesting pharmaceutical activities. Hence, a novel brown algae, *Sargassum ilicifolium* (Turner) C.Agardh belonging to *Sargassaceae* family has selected and the study was aimed to investigate the *in vitro* anti-proliferative activity of ethanolic extract and chloroform fraction of *Sargassum ilicifolium* (Turner) C.Agardh. against colon cancer (HT-29) and lung cancer (A549) cell lines. Extraction of seaweed using 70% ethanol and fractionated with various solvents. Based on phytochemical screening, chloroform fraction was selected, then it was subjected to GC-MS analysis. The 70% ethanolic exract and chloroform fraction were selected to *in vitro* MTT cell line assay using colon cancer cell line (HT-29) and lung cancer cell line (A549). The GC-MS analysis report confirmed the presence of 21 compounds and in the the *invitro* assay, IC_{50}value showed the effective anti-proliferative activity against colon and lung cancer cell lines. The results of the present study proved the anti-proliferative effect of *Sargassum ilicifolium*. Further, the active biomolecule in the fraction has to be isolated and charecterized the formulated product subjected to *in vivo* study to strengthen the anticancer activity.

Key Words: Antiproliferative effect, colon cancer, lung cancer, *Sargassum ilicifolium*, GC-MS analysis.

1. INTRODUCTION

Today, cancer still remains an aggressive killing disease worldwide, however novel synthetic chemotherapeutic agents are use clinically but have not succeeded in fulfilling the expectations, so development of new anti-cancer drugs are needed [1]. Past 30 years the natural sources have received more attention for their active constituents for developing a new anti-cancer drugs [2,3]. In another side there is increasing the strong evidence for plant derived compounds inhibiting the various stages of cancer and associated inflammatory process. In cancer treatment 60% of drugs are isolated from the natural sources [4]. Out of 121 prescription drugs in use today for cancer treatment, 9 are derived from plant species. In the period of 1981 to 2002, 48 of 65 drugs approved for the therapy of cancer were based on natural products [5]. High

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Submitted / Gönderilme: 22.08.2016 **Revised / Düzeltme:** 03.11.2016
Accepted / Kabul: 22.11.2016

death rate associated with cancer and serious side effects of chemotherapy and radiation therapy many cancer patients seek alternative medicine. Now a days various therapies are available for treatment of cancer such as chemotherapy, radiotherapy but plant phytotherapy plays important role in the treatment of cancer.

Sargassum ilicifolium (Turner) C.Agardh belongs to the family *Sargassaceae* is a brown algae having photosynthetic pigments such as chlorophyll, carotene, steroids, terpenoids mannitol, polysaccharides, alginic acid and it also contains algin in cell wall and the cells shows plastid of thyalkoidal lamellae [6-8]. Marine algae recognized as rich sources of structurally diverse biologically active compounds with great pharmaceutical and biomedical potential. Many literature survey have been reported that marine algae have various important pharmacological activities such as neuroprotective activity, anti-viral activity, cholinesterase inhibitor activity [9-11].

In the current study we examined the effect of ethanolic extract and chloroform fraction of brown marine algae *S. ilicifolium* against colon cancer cell line (HT-29), and lung cancer (A549) cell lines over MTT assay. The chloroform fraction were subjected into spectral analysis for find out the phytochonstituents present in it.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

MEM was purchased from Hi Media Laboratories. Fetal Bovine Serum (FBS) was purchased from Cistron Laboratories. Trypsin, methyl thiazolydiphenyltetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were purchased form Sisco Research Laboratory Chemicals (Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich (Mumbai).

2.2 Pharmacognostic study

Sargassum ilicifolium (Turner) C.Agardh was collected form Mandabam coast, Rameshwaram and aunthetified by Professor. Jeyaraman, Director, Plant Anatomy Research Centre, Tambaram, Chennai. Certificate No. PARC/2011/1032.

