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Research Article

ANTI-INFLAMMATORY, ANTI-OXIDANT, PHYTOCHEMICAL AND GC-MS ANALYSIS OF MARINE BROWN MACROALGA, *SARGASSUM WIGHTI*

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ABSTRACT

Marine brown alga, *Sargassumwightii*extracts was analyzedfor the anti-inflammatory and anti-oxidant activity by *in vitro*methods and the active ethyl acetate fraction has been subjected to GC-MS analysis. At 50 µg/ml RBCs membrane showed an inhibition of 55.7% followed by methanol (54.3%) and hexane (52.2%), whereas in hypotonicity induced haemolysis, it is found toprotect the erythrocyte membrane against lysis induced by hypotonic solution compared to diclofenac sodium, a standard drug. It also showed maximum inhibition of 52.9% compared to methanol and hexane in its proteinase and albumin denaturationtest (70.8%) at 50 µg/ml. Free radical scavenging assay showed that ethyl acetate extract has significant scavenging effect on DPPH (64.9%) at 200 µg/ml. GC-MS analysis revealed that ethyl acetate fraction of *S. wightii* contains seventeen compounds and some of the major compounds detected wereBromoacetic acid, hexadecyl ester (94.98%),1,4-Eicosadiene (87.16%), Eicosane (73.97%), 6-Octadecenoic acid, (Z)- (72.17%), n-Hexadecanoic acid (62.97%), Benzene, 1,2-dimethoxy-4-(1-propenyl)- (62.92%), Stigmasta-5,24(28)-dien-3-ol, (3.beta)- (61.06%), Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(Phenyl methyl)- (56.62%) 2*(*1H*)*-* Pyrimidinone*,* 4-amino-5-methyl-(41.97%) and 4-Methoxy-3-Propoxy-Benzaldehyde (40.18%).

Keywords: *Sargassumwightii*, antioxidant, anti-inflammatory, macroalgae

INTRODUCTION

The marine environment represents extensive biodiversity of organisms and huge resource potential to provide many complex chemical compounds with functional materials including polyunsaturated fatty acids (PUFA), polysaccharides, essential minerals and vitamins, antioxidants, enzymes and bioactive peptides compared to terrestrial environment[1]. Today, with the modern tools of molecular biology and advanced technology, marine organisms have provided numerous medical drugsthat can provide new insights and understanding of human diseases and their treatment[2]. The metabolic and physiological capabilities of marine organisms that allow them to survive in complex habitat provide a great potential for production of secondary metabolites which are not found in terrestrial environments. Marine organisms are currently undergoing detailed investigations with the objective of isolating biologically active molecules along with the search for new compounds. Among marine organisms, macroalgae are rich sources of structurally diverse bioactive compounds with different bioactivity spectra and biomedical value[3]. The development of ways to obtain large quantities of the natural metabolites is currently the most important quest[4]. Macroalgal substances serve as the viable source of drugs for the world population and several algae-based drugs are in extensive clinical use.

Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics (Opiods) or non-narcotics (Salicylates, Corticosteroids and Hydrocortisone) and all of the drugs possess

well known toxic and side effects. Aspirin can cause stomach bleeding, acetaminophen can cause liver damage, Cox-2 inhbitorVioxx and Celebrex can cause heart problem and non-steroidal anti-inflammatory drugs (NSAID's) was reported to contribute to numerous death yearly[5]. Marine organisms have been tested as source of anti-inflammatory compounds and analysis of macroalgae indicated the presence of bioactive compounds including caulerpin, fucoidans, galactofucanwith anti-inflammatory activities[6].

The reactive oxygen species (ROS) generated in the human body can cause oxidative damages associated with many degenerative diseases such as atherosclerosis, coronary heart diseases, cancer, mutagenesis, arthritis, diabetics, inflammation, aging and genotoxicity. Many commercial synthetic antioxidants, such as butylatedhydroxyanisole (BHA) and butylatedhydroxytoluene (BHT) are used under strict regulation due to their potential health hazards. Macroalgae are known to contain reactive antioxidant molecules such as ascorbate and glutathione (GSH) when fresh, as well as secondary metabolites, including carotenoids (alpha and beta carotene, fucoxanthin, astaxanthin), mycosporine-like amino acids (mycosporine-glycine) and catechins (epigallocatechin), gallate, phlorotannins (phloroglucinol), eckol and tocopherols[7].

