International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (2): 379-387
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Evaluation of Anticancer and Anti-inflammatory potential of methanolic extract of green seaweed *Ulva fasciata*

J K. Mini Alphonse*[1], K R T. Asha[2] and G. Immanuel[3]
[1]Department of Biochemistry, Malankara Catholic College, Mariagiri, Kaliyakkavilai - 629153, Tamilnadu, India.
[2]Department of Biochemistry, Government Arts College, Paramakudi - 623707, Tamilnadu, India.
[3]Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam, Kanyakumari - 629502, Tamilnadu, India.

Received: 16 Jan 2019 / Accepted: 15 Mar 2019 / Published online: 1 Apr 2019
Corresponding Author Email: minialphonsebiochem@gmail.com

Abstract
In the recent times, discovery of metabolites with biological activities from the marine seaweeds has increased significantly to determine their efficacy against the various human diseases. In the present study was conducted to determine the anticancer and anti-inflammatory efficacy of methanolic extract of green seaweed *Ulva fasciata*. The methanolic extract was characterized by FTIR and GC-MS analysis for determining the functional groups and secondary metabolites present in the extract. The methanolic extract showed the potential activity against the *in vitro* anti-inflammatory assays (albumin denaturation, antiproteinase, hypotonicity-induced haemolysis and anti-lipoxygenase activities). The Hep2 cancer cells inhibition of the *U. fasciata* methanolic extract was observed in a dose dependent manner and increasing of activity with increasing of concentrations. The maximum activity was displayed in 500µg/ml for 65% and the minimum activity was noticed in 100µg/ml. The IC_{50} value *U. fasciata* was documented as 402.16µg/ml. This result suggested that the active fraction can be used in pharmaceutical applications.

Keywords
Anticancer, Anti-inflammation, *Ulva fasciata,* Seaweed, liver cancer.

*****

INTRODUCTION
Marine seaweeds are rich in bioactive compounds that could potentially be exploited as functional ingredients for both human and animal health applications. In the recent times, discovery of metabolites with biological activities from the marine seaweeds has increased significantly to determine their efficacy against the various human diseases. Moreover, the recent research has focused on the natural products as an anti-inflammatory agent due to the no side effect properties. Pharmaceutical anti-inflammatory drugs are generally used to treat inflammation and pain. In general, these drugs reduce inflammatory response

by suppressing the production of pro-inflammatory mediators, which are involved in the pathogenesis of inflammatory diseases [1]. Nevertheless, reports on the increased risk of digestive, cardiovascular and renal diseases with long-term use of several non-steroidal anti-inflammatory drugs and the serious systemic side effects of gluco corticoids, have raised concerns about using the drugs. To overcome this limitation, a considerable amount of researches have promoted the discovery and development of new bioactive natural products with anti-inflammatory and antinociceptive properties which do not show any side effects. An increasing number of studies have demonstrated that certain extract and compound from seaweeds have potential anti-inflammatory uses. For example, Ganovski et al. [2] reported the anti-inflammatory effect of an aqueous extract from *Cystosina barbata, Ulva lactuca* and *Zostera nona*. Also, Payá et al [3] studied the anti-inflammatory potential of methanol and dichloromethane extracts of seven species of seaweeds.

On the other hand, carcinogenesis or 'creation' of cancer is the second leading cause of death world-wide right next to heart disease [4]. It is a class of disease, in which a group of cells display uncontrolled growth, invades and destroys adjacent tissues via lymph or blood. The formation of a cancer is a multi-step process in which multiple genetic alterations occur usually over the span of years to deregulate the control of cell growth, cell division and cell differentiation [5]. Among human tumors, most of the genetic alterations are acquired in the form of chromosomal translocation, deletion, inversion, amplification and point mutation. Certain oncogenic viruses also play an important role in a few human tumors [6].

Cancer cells often exhibit more rapid rates of uptake of nutrients than their normal counterparts, reflecting changes in the activity of membrane transport mechanisms [7]. However, cancer occurs when clones of mutated cells survive and proliferate inappropriately. The plasma membrane is the site of many specialized receptors for hormones that regulate growth and metabolism. Changes in membrane functions usually accompany with neoplastic transformation [8]. The World Health Organization (WHO) says that liver cancer as a cause of death is reported at more than 30 cases per 100,000 people worldwide. Liver cancer (or) hepatic cancer, or hepatic cellular carcinoma or primary liver cancer or hepatoma is called as 'carcinoma of the king' complex disease symptoms are numerous. The liver is made up of different cell types like, bile duct, blood vessels and fact storing cell. Liver cells (heptocytes) make up 80% of the liver tissue. The majority of primary liver cancer (over 90-95%) arises from liver cells and it is called hepato cellular carcinoma or cancer. In this, the liver cells become abnormal, grow out of control and form a cancerous tumor. Very young children may develop another form of liver cancer known as hepatoblastoma. In advanced liver cancer, the tumor can spread locally to neighboring tissues or through the blood vessels, elsewhere in the body locally, and can invade the vein that drains the liver. The tumor can then block these veins, which results in congestion of the liver. The congestion occurs because the blocked veins cannot drain the blood out of the liver [9]. In 2010 survey of 1000 plants, 356 had clinical trials published evaluating their pharmacological activity [10]. In this line, seaweeds are large subset of a group known as algae. They are marine algae, and ecologically and biologically Important. El-Saharty et al. [11] who, studied the anticancer ability of four seaweeds of red and brown namely, *Jania rubens, Scinaia fascularis, Hydroclathrus clathratus* and *Sargassum cinereum* against HepG2 cell lines by MTT assay and proved the maximum effect. With this background, the present study was aimed to investigate the anti-inflammatory and anti-cancer potential of methanolic extract of *Ulva fasciata.*