2.3 Extraction

The Coarse powder was extracted using ethanol (70% v/v) by percolation method and the percentage yield was calculated. The ethanolic extract was subjected to defatting with petroleum ether then fractionated with chloroform. The chloroform fraction was subjected to GC-MS analysis.

2.4 GC-MS Analysis [12]

2µl of the liquid chloroform fraction of *Sargassum ilicifolium* employed for GC-MS for analysis of different compounds. Instruments and chromatographic conditions was carried out on a clarus 500 Perkin Elmer system comparising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the standard protocol and the interpretation of phytomolecule present was performed using National Institute Standard and Technology (NIST) data base.

3 Pharmacological screening

3.1 In vitro cytotoxic studies

3.1.1 Cell line and culture

HT-29 and A 549 cell lines were purchased from National Centre for Cell Science, Pune (NCCS). The cells were maintained in Minimal Essential Medium supplemented with 10% FBS, penicillin (100u/ml) and streptomycin (100µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37°C.

3.1.2 In vitro assay for Anti-cancer activity (MTT assay) (Moamann, 1983) [13]

Cells (1 x 10^5/well) were plated in 24-well plates and incubated in 37^0C with 5% of CO_2 condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24 hrs. 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-dimethlyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) wsas added and incubated for hours. After incubation, 1ml of DMSO was added in all the wells. The absorbance was measured at 570nm with UV-spectrophotmeter using DMSO as the

blank. All the measurements were performed and the concentration required for a 50% inhibition (IC_{50}) was determined graphically. The % cell viability was calculated using the following formula

%cell viability=A570 of treated cells/A570 of control cells x 100

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

4. RESULTS & DISCUSSION

The percentage yield of ethanolic extract and chloroform fraction of *Sargassum ilicifolium* were found to be 5.03% and 0.48% respectively. Phytochemical analysis revealed the presence of alkaloids, steroids, terpenoids, fatty acid, saponins and tannins in the ethanolic extract and in chloroform fraction steroids, fatty acid and tannins were present.

Literature study supports that steroid and fatty acids are having potent role in cytotoxicity. The chloroform fraction subjected to GC-MS analysis confirmed the presence of 21 compounds namely 1-hexadecene, 1-octadecene,16-octadecenoic acid methyl ester, 10-nonadecanone, 9-hexadecenoic acid methyl ester, 14-methylpentadecanoic acid methyl ester, 10-octadecenoic acid methyl ester, 5,8,11,14-eicosatetraenoic acid methyl ester, 11-eicosenoic acid methyl ester, 1-tricosene, 18-nonadecenaic acid, 17-methly octadecanoic acid methyl ester, 2-hexadecanol, 5-hydroxypentanoicacid 2,4-di-*t*-butylphenyl esters, hexadecane, 9-hexylheptadecane, 2,6,10-trimethyltetradecane, 7,10-octadecanoic acid methyl ester, 1-monolinoleoyl glycerol trimethylsilyl ether, 6,17,21-*tris*[{trimethylsilyl}]-3,20-*bis*(*O*-methyloxime) pregn-4-ene-3,11,20-trione (Table1). The GC-MS spectroscopic studies confirmed the presence of eicosaenoic acid derivatives and steroids which are of biological importance.

MTT assay is performed to assess the cell viability. The principle involved in the assay is the reduction of MTT tetrazolium dye to insoluble purple colour formazan by NAD(P)H-dependent cellular oxido-reductase enzymes under defined conditions and it directly reflects the number of viable cells present in it. The quantity of formazan formed was measured at 570 nm using spectrophotometer [14].

The percentage cell viability by the extract and fraction of *S. ilicifolium* against HT-29 (colon cancer cell line) were listed in the Table 2 and Figure 2 and the A549 cell line (lung cancer cell line) result were listed out in the Table 3 and Figure 3 for various concentrations respectively. The IC_{50}value for ethanolic extract and chloroform fraction of *S. ilicifolium* against HT-29 cell line was found to be 14.14µg/ml and 15.22µg/ml respectively and the IC_{50} value against A549 was found to be 31.2µg/ml and 7.8µg/ml respectively. The 50% inhibition of human cancer cell lines by the extract and chloroform fraction of *S. ilicifolium* elicits the cytotoxicity role against colon and breast cancer cell lines. The results concludes that the chloroform fraction was very effective against lung cancer cell line than colon cancer.

