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Phytoconstituents Screening by GC-MS and Evaluation of *In Vitro* Gastroprotective and Antioxidant Activity of *Ulva lactuca*

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Abstract

Background: The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases. Seaweeds are rich source of original and bioactive natural substances. In particular, Green seaweed, *Ulva lactuca* have been demonstrated to metabolize biomolecules with pharmacological potential. **Aim:** The ethanolic extract of collected alga, *ulva lactuca* was subjected to screen phytoconstituents, antioxidant activity and cytotoxicity assays. The bioactive compounds were identified by GC–MS analysis. **Materials and methods:** The antioxidant activity was carried out by reducing power assay, DPPH activity, superoxide and nitric oxide radical scavenging activity, evaluation of total antioxidant capacity of the extract. The phytochemical analysis was carried out as per standard methods and Agilent GC-MS 680 analysis performed using a fused silica capillary column. Cytotoxic activity also employed against gastrointestinal protection in cell line for three different solvent extracts. **Results:** Ethanolic extract of *ulva lactuca* showed higher antioxidant activity and the presence of phytochemicals like alkaloids, flavonoids steroids, tannins, terpenoids, phenol, carbohydrates and saponins and also possess cytotoxic activity in cell line, ethanolic extract showed highest activity. The GC-MS analysis provided different peaks determining the presence of 20 different compounds, among that 13 major compounds viz., Beta Carotene, Eicosatetraenoic acid Trans-Traumatic acid 1-Naphthalenepropanol, which exhibit the antioxidant, antibacterial, antimicrobial, anti-inflammatory, antiulcer and anticancer activity was present in this algae. **Conclusion:** In the present study it was concluded that the *Ulva lactuca* is a rich source of antioxidant and pharmaceutically important by the presence of various bioactive compounds used for the treatment of human pathogenic diseases.

Keywords
Antioxidant, Gastroprotective, GC-MS, Cytotoxicity, Phytochemicals and *Ulva lactuca.*

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1. INTRODUCTION

Ulva Linnaeus genus (Ulvaceae, Ulvales) is a ubiquitous genus widely distributed in oceans and estuaries. Currently, 128 species (accepted taxonomically) have been listed all around the world[1]. *Ulva lactuca* belongs to chlorophyta and is a unicellular green alga. The genus Ulva (Phylum Chlorophyta, Class Ulvophyceae, Order Ulvales, Family Ulvaceae) was first identified by Linnaeus in 1753[2].Morphology of Ulva lactuca resembles bright green sheets[3], heavily influenced by environmental conditions, age of the thallus, and life style, making difficult the delineation of species by morphological features alone[4]. Individuals of this genus are characterized by a broad range of environmental tolerance, high growth rate and photosynthetic activity leading to a relatively abundant natural biomass. Additionally, in a rich nutrient environment, these species can proliferate into green tides, making available important amounts of biomass[5]. On the other hand, the successful results obtained for Ulva spp. cultivation in integrated multitrophic aquaculture (IMTA) systems[6] or in land based aquaculture coupled with waste water bioremediation[7] allows promising development for sustainable raw material supply. This last decade, scientific interest for this taxonomic genus has increased[2]. From an economic perspective, the use of Ulva species for different applications has been largely described: bioremediation[8], bioenergy[9], food and feed[10]. A biorefinery approach for industrial exploitation of Ulva constituents have been proposed[11].

Some of the beneficial bioactivities demonstrated by the Ulvaceae and specially their species *ulva lactuca*, either in vitro or in vivo, upon various kinds of cell-lines and animal models, include anticoagulant and/ or antithrombotic properties, immunomodulatory ability, antitumor and cancer preventive activity (as anti-proliferative agents, tumour suppressors or natural cell-killers). They are also good antidislipidaemic and hypoglycaemic agents and can be powerful antioxidants, antibiotics, anti-inflammatory, gastro-and cardioprotective bioactivities[12,13,14,15,16,17,18,19]. Various bioactive compounds from marine organisms have been experimentally tested to comprehensively study the biological effects of recently developed drugs[20]. Marine algae are rich in dietary fiber, minerals, lipids, proteins, omega-3 fatty acids, essential amino acids, polysaccharides, and vitamins A, B, C, and E[21, 22,23,24,25].

GC-MS is a simple, rapid and accurate method for analyzing seaweed material[26]**.** The GC-MS method can be used for phytochemical profiling of seaweeds and quantification of compounds present in algae, with increasing demand for natural products as medicines and cosmetics there is an urgent need for standardization of algae products[27]**.** It can serve as a tool for identification, authentication and quality control of herbal drug[28].

Cell culture methods have been widely used in toxicological studies, primarily in basal cytotoxicity assays, the results of which can be used to design more specific studies. Natural medicinal products have been used for millennia for the treatment of multiple ailments. Although many have been super sided by conventional pharmaceutical approaches, there is currently a resurgence of interest in the use of natural bioactive products by the general public, with many healthy subjects and patients taking them for the prevention and treatment of multiple conditions, including gastrointestinal disorders and postoperative recovery[28].