Brown algae have been shown to possess the ability to produce a great variety of secondary metabolites with very different skeleton types and functionalities[8]. *Sargassum*, one of the marine macroalgal genera belonging to the class Phaeophyta, is widely distributed in tropical and temperate oceans and a wide range of bioactive properties have been reported from this species[9]. It belongs to the family *Sargassaceae*and order *Fucales*. *S. wightii*shows a good amount of flavonoids in support of its antioxidant activityindicate that this genus is an ideal target for investigating presence of bio-molecules for various medical and industrial applications[10]. The aim of this study was to analyze the anti-inflammatory and antioxidentactivities of *Sargassumwightii*extracts and characterize the composition of active phytoconstituents by Gas chromatography-mass spectrometry.

MATERIALS AND METHODS

Sample collection and preparation of algal extracts

Samples of marine macroalga, *Sargassumwightii*was collected from the coastal region of Thiruchendur (Lat. 08°30' N; Long. 78°11'E), Southeast coast of India. Immediately after collection, the macroalgae were washed in filtered fresh seawater to remove the epiphytes, sand particles, pebbles, shells and other extraneous matter. The completely shade dried material was weighed and cut into small pieces and ground finely in a mechanical grinder and extracted with methanol, ethyl acetateand hexane. For this, 10 g of dried algal powder was weighed and submerged in a flask containing 100 ml of solvents and placed in a shaker at 120 rpm for one day overnight at room temperature. The algal material was re-extracted twice for the complete extraction of methanol-soluble compounds and all the three methanol extracts were pooled and filtered using Whatmann No. 1 filter paper. . The extraction procedure was repeated for the ethyl acetate and hexane solvents. The obtained filtrates were concentrated under rotary evaporator under reduced pressure and the residues were finally dried in a vacuum desiccator and stored in the refrigerator[11].

*In-vitro*anti inflammatory activity

To test anti-inflammatory activity, prepared stock solutions of red blood cells (RBCs) suspension by collecting fresh whole human blood (5 ml) and transferred to the centrifuge tubes containing EDTA. The tubes were centrifuged at 3000 rpm for 10 minutes and were washed three times with equal volume of normal saline (0.85% sodium chloride). Discarded the supernatant and collected 2 ml RBC cells and mixed with 2 ml normal saline. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline. 10% stock solutions were prepared by adding 1 ml RBC suspension along with 9 ml distilled water.

Heat induced haemolysis

To conduct heat induced haemolysis, prepared the stock solutions in 2 ml eppendorff tubes by adding 0.001 g condensed seaweed sample and 1 ml DMSO. The reaction mixture (2 ml) consisted of 1ml test sample of different concentrations (10-50 µg/ml) and 1ml of 10% RBCs suspensions, instead of test sample only saline was added to the control test tube. Aspirin was used as a standard drug (100µg/ml). All the centrifuge tubes containing reaction mixture were incubated in water bath at 56^{0}C for 30 minutes. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 minutes and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in

triplicates for all the test samples. The percentage inhibition of haemolysis was calculated as follows:

Percentage inhibition = (Abs control - Abs sample) × 100 /Abs control

Hypotonicity- induced haemolysis

The hypotonicity- induced haemolysis test was conducted by preparing **d**ifferent concentrations of extract (10-50µg/ml), reference sample, and control were separately mixed with 1ml of phosphate buffer (pH 7.4), 2 ml of hyposaline (0.36% Sodium chloride) and 0.5 ml of HRBC suspension. Diclofenac sodium (100µg/ml) was used as a standard drug. All the assay mixture were incubated at 37^{0}C for 30 minutes and centrifuged at 3000 rpm. The supernatant liquid was decanted and the haemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage haemolysis was estimated by assuming the haemolysis produced in the control as 100%.