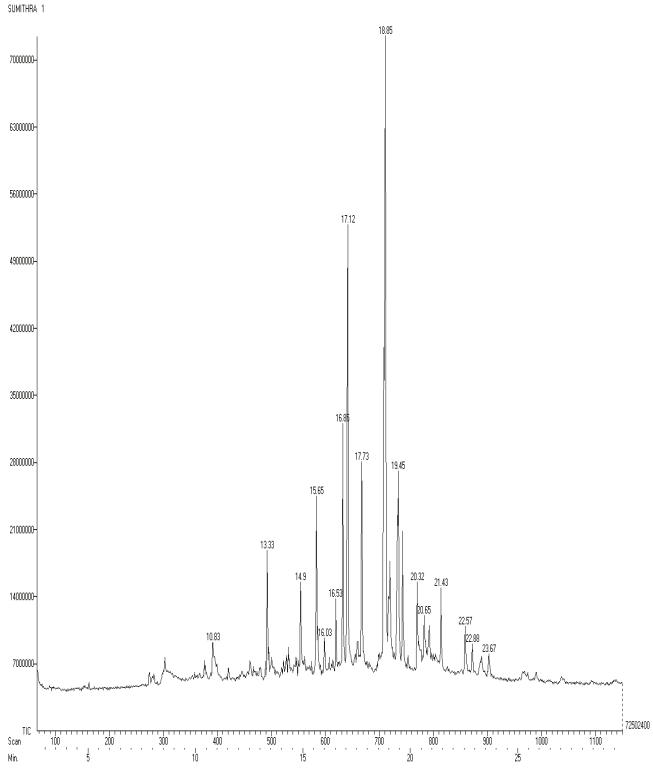
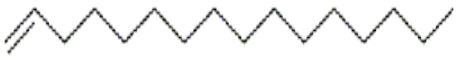
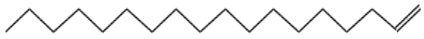
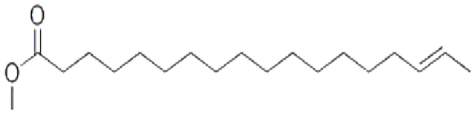
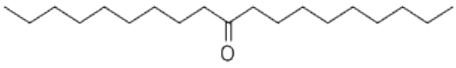
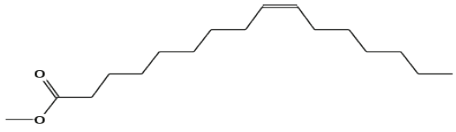
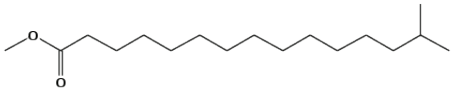
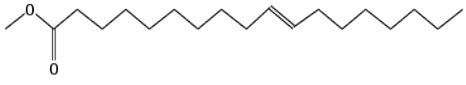
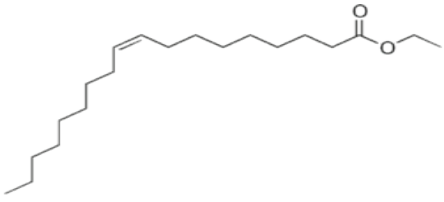
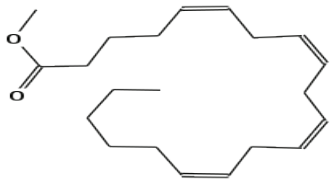
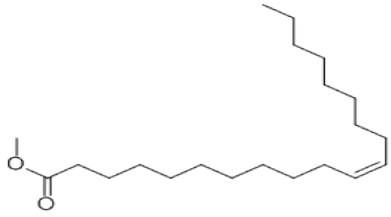
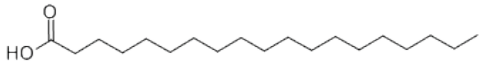
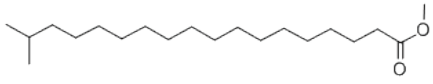
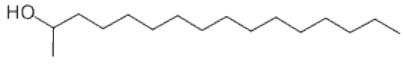
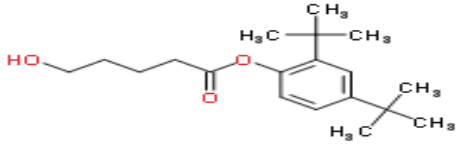
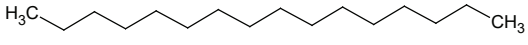