MATERIALS AND METHODS

Seaweed Collection

Seaweed *Ulva fasciata* was collected from the Tamilnadu coastal waters Muttom (Lat. 8° 07' N and Long. 77° 18' E), Southeast coast of India. The collected seaweed was immediately rinsed with filtered seawater and then washed with 5% ethanol, added fresh water to remove sand particles, prevent salt formation and also to expel epibionts. The cleaned seaweeds were dried in shade at room temperature until a constant weight is obtained. The dried seaweed material was made into coarse powder and packed in vacuum packet.

Extraction of seaweeds

Briefly, 100g of seaweed powder was extracted individually in 500 ml of methanol. The extraction was performed in darkness at room temperature 35±2°C. The process was repeated thrice and filtered using Whatman No.1 filter paper and pooled together. Each filtrate was concentrated to dryness under reduced pressure using a rotary evaporator and stored in screw cap vials for further study.

Characterization of bioassay guided fractions

Based on our previous results (data not shown), we have chosen 17th fraction of methanolic extract of *U.*

fasciata obtained by column chromatography was used for this study

Fourier Transmission Infra-Red Spectrum

The functional groups of the active methanolic fraction (F17) of *U. fasciata* were analyzed by using Fourier Transmission Infra-Red (FTIR)). The instrument used for FTIR analysis was Shimadzu, Japan at USIC, CAS in Marine Biology, Annamalai University, Parangipettai. Briefly, KBr discs were prepared by grinding the pure compound (2.0 mg by weight) with KBr and compressing the whole into a transparent wafer or discs. The KBr was dried and it is an advantage to carry out grinding under an infrared lamp to avoid condensation of atmospheric moisture which gives to broad adsorption at 3500 cm^{-1}. The particle size of the grinding was achieved by grinding KBr sample complex to < 2 µm to avoid wavelength scaling. High pressure was given to KBr disc to condense fairly to 13 mm in diameter and 0.3 mm in thickness.

Gas Chromatography Mass Spectroscopy (GC-MS) analysis of active fractions of *U. fasciata*

GC-MS analysis of the bioassay guided $F17^{th}$ fraction of crude methanolic extract of *U. fasctiata* was analyzed individually using Agilent GC-MS 5975 Inert XL MSD (United States) gas chromatography equipped with J&W 122 – 5532G DB – 5 ms 30 × 0.25mm × 0.25 µm and mass detector (EM with replaceable horn) was operated in EMV mode. Helium was used as carrier gas with the flow rate of 1.0 ml /min. The injection port temperature was operated at 250°C. The column oven temperature was held at 80°C for 2 min then programmed at 10° C to 250°C, which was held for 0 min, and then at 5°C to 280°C which was held for 9 min. Electron impact spectra in positive ionization mode were identified.

***In vitro* anti-inflammatory assays**

The *in vitro* anti-inflammatory activity of *U. fasciata* methanolic extract was estimated by inhibition of albumin denaturation, antiproteinase, hypotonicity-induced haemolysis and anti-lipoxygenase activities. The albumin denaturation inhibition assay was performed spectroscopically by Mizushima et al. [12] the sample reaction mixture (100-500µg/ml) was read at the wavelength of 660 nm and the activity was expressed in percentage aspirin used as standard. The antiproteinase activity was measured spectroscopically following the method of Oyedepo et al. [13], the sample was used in different concentrations (100-500 µg/ml) and the absorbance was measured at 210 nm and the results were expressed in percentage and the aspirin was used as a standard. The hypotonicity-induced haemolysis assay was determined by spectroscopic method following the method of Azeem et al. [14]. The haemoglobin content was estimated by a spectrophotometer (Shimadzu-1800, Japan) at 560nm and the sample was taken at the different concentrations (100-500 µg/ml), diclofenac was applied for standard. Anti-lipoxinase activity was carried out by Shinde et al. [15], in this assay the anti-lipoxinase effect was estimated by using linoleic acid as substrate and lipoxidase as enzyme for different concentrations of sample (100-500 µg/ml) and Indomethacin was used as a standard. The absorbance was measured at 234 nm and the results were expressed in percentage.

Anticancer activity

Cells lines and culture

Vero cells and Hep2 cell lines were procured from NCCS, Pune, India. The cells were maintained under optimum condition. All the cells were cultured in 50 ml cell culture flasks containing growth medium (GM), i.e., Minimum Essential Medium (MEM, GIBCO™, USA) supplemented with 10% (v/v) Fetal Calf Serum (FCS, GIBCO™, USA) and incubated at 37°C in 95% humidified atmosphere and 5% (v/v) CO_2 incubator. Maintenance Medium (MM) was the same MEM but containing 2% (v/v) FCS.