Indomethacin-induced small intestinal injury has been assessed *in vitro* using Rat intestinal epithelial cells (IEC-6). IEC-6 cell lines have been proposed as an alternative model to the intestine and are used in various metabolic and drug toxicity studies[29]. The intestinal epithelial cells (IECs) form a selective permeability barrier separating luminal content from underlying tissues[30]. Upon injury, the intestinal epithelium undergoes a wound healing process. Intestinal wound healing is dependent on the balance of three cellular events; restitution, proliferation, and differentiation of epithelial cells adjacent to the wounded area. Previous studies have shown that various regulatory peptides, including growth factors and cytokines, modulate intestinal epithelial wound healing[31]. Recent studies have revealed that novel factors, which include toll-like receptors (TLRs), regulatory peptides, particularly dietary factors, and some gastroprotective agents, also modulate intestinal epithelial wound repair[30]. Since it retains many of the phenotypic and genotypic characteristics of intestinal cells, this cell line has been used in various studies related to marine algae for their gastroprotective property.

2. MATERIALS AND METHODS

2.1 Collection of *Ulva lactuca*

The fresh samples of *Ulva lactuca* were collected from the Marine department, Annamalai University, Chidambaram, Tamil Nadu and authenticated by Dr. Deivasigamani, Marine department, Annamalai University, Chidambaram, Tamil Nadu. Sample materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles in refrigerator.

2.2 Preparation of Extract

Crude sample extract was prepared by Soxhlet extraction method. About 20 gm of powdered material was uniformly packed into a thimble and extracted with 250 ml of ethanol extract separately. The process of extraction has to be continued for 24 hours or till the solvent in siphon tube of extractor become colourless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40˚C till all the solvent got evaporated. Dried extract was kept in refrigerator at 4˚C till future use.

2.3 GC-MS ANALYSIS OF *Ulva lactuca*

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df). The components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min−1; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

2.4 CYTOTOXICITY ASSAYS

2.4.1 IEC6 (Rat intestinal epithelial) cells

The rat intestinal epithelial cell line (IEC6) was obtained from NCCS Pune. Cells were maintained in RPMI -1640 medium supplemented with heat inactivated fetal calf serum (FCS 10%), penicillin (100U/ml) and streptomycin (100 µg/ml). The cells were grown in 25 cm^2 tissue culture flasks and maintained at 37˚C in a humidified, 5% CO_2 - atmosphere throughout the experiment.

2.4.2 Experimental design

A total of 6 groups were used for the experimental studies for cell viability, including trypan blue staining and MTT assay.

Group 1: (DMSO control 0.25% v/v): These cells were treated with DMSO alone.

Group 2: (*ulva lactuca* Extract alone): These cells were treated with 600µg/ml extract alone.

Group 3: In this group cells was treated with 10mM indomethacin (IND) only[32].

Group 4: (200 µg/ml *ulva lactuca* extract + IND): These cells were treated with 200 µg/ml extract combined with 10 mM IND.

Group 5: (400 µg/ml *ulva lactuca* extract + IND): In this group cells was treated with 400 µg/ml extract with 10 mM IND.

Group 6: (600 µg/ml *ulva lactuca* extract + IND) in this group cells was treated with 600 µg/ml extract with 10 mM IND.

The *ulva lactuca* extracts were incubated for 24h for trypan blue staining and 72h for MTT assay. The same kind of experimental groups was designed for three different solvent extracts (Chloroform, ethyl acetate and ethanol) of *Ulva lactuca*.

2.4.3 Cell viability

Of the monolayer cultures, 50 µl of 10mM Indomethacin was added to each well followed by the addition of 50 µl of three different solvents (chloroform, ethyl acetate and ethanol) extracts of *Ulva lactuca* at different concentrations (200, 400 and 600 µg/ml) and incubated at 37˚C for 24 hours. After incubation, the cell viability was determined by trypan blue dye exclusion method. MTT assay and the minimum effective concentration and effective solvent extract of *ulva lactuca* were also determined.

2.4.3.1 Trypan Blue Staining

Trypan blue is one of the several stains recommended for use in dye exclusion procedures for viable cell counting. This method is based on the principle that live cells do not take up dye unlike the dead cell. After 24h incubation with the extracts and acetaminophen, cells were trypsinised and resuspended in MEM.

A cell suspension containing approximately 2.5×10^5 cells/ ml was prepared in MEM and 0.2 ml of cell suspension was added, and mixed thoroughly with 0.4% trypan blue. The mixture was allowed to stand for 5 minutes. The suspension was viewed in a hemocytometer and analysed for viable cells. The Viable cell count was determined as per the method described previously[33] by using the following calculations:

Cells / ml=Average cell count per square × dilution factor$\times10^4$

Total cells=cells / ml original volume of fluid from which cell sample was removed.

% cell viability=Total viable (Unstained) / Total cells (stained and unstained) × 100

2.4.3.2 MTT (3-(4, 5-Dimethylthiazol-2-Yl)-2, 5-Diphenyltetrazolium Bromide) Assay

The inhibitory effect of different solvent extracts on IEC6 cells was determined by MTT assay[34]. The MTT test is based on the enzymatic reduction of the tetrazolium salt in MTT in viable / metabolically active cells. IEC6 monolayer cells were trypsinized, collected and the cell count was adjusted to 1×10^5 cells/ml with MEM containing 10% fetal calf serum. 0.1 ml of the diluted cell suspension (10,000 cells) was added to each well of the 96 well microtitre plates. After 24 hours, when a partial monolayer formed the supernatant was flicked off, washed once with PBS, different extracts with different concentrations (200, 400 and 600µg) and 10 mm Indomethacin (in 0.25% DMSO prepared in serum free culture medium) was added to the cells.