Figure 1: GC-MS analysis data of chloroform fraction of *Sargassum ilicifolium*

Table 1: Phytochemical identified in the ethanol extract of *Sargassum ilicifolium* by GC-MS peak report.

S. No	Compounds	RT	M+	Mass spectral data, m/z	Mol. Formula
1	1-Hexadecene 	13.33	224	224, 196, 154, 140, 125, 111, 97, 55	$C_{16}H_{32}$
2	1-Octadecene 	15.65	252	252, 224, 154, 111, 97, 83, 69, 55	$C_{18}H_{36}$
3	16-Octadecenoic acid methyl ester 	16.03	296	296, 267, 256, 221, 207, 147, 111, 87, 73	$C_{19}H_{36}O_2$
4	10-Nonadecanone 	16.53	282	282, 254, 223, 183, 170, 155, 149, 127, 110, 85, 71, 57	$C_{19}H_{38}O$
5	9-Hexadecenoic acid methyl ester 	16.85	268	268, 236, 194, 152, 123, 110, 97, 87, 69, 55	$C_{17}H_{32}O_2$
6	14-methylpentadecanoic acid methyl ester 	17.73	270	270, 239, 227, 111, 87, 74, 57	$C_{17}H_{34}O_2$
7	10-Octadecenoic acid methyl ester 	18.87	296	292, 264, 222, 123, 98, 83, 69, 55	$C_{19}H_{36}O_2$
8	Ethyl oleate 	19.45	310	310, 264, 180, 123, 101, 88, 69, 55	$C_{20}H_{38}O_2$

S. No	Compounds	RT	M+	Mass spectral data, m/z	Mol. Formula
9	5,8,11,14-Eicosatetraenoic acid methyl ester 	20.32	318	318, 247, 180, 150, 105, 91, 79, 67	$C_{21}H_{34}O_2$
10	11-Eicosenoic acid methyl ester 	20.65	324	324, 292, 250, 208, 111, 97, 69, 55	$C_{21}H_{40}O_2$
11	1-Tricosene 	21.43	336	336, 322, 125, 111, 97, 83, 69, 57	$C_{23}H_{46}$
12	18-Nonadecenaic acid 	22.57	278	278, 236, 194, 125, 111, 97, 83, 69, 55	$C_{19}H_{38}O_2$
13	17-methyloctadecanoic acid methyl ester 	22.88	312	312, 269, 199, 143, 111, 87, 74	$C_{20}H_{40}O_2$
14	2-Hexadecanol 	23.67	242	242, 176, 125, 111, 97, 83, 69	$C_{16}H_{34}O$
15	5-hydroxypentanoic acid 2,4-di-*t*-butylphenyl ester 	12.52	306	306, 191, 163, 91, 74, 57	$C_{19}H_{30}O_3$
16	Hexadecane 	13.43	226	226, 183, 141, 99, 85, 71, 57	$C_{16}H_{34}$