MTT assay

The proliferation activity of cell populations (Vero and Hep2) untreated and treated with *U. fasciata* methanolic extract was determined by the MTT assay based on the detection of mitochondrial dehydrogenase activity in living cells [16]. MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide) assay, in which the yellow tetrazolium salt is metabolized by NAD-dependent dehydrogenase (in active mitochondria) to form a dark blue formazan product. Yellow color MTT is converted to the blue formazan product only by metabolically active mitochondria, and the absorbance is directly proportional to the number of viable cells. MTT solution (5 mg/ml) was added to each culture well 24 h after treated with *U. fasciata* methanolic extract and the color was allowed to develop for additional 4 h incubation. An equal volume of DMSO was added to stop the reaction and to solubilize the blue crystals. Samples were transferred into culture plates and the absorbance was measured calorimetrically at 490 nm.

RESULTS AND DISCUSSION

Edible seaweeds are previously used as gelling and thickening agents in the food or pharmaceutical industries. Current researches have revealed their potential as complementary medicine due to their

therapeutic properties for health and disease management, such as anticancer, anti-obesity, anti-diabetic, antihypertensive, anti-hyperlipidemic, antioxidant, anticoagulant, anti-inflammatory, immunomodulatory, anti-estrogenic, thyroid stimulating, neuroprotective, antiviral, antifungal, antibacterial and tissue healing properties *in vivo*.

In the present study, FT-IR analysis of bioassay guided active fraction of *U. fasciata* revealed the presence of functional groups ranged from 500 to 4000 cm^{-1}. A medium stretch observed at 1458.18 cm^{-1} may be due to the presence of C-C bond of aromatic compounds. A strong peak was observed at 1730.15 cm^{-1} attributed to the occurrence of C=O bond of alpha, β unsaturated esters. A medium stretch observed at 2858.51cm^{-1} may be due to the C=H bond of alkane groups. A strong and broad band was observed at 3441.01 cm^{-1} corresponds to Oxygen and Hydrogen bonding (O=H) of alcohol and phenol groups (Fig. 1). The similar characteristic features and functional groups were reported in several seaweeds [17]. The GC-MS profile of the bioassay guided active fraction of *U. fasciata* showed the presence of 9 compounds and their closely matching molecules in the library, their molecular formula and molecular weight extracted from the databases have been listed in table 2 and Fig. 2. Observation showed that the estimated molecular weights of the compounds ranged from 168 to 402 with varying retention time (14.30 to 31.44 minutes) and percentage composition (area %) 0.45 to 34.90. Based on the spectral match of the obtained compound with NIST 2005 library and relative percentage of the compound, the major phytoconstituents observed were Tetradecanoic acid, n-Hexadecanoic acid, 9-Octadecanoic Acid, Eicosanoic acid, Bis (2 Ethylhexyl) phthalate, Fenretinide, 2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trim ethyltridecyl)-, [2R-[2R*(4R*,8R*)]]-Pregn-5-ene-3,20-dione, Pregnenolone. Similarly, Horincar et al. [18] have studied GC-MS analysis of volatile compounds content in macroalgae from the Romanian Black Sea coast. The major components in the extract of *Ceramium virgatum* were 3-hexen-2-one, acetone, hexanal and o-cymene.

The anti-inflammatory potential of *U. fasciata* methanolic extract has been screened by the inhibition of albumin denaturation, antiproteinase, hypotonicity-induced haemolysis and anti-lipoxygenase activities through *in vitro* spectroscopic method. In the present study, the protein inhibitory effect of *U. fasciata* methanolic extract has shown dose dependent and it was found to be 9, 16, 37, 44 and 56% at the concentrations of 100, 200, 300, 400 and 500 µg/ml and the maximum protein inhibitory activity was recorded in 500µg/ml, and found to be 56% in *U. fasciata* followed by 44% inhibition at 400µg/ml (Fig. 3). The proteinase enzyme inhibitory potential of *U. fasciata* methanolic extract and aspirin was noticed concentration dependent and the results was found to be 7, 16, 21, 35 and 51% at the concentrations of 100, 200, 300, 400 and 500 µg/ml and the highest proteinase enzyme inhibition of *U. fasciata* was noticed in 500µg/ml and it was found to be 51% followed by 35% inhibition in 400µg/ml (Fig. 4). The hemolysis inhibitory effect of *U. fasciata* methanolic extract and aspirin was documented in dose dependent and the results was found to be 21, 31, 51, 64 and 72% at the concentration of 100, 200, 300, 400 and 500µg/ml and the highest hemolysis inhibition of *U. fasciata* was noticed in 500µg/ml and it was found to be 72% followed by 64% inhibition in 400µg/ml (Fig. 5). The anti-lipoxigenase effect of methanolic extract of *U. fasciata* and aspirin was documented in dose dependent and the results was found to be 6, 17, 32, 58 and 69% at the concentration of 100, 200, 300, 400 and 500µg/ml and the highest hemolysis inhibition of *U. fasciata* was noticed in 500µg/ml and it was found to be 69% followed by 58% inhibition in 400µg/ml (Fig. 6). Similarly, Radhika et al. [19] have been studied the anti-inflammatory effect of marine macroalgae (Seaweeds) such as *Padina tertastomatica*, *Sargassum wightii*, *Gracilaria edulis* and *Caulerpa racemosa* and reported lower anti-inflammatory response of all seaweeds than the *U. fasciata* methanolic extract. Likewise, Eman et al. [20] have described the anti-inflammatory activity two seaweeds namely *D. fasciola* and *G. cylindrica*by *in vitro* screening and they recorded inhibition percent reached to 40.62 and43.64% at the dose of inhibitor 200µg/ml respectively. Similarly, Oumaskour et al. [21] studied the anti-inflammatory potential of 23 red seaweeds by *in vitro* enzyme inhibition namely PLA2 inhibition assay and Elastase Inhibition assay and showed maximum enzyme inhibition in *Chondrus crispus* and *Gelidium sesquipedale* extracts respectively. Correspondingly, Chaliniet al. [22] have been investigated the anti-inflammatory response of *Gracilaria edulis*, *Gracilaria corticata*, *Gracilaria fergusonii* and *Gracilaria verrucosa* aqueous extract and reported the highest enzyme inhibition for 95.55% at the concentration of 250µg/ml for *G. edulis*. Likewise, Hwang et al. [23] exhibited the anti-inflammatory activity of the seaweed *Undaria pinnatifida* from different region and the activity was found to be high

