ASSESSING THE ANTICANCER, ANTIINFLAMMATORY, ANTIMICROBIAL AND ANTIOXIDANT POTENTIAL OF BIOACTIVE COMPOUNDS PRESENT IN MARINE ALGAE 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. The present study was performed to investigate the bio activities of the marine algae, Ulva lactuca collected from south west coast of India. Objective: The collected alga was subjected to screen phytochemicals, antimicrobial activity, and antioxidant, anti inflammatory and anticancer activities. The bioactive compounds were identified by GC–MS analysis. Extracts of the algae was prepared using methanol, ethyl acetate, chloroform, petroleum ether, acetone and hexane. Materials and methods: The antibacterial activity was carried out by disc diffusion method, antioxidant activity by diphenyl picrylhydrazyl and reducing power method, anti inflammatory activity was determined by COX inhibitory assay, anticancer activity by dimethylthiazol diphenyl tetrazolium bromide (MTT) and GC-MS analysis performed using an Agilent GC-MS 5973 assembly equipped with a HP-5cross-linked fused silica capillary column. Result: phytochemical analysis shows the presence of carbohydrate, saponin, flavanoids steroids, tannins, alkaloids, terpenoids and phenol. Antimicrobial activity of algal extract showed highest activity against Bacillus subtilis (12mm) and Candida tropicalis (20mm). Methanol extract of Ulva lactuca showed highest antioxidant activity compared to other extracts and also methanol extract possess cytotoxic activity against SKMEL skin cancer cell line. Through GC-MS analysis twelve organic...
compounds were separated among that Cyclo (L-Pro-L-Val) was more abundant in chromatogram. In the present study it was concluded that the Ulva lactuca is a source of pharmaceutical important compounds used for the treatment of human pathogenic diseases, anti-inflammatory and cancer.

**KEYWORDS:** Ulva lactuca, phytochemical analysis, antimicrobial activity, antioxidant activity, anti-inflammatory activity, anticancer activity, GC-MS analysis.

**INTRODUCTION**

Marine ecosystems usually have a large biodiversity. Marine algae are one of the largest producers of biomass in the marine environment.[1] Seaweeds are considered as source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by broad spectrum of biological activities. Marine macro algae compose a natural source of a variety of drugs for pharmaceutical, food and cosmetic applications including carotenoids, terpenoids, steroids, amino acids, phenolic compounds, halogenated ketones, alkanes and cyclic polysulphides.[2,3] Marine macro algae are considered as an excellent source of bioactive compounds which has a broad range of biological activities including antibacterial,[4,5] antifungal,[6] antiviral,[7] antitumorals,[8] antioxidant,[9-11] and anti-inflammatory.[12-14] Therefore, algae have been used in traditional medicine for a long time. Recently, there is a growing interest on the discovery of natural phytochemicals which are generally safer than synthetic chemicals. The present study intended to screen various phytochemicals, antimicrobial, antioxidant, anti-inflammatory, anticancer and GC-MS analysis of Ulva lactuca collected from the southern coast of Kerala.

**MATERIALS AND METHODS**

**Collection of samples**

Marine algae were collected from the south west coast of India. The algal residues were thoroughly washed with sea water to remove extraneous materials and then brought to the laboratory in polythene bags. Samples were again washed thoroughly with distilled water, air dried in shade. Then cut into small pieces and ground in a tissue grinder until reach fine powder.[15]

**Preparation of crude extract from marine algae**

The samples of marine algae are dried at room temperature under shade and dried. Algal material (5g) was soaked in 50 ml of solvents such as methanol, ethyl acetate, chloroform,
acetone, petroleum ether and hexane. These were kept for 48 hours and filtered through Whatman filter paper to remove all extractable material. The filtrate was concentrated under reduced pressure by using a rotator evaporator. The extracts were transferred to a hot air oven, where it was dried at 40°C.

**Phytochemical analysis**
A qualitative analysis was carried out on different extract of *Ulva lactuca* (methanol, ethyl acetate, chloroform, petroleum ether, acetone and hexane) in order to determine its phytochemical constituents and other secondary metabolites.