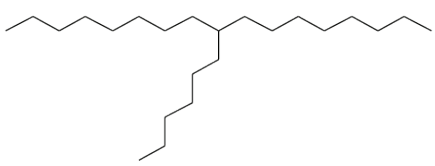
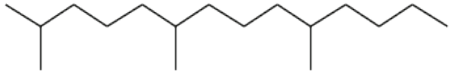
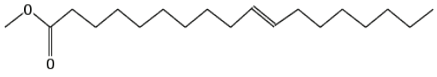
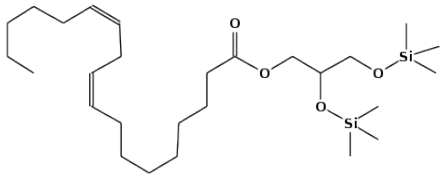
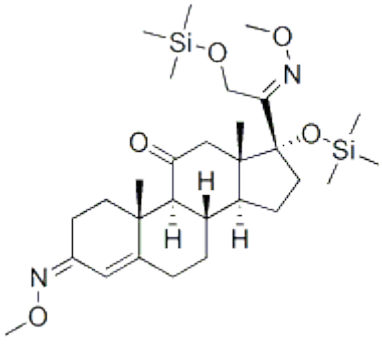
S. No	Compounds	RT	M+	Mass spectral data, m/z	Mol. Formula
17	9-hexylheptadecane 	15.22	291	291, 276, 252, 230, 197, 183, 163, 127, 113, 99, 85, 71, 57	$C_{23}H_{48}$
18	2,6,10-trimethyltetradecane 	16.78	240	240, 225, 155, 113, 99, 85, 71, 57	$C_{17}H_{36}$
19	10-Octadecenoic acid methyl ester 	18.8	296	296, 263, 220, 178, 150, 121, 109, 95, 81, 67	$C_{19}H_{36}O_2$
20	1-Monolinoleoyl glycerol trimethylsilyl ether 	17.63	498	498, 383, 355, 341, 295, 281, 207, 147, 113, 99, 73	$C_{27}H_{54}O_4Si_2$
21	**Steroids** 6,17,21-*tris*[{trimethylsilyl}oxyl]-3,20-*bis*(*O*-methyloxime) pregn-4-ene-3,11,20-trione 	23.37	529	529, 439, 355, 246, 168, 147, 113, 85, 73	$C_{32}H_{58}N_2O_6Si_3$

Table 2: Anti-cancer activity of chloroform fraction and ethanolic extract of *Sargassum ilicifolium* on HT-29 cell line

S. No	Concentration (µg/ml)	Dilutions	Absorbance (O.D) of chloroform fraction	Cell viability (%)of chloroform fraction	Absorbance (O.D) of ethanolic extract	Cell viability (%) of ethanolic extract.
1	1000	Neat	0.09	14.28	0.05	7.93
2	500	1:1	0.12	19.04	0.11	17.46
3	250	1:2	0.15	23.80	0.17	26.98
4	125	1:4	0.19	30.15	0.21	33.33
5	62.5	1:8	0.22	34.92	0.28	44.44
6	31.2	1:16	0.27	42.85	0.30	47.61
7	15.6	1:32	0.31	49.20	0.33	52.38
8	7.8	1:64	0.34	53.96	0.35	55.55
9	Cell control	-	0.63	100	0.63	100

Table 3: Anti-cancer activity of ethanolic extract and chloroform fraction of *Sargassum ilicifolium* on A549 cell line

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)ethanolic extract	Cell viability (%) of ethanolic extract	Absorbance (O.D) of chloroform fraction	Cel viability (%) of chloroform fraction
1	1000	Neat	0.06	10.16	0.02	3.38
2	500	1:1	0.10	16.94	0.05	8.47
3	250	1:2	0.14	23.72	0.00	15.25
4	125	1:4	0.20	33.89	0.13	22.03
5	62.5	1:8	0.26	44.06	0.19	32.20
6	31.2	1:16	0.30	50.84	0.23	38.98
7	15.6	1:32	0.33	55.93	0.28	47.45
8	7.8	1:64	0.37	62.71	0.31	52.54
9	Cell control	-	0.59	100	0.59	100