The plates were incubated at 37ºC for 3 days in 5% CO_2 atmosphere and the medium in the well was discarded and 50 µl of 5 mm MTT was added to each well. The plates were gently shaken and incubated for 4 hours. The supernatant was removed and 50 µl of propanol was added and gently solubilized the formation. The absorbance was measured using microtitre plate at 540 nm. The inhibitory rate of cell growth was calculated with the following formula.

% growth inhibition = 1 - OD extract treated / OD negative control × 100

The same kind of experimental groups was designed for three different solvent extracts (Chloroform, ethyl acetate and ethanol).

2.5 INVITRO ANTIOXIDANT ASSAY

2.5.1 Reducing Power Assay

A method developed by Oyaizu, 1986 for reducing power test was used. The above sample including extract together with Ascorbic acid solutions were spiked with 2.5ml of phosphate buffer (0.2 M, pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was then kept in a 50°C water-bath for 20min. The resulting solution was then cooled rapidly, spiked with 2.5ml of 10% trichloroacetic acid, and centrifuged at 3000rpm for 10 min. The supernatant (5ml) was then mixed with 5ml of distilled water and 1ml of 0.1% ferric chloride. The absorbance at 700nm was then detected after reaction for 10min. The higher the absorbance represents the stronger the reducing power. The reducing power assay was expressed in terms of Ascorbic acid equivalent per gram of dry weight basis.

2.5.2 DPPH (1, 1'-Diphenyl-2-picryl hydrazyl) Activity

DPPH radical scavenging activity was carried out by the method of Molyneux (2004). To 1.0 ml of 100.0 µM DPPH solution in methanol, equal volume of the sample in methanol of different concentration was added and incubated in dark for 30 minutes. The change in colouration was observed in terms of absorbance using a spectrophotometer at 514 nm. 1.0 ml of methanol instead of test sample was added to the control tube. The different concentration of ascorbic acid was used as reference compound. Percentage of inhibition was calculated from the equation [(Absorbance of control - Absorbance of test)/ Absorbance of control)] × 100. IC50 value was calculated using Graph pad prism 5.0.

2.5.3 Superoxide Radical Scavenging Activity

The superoxide radical scavenging activity of the test sample was studied using the method of Liu et al. (1997) with slight modifications. Superoxide radicals are generated in phenazine methosulphate (PMS) - (Nicotinamide adenine dinucleotide (NADH) systems by oxidation of NADH and assayed by the reduction of Nitro Blue Tetrazolium (NBT). 200.0 µl of test samples of different concentrations were taken in a series of test tube. Superoxide radicals were generated by 1.0 ml of Tris-HCl buffer (16.0 mM, pH-8.0), 1.0 ml of NBT (50.0 µM), 1.0 ml NADH (78.0 µM) solution and 1.0 ml of PMS (10 µM). The reaction mixture was incubated at 25°C for 5 min and the absorbance at 560 nm was measured. A control tube containing Tris-HCl buffer was also processed in the same way without test sample. Different concentration of ascorbic acid was used as reference compound.

2.5.4 Nitric Oxide Radical Scavenging

Nitric oxide radical scavenging activity was measured spectrophotometrically (Govindharajan et al., 2003). 1.0 ml of Sodium nitroprusside (5 mmol) in phosphate buffer (p H 7.4, 0.1 M) was mixed with different concentrations of the extract (100 – 500 microgram/ml in phosphate buffer (pH 7.4, 0.1 M). The tubes were then incubated at 250˚C for two hours. At the end of second hour 1.5 ml of reaction mixture was removed and diluted with 1.5 ml of Greiss reagent (1% sulphanilamide, 2% o-phosphoric acid, 0.1% of naphthyl ethylenediamins dihydrochloride) The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphtylethylene diamine dihydrochloride was measured at 546 nm. Control tube contain all chemicals except algal extract.

2.5.5 Evaluation of Lipid peroxidation

Lipid hydroperoxide was estimated by the method of Jiang *et al.* (1992). In this method, oxidation of ferrous ions (Fe^{2+}) under acidic conditions in the presence of xylenol orange leads to the formation of a chromophore with an absorbance maximum at 560nm.The Standard is 0.2 M H_2O_2 was prepared .0.2ml of test sample (in phosphate buffer pH – 7.5) was taken; 1.8ml of the Fox reagent(88mg Butylated Hydroxy Toluene (BHT), 7.6 mg of xylenol orange and 9.8 mg of ferrous ammonium sulphate were taken and mixed with 90ml of methanol and then 10ml of 250 mM sulphuric acid) was added and then incubated for 30 minutes at room temperature and read at 560nm.Lipid hydroperoxide were expressed as mmoles/100 mg extract.

2.5.6 Hydroxyl radical scavenging assay

All solutions were prepared freshly. 1.0 ml of the reaction mixture contained 100 µl of 28 mM 2-deoxy-2-ribose (dissolved in phosphate buffer, pH 7.4), 500 µl solution of various concentrations of test sample (10 to 80 µg), 200 µl of 200 µM FeCl3 and 1.04 mM EDTA (1:1 v/v), 100 µl H2O2 (1.0 mM) and 100 µl ascorbic acid (1.0 mM). After an incubation period of 1 hour at 37°C the extent of deoxyribose degradation was measured by the TBA reaction. Measure the absorbance at about 532 nm against the blank solution. Vitamin E was used as a positive control.