**Antibacterial activity**
The antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method. Pathogenic bacteria (Gram positive and Gram negative) were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, and Chandigarh. The bacterial strains were Bacillus subtilis (MTCC 121), Klebsiella pneumonia (MTCC 4030), Clostridium perferinges (MTCC 450), Pseudomonas aeruginosa (MTCC 4676), Pseudomonas putida (MTCC 4910), Bacillus megaterium (MTCC 456), Enterococcus faecalis (MTCC 439), Staphylococcus epidermis (MTCC 6810) and Enterococcus aeruginosa (MTCC 111) are spread over the medium. Filter paper disc of uniform size (6mm) are impregnated with specified concentrations of marine algal extract and then placed on the surface of Muller Hinton agar plates that has been seeded with organism to be tested. Label the each plate with the name of the test organism to be inoculated. Bacterial colonies were allowed to grow overnight at 37°C, then the inhibition zone around the disc was measured.

**Antifungal activity**
Antifungal screening was followed by the method Cappuciino et al., 2004. Fungal cultures were obtained from Microbial Type Culture Collection (MTCC), inoculated with respective fungi. The pathogenic strains include Candida tropicalis (MTCC 184), Candida albicans (MTCC 183), Aspergillus niger (MTCC 961), Candida glabarata (MTCC 183), Ashbaya gossypii (MTCC 358), Aspergillus fumigates (MTCC 4333), Rhizomucor miehei (MTCC 546) and Aspergillus aculeatus (MTCC 1331). The filter paper disc of uniform size (6mm) are impregnated with specified concentrations of methanol, ethyl acetate, chloroform, petroleum ether, acetone and hexane, algal extract and then placed on the surface of Rose Bengal agar plates that has been seeded with organism to be tested. Label the each plate with
the name of the test organism to be inoculated. Fungal colonies were allowed to grow 7 days at 20°C and then the inhibition around the disc was measured.

**Antioxidant activity**

**Antioxidant activity by DPPH method**

The scavenging effect of DPPH radical (1, 1-diphenyl-2-picrylhydrazyl) was investigated by the method described by Blois, 1958.[21] Stock solution of the whole plant extracts was prepared to the concentration of 1 mg/ml. 100μg of each extracts were added, at an equal volume, to methanolic solution of DPPH (0.1 mM). The reaction mixture was incubated for 30 minute at room temperature; the absorbance was recorded at 517 nm. The experiment was repeated for three times. BHT was used as standard controls.

**Antioxidant activity by reducing sugar method**

Extraction of different concentration in 1ml distilled water was mixed with phosphate buffer (2.5ml, 2M, PH 6.6) and potassium ferric cyanide (2.5ml, 1%); the mixture was then incubated at 50°C for 20 minutes. A portion (2.5ml) of trichloro acetic acid (TCA 10%) was added to the mixture which was then centrifuged at 1500 rpm for 10 minutes. The upper layer solution (2.5ml) was mixed with distilled water (2.5ml) and Fecl2 (0.5ml, 0.1%) and the absorbance was measured at 700nm. Increased absorbance of reaction mixture indicated increased reducing power. Ascorbic acid was taken as a reference.[22-23]

**In vitro Anti inflammatory activity effect of Ulva lactuca extract on THP1 cell lines**

In vitro anti-inflammatory activity of Ulva lactuca extract on THP1 cell lines was performed using method such as COX inhibitory, cyclooxygenase and 5-lipoxygenase assay.[24] Estimation of myeloperoxidase and nitrate levels calculated by the method of Lepoivre et al., 1990.[25]

**Anticancer activity**

SKMEL skin cancer cell line was purchased from NCCS Pune was maintained in Dulbeco’s modified eagles media supplemented with 10% FBS and grown to confluency at 37°C in 5% CO2 in a humified atmosphere in CO2 incubator. The cells were trypsinized for 2 minutes and passage to T flasks in a complete aseptic condition. Extracts were added to grown cells at concentration of 10μg, 50μg and 100μg from a stock of 10mg/ml 0.1% DMSO and incubated for 24 hours. The percentage difference in viability was determined by using MTT assay after 24 hours of incubation.
MTT assay

MTT assay was performed based on the method followed by Arung et al., 2000. [26] MTT is a colorimetric assay that measure the reduction of yellow 3-(4, 5dimethyl-2-yl)-2, 5- diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes in to the mitochondria where it is reduced to an insoluble colored (dark purple) formazen product. The cells are then solubilised with an organic solvent (eg.isopropanol) and then released, solubilised formazen reagent. Since reduction of MTT can only occur in metabolically active cells. The level of activity is a measure of the viability of cells.

