Extraction Of Metabolites And Screening Their Antioxidant Potential From Marine Macro Algae

M. Elangovan, A. Noorjahan and P. Anantharaman

Abstract: Antioxidant potential of different extracts of macroalgal species of south east coast of Tamilnadu were examined. The organic solvents such as Acetone, Methanol, Ethanol and Hexane were used to extract the macroalgae such as C. clavulatum, G. edulis of red algae and E. intestinalis belongs to green algae which were taken for the study. Methanolic extracts of all the test species exhibit higher antioxidant activity (DPPH) when compared to other solvents comparing C. clavulatum shows 97% followed by the E. intestinalis 91.17% and G. edulis shows 90.23%. In Hydrogen peroxide scavenging assay Acetone extract of C. clavulatum shows 91.34% followed by G. edulis 90.85% and E. intestinalis 87.59%. Hexane shows maximum activity in FRAP. The results suggest that Methanolic extracts have potent antioxidant ability.

Keywords: Antioxidant activity, Macro algae, Radical scavenging activity, FRAP

1. INTRODUCTION:
The macroalgae are present in 2400 natural product belonging to the classes Chlorophyceae, Phaeophyceae and Rhodophyceae [1]. The macroalgae are antimicrobial activity indicator to detect the potent pharmaceutical capacity due to the synthesis of bioactive secondary metabolites [2]. In recent years, there are many reports of macroalgae derived compounds that have a wide range of biological activities, such as antiviral [3], [4], antifungal [5] antioxidant [6], [7], [8], antitumors [9], [10], and anti-inflammatory activity [11], [12], [13]. The use of antimicrobial drugs has certain limitations due to changing patterns of resistance in pathogens and side effects they produce. Natural products are present in the antioxidant activity by using marine fresh water algae [14]. Marine algae are photosynthetic plants; it's showed to complain of oxygen and light. They are present lead to the formation of oxidizing agents and free radicals. The photodynamic damage is vulnerable due to the elements of the photosynthetic element, because thylakoid membrane is present in important compounds of the polyunsaturated fatty acids [15]. Several studies on compounds from specific type of organisms, such as anticoagulants from marine algae [16], medicinal and pharmaceutical products from macroalgae [17], cytotoxic metabolites from marine algae [18], antimicrobials and antifungals from marine microorganisms [19], metabolites of marine-derived fungi [20], toxins from microalgae [21] and enzyme inhibitors from marine microbes [22]. In higher plant, the natural antioxidant such as α-tocopherol, phenols and β-carotene found to be used in the food industry to inhibit lipid peroxidation which can protect against free radicals and retard the progress of many chronic diseases in the human body [23], [24]. Herein, we have found the synergistic action of wide spectrum of antioxidant is far better than the activity of a single antioxidant derived from natural source (primarily foods) which have a higher bioavailability. Therefore, the higher protective efficacy against oxidative stress than synthetic antioxidants [25]. Recently, due to the evidence of consuming vegetables and fruits reduced the risk of developing chronic disease, there is increased the interest of the discovery of natural antioxidants. Also, the phytochemicals are generally safer than synthetic chemicals. The herbs and spices are the source of antioxidant compounds. In addition, the seaweeds are cheap resources and the rich source of highly bioactive compounds such as carotenoids, dietary fiber, protein, essential fatty acids, vitamins and minerals. Considering the availability and bioactivity, the present study aimed to extract the metabolites from the seaweed and investigate their antioxidant potential by in vitro method.

2. MATERIALS AND METHODS

2.1 Collection of Macro algae
The seaweeds were collected from the station in Rameshwaram the olaikuda (N.Lat.09°18.300’ and E Long. 079°20.096’) southern coast of India (Fig. 1). The sample was washed in seawater to remove all the extraneous sand particles, and impurities. Samples are collected in laboratory plastic bags aseptically in the ice box (4°C). Then the samples are washed with tap water followed by distilled water. Washed samples were blotted in blotting paper then shadow dry in 15 days after samples grounded into a fine powder. Then sample was stored in refrigerator (4°C) for further use.