3. RESULTS AND DISCUSSION

3.1 Identification of Phytochemicals in ethanolic extract of *Ulva lactuca by GC-MS study*

In this present study the GC-MS analysis leads to the prediction of chemical constituents present in the ethanolic extract of *Ulva lactuca*. 20 compounds were found in the ethanolic extracts of *U. lactuca*, among that 13 major compounds which exhibit the antioxidant, antibacterial, antimicrobial, anti-inflammatory, antiulcer and anticancer activity was present in this alga are presented in Table 1 and Figure 1. The identified compounds bioactivities were predicted using Dr. Duke's Phytochemical & Ethnobotanical Databases prediction. The results revealed the presence of 20 different phytocompoundsviz.,Heptanal,Pseduosarsasapogenin-5, 20-Dien, 3-Chloro-5-Cholestene, L-Gala-L-ido-octose, Glutaraldehyde, 8, 11, 14-Eicosatrienoic acid, (Z,Z,Z)-, Cholest-8-Ene-3,6-Diol, 14-Methyl-, (3.Beta., 5.Alpha.,6.Alpha.), 2-Methyl-6-Methylene-octa-1, 7-Dien-3-ol, 3-Decyn-2-ol, 3-Nonyn-2-ol, Pentanoic Acid, 2-(Aminooxy), 17-Octadecynoic Acid, Z, Z-6, 13-Octadecadien-1-olAcetate,Spiro[Androst-5-Ene-17,1'-Cyclobutan]-2'-One, 3-Hydroxy-, (3.Beta.,17.Beta.), Trans-Traumatic Acid,5,8,11,14-Eicosatetraenoic Acid, Methyl Ester, (All-Z), 1-Naphthalenepropanol, Alpha-Ethyldecahydro-5-(Hydroxymethyl)-.Alpha., 5, 8a-Trimethyl-2-Methyl,1-Heptatriacotanol, Cholesta-8, 24-Dien-3-ol, 4-Methyl-, (3.Beta.,4.Alpha.), Beta Carotene. We observed boundless activity for the major constituents in the ethanolic extracts of U. lactuca. The presence of various bioactive compounds confirms the application of U. lactuca for various ailments by traditional practitioners.

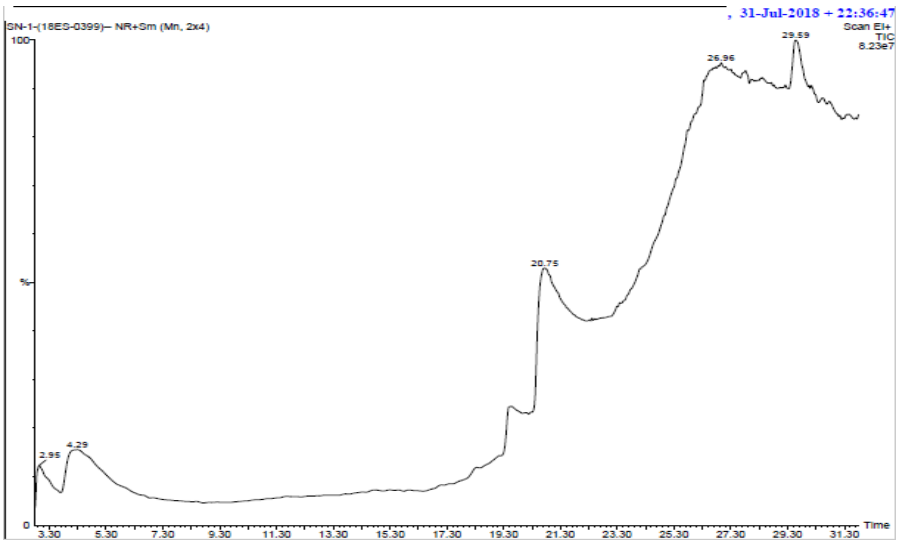

Figure 1. The chromatogram showing different compounds as peaks detected by Gas chromatography–Mass spectrophotometry in ethanolic extract of *Ulva lactuca*

Table -1: Components identified in Ethanolic extract of *ulva lactuca* by GC-MS analysis