The cell culture suspension was washed with 1X PBS and then added 30µl of MTT solutions to culture flask (MTT 5 mg/volume dissolved in PBS filtered through a 0.2 µm filter before use). Then incubated at 37°C for 3 hours, removed all MTT solution by washed with 1x PBS and 200µl of DMSO was added to each culture flask, incubated at room temperature for 30 minutes until all cells get lyses and homogenous colour was obtained. The solution was then transferred to centrifuge tubes and centrifuged at top speed for 2 minutes to precipitate cell debris. OD was measured at 540 nm using DMSO blank. Then the precipitate viability was calculated using the following formulae.

\[
\text{Percentage of viability} = \left( \frac{\text{OD of test}}{\text{OD of control}} \right) \times 100
\]

GC-MS Analysis

GCMS analysis was performed using an Agilent GC-MS 5973 assembly equipped with a HP-5cross-linked fused silica capillary column. Identification of chemical compounds was carried out by the modified procedure of Ismet Ara et al., 2012. [27] Helium was used as carrier gas. The column total flow rate was 1ml/min. General temperature conditions split less injector at 280°C, source 230°C transfer line at 280°C and column temperature program of 10°C/min. [28]

RESULTS

Phytochemical analysis

In the preliminary phytochemical screening eleven compounds (carbohydrate, saponin, flavanoids, steroids, tannin, alkaloids, terpenoids, protein, phenol, amino acid and cardiac glycoside) were tested for their presence or absorbance in six different extracts of Ulva lactuca. The results were summarized in table 1.
Table 1: Phytochemical screening of various extract of Ulva lactuca

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Test</th>
<th>Methanol</th>
<th>Ethyl acetate</th>
<th>Chloroform</th>
<th>Petroleum ether</th>
<th>Acetone</th>
<th>Hexane</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbohydrate</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Saponin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Flavanoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Steroids</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Tannin</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Terpenoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Protein</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Phenol</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Amino acid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>Cardiac glycoside</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*+* indicates a positive result, *-* indicates a negative result.

**Antibacterial activity**

The results of antibacterial activities of seaweed Ulva lactuca using six different solvents (methanol, ethyl acetate, chloroform, acetone, petroleum ether and hexane) were studied against nine human pathogenic bacteria. Among the six extracts acetone extract of Ulva lactuca show highest antibacterial activity against Clostridium perferinges and Staphylococcus epidermis (20mm). The results of antibacterial screening are summarized below in figure 1.

![Figure 1: Histogram showing antibacterial activity of different extracts of Ulva lactuca against human pathogens.](image-url)
Antifungal activity
The bioactive extracts prepared from selected algae were tested against human fungal pathogens such as Candida tropicalis, Candida albicans, Aspergillus niger, Candida glabrata, Ashbaya gossypii, Aspergillus fumigatus, Rhizomucor miehei and Aspergillus aculeatus. The algal extract showed potential antifungal activity against a panel of clinical fungal pathogens. Among the six extracts, ethyl acetate showed highest activity. When tested by the disc diffusion method, ethyl acetate extract of Ulva lactuca showed highest activity against Candida tropicalis (20mm) and Aspergillus niger (20mm). Results showed in figure 2.

![Antifungal activity](image)

**Figure 2:** showing antifungal activity of different extracts of Ulva lactuca.

Antioxidant activity of different extracts of Ulva lactuca
**In vitro evaluation of antioxidant activity by DPPH method**
In the present study in vitro antioxidant activity of algal extracts (methanol, ethyl acetate, chloroform, petroleum ether, acetone and hexane) showed potential free radical scavenging activities such as 0.0074%, 0.0057%, 0.0061%, 0.001%, 0.0067% and 0.0060% respectively. Methanol extract showed highest activity, and was subjected to anti-inflammatory activity. Results were showed in figure 3.
Antioxidant activity of Ulva lactuca by reducing power method
In the present study in vitro antioxidant activity of algal extracts (methanol, ethyl acetate, chloroform, petroleum ether, acetone and hexane) showed potential free radical scavenging activities such as 0.987%, 0.490%, 0.560%, 0.372, 0.827% and 0.436% respectively. Results were showed in figure 4.