with an IC_{50} value of 78.5 µg/mL as Jeju exhibited the highest antioxidant activity by 208.1µmol TE/100 µg, followed by Gijang (112.8 µmol TE/100 µg) and Wando (94.8 µmol TE/100 µg). Ananthi et al. [24] have reported the good anti-inflammatory activity of brown algae *Turbinaria ornate in vivo* method of wistar rats. Similarly, Mhadhebi et al. [25] analyzed the in vivo anti-inflammatory effect of three mediterranean brown seaweeds namely, *Cystoseira crinita*, *Cystoseira sedoides* and *Cystoseira compressa* by aqueous extraction and reported the excellent anti-inflammatory effect in all seaweeds species respectively. Likewise, Vijayalakshmi [26] investigated the anti-inflammatory activity of the polar solvents namely methanolic and aqueous and extracted the metabolites from seaweed *Gracillaria edulis* pointed out the maximum effect in aqueous extract metabolites than the methanol.

Liver cancer is the third cause of death in worldwide with 5-year survival rate less than 15% [27]. In the latter two decades the death rate was increased due to liver cancer, nevertheless, the death rate of all types of cancer reduced [28]. The increased death rate due to liver cancer started for numerous factors, one of these factors is the very limited treatment options [29]. MTT assay is a most accessible *in vitro* method for detection of the anticancer potential of a drugs and natural compounds. The anticancer activity of *U. fasciata* methanolic extract against Hep-G2 cell lines through MTT assay was studied. The cancer cells inhibition of the *U. fasciata* methanolic extract was observed in a dose dependent manner and increasing of activity with increasing of concentrations. The maximum activity was displayed in 500µg/ml for 65% and the minimum activity was noticed in 100µg/ml (Figs. 7). The IC_{50} value (drug required for half inhibition) of *U. fasciata* was documented as 402.16µg/ml (Table 3)

In the present study is an agreement with El-Saharty et al. [30] who, studied the anticancer ability of four seaweeds of red and brown namely, *Jania rubens, Scinaia fascularis, Hydroclathrus clathratus* and *Sargassum cinereum* against HepG2 cell lines by MTT assay and reported the maximum activity in *Jania rubens* with an LC_{50} at 8.61µg/mL followed by *Hydroclathrus clathratus* 17 µg/mL, *Sargassum cinereum* 18.2 µg/mL and *Scinaia fascularis* for 43 µg/mL respectively. Likewise, Taskin et al. [31] have been reported the crude methanol extract of marine algae isolated from Aegean Shores of Turkey have potent anticancer effect (90%) of *Padina pavonica* and *Cystoseira mediterranea* brown algae against human breast adenocarcinoma (MCF-7) and human prostate cancer cells. Similarly, Athukorala et al. [32] have explored the enzymatic extract of *Ecklonia cava* composed with its crude polyphenolic and polysaccharide fractions strongly reflects the antiproliferative activity against murine colon cancer cell line, human leukemia, mouse melanoma and human leukemia cells through MTT assay. Additionally, Zandi et al. [33] have been reported the antiproliferative effect of *Sargassum oligocystum* water extract against human cancer cell lines by MTT assay and showed the effective anticancer effect against Daudi and K562 cell lines at the concentration of 400 and 500µg/mL respectively. From the study of Namvar et al. [34] who, described the anticancer effect of different seaweeds from Persian gulf namely, *Gracillaria corticata*, *Ulva fasciata* and *Sargassum ilicifolium* against different human cancer cell lines such as MCF-7, MDA-MB-231, HeLa, HepG2 and HT-29 and all the methanolic extracts of seaweeds were showed antiproliferative against all the cancer cell lines with dose-dependently and with *G. corticata* methanol extract have displayed the greatest inhibition activity against MCF-7 cell line with apoptosis increased from 18 to 78 %.

Table 1. Anticancer activity of methanolic extract of *U. fasciata* Hep-G2 cell lines

S.No	Concentration (µg/ml)	Cell inhibition (%)	IC_{50} (µg/ml)
1	100	11	
2	200	23	
3	300	35	
4	400	49	402.16
5	500	65	
6	Cell control	100	

Table 2. Results of the GC-MS analysis of bioassay guided active fraction of *U. fasciata* showing retention time, area% and the name of the closely matching compounds

Retention Time (RT)	Fatty Acids	Molecular Formula	Molecular Weight	Area %
14.30	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	8.30
16.39	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	34.90
18.05	9-Octadecanoic Acid	$C_{18}H_{34}O_2$	282	18.31
19.95	Eicosanoic acid	$C_{20}H_{40}O_2$	312	1.62
21.50	Bis (2 Ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	3.09
23.14	Fenretinide	$C_{26}H_{33}NO_2$	391	1.60
23.97	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R*(4R*,8R*)]]-	$C_{27}H_{46}O_2$	402	1.02
28.42	Pregn-5-ene-3,20-dione	$C_{21}H_{30}O_2$	314	14.71
31.44	Pregnenolone	$C_{21}H_{32}O_2$	316	2.36