S.NO.	RT	Compound Name	M.F.	M.W.	**Biological Activity
1.	18.660	Heptanal	$C_7H_{14}O$	114	**Antioxidant activity**, Antibacterial Activity
2.	18.660	Pseduosarsasapogenin-5,20-Dien	$C_{27}H_{42}O_3$	414	Treatment Of Amyotrophic Lateral Sclerosis
3.	19.795	3-Chloro-5-Cholestene	$C_{27}H_{45}Cl$	404	Antimicrobial Activity
4.	18.660	L-Gala-L-ido-octose	$C_8H_{16}O_8$	240	Synthesis Of Higher Sugar, Production Of Drugs, Particularly To Prevent The Cognitive Deficits Associated With Dementias
5.	18.660	Glutaraldehyde	$C_5H_8O_2$	100	Leather Tanning, Sterilization And Disinfection, And Tissue Fixation For Electron Microscopy
6.	18.660	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	Anti-Thrombogenic, Anti-Inflammatory, **anti-ulcer** and Anti-Atherogenic, **Antioxidant** Properties
7.	19.630	Cholest-8-Ene-3,6-Diol, 14-Methyl-, (3.Beta., 5.Alpha.,6.Alpha.)-	$C_{28}H_{48}O_2$	416	Antimicrobial, Diuretic, Anti-inflammatory, Anti-asthma
8.	19.795	2-Methyl-6-Methylene-octa-1,7-Dien-3-ol	$C_{10}H_{16}O$	152	**Antioxidant activity**, Antimicrobial Activity
9.	18.660	3-Decyn-2-ol	$C_{10}H_{18}O$	154	Antibacterial Activity
10.	18.660	3-Nonyn-2-ol	$C_9H_{16}O$	140	anti –septic, **antioxidant** and anti-inflammatory activity
11.	19.630	Pentanoic Acid, 2-(Aminooxy)-	$C_5H_{11}O_3N$	133	**Antioxidant activity**, Anti-bacterial Activity
12.	20.801	17-Octadecynoic Acid	$C_{18}H_{32}O_2$	280	Potent suicide inhibitor of LTB 4 ω -hydroxylase. Antimicrobial, **Antioxidant activity**
13.	21.796	Z,Z-6,13-Octadecadien-1-ol Acetate	$C_{20}H_{36}O_2$	308	Anti-tumor, Fungicide, Analgesic, Sedative, Antibacterial, **Antioxidant**, Anti-inflammatory activity
14.	23.577	Spiro[Androst-5-Ene-17,1'-Cyclobutan]-2'-One, 3-Hydroxy-, (3.Beta.,17.Beta.)-	$C_{22}H_{32}O2$	328	Antiarthritic, Diuretic, Hepatoprotective, Antiasthma, Anti-inflammatory, **Antioxidant activity**, Cancer preventive
15.	23.577	Trans-Traumatic Acid	$C_{12}H_{20}O_4$	228	Tissue Repair, **Antioxidant activity**
16.	25.963	5,8,11,14-Eicosatetraenoic Acid, Methyl Ester, (All-Z)-	$C_{21}H_{34}O_2$	318	Strong antimicrobial activity against oral microorganisms. **Antioxidant activity** and also used for **preventing and treating gastric disorders**
17.	26.003	1-Naphthalenepropanol, .Alpha.-Ethyldecahydro-5-(Hydroxymethyl)-.Alpha.,5,8a-Trimethyl-2-Methyl	$C_{20}H_{36}O_2$	308	Antimicrobial, Anti-inflammatory, **Antioxidant activity**
18.	26.003	1-Heptatriacotanol	$C_{37}H_{76}O$	536	Fungicide, Analgesic, **Antioxidant**, Antimicrobial activity
19.	26.273	Cholesta-8,24-Dien-3-ol, 4-Methyl-, (3.Beta.,4.Alpha.)	$C_{28}H_{46}O$	398	Strong antimicrobial activity, Diuretic, **Antioxidant activity**
20.	26.273	Beta Carotene	$C_{40}H_{56}$	536	**Anti-ulcer activity, Antioxidant activity**

****Source: Dr. Duke's Phytochemical and Ethnobotanical Databases**

3.2 CYTOTOXICITY ASSAYS

3.2.1 Effect of *U. lactuca* extracts on the cell viability by Trypan blue staining assay

Figure-2 depicts the cell viability by trypan blue assay in terms of percentage of cell death. The percentage of cell death significantly increased (P<0.001) in Indomethacin induced group (Group 3) when compared to control (Group 1). All the solvent extracts of *U. lactuca* showed decreases in the cell death after inducement with the IND. The ethanol extract treatment showed a better effect than chloroform and ethyl acetate extracts (P<0.01). Of the three concentrations of the ethanol extract, 600 µg/ml were more effective (P<0.001) than the other concentrations 200 µg/ml (Group 4) and 400 µg/ml (Group 5). Treatment with extract alone (Group 2) did not change the cell viability when compared to control (Group 1).

3.2.2 Effect of *U. lactuca* extracts on the cell viability by MTT assay

Figure-3 depicted the effect of *U. lactuca* extract on the cytotoxicity of IEC-6 cell lines as determined by MTT assay.

Treatment with Indomethacin (1mM) caused significant loss of viability of cells as measured by this assay. Pretreatment with *U. lactuca* extracts (200 µg - 600 µg) before adding acetaminophen caused significant increment in viability of cells in a dose dependent manner. Both *U. lactuca* extract treatments along with Indomethacin significantly increased cell viability by MTT assay. The highest concentration of *U. lactuca* (600 µg) (Group 6) was most effective as (P<0.01) compared to other concentrations of extract. Efficacies of different solvent extracts of *U. lactuca* were tested. Of these, an ethanol extract of *U. lactuca* showed the most efficiency (P<0.001) in protecting the cells against Indomethacin toxicity.

There was no significant difference in cell viability between cells incubated with algae extract (600 µg/ml) for 12 hr and control cells, which indicates that extracts of *U. lactuca* no toxic effect up to 600 µg/ml.