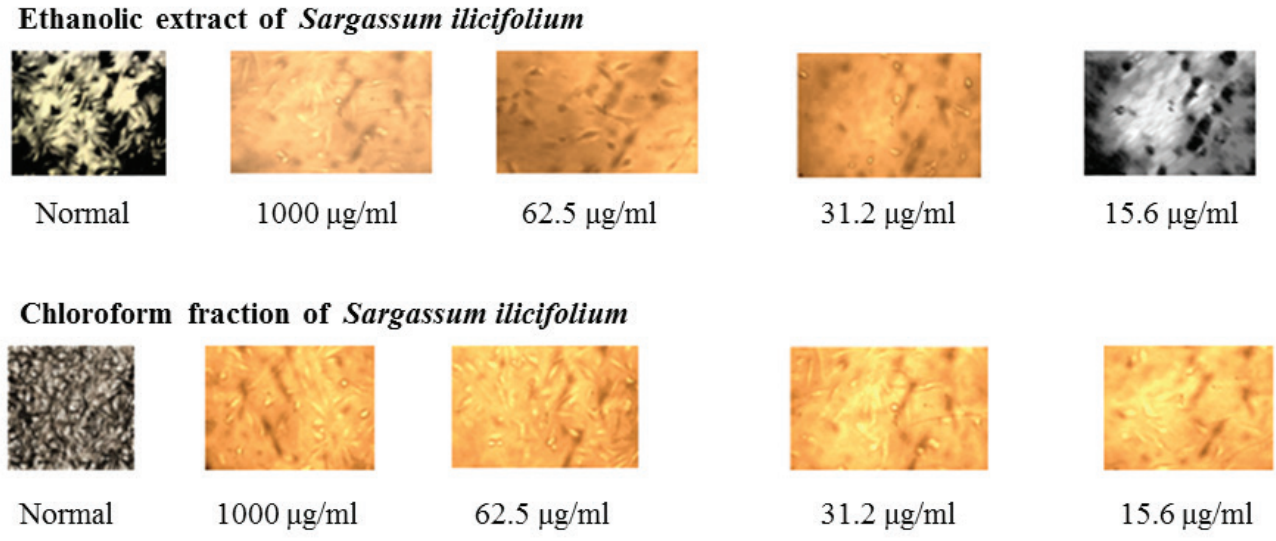


Figure 2. Anticancer effects of ethanolic extract and chloroform fraction of Sargassum ilicifolium on HT-29 cell line.