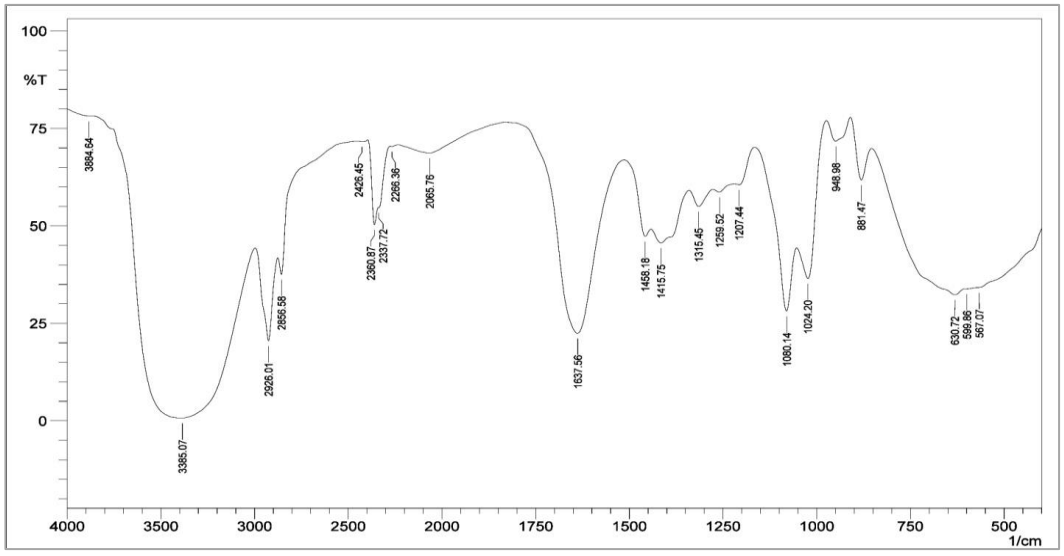

Fig. 1 FTIR spectra of methanolic extract of *U. fasciata*

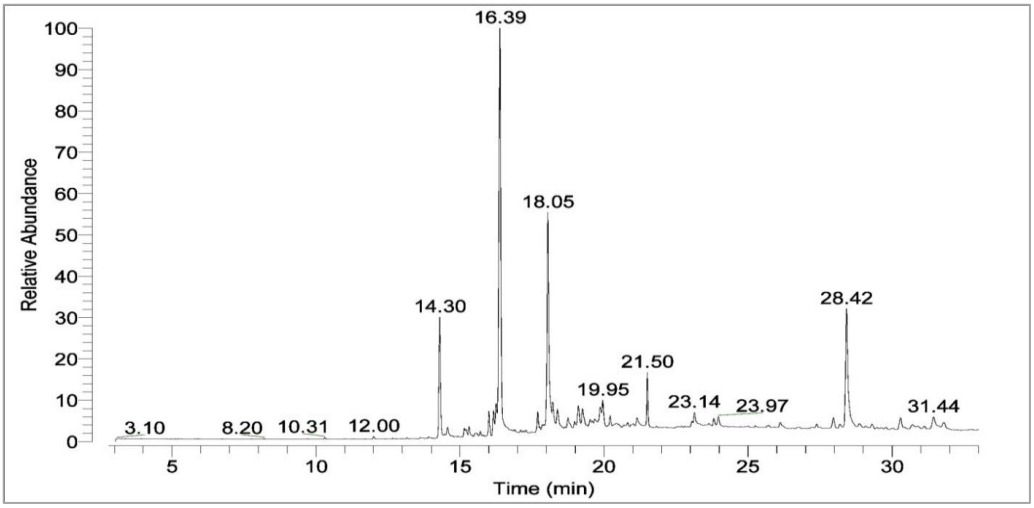

Fig. 2 GC-MS spectrum of active fraction of *U. fasciata*