Determination of in vitro anti-inflammatory activity of methanol extract of Ulva lactuca on cultured THP1 cell lines
Assay of cycloxygenase
The anti-inflammatory effect of algal extract on THP1 was tested by cycloxygenase. The algal extract showed 59.66% of human monocytic cell line inhibition at 10µg/ml, 62.625% of inhibition at 50µg/ml and 72.25% of inhibition at 100µg/ml.
Table 2: anti inflammatory activity of Ulva lactuca by cyclooxygenase Assay of 5-lipoxygenase.

<table>
<thead>
<tr>
<th>Sample concentration (µg/ml)</th>
<th>OD 632 nm</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.240</td>
<td>0.00</td>
</tr>
<tr>
<td>10µg/ml</td>
<td>0.0968</td>
<td>59.66</td>
</tr>
<tr>
<td>50µg/ml</td>
<td>0.0897</td>
<td>62.625</td>
</tr>
<tr>
<td>100µg/ml</td>
<td>0.066</td>
<td>72.25</td>
</tr>
</tbody>
</table>

The anti-inflammatory effects of methanol extract on THP1 were tested by 5-lipoxygenase assay. The methanol extract showed 12.64% of human monocytic cell line inhibition at 10µg/ml, 21.82% of inhibition at 50µg/ml and 29.10% of inhibition at 100µg/ml. The results were showed in table 4.

Table 3: anti inflammatory activity of Ulva lactuca by 5-lipoxygenase.

<table>
<thead>
<tr>
<th>Sample concentration (µg/ml)</th>
<th>OD at 234 nm</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.515</td>
<td>0.00</td>
</tr>
<tr>
<td>10µg/ml</td>
<td>2.197</td>
<td>12.64</td>
</tr>
<tr>
<td>50µg/ml</td>
<td>1.966</td>
<td>21.82</td>
</tr>
<tr>
<td>100µg/ml</td>
<td>1.783</td>
<td>29.10</td>
</tr>
</tbody>
</table>

Estimation of myeloperoxidase

The anti-inflammatory effects of algal extract on human monocytic cell lines were tested by myeloperoxidase estimation. One unit of myeloperoxidase activity was defined as degrading 1m mol peroxidase degraded per minute at 25°C. The algal extract contain 0.038 unit enzyme/ml at 10µg/ml, 0.018 unit enzyme/ml at 50µg/ml and 0.004 unit enzyme/ml at 100µg/ml.

Table 4: Anti-inflammatory activity of Ulva lactuca by myeloperoxidase activity.

<table>
<thead>
<tr>
<th>Sample concentration (µg/ml)</th>
<th>OD at 340 nm</th>
<th>Enzyme (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.048</td>
<td>0.0638</td>
</tr>
<tr>
<td>10µg/ml</td>
<td>0.029</td>
<td>0.038</td>
</tr>
<tr>
<td>50µg/ml</td>
<td>0.0141</td>
<td>0.018</td>
</tr>
<tr>
<td>100µg/ml</td>
<td>0.0032</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Estimation of cellular nitrate levels

The anti-inflammatory effects of algal extracts on human monocytic cell lines were tested by the estimation of cellular nitrate levels. The cellular nitrate level of control was 760µg. The concentration of cellular nitrate level at 10µg/ml of algal extract was 498.54µg, nitrate level at 50µg/ml was 484.63 and 365.33µg of nitrate level at 100µg/ml.
Table 5: Anti-inflammatory activity of Ulva lactuca by Estimation of cellular nitrate levels.

<table>
<thead>
<tr>
<th>Sample concentration (µg/ml)</th>
<th>OD at 540nm</th>
<th>Concentration (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.1529</td>
<td>760</td>
</tr>
<tr>
<td>10µg/ml</td>
<td>0.1003</td>
<td>498.54</td>
</tr>
<tr>
<td>50µg/ml</td>
<td>0.0975</td>
<td>484.63</td>
</tr>
<tr>
<td>100µg/ml</td>
<td>0.0735</td>
<td>365.33</td>
</tr>
</tbody>
</table>

Anticancer activity
The cytotoxic effects of marine algal extracts on SKMEL cell line was tested by MTT cell viability assay. The methanol extract of algae at concentration ranging from 10µg/ml, 50µg/ml, 100µg/ml and then percentage of cell viability was analyzed. The result showed a dose dependent inhibition of the algal extract towards SKMEL skin cancer cell line. Results were showed figure 5 and plate 1.