Percentage of haemolysis = 100 - (OD sample/OD control) x 100

Proteinase inhibitory activity

In proteinase inhibitory test, the reaction mixture (2 ml) containing 0.06 mg trypsin, 1ml 20 mMTrisHCl buffer (pH 7.4) and 1 ml test sample of different concentrations (10-50 µg/ml) were incubated at 37^{0}C for 5 minutes and then 1ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 minutes. 2ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated.

Percentage inhibition = (Abs control - Abs sample) × 100 /Abs control.

Albumin denaturation test

In inhibition of albumin denaturation, the reaction mixture consisting of test extract at different concentrations (10-50 µg/ ml) and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture (7.4) was adjusted using 1N HCl. The sample were incubated at 37^{0}C for 20 minutes and then heated at 57^{0}C for 20 minutes. After cooling the samples, the turbidity was measured at 660 nm (UV-visible spectrophotometer). The experiment was performed in triplicate. Percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition = Abs control-Abs sample × 100/Abs control

***In-vitro* antioxidant activity**

In-vitro antioxidant activity of extracts on DPPH radicals were estimated according to the method of Brand-Williams with minor modifications[12]. Free radical scavenging activity of different algal extract concentrations were evaluated spectrophotometrically (at 517 nm) against the absorbance of the indicator 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) solution (2.96 ml). 20-200 µg /ml seaweed stock were mixed with different concentrations of DMSO (38-20 µg /ml) along with 2.96 ml of DPPH solution was mixed and conducted DPPH assay. The reaction mixture was vortex mixed thoroughly and incubated at room temperature in the dark for 30 min. Reduction in the absorbance of the mixture was measured at 517 nm using ascorbic acid as a control. All reactions were carried out in triplicates and the degree of decolourization indicates the free radical scavenging activities of the algal extracts. Scavenging of DPPH radicals by the extract was calculated using the following formula:

Percentage of scavenging = $[(A_0-A_1) / A_0] \times 100$;

Where A_0 is the absorbance of control and A_1 is the absorbance of test sample.

Phytochemical screening, TLC and GC-MS analysis

Phytochemical screening of the extracts was carried out according to the standard method described by Harborne[13]. The active ethyl acetate extracts were tested for alkaloids, carbohydrates, saponins, aminoacids, phenolic compounds, steroids, flavanoides and tannins.

Concentrated ethyl acetate extracts of *S. wightii* was purified by thin layer chromatography using chloroform: ethyl acetate (9:1) as solvent systems. TLC was carried out on 10 x 20 cm silica gel plates (Merck, Germany) using capillary tube (2-5 l) with silica gel 60 as stationary phase and allowed the plates to dry. The colour change indicates the presence of bioactive components separated in TLC plates. The eluted spots, representing various compounds were visualized under UV transilluminator at wavelength 254 nm and also in the iodine chamber.

GC-MS technique was used in this study to identify the phyto components present in the extract. GC-MS technique was carried out at Sargam laboratory, Chennai, Tamil Nadu. GC-MS analysis was performed using GC SHIMADZU

QP2010 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1 fused silica capillary column (Length : 30.0 m, Diameter : 0.25 mm, Film thickness : 0.25 µm Composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization energy system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1.51ml/min and an injection volume of 1ml was employed (split ratio: 10). Injector temperature 240°C andion source temperature 200°C. The oven temperature was programmed from 70°C (isothermal for 2 min.), with an increase of 300°C for 10 min. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds with scan range of 40 – 1000 m/z. Total GC running time was 35 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a GC MS solution ver. 2.53. Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique (NIST08s), WILEY8 and FAME having more patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST08s, WILEY8 and FAME library. The Name, Molecular weight, Molecular formula and Structure of the component of the test material was ascertained.

All data were statisticallyanalyzed using SPSS version 17. Statistical differences between the three extracts activity were determined by using students's t test for paired data. Differences were considered statistically significant when $p<0.05$. Error mean square (ANOVA) is made use by applying Dunnet's t test to determine the effect of *Sargassumwightii*organic extracts against control.