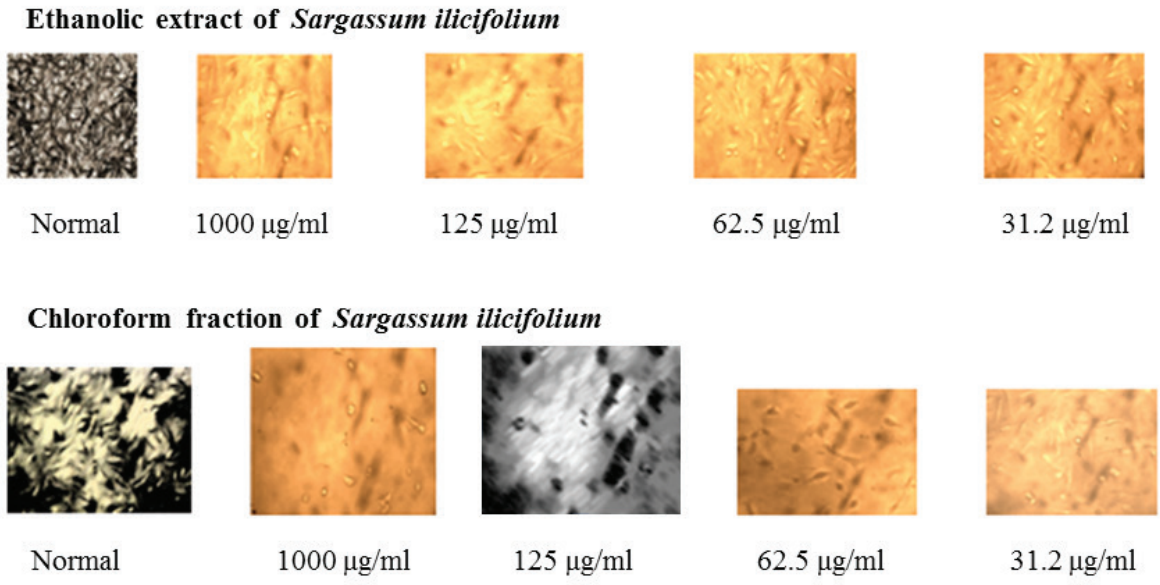


Figure 3. Anticancer effects of ethanolic extract and chloroform fraction of Sargassum ilicifolium on A-549 cell line.

5.Conclusion

In vitro cytotoxic studies of the ethanolic extract and chloroform fraction against HT-29 (colon cancer cell line) and A549 (lung cancer cell line) cell lines supports the anti-cancer activity of *Sargassum ilicifolium*. The IC_{50} value of MTT assay on extract and fraction confirms the potency against cancer cell lines. Thus further studies has to be carried out to strengthen the result by promoting *in vivo* studies and to formulate the isolated compound responsible for anticancer activity.

Acknowledgement

Our sincere thanks to the ChancellarParivendhar**,** SRM University, Kattangulathur for supporting research work in this premises and Dr.K.Lakshmi, Dean SRM College of Pharmacy for her inspiration and motivational approach.

Conflict of interest

There is no conflict of interest

***Sargassum ilicifolium* (Turner) C.Agardh'ın Spektral Analizi ve Kolon Kanseri (HT-29) ve Akciğer Kanseri (A549) Hücre Serilerine Karşı Etanolik Ekstre ve Kloroform Fraksiyonunun *in vitro* Anti-proliferatif Çalışması**

ÖZ

Tıbbi bitkilerden ilaç keşfi ile ilgili güncel araştırmalar, hastalıklara karşı güvenli ve ucuz öncü molekül tanımlanmasını gerektirir. Kanser ölüme neden olan ikinci büyük ulusal bir yüktür. "Kanser Savaşı" nı kazanmak için ulusun kanser yükünü azaltmak, büyük bir mücadeledir. Son on yılda, deniz organizmalarından ilginç farmasötik aktiviteleri ile çok sayıda yeni bileşik bulunmuştur. Bu nedenle, bu çalışmada Sargassaceae familyasına ait yeni bir kahverengi yosun olan *Sargassum ilicifolium* (Turner) C.Agardh seçilmiş ve *Sargassum ilicifolium* (Turner) C.Agardh'ın etanolik ekstraktı ve kloroform fraksiyonunun kolon kanseri (HT-29) ve akciğer kanseri (A549) hücre serilerine karşı *in vitro* anti-proliferatif etkinliğinin araştırılması amaçlamıştır. Deniz yosununun ekstraksiyonu %70 etanol ve çeşitli çözücüler kullanarak fraksiyonlanmıştır. Fitokimyasal taramaya dayanılarak, kloroform fraksiyonu seçildi, sonra GC-MS analizine tabi tutuldu. İ*n vitro* MTT hücre hattı deneyi için, kolon kanseri hücre hattı (HT-29) ve akciğer kanseri hücre hattı (A549) kullanılarak % 70 etanolik ekstrakt ve kloroform fraksiyonu seçildi. GC-MS analizi raporunda 21 bileşiğin varlığı doğrulandı ve *in vitro* kolon ve akciğer kanseri hücre hatlarına karşı anti-proliferatif aktivite etkisi IC50 değeri ile gösterildi. İleride fraksiyondaki aktif biyomolekül izole edilmeli ve antikanser aktivitesini güçlendirmek için *in vivo* incelemeye tabi tutulan formüle edilmiş ürün karakterize edilmelidir.

Anahtar Kelimeler: Antiproliferatif etki, kolon kanseri, akciğer kanseri, *Sargassum ilicifolium*, GC-MS analizi.

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