The result indicates that moderate to good protection is offered by the extracts of *U. lactuca*. The highest protection is observed in the ethanolic extracts of *U. lactuca*. Hence ethanolic extract of *U. lactuca* were used for the *in vivo* Gastroprotective studies. Cells were treated with 1 mM indomethacin, a concentration previously associated with cytotoxicity and extensively used in in *vitro* cell culture system[33,36] also reported that indomethacin (5-30 mM) caused a significant concentration dependent decrease of cell viability (MTT assay) and depletion of intracellular GSH after 24 hr. Since indomethacin inhibits both COX-1 and COX-2, it inhibits the production of prostaglandins in the stomach and intestines, which maintain the mucous lining of the gastrointestinal tract. Indomethacin, therefore, like other non-selective COX inhibitors can cause peptic ulcers. These ulcers can result in serious bleeding.

In recent years, considerable attention has been directed on Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and indomethacin are the most commonly prescribed drugs for arthritis, inflammation, and cardiovascular protection. However, they cause gastrointestinal complications such as ulcers and erosions. The pathophysiology of these complications has mostly been ascribed to NSAID's action on the cyclooxygenase (COX) inhibition and the subsequent prostaglandin (PG) deficiency[35, 37]. Treatment with the algae extracts reduced toxicity induced by indomethacin.

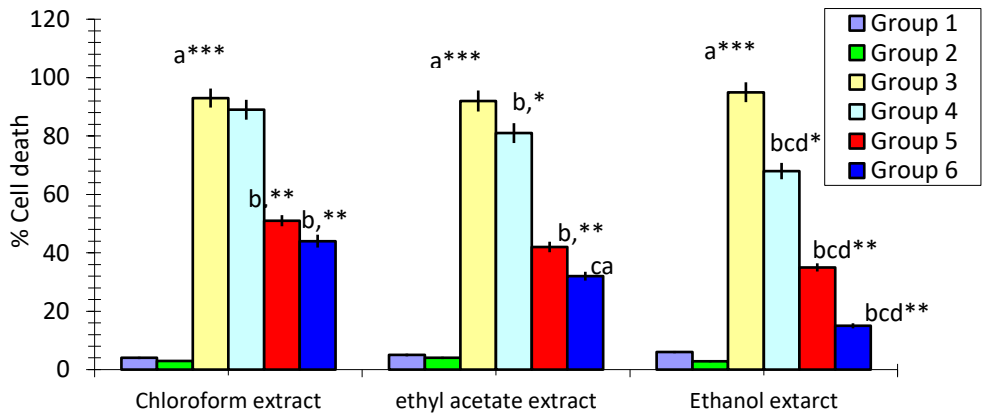

Figure – 2 Effect of different solvent extracts of *U.lactuca* on Indomethacin -induced toxicity in IEC-6 cell by trypan blue staining.

Group 1: DMSO **Group 2:** U.lactuca extract alone **Group 3:** Indomethacin **Group 4** : U.lactuca extract 200µg/ml + Indomethacin **Group 5:** U.lactuca extract 400µg/ml + Indomethacin **Group 6:** U.lactuca extract 600µg/ml + Indomethacin.

The percentage of cell death is significantly (P<0.001) increased in Indomethacin induced group when compared with control group and the percentage of cell death is decreased in U.lactuca ethanol extract of 600 µg/ml (Group 6), when compared with the Indomethacin induced group (Group 3).

Data are expressed as mean ±SD (n=6). *** (P<0.001), ** (P<0.01), *(P<0.05) with respect to control (one-way ANOVA followed by Dennett's t-test). Comparisons were made as follows. a-Compared with control, b-compared with Indomethacin, c-compared with chloroform extract with respective dose, d-compared with ethylacetate extract with respective dose.

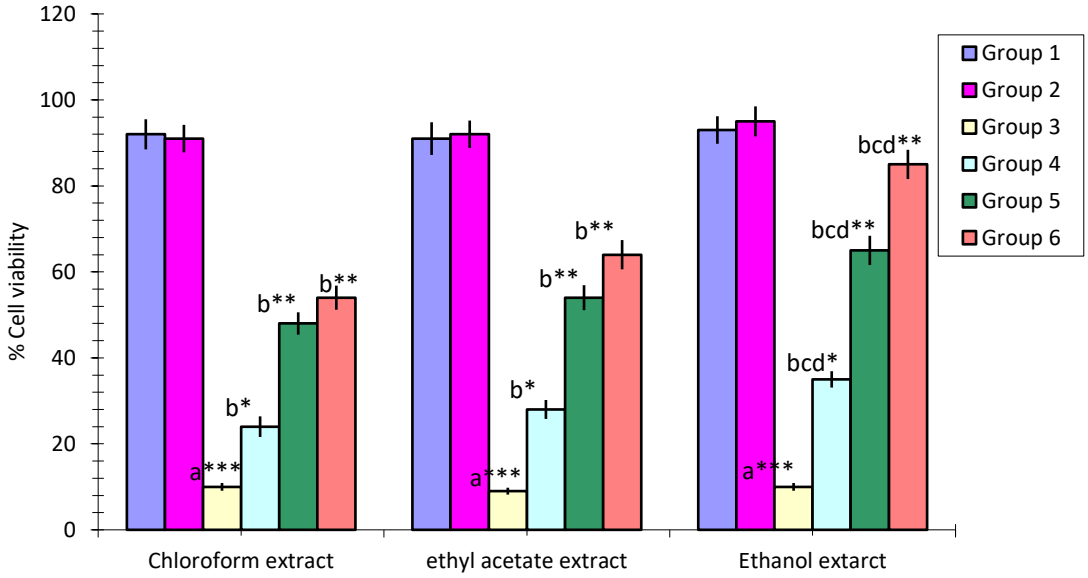

Figure – 3 Effect of different solvent extracts of U.lactuca on Indomethacin -induced toxicity in IEC-6 cell by MTT assay.