RESULTS AND DISCUSSION

In heat induced haemolysis, the ethyl acetate extract showed the maximum inhibition of 55.7% followed by methanol (54.3%) and hexane (52.2%) at 50 µg/ml. Whereas, aspirin, standard drug showed 70.3% inhibition at 100 µg/ml. The results showed that *S. wightii* ethyl acetate extract at concentration 10-50 µg/ml protect the erythrocyte membrane against lysis induced by heat. The extracts are found to be effective in inhibiting the heat induced haemolysis at different concentrations. Macroalgae*Ecklonia cava, E. kurome* and *Ishigesinicola* possessed strong anti-inflammatory activity against acne inducing bacteria, *Propionibacterium acnes*[14]. The extract inhibits the release of lysosomal content of neutrophils at the site of inflammation. These neutrophil lysosomal constituents include bactericidal enzymes and protease, which upon extracellular release cause further tissue inflammation and damage. The non steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. Methanol extracts of the seaweeds were tested against mouse ear edema and erythema induced by phorbolmyristate acetate and showed the greatest suppression of erythema, with inhibition of 78 and 70% respectively[15]. Previous studies also reported that *Symphyocladialatiuscula* extract reduced cell viability by 55% at 200 µg/ml and contained high concentrations of bromophenols[16].

The inhibition of hypotonicity induced membrane lysis was also taken as a measure of the anti-inflammatory activity and the results showed that *S. wightii* ethyl acetate extract at concentration range of 10-50 µg/ml protect the erythrocyte membrane against lysis induced by hypotonic solution. At the concentration of 50 µg/ml ethyl acetate extract showed maximum of 73.4% protection, whereas, diclofenac sodium (100 µg/ml) showed only 50.8% inhibition of RBC haemolysis. Methanol and hexane extracts showed inhibition protection of 70.8% and 67.7% at 50 µg/ml respectively.These results indicated that the stated macroalga could be used as an effective remedy for inflammation-related symptoms and can be good criteria for reducing effect of inflammation as well as inducing analgesic effects[17].

Ethyl acetate showed maximum proteinase inhibition of 52.9 % at 50 µg/ml compared to methanol and hexane. At the concentration of 40 µg/ml, inhibition of 43%, 41.8% and 40.2% were produced by ethyl acetate, methanol and hexane extracts, but at the concentration of 10 and 20 µg/ml did not show much activity. Neutrophils are known to be a rich source of serine proteinase and are localized at lysosomes. It was previously reported that leukocytes proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors. *Sargassumwightii* extracts also exhibited anti proteinase activity at different concentrations and the ethyl acetate showed maximum inhibition of 52.9 % at 50 µg/ml compared to methanol and hexane. Proteinase activity was detected in the methanol and hexane extracts as 49.3% and 46.8% compared to aspirin which showed the maximum inhibition of 55.4 % at 100µg/ml.

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well documented cause of inflammation. As part of the imvestigation on the mechanism of the anti-inflammation activity, ability of extract to inhibit protein denaturation was studied. The ethyl acetate extract was found to be effective in inhibiting heat induced albumin denaturation with 70.8% at 50 µg/ml.Maximum inhibition of 68.7% and 67.8% was observed in methanol and hexane extracts at 50µg/ml compared to standard anti-inflammatory drug aspirin (67.3%) at 100 µg/ml (Table 1).Anti-inflammatory activity of brown alga *Dictyotadichotoma* in murine macrophage RAW 264.7 cells are also reported[18].

Antioxidant is the first line of defence against free radical damage, and is critical for maintaining optimum health and well being[19]. 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) has been used extensively as a stable free radical to evaluate reducing substance and is a useful reagent for investigating free radical scavenging activity of the component. Studies indicated that*Fucusvesiculosus* and *Ascophyllumnodosum* scavenged DPPH radicals by 31.2% and 25.6% while ethyl acetate extract of *Sargassum*sp exhibited highest free radical scavenging activity of 30.10±3.62 mg/ml[20]. *Laurenciaobtusa* and *L. obtusa* var. *pyramidata* showed antioxidant activity raging from about 20 to 45%[21]. The present study indicates the DPPH radical scavenging activity of *Sargasssumwightii* in different solvent extracts in a concentration-dependent manner. Screening the antioxidant activity by free radical scavenging assay showed that ethyl acetate extract has significant scavenging effect on DPPH (64.9%) at 200 µg/ml. The scavenging activity was compared with that of ascorbic acid, used as reference control, which scavenged 79% of the free radicals (Table 2).