Plate 1: anticancer activity of Ulva lactuca by MTT cell viability assay

Anticancer activity of control (DMSO) 10µg/ml of on SKMEL cell lines

50µg/ml of on SKMEL cell lines 100µg/ml of on SKMEL cell lines

Figure 5: anticancer activity of Ulva lactuca by MTT cell viability assay
GC-MS analysis of methanol extract of Ulva lactuca

Volatile organic compounds were analyzed by gas chromatography mass spectrometry. Among the volatile compound Cyclo (L-Pro-L-Val) was more abundant. Results were showed in table 6 and figure 6.

Table 6: Shows compounds in methanol extract of marine algae by GC-MS analysis.

<table>
<thead>
<tr>
<th>Number of peaks</th>
<th>Retention time</th>
<th>Compounds</th>
<th>Abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.16</td>
<td>Methane sulfonic acid methyl ester</td>
<td>1.09</td>
</tr>
<tr>
<td>2</td>
<td>6.16</td>
<td>2-Methoxy-4-(2-propenyl) phenol</td>
<td>1.72</td>
</tr>
<tr>
<td>3</td>
<td>12.52</td>
<td>6-aminohexanoic acid</td>
<td>1.13</td>
</tr>
<tr>
<td>4</td>
<td>12.52</td>
<td>Ethane</td>
<td>1.90</td>
</tr>
<tr>
<td>5</td>
<td>23.89</td>
<td>Cis-1,2-cyclohexanedicarboxylic anhydride</td>
<td>1.09</td>
</tr>
<tr>
<td>6</td>
<td>24.35</td>
<td>Styrene</td>
<td>2.41</td>
</tr>
<tr>
<td>7</td>
<td>25.89</td>
<td>Cyclo (L-Pro-L-Val)</td>
<td>7.13</td>
</tr>
<tr>
<td>8</td>
<td>26.07</td>
<td>n-[2-(1-cyclic hexen-1-yl) ethyl pyrroline n-oxide]</td>
<td>0.96</td>
</tr>
<tr>
<td>9</td>
<td>31.16</td>
<td>4(N,N-dimethylcarbomyl)-2-oxo1,2,3,4 tetrahydroquinoline</td>
<td>0.22</td>
</tr>
<tr>
<td>10</td>
<td>31.16</td>
<td>Pyrrole-2-methanamine</td>
<td>0.32</td>
</tr>
<tr>
<td>11</td>
<td>36.5</td>
<td>1-phenyl-N-cyclohexyl-1,2-benzene dicarboxylic acid</td>
<td>2.39</td>
</tr>
<tr>
<td>12</td>
<td>37.5</td>
<td>Bis-2- nonadecane</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Figure 6: shows chromatogram of methanol extract of marine algae

DISCUSSION

Marine environment has been described as a source of novel chemical diversity for drug discovery because many bioactive substances are isolated from marine organisms, including phytoplankton, algae, sponges, tunicates and mollusks. Algae play a vital role in the aquatic ecosystem. They provide food and shelter for other organism and are important in the process of absorbing nutrients and toxins. Marine algae are considered as excellent source of bioactive compounds which has a broad range of biological activities including
antibacterial and antioxidant. Different types of solvents such as acetone, ethanol, methanol, ethyl acetate, diethyl ether, petroleum ether, hexane, chloroform, aqueous, benzene were used earlier to extract bioactive principles from seaweeds.[32-37] The present investigation was carried out in methanol, ethyl acetate, chloroform, petroleum ether, acetone, and hexane extracts. Using organic solvents always provides a higher efficiency in extracting compounds for antimicrobial activity.[38]

Marine algae are considered to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activated. Green algae belonging to the genus Ulva contain 18-26% protein.[39-40] Compounds with cytotoxic, antiviral, anthelmintic, antifungal and antibacterial activities have been detected in green, brown and red algae.[41-42] The present investigation report the presence of carbohydrate, saponin, steroids and tannin. Ethyl acetate extract showed the presence of saponin, alkaloids and terpenoids in methanol extract of Ulva lactuca. Chloroform extract showed the presence of alkaloids, terpenoids and phenol. Petroleum ether extract showed the presence of saponin, flavanoids, terpenoids and phenol. Acetone extract showed the presence of saponin, flavanoids and terpenoids. Hexane extract showed the presence of saponin, flavanoids, terpenoids and phenol. These bioactive compounds show bioactivities in Ulva lactuca.