Group1: DMSO **Group 2:** U.lactuca extract alone **Group 3:** Indomethacin **Group 4** : U.lactuca extract 200µg/ml + Indomethacin **Group 5:** U.lactuca extract 400µg/ml + Indomethacin **Group 6:** U.lactuca extract 600µg/ml + Indomethacin.

The percentage of cell viability is significantly (P<0.001) reduced in Indomethacin induced group when compared with control group and the percentage of cell viability is increased in U.lactuca ethanol extract of 600 µg/ml (Group 6), when compared with Indomethacin induced group (Group 3).

Data are expressed as mean ±SD (n=6). *** (P<0.001), ** (P<0.01), *(P<0.05) with respect to control (one-way ANOVA followed by Dunnett's t-test). Comparisons were made as follows. a-Compared with control, b-compared with Indomethacin, c-compared with chloroform extract with respective dose, d-compared with ethylacetate extract with respective dose.

3.3 Antioxidant Activity in ethanolic extract of *Ulva lactuca*

3.3.1 DPPH radical scavenging activity

The hydro alcoholic extract of *Ulva lactuca* exhibited a significant dose dependent inhibition of DPPH activity, with a 50% inhibition (IC50) at a concentration of 1.5 µg/ml. The result was mentioned in figure 4. The IC50 value of the extract was found to be lesser than the standard, vitamin C (IC50 3.0 µg/ml).

3.3.2 Nitric oxide radical inhibition assay

The scavenging of nitric oxide by algae extract was increased in a dose-dependent manner as illustrated in figure 5. At concentration of 116.0 µg/ml of extract 50% of nitric oxide generated by incubation was scavenged. This IC50 value of extract found to be lesser than the standard, rutin (IC50 160.0 µg/ml).

3.3.3 Superoxide anion scavenging activity

The superoxide anion derived from dissolved oxygen by Phenazine methosulphate/NADH coupling reaction reduces nitro blue tetrazolium. The decrease the absorbance at 560 nm with the algae

extract thus indicates the consumption of superoxide anion in the reaction mixture. As mentioned in figure 6, the algae extract showed the scavenging activity; IC50 values, 4.7 µg/ ml and 5.84 µg/ml, respectively.

3.3.4 Lipid peroxidation assay

Activity of algae extract against non-enzymatic lipid peroxidation in rat liver microsomes has been shown in figure 7. Addition of Fe 2+/ascorbate to the liver microsomes cause increase in lipid peroxidation. The extract showed inhibition of peroxidation effect in all concentrations which showed 50% inhibition effect at 104.0µg/ml. The extract inhibition value was found to be lesser than the standard, vitamin E (IC50 120.5 µg/ml).

3.3.5 Hydroxyl radical scavenging assay

To attack the substrate deoxyribose hydroxyl radicals were generated by reaction of Ferric-EDTA together with H2O2 and ascorbic acid. When the algae extract was incubated with the above reaction mixture, it could prevent the damage against sugar. The results are shown in figure 8, the concentrations of 50% inhibition were found to be 27.0 µg/ml and 32.5 µg/ml for the extract and standard of vitamin E, respectively. The extract inhibition value was found to be lesser than the standard.

3.3.6 Reducing power

Figure 9 shows the reductive capabilities of the algae extract compared to butylated hydroxy toluene. The reducing power of extract of *Ulva lactuca* was very potent and the power of the extract was increased with quantity of sample. The algae extract could reduce the most Fe3+ ions, which had a lesser reductive activity than the standard of butylated hydroxy toluene.

Scavenging effect of Ulva lactuca Extract and standard vitamin C on 1, 1'-Diphenyl-2-picryl hydrazyl (DPPH) radical. Results are mean ± S.D of five parallel measurements

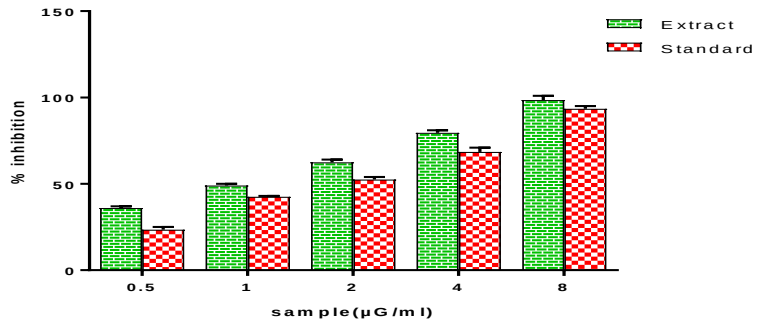

Figure- 4

Scavenging effect of Ulva lactuca Extract and standard rutin on Nitric oxide radical. Results are mean ± S.D of five parallel measurements.