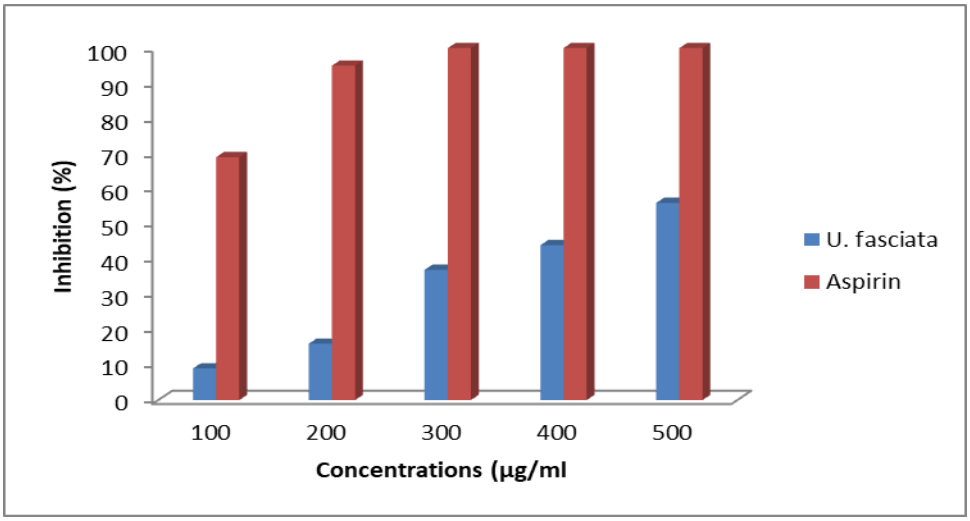

Fig. 3. Protein inhibition activity of methanolic extract of *U. fasciata*

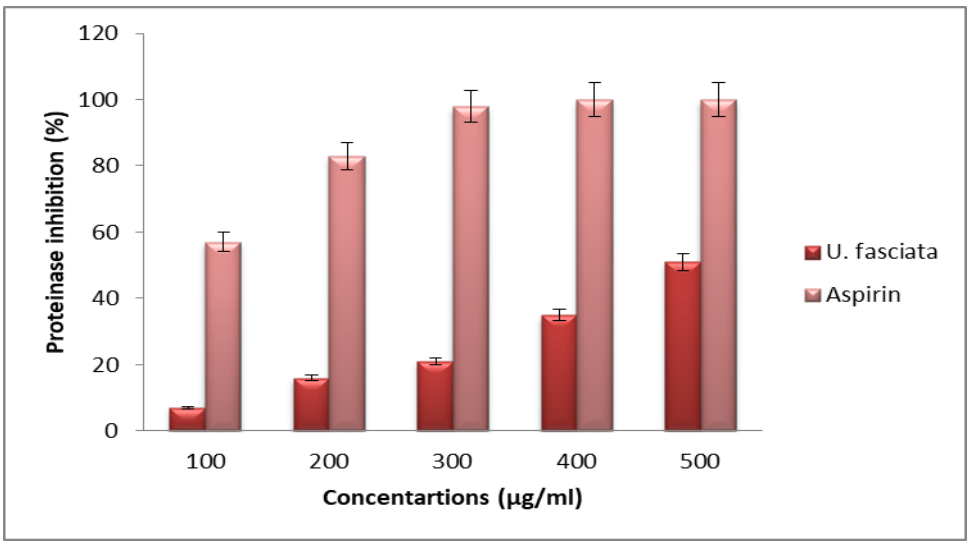

Fig. 4. Proteinase enzyme inhibitory effect of methanolic extract of *U. fasciata*