The percentage of inhibition of methanol were found to be 50.2% and low polar hexane extract yielded antioxidant value of 48.5% at 200 µg/ml.Studies revealed that DPPH assay conducted on extracts of *Stoechospermummarginatum* (C. Agardh) showed radical scavenging activity by electron and hydrogen transfer mechanisms[22]. Earlier reports revealed that marine macroalgae extracts containing polyphenols have antioxidant activity[23]. According to the previous studies, the antioxidant properties of tropical marine macroalgae viz., *Avrainvillealongicaulis*, *Chondriabaileyana* and *Lobophoravariegata*have great antioxidant potential with very low oxidation index EC_{50} (1.44±0.01, 2.84±0.07and 0.32±0.01 mg/ml^{-1})[24]. Hexane extracts of *Dictyotadichotoma* var. *implexa*were found to have the highest phenolic contents. Several studies had shown a highly significant correlation between the phenolic content and the antioxidant activity in seaweed exrtracts. In addition, some studies described the antioxidant activity of some phenolic compounds purified from *Eiseniabicyclis* and *Sargassum kjellmanianum*[21]. It was also noted that phenolic compounds have more effective antioxidant properties than α-tocopherol and an activity comparable to that of synthetic antioxidants, BHA and BHT[25].Based on the Dunnet's t test results, it would be said that ethyl acetate and methanol extracts differ significantly($p<0.05$) from positive control.

Earlier reports revealed that marine macroalgal extracts, especially polyphenols have antioxidant activity[23]. Alkaloids are commonly found to have antimicrobial properties against both Gram-positive and Gram-negative bacteria[26]. Flavonoids are known as nature's tender drug which possesses numerous biological and pharmacological activities. Antiviral, anti-fungal, antioxidant, anti-inflammatory, antiallergenic, antithrombic, anticarcinogenic, hepatoprotective and cytotoxic activities of flavonoids are recently reported[27]. Steroids may serve as an intermediate for the biosynthesis of downstream secondary natural products and it is believed to be a biosynthetic precursor for carotenoides in plants. Marine algae have shown to be good sources of unsaponofiable, non toxic sterols that have medicinal value[28]. Saponins possess numerous biological properties which include antimicrobial, anti-inflammatory, anti-feedent and hemolytic effects[29]. The result of total phenol content and flavonoids indicated that phenolic content (0.613±0.716) was more in ethyl acetate extract followed by flavonoides (0.461±0.361). Previous phytochemical studies indicated the presence of sterols, alkaloid, phenolics and flavanoids in macroalga*Gayraliaoxysperma*extracts[11]. The presence of alkaloids, saponins, phenolic compounds, flavonoids and tannins in the ethyl acetate extracts of *Sargassumwightii*suggest that macroalgae can be used as antimicrobial, anti-parasitic, anti-inflammatory, anti-oxidant, anti-thrombic, anti-carcinogenic and anti-ulcer agents in the near future. A number of studies explained the separation of phenolics and

steroids using TLC[30]. In the present study also we developed the TLC profile for *S. wightii* which demonstrated four distinct spots with different RF values such as 0.83, 0.66, 0.51 and 0.31.