![Sample collection area](image)

2.2 Preparation of organic algae extracts
The extraction was done by Soxhlet extraction techniques. Different solvents were used successively with gradient polarity (chloroform, ethyl acetate, methanol and ethanol). The extracts were evaporated to complete dryness by vacuum distillation and stored in refrigerator for further use [26], [27], [28].
2.3 Radical scavenging activity
DPPH radical scavenging activity of each solvent extract of three algal species was determined according to [29]. Briefly, 1 ml of each lyophilized extract of all the three species were taken at variable concentrations (25-400 μg/ml), was added to 1 ml of DPPH (1,1 diphenyl-2-picryl hydrazyl) solution (0.1mM in methanol) as the free radical source. The mixture was shaken and kept for 30 minutes at room temperature. The decrease of solution absorbance due to proton donating activity of components of each extract was determined at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Vitamin C was used as the positive control. The DPPH Radical Scavenging activity was calculated using the following formula:

\[ \% = \left[ \left( \frac{A_0 - A_t}{A_0} \right) \right] \times 100 \]

where \( A_0 \) is the absorbance of the control and \( A_t \) is the absorbance of extract or standard sample.

2.4 Hydrogen peroxide scavenging assay
Hydrogen peroxide solution (2 mM/L) was prepared with standard phosphate buffer (pH 7.4). Different concentration of the fractions (25-400 μg/ml) in distilled water was added to 0.6 ml of hydrogen peroxide solution. Absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging activity at different concentrations of the fractions was determined and the IC50 values were compared with the standard, α-tocopherol [30].

2.5 Ferrous reducing assay
The reducing power was determined by using Ferrous reducing assay (FRAP) described by [31] with some modifications. Briefly, the FRAP reagent contain 2.5mL of 10 mM tripyridyltriazine (TPTZ) solution in 40 mM HCL plus 2.5mL of 0.05M FeCl3 and 25mL of 0.3M acetate buffer, pH 3.6, was freshly prepared. All the extracts were dissolved in water at a concentration of 1mg/mL and diluted to 10, 20, 30, 50 and 100 μg/mL. Aliquots (0.1mL) of each diluted extract mixed with 2.9mL of FRAP reagent. The absorption of the reaction mixture was measured at 700 nm. The FRAP value was defined as the conditions as a standard antioxidant compound. The FRAP value was defined as the concentration of antioxidant having a ferric reducing ability equivalent to that of 1μM FeSO4.

2.6 Statistical analysis
The ggplot2 software package was used for the line diagrams and Box plot [32].

3. RESULTS
By now, it is well established that algae species are alternate sources for natural occurring antioxidants. In this study we compared the antioxidant ability of various polar solvents of three different Macro algae such as Gracilaria edulis, Centerocerous clavulatum, Enteromorpha intestinalis. There are several assays commonly used for the measurement of antioxidant activity, including DPPH, FRAP and Hydrogen peroxide assays. DPPH is a stable electron containing free radical which is used for detecting radical scavenging activity in chemical analyses. The antioxidant properties of marine algae have been investigated in recent years, by the identification and quantification of capacities and compounds such as polyphenols, Fe-reducing power and ascorbic acid content, among many others [33], [34], [35]. However, as the concentration of the compound in the assay system increased, the differences in scavenging activities between the extracts become less significant.

3.1 Radical scavenging ability
The studied extracts exhibited the scavenging activity of various strengths and were dose dependent in all extracts. In addition positive controls with ascorbic acid were tested for their DPPH radical scavenging. The calculated EC50 for 30 min incubation time are reported in Table (1), Fig (2). DPPH radical scavenging activity of Acetone, methanol, Hexane extracts of the algal species Centerocerous clavulatum, Gracillaria edulis, Enteromorpha intestinalis revealed antioxidant potency considering the fact that the IC50 values. A lower value of IC50 indicates a higher antioxidant activity. The different in antioxidant activities among Algae species (EI, GE and CC) extracts due to multiple factors including concentration of the extracts and quantitative profile of extracts. The solvent extracts which has high antioxidant activity in G.edulis species were to be in the order Methanol 90.23%> Acetone 87.03%> Hexane 86.75%> Ethanol 85.53%. Depicts the presence of E.intestenalis to scavenge DPPH was determined and compared with the standard ascorbic acid. The extraction results exhibited in the order Methanol 91.17%> Ethane 86.26%> Ethanol 87.50%> Acetone 73.78%. Table 1c C.clavulatum shows the activity in the order Methanol 97%> Ethane 87.60%> Acetone 86.03%> Hexane 85.25%. Remarkable activity were seen in the Methanolic extract on all the species on comparing c.clavulatum shows 97% followed by the E.intestenalis 91.17% and G.edulis shows 90.23% respectively.