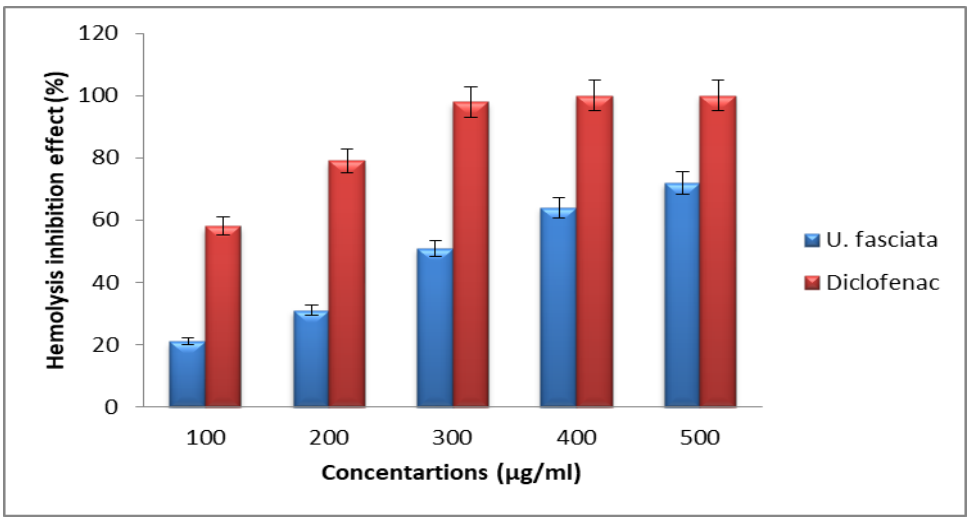

Fig. 5. Hemolysis inhibitory effect of methanolic extract of *U. fasciata*

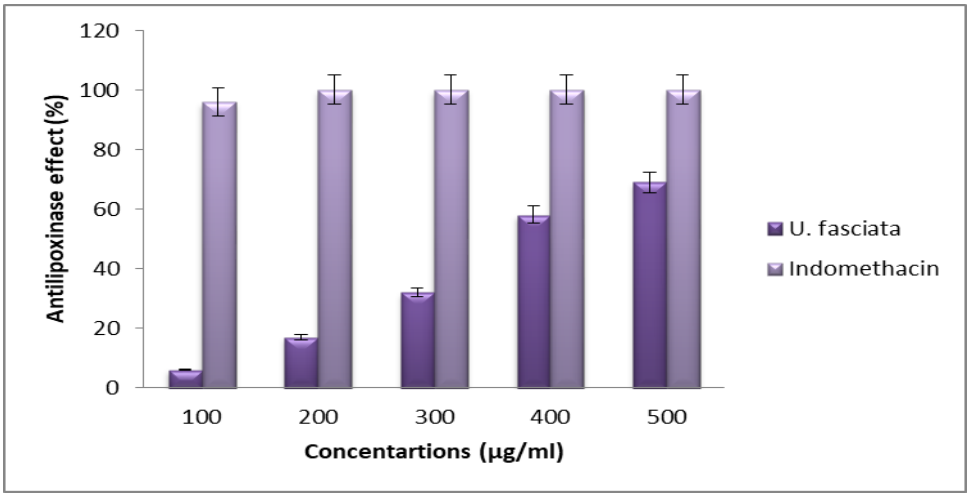

Fig. 6. Anti-lipoxigenase effect of methanolic extract of *U. fasciata*

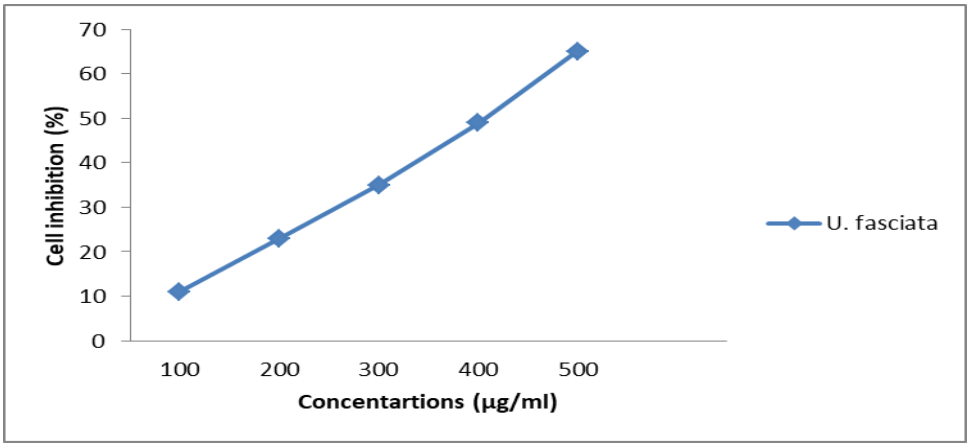

Fig. 7. Anticancer effect of methanolic extract of *U. fasciata*

CONCLUSION

From the results and previous reports, the seaweeds have been showed good anticancer and effect against all type of human cancer lines, especially for the polar solvent extracted molecules have been more potent and the study suggested that the methanolic extract of *U. fasciata* having more anti-inflammatory pharmaceutical importance.

ACKNOWLEDGMENT

The authors are grateful to the Management of Malankara Catholic College, Mariagiri for providing the laboratory facilities to carry out the work successfully.

REFERENCES

1. Guslandi M., Nitric oxide and inflammatory bowel diseases. Eur. J. Clin. Invest., 28: 904-907, (1998).
2. Ganovski KH., Shipochliev T., Bratova K., Antiinflammatoryaction of extracts from marine algae collectedin the area of Burgas seacoast. Vet. Med. Nauki, 16 (7): 54-61, (1979).
3. Payá M., Ferrándiz ML., Sanz MJ., Bustos G., Blasco R., Rios JL., Alcaraz MJ., Study of theantioedema activity of some seaweed and sponge extracts from the mediterranean coast in mice. Phytother. Res., .7 (159): 162 (1993).
4. Jemal Ahmedin DVM., Bray F., Melissa M., Jacques Ferlay ME., Elizabeth W., Forman D., Global cancer statistics. CA CANCER J CLIN. 61:69–90, (2011).
5. Suresh V., Senthilkumar N., Thangam R., Rajkumar M., Anbazhalagan C., Rengaswamy R., Gunasekeran P., Kannan S., Palani P. Separation, purification and preliminary characterization of sulphated polysaccharides from *Sargassum plagiophyllum* and its in vitro anticancer and antioxidant activity. Proc. Biochem. 48: 364-373, (2013).
6. Ding X., Zhu F., Gao S., Purification, antitumour and immunomodulatory activity of water-extractable and alkaliextractablepolysaccharides from *Solanum nigrum* L. Food chem. 131:677-684, (2012).
7. Amira M., Eldeen G., Ahmed EF., Mona A., and Zeid, A., Invitro cancer chemo preventive properties of polysaccharide extractfrom the brown alga, *Sargassum latifolium.* Food Chem. Tech. 47:1378-1384, (2009).