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. Detected bioactive compounds are Bromoacetic acid, hexadecyl ester (94.98%) followed by 1,4-Eicosadiene, Eicosane, 6-Octadecenoic acid, (Z), n-Hexadecanoic acid, Benzene, 1,2-dimethoxy-4-(1-propenyl), Stigmasta-5,24(28)-dien-3-ol, (3.beta), Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(Phenyl methyl), 2(1H)-Pyrimidinone,4-amino-5-methyl, 4-Methoxy-3-Propoxy-Benzaldehyde, Adamantine,1-Isothiocyanato-3-methyl, Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl, Benzene, 1-methoxy-3-(methylthio), Bis(2-ethylhexyl) phthalate, Diisooctyl phthalate, N,N'-Dibutyl-N,N'-dimethyl-succinamide and Diethyl phthalate. It is possible that bioactive compounds primarily consisting of Bromoacetic acid, hexadecyl ester (94.98%). Flora and Rani identified twenty four chemical constituents of methanolic extract of *Acanthophoraspicifera* by GC-MS analysis and the major constituents reported are octanol, piperazine, benzoic acid and octadecenoic acid[4]. The fatty acid composition of marine macroalga, *Acanthiphoraspicifera* revealed that palmitic acid, arachidonic acid and eicosapentanoic acid as the dominant fatty acids[31]. Earlier studies detected a fatty acid 9,12-Octadecadienoic acid (Z,Z)- (49.75%) from a red alga, *Laurenciabrandenii*byGC-MS[32]. Based on the GC-MS analysis of the phytochemical constituents, it can be said that unsaturated fatty acids and hydrocarbons do contributed to the antioxidative and anti-inflammatory effects of ethyl acetate extracts. The capability of oleic acids and hexadecane that exhibited anti-oxidant capabilities have also been reported[33].

Straight chain paraffins (n-alkanes), branched chain paraffins (alkyl-alkanes) and unsaturated hydrocarbons (alkenes) were already reported from many marine algae[34]. Therefore in the present study, biological activity of *Sargassumwightii* might be due to the presence of fatty acid, Bromoacetic acid, hexadecyl ester (94.98%) in higher percentage. Based on the GC-MS results, the chemical constituents of main active fraction was extrapolated as low molecular weight lipophilic compound composed of mixture of volatile metabolites and fatty acids. These compounds are invariably having pesticidal, anthelmentic, antifungal, antimicrobial, antioxidant, insect repellent, nematicidal and cancer preventive properties, which could be further isolated, purified and confirmed to be utilized in medical and agricultural industries.

Table 1: Inhibitory percentages of *S. wightii* extracts on anti inflammatory activity

Concentration (µg/mL)	Extracts	Heat induced haemolysis	Hypotonicity induced haemolysis	Proteinase inhibition	Albumin inhibition
10	Ethyl acetate	23.8	31.7	20.8	31.2
	Methanol	21.5	28.3	19.7	30.1
	Hexane	18.7	26.5	17.5	28.6
20	Ethyl acetate	32.3	46.8	27.3	49.5
	Methanol	29.2	43.6	25.8	46.3
	Hexane	27.9	41.8	22.3	43.7
30	Ethyl acetate	37.7	54.5	38.5	57.8
	Methanol	35.5	51.7	37.4	54.8
	Hexane	33.2	49.2	35.6	52.6
40	Ethyl acetate	45.4	60.3	38.5	64.3
	Methanol	43.5	58.4	37.4	60.4
	Hexane	40.9	55.2	35.6	58.6
50	Ethyl acetate	55.7	73.4	52.9	70.8
	Methanol	54.3	70.8	49.3	68.7
	Hexane	52.2	67.7	46.8	67.8
Aspirin / Diclofenac sodium	Ethyl acetate	70.3	50.8	55.4	67.3
	Methanol	70.3	50.8	55.4	67.3
	Hexane	70.3	50.8	55.4	67.3

Table 2: Effect of *Sargassumwightii* extracts on DPPH assay

Concentrations (µg/mL)	Ethyl acetate	
	OD	%
Control	0.79±0.04	-
20	0.69±0.02	12.2
40	0.67±0.01	15.5
60	0.65±0.03	18.7
80	0.58±0.03	26.2
100	0.54±0.02	30.8
120	0.50±0.05	36.7
140	0.48±0.03	38.7
160	0.42±0.04	47
180	0.38±0.04	51.5
200	0.27±0.05	64.9