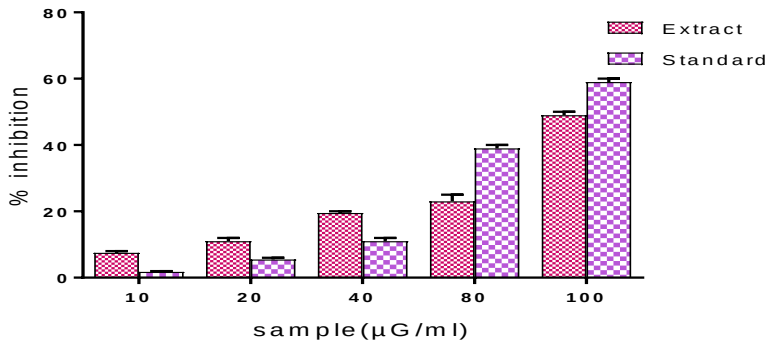

Figure- 5

Effect of Ulva lactuca Extract and curcumin on scavenging of superoxide anion radical formation. Results are mean ± S.D of five parallel measurements.

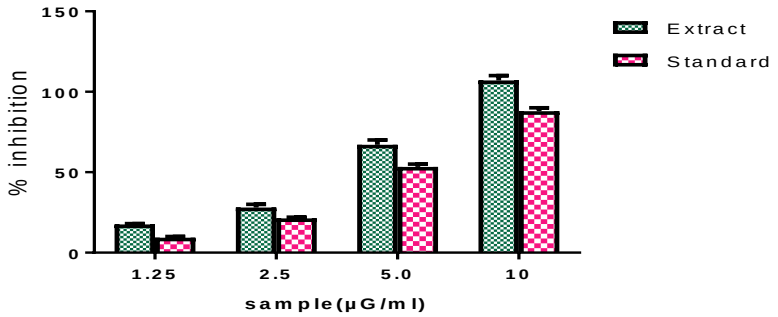

Figure- 6

Effect of Ulva lactuca Extract and vitamin E on lipid peroxidation of liver microsome induced by Fe2+/ascorbate. Results are mean ± S.D of five parallel measurements

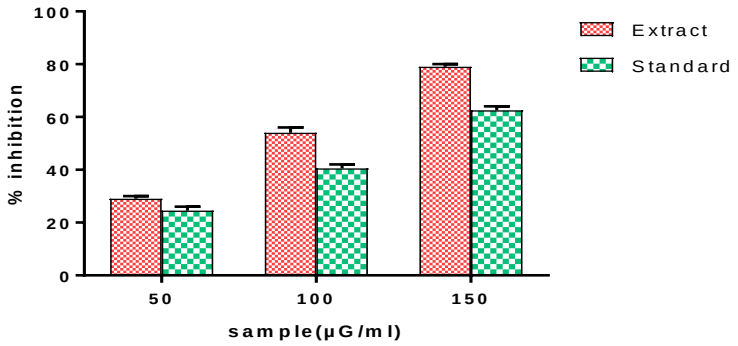

Figure- 7

Hydroxy radical scavenging activity
Effect of Ulva lactuca Extract and vitamin E on deoxyribose degradation assay. Results are mean ± S.D of five parallel measurements.

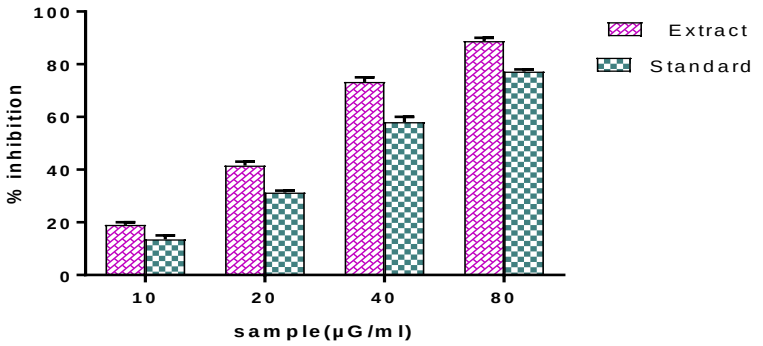

Figure- 8

Reducing power
The reductive ability of Ulva lactuca Extract and butylated hydroxy toluene. Results are mean ± S.D of five parallel measurements.

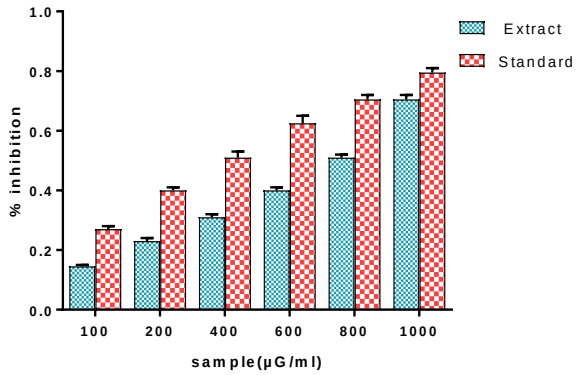

Figure- 9

4. CONCLUSION

At the end of this study it was revealed that the phytochemical constituents present in the ethanolic extracts of *Ulva lactuca* have highest antioxidant activity. It can be interesting to use algae in our lives because along with their antioxidant effect, they also contain great amounts of nutrients that are essential for a strong health. Also the highest gastrointestinal protection is observed in the ethanolic extracts of *U. lactuca* in cytotoxicity assays. Hence ethanolic extract of *U. lactuca* were used for the further *in vivo* Gastroprotective studies. Finally, the present investigation brings out adequate data on the phytochemical constitute and emphasize the significance of Ulva lactuca as a potential source of powerful broad spectrum of bio activities.

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