8. Surh YJ., Kundu JK., Cancer preventive phytochemicals asspeed breakers in inflammatory signaling involved in aberrant COX-2 expression. Curr. Cancer Drug Target. 7: 447–458, (2007).
9. Garden O.J., Ree SM., Poston GJ., Mirza D., Saunders M., Ledermann J., Prim rose JN., and Parks RW. Guide lines for resection ofcolorectal cancer liver metastases. Gut, 55 (3): 1-8, (2006).
10. Cravotto G., Boffa L., Genzins L., and Gareklla D. (2010). "Phytotherapeutics: an evalution of the potential of 1000 plants" J.Clini. pharm Ther, 35(1): 11-48.
11. El-Saharty A., Osman A., Farghaly., Abdel-Rahman M., Hamed, Hamada Abdel-Fattah A., Noreldeen., Anticancer Activity of Some Marine Macroalgae in Hepatocellular Carcinoma Cell Lines (HepG2). Int J Ecotoxicol Ecobiol. 3(1): 22-30 (2018).
12. Mizushima Y., Kobayashi M., Interaction of anti-inflammatory drugs with serum preoteins, especially with some biologically active proteins. J of Pharma Pharmacol 20:169-173, (1968).
13. Oyedepo OO., Femurewa AJ., Anti-protease and membrane stabilizing activities of extracts of Fagra zanthoxiloides, Olax subscorpioides and *Tetrapleura tetraptera*. Int J of Pharmacong. 33: 65-69, (1995).
14. Azeem AK, Dilip C, Prasanth SS, Junise V, Hanan Shahima. Anti-inflammatory activity of the glandular extracts of *Thunnus alalunga*. Asia Pac J for Med. 3(10): 412-20, (2010).
15. Shinde UA, KR Kulkarni, A S Phadke, A M Nair, Dikshit V J, Mungantiwar and Saraf MN. Mast cell stabilizing and lipoxygenase inhibitory activity of *Cedrus deodara* (Roxb.) Loud. Wood Oil. Indian J Exp Biol. 37(3): 258-261, (1999).
16. Mosmann T., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immuno. Met. 65: 55–63, (1983)
17. Sumayya SS., and Murugan K., Phytochemical screening, RP-HPLC and FTIR Analysis of Kappaphycus alvarezii (Doty) Doty EX P.C Silva: Macro red algae. J Pharmacogn Phytochem. 6(1): 325-330, (2017).
18. Horincar VB., Parfene G., Tyagi AK., Gottardi D., Dinica R., Guerzoni ME., Bahrim G., Extraction and characterization of volatile compounds and fatty acids from red and green macroalgae from the Romanian Black Sea in order to obtain valuable bio additives and bio preservatives. J. Appl. Phycol. 26: 551–559, (2014).
19. Radhika D., Veerabahu C., Priya R., Anti-inflammatory activities of some seaweed collected from the gulf of mannar coast, tuticorin, south India. Int. J.Phar. Biosci. 4(1): 39-44. (2013).
20. Eman A., Ibrahim., Hanan F., Aly and Farouk K., El-Baz., Anticoagulant and Anti-Inflammatory Potentials of some Egyptian Marine Algae. Int J Pharma Bio Sci., 7(2): 89-94, (2016).
21. Oumaskour K., Boujaber N., Etahiri S., and Assobhei O., Anti-inflammatory and antimicrobial activities of twenty-three marine red algae from the coast of Sidi Bouzid. International Journal of Pharmacy and Pharmaceutical Sciences. 5(3): 145-149, (2013).
22. Chalini K., Johnson M. Adaikalaraj G., Vidyarani George., Ramakrishnan P., Anti-inflammatory activity of aqueous extracts of *Gracilaria.* Int J Curr Pharm Res. 9(5):17-19, (2017).
23. Hwang JH., You-Sung Oh, and Sang-Bin Lim. Anti-inflammatory Activities of Some Brown Marine Algae in LPS-Stimulated RAW 264.7 Cells Food Sci. Biotechnol. 23(3): 865-871, (2014).
24. Ananthi S., Gayathri V., Chandronitha C., Lakshmisundaram R., Vasanthi HR., Free radical scavenging and anti-inflammatory potential of marine brown algae *Taurbinaria ornate*. Indian J Geomarine Sci. 40(5): 664-670, (2011).
25. Mhadhebia L., Amel Mhadhebic., Jacques Robertb., and Abderrahman Bouraouia., Antioxidant, Anti-inflammatory and Antiproliferative Effects of Aqueous Extracts of Three Mediterranean Brown Seaweeds of the Genus *Cystoseira.* Iran J Pharm Res. 13 (1): 207-220, (2014).
26. Vijayalakshmi S., Screening and anti-inflammatory activity of methanolic and aqueous extracts of seaweed *Gracillaria edulis.* Int. j. modern chem. appl. sci. 2(4): 248-250, (2015)
27. Arzumanyan A., Reis HM., Feitelson MA., Pathogenic mechanisms in HBV-and HCV-associated hepatocellular carcinoma. Nat. Rev. Cancer. 13: 123–135, (2013).
28. Siegel R., Ma J., Zou Z., Jemal A., Cancer statistics 2014, CA Cancer J. Clin. 64: 9–29. (2014).
29. Kimhofer T., Fye H., Taylor-Robinson S., Thursz M., and EHolmes., Proteomic and metabonomic biomarkers for hepatocellular carcinoma: a comprehensive review, Br J Cancer. 112: 1141–1156, (2015).
30. El-Saharty A., Osman A., Farghaly Abdel-Rahman M., Hamada Abdel-Fattah AH., Noreldeen., Anticancer Activity of Some Marine Macroalgae in Hepatocellular Carcinoma Cell Lines (HepG2). Int J Ecotoxicol Ecobiol. 3(1): 22-30, (2018).
31. Taskin E., Z Caki., M. Ozturk., and Taskin E., Assessment of invitro antitumoral and antimicrobial activities of marine algae harvested from the eastern Mediterranean Sea," Afr. J. Biotechnol. 9 (27): 4272–4277, (2010).
32. Athukorala Y., Kim KN., and Jeon YJ., "Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brownalga, *Ecklonia cava*. Food Chem Toxicol. 44(7): 1065–1074, (2006).
33. Zandi K., Ahmadzadeh S., Tajbakhsh S., Rastian Z., Yousefi F., Farshadpour F., Sartavi K., Anticancer activity of *Sargassum oligocystum* water extract against human cancer cell lines. Eur Rev Med Pharmacol Sci. 14: 669-673, (2010).
34. Namvar F., Baharara J., Mahdi A., Antioxidant and Anticancer Activities of Selected Persian Gulf Algae. Ind J Clin Biochem., 29(1): 13–20, (2014).