Table 3: GC-MS data of ethyl acetate extract of *Sargassumwightii*

S.No	RT	Name of the Compound	Molecular formula	Molecular weight	Compound Nature
1	150	Diethyl phthalate	$C_{12}H_{14}O_4$	222.24	Phthalate ester
2	43	4-Methoxy-3-Propoxy-Benzaldehyde	$C_{11}H_{14}O_3$	194.23	Aromatic aldehydes
3	163	Benzene, 1,2-dimethoxy-4-(1-propenyl)-	$C_{11}H_{14}O_2$	178.227	Methyl ester
4	71	Eicosane	$C_{20}H_{42}$	282.547	Alkane
5	82	1,4-Eicosadiene	C20H38	278.515	Alkene
6	207	Benzene, 1-methoxy-3-(methylthio)-	$C_8H_{10}O_2$	154.229	-
7	43	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.424	Methyl ester
8	55	Bromoacetic acid, hexadecyl ester	$C_{18}H_{35}BrO_2$	363.373	Ester
9	41	6-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$	282.461	Ester
10	55	N,N'-Dibutyl-N,N'-dimethyl-succinamide	C14H28N2O2	256.384	-
11	91	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(Phenyl methyl)-	$C_{14}H_{16}N_2O_2$	244.289	Acyclic
12	70	2(1H)-Pyrimidinone, 4-amino-5-methyl-	$C_5H_7N_3O$	125.128	-
13	57	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390.56	Phthalate ester
14	57	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.56	Diester
15	281	Adamantine,1-Isothiocyanato-3-methyl-	C12H17NS	207.335	Cycloalkane
16	281	Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	C11H13NO3	207.225	Aromatic
17	281	Stigmasta-5,24(28)-dien-3-ol, (3.beta)-	$C_{29}H_{48}O$	412.69	-

REFERENCES

1. Murty Y and Agrawal T. Marine derived pharmaceuticals-Development of natural health products from marine biodiversity. Inter J Chem Tech Res. 2010;2(4):2198-2217.
2. Kolanjinathan K and Stella D. Pharmacological effect of Gracilariacorticata solvent extracts against human pathogenic bacteria and fungi. International Journal of pharmaceutical & biological Achives. 2011;2:1722-1728.
3. Yuvaraj N, Kanmani P, Satishkumar R, Paari KA, Pattukumar V and Arul V. Extraction, purification and partial characterization of Cladophoraglomerata against multidrug resistant human pathogen Acinetobacterbaumannii and fish pathogens. World Journal of fish and marine sciences. 2011;3:51-57.
4. Flora G and Rani MVS. GC-MS analysis of Acanthophoraspicifera. International Journal of Pharma and Bio Sciences. 2013;4(1):649-653.
5. Wolf M, Lichtensteein D and Singh G. Gastrointestinal toxicity on nonsteroidal anti-inflammatory drugs. N Engl J Med. 1999;340(24):1888-1889.
6. Kasim CG, Aline P and Ekrem S. Alkaloids in marine Algae. Mar Drugs. 2010; 8:269-284.
7. Yuan YV, Bone DE and Carrington MF. Antioxidant activity of (Palamariapalmata) extract evaluated in vitro. Food Chem. 2005;91:485-494.
8. Blunt JW, Copp BR, Hu WP and Munro MHG. Marine Natural Products. Nat Prod Rep. 2007;24:31-86.
9. Mizukoshi S, Matsuoka H, Katou H and Noda H. Search for bioactive substances from marine algae. Bulletin Fac. Bioresource Mie Univ.