Antibacterial effects of crude methanol extracts obtained from 32 marine macro algae species (13 Chlorophyceae and 19 phaeophyceae) harvested from Atlantic and Mediterranean coast of Morocco were found to be effective against pathogenic bacteria namely Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia and Enterococcus faecalis.[43] The present study also shows the marine algal extract Ulva lactuca was tested against nine species of pathogenic bacterial strains and eight fungal strains by disc diffusion method. The extract released only on the active principles but it may change due to the assay methods, incubation temperature and culture media. The marine algal extract showed highest activity against Bacillus subtilis and Staphylococcus epidermis (20mm) and lowest activity against Candida tropicalis and Aspergillus Niger (20mm) and lowest activity against Candida albicans (6mm). This study revealed that all the extracts showed antimicrobial activity against variety of pathogens and evaluation of the potential usefulness of natural products in the development of new antimicrobials.

Antioxidant in biological system have multiple fractions, including defending against oxidative damage in the major signaling pathways of cells. Several synthetic antioxidants
such as butylated hydroxyanisol, butylated hydroxyquinones and tert butylhydroquinone are commercially available and are currently in use. However their use is now restricted due to their side effects. Marine algae are also being studied as a source of antioxidants.\[44\] Meenakshi et al 2009 in their study evaluated in vitro antioxidant activity of two sea weeds Ulva lactuca and Sargassum wightii.\[45\] Antioxidant activity of methanolic extract of these two sea weeds was 1.16, 0.11 respectively. Present study shows that antioxidant activity of algal extracts (methanol, ethyl acetate, chloroform, petroleum ether, acetone and hexane) has potential free radical scavenging activities such as 0.0074%, 0.0057%, 0.0061%, 0.001%, 0.0067% and 0.0060% respectively. Methanol extract showed highest activity.

Seaweeds are also known to have a rich source of structurally diverse bioactive compounds with valuable pharmaceutical potential.\[46-47\] Studies with rats have confirmed its protective effects against acetic acid-induced small bowel inflammation.\[48\] Methanol extracts of Ulva conglobata and U. lactuca have shown anti-inflammatory effects in experiments that used a murine hippocampal HT22 cell line and rats.\[49-50\] In the present study anti-inflammatory effect of algae was done to by different methods it shows that marine algae posses potential anti-inflammatory activity.

Seaweeds contain powerful antioxidant and anticancer properties to arrest the proliferation of cancer cells.\[51-52\] Chemo preventive effects of Walkame seaweed on breast cancer and reported a suppressive effect on the proliferation of DMBA (Di Methyl Benz Anthracene) Induced rat mammary tumors. The effects were better than that of a chemo therapeutic agent widely used to treat human breast cancer. The antiproliferative activity exhibited by the seaweed extracts were positively correlated with the total polyphenol suggesting a link related to the content of phlorotannins and polyphenols including mycospirine like amino acids and phenolic acids present in dulse and kelp. In this study the methanolic algal extracts exhibited 75.25% cell viability at 10µg/ml, 54.22% cell viability at 50µg/ml, 39.47% cell viability at 100µg/ml concentration towards SKMEL skin cancer cells.

Marine algae contain more than 600 secondary metabolites it has been reported by Faulkner DJ, 1984.\[53\] Many of these compounds are bioactive and have been extensively studied using laboratory and pharmacological assays.\[54-55\] However, their natural functions under ecologically realistic conditions have been investigated only recently.\[56-58\] In the present study shows that certain compounds present in Ulva lactuca identified by GC-MS analysis. In the algal extract, the chromatogram showed six peaks and mass spectrum detected the
compounds present in the respective peak areas. Cyclo (L-Pro-L-Val) was more abundant (7.13) with retention time of 23.89. Among the volatile compounds alkanes, alkenes, esters, ketones, sulfur compounds, alcohols and isoprenoid compounds were present.

CONCLUSION
The phytochemical screening of seaweeds showed the presence of carbohydrate, saponin, flavanoids, steroids, tannin, alkaloids, terpenoids, protein, phenol, amino acid and cardiac glycosides .Antimicrobial screening of algal extract showed highest activity against Bacillus subtilis, Candida tropicalis and Aspergillus niger which make them interesting for programs of screening for antibiotics. Methanolic extract showed potential antioxidant activity, anti-inflammatory activity THP1 cell lines in and anticancer activity in SKMEL cell line. GC-MS analysis revealed the presence of different compounds of varying abundance, among the twelve compounds separated, Cyclo (L-Pro-L-Val) was more abundant. 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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