MiediaSeibutsushigen Kiyo. 1993;8:27-34.
10. Meenakshi S, Manicka GD, Tamilmozhi S, Arumugam M and Balasubramanian T. Total flavonoid and in vitro antioxidant activity of two seaweeds of Rameshwaram Coast. Global J Pharmacol. 2009;3:59-62.
11. Pise NM, Verlecar XN, Gaikwad DK and Jagtap TG. Nutraceutical properties of the marine macroalgaGayraliaoxysperma. Bot Mar. 2012;55(6):581-589.
12. Brand williams W, Cuvelier ME and Berset C. Use of a free radical method to evaluate antioxidant activity. Lebensm-Wiss U- Technol. 1995;28:25-30.
13. Harborne JB. Phytochemical methods. A guide to modern techniques of plant analysis, 3rdEdn. Chapman and Hall, New York. 1998;1-150.
14. Choi J, Bae H, Kim S and Choi I. In vitro antibacterial and anti-inflammatory properties of seaweed extracts against acne inducing bacteria, Propionibacterium acnes. J Environ Biol. 2011;32:313-318.
15. Kang S.M, Kim KN, Lee SH, Ahn G, Cha SH, Kim AD, Yang XD, Kang MC and Jeon YJ. Anti-inflammatory activity of polysaccharide purified from AMG-assistant extract of Eckionia cava in LPS-Stimulated RAW 264.7 macrophages. Carbohydr Polym. 2011;85:80-85.
16. Wang T, Jonsdottir R and Olafsdottir G. Food Chem. 2009;116(1):240-248.
17. Shin HC, Hwang HJ, Kang KJ and Lee BH. An anti oxidative and anti-inflammatory agent for potential treatment of osteoarthritis from Ecklonia cava. Arch pharm Res. 2006;29:165-171.
18. Yoon W, Ham YM, Kim KN, Park SY, Lee NH, Hyun CG and Lee WJ. Anti-inflammatory activity of brown alga Dictyotadichotoma in murine macrophage RAW 264. 7 cells. J Medicinal Plants Research. 2009;3(1):001-008.
19. Shanab SMM. Antioxidant and antibiotic activities of some seaweeds (Egyptian Isolates). International Journal of Agriculture & Biology. 2007;9(2):1560-8530.
20. Bhaigyabati T, Kirithika T, Shiny K and Usha K. Phytochemical screening and antioxidant activity of various extracts of Sargassummuticum. IJPRD. 2011;3(10):25-30.
21. Larson RA. The antioxidants from higher plants. Phytochem. 1988;27:969-78.
22. Esmaeili A and Khodadadi A. Antioxidant activity of a solution of thymol in ethanol. Zabedan J Res Med Sci. 2012;14:14-18.
23. Ganesan P, Chandini SK and Bhaskar N. Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. Biores Tech. 2008;99:2717-2723.
24. Zubia M, Robledo D and Freile-Pelegrin Y. Antioxidant activities in tropical marine macroalgae from the Yucatan peninsula. Mexico. J Appl Phycol. 2007;19:449-458.
25. Lynn Cornish M and David JG. Antioxidants from macroalgae: potential applications in human health and nutrition. Algae. 2010;25(4):155-171.
26. Cowan MM. Plants products as antimicrobial agents. Clinical Microbiology Review. 1999;12:564-582.
27. Rajasulochana P, Dhamotharan R and Krishnamoorthy P. Primary phytochemical analysis of KappaphycusSp. Journal of American science. 2009;5(2):91-96.
28. Sanchez-Machado DJ, lopez-Hernandez J, PaseiroLosada P and Lopez-Cervantes J. An HPLC method for the quantification of sterols in edible seaweeds. Biomed Chromarogr. 2004;18:183-190.
29. Xu SH, Ding LS, Wang MK, Peng SL and Liao X. Studies on the chemical constituents of the algae Sargassumpolycystum. Chinese J Org Chem. 2002; 22:138-140.
30. HajimehdipoorH, Khanavi M, Zahedi H, Abedi Z and Kalantari KN. Fingerprint study of Thymus spp. by TLC. Journal of Medicinal Plants. 2009, 8:19-24.
31. Zakaria NA, Ibrahim D, Shaida SF and Supardy AA. Phytochemical composition and antibacterial potential of hexane extract from Malaysian red algae, Acanthophoraspicifera (Vahl) Borgesen. World applied Sciences Journal. 2011;15(4):496-501.
32. Manilal A, Sujith S, SeghalKiran G, Joseph Selvin and Shakir C. Cytotoxic potentials of Red alga, Laurenciabrandenii collected from the Indian coast. Global Journal of Pharmacology. 2009;3(2):90-94.

33. Bhaskar N, Kinami K, Miyashita K, Park SB, Endo Y and Fujimoto K. Occurrence of conjugated polyenoic fatty acids in seaweeds from the Indian ocean. Z Naturforsch. 2004;59:310-314.

34. Weber PC and Leaf A. Cardiovascular effects of O-3 fatty acids. World. Rev Nutr Diet. 1991;66:2318-232.