3.2 Hydrogen peroxide scavenging assay
The ability of the three species of various solvent extracts subjected to scavenge hydrogen peroxide was determined and was compared to that of the standard α-tocopherol. G.edulis shows Acetone 90.85%> Ethanol 86.24%> Methanol 86.12%> Hexane 77.34%. E. intestinalis exhibits reducing capacity in the order Methanol 88.15%> Acetone 87.59%> Hexane 81.82%> Methanol 79.98%> Ethanol 78.94%. C. clavulatum s depicts the presence of reduction activity in the order Acetone 91.34%> Hexane 83.60%> Ethanol 83.36%> Methanol 79.98%. The reduction activity increased with the increasing concentration. The scavenging effect in Acetone extraction of C.clavulatum shows 91.34% followed by G.edulis 90.85% and E.intestenalis 87.59% Table (2), Fig (3).

3.3 Ferrous reducing assay
G.edulis depicts the value in the order Ethanol 7.01%> Methanol 32.98%> Hexane 57.19%> Acetone 58.94%. The reductive effect similar to the antioxidant activity, the reducing power increased with increase in concentration. All concentrations showed higher activities than the control. C.clavulatum exhibits in the order Acetone 7.01%> Methanol 45.40%> Ethanol 45.43%> Hexane 57.01%. E. intestinalis shows higher activity in Methanol 30.70%> Ethanol 37.8%> Acetone 61.22%> Hexane 66.84%. Table (3), Fig (4)
**Fig 2.** DPPH radical scavenging activity in seaweed extract

**Fig 3.** Hydrogen peroxide scavenging activity in seaweed extract

**Fig 4.** Ferrous reducing activity in seaweed extract

### Table 1 DPPH radical scavenging activity in seaweed extract

<table>
<thead>
<tr>
<th>Seaweed</th>
<th>% of Scavenging to DPPH free radical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gracillaria edulis</td>
<td>Ethanol (85.53) Methanol (90.23) Acetone (87.03) Hexane (86.75)</td>
</tr>
<tr>
<td>Centerocerus clavulatum</td>
<td>Ethanol (87.60) Methanol (97) Acetone (86.03) Hexane (85.25)</td>
</tr>
<tr>
<td>Enteromorpha intestinalis</td>
<td>Ethanol (87.50) Methanol (91.17) Acetone (73.78) Hexane (88.26)</td>
</tr>
</tbody>
</table>

### Table 2 Hydrogen peroxide scavenging activity in seaweed extract

<table>
<thead>
<tr>
<th>Seaweed</th>
<th>Percentage (%) of H₂O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gracillaria edulis</td>
<td>Ethanol (86.85) Methanol (86.12) Acetone (90.85) Hexane (77.34)</td>
</tr>
<tr>
<td>Centerocerus clavulatum</td>
<td>Ethanol (83.36) Methanol (79.98) Acetone (91.34) Hexane (83.60)</td>
</tr>
<tr>
<td>Enteromorpha intestinalis</td>
<td>Ethanol (78.94) Methanol (88.15) Acetone (87.59) Hexane (81.82)</td>
</tr>
</tbody>
</table>
4. DISCUSSION

Marine algae are present in flavonoids the many reports and it's have been investigate for the biological activity [36],[37]. The macroalgae (green and brown) are persent in antibacterial activity from the morocco coast and cystoseira does not inhibit from Staphylococcus aureus the methanolic extract [38].The tested bacteria are all algal extract be the most senstive zone of inhibition to the S. aureus. All the seaweed extract are resistant to the escherchia coli. They reported that the Gram-positive bacterial strains were comper then more susceptable to seaweeds extract than Gram-negative bacterial strains [39]. All over the world are antioxidant and antiproliferative studies have been performed to the marine algae [35], [40], [41]. The antioxidant activity reports are present in marine algae found along the Indian coastline but few reports discribed in antiproliferative activity [42], [43], [44]. The antioxidant and antiproliferative activities of methanol, chloroform and ethyl acetate extracts of Gracilaria edulis and Enteromorpha lingulata marine algae from [45]. Enteromorpha lingulata and Gracilaria edulis are edible marine algae. The in vitro antiproliferative activity of Gracilaria edulis are no reports but Gracilaria edulis and Aganthaphora spicifera are present three fatal poisoning cases have reported during at 2002-2003 in philipines [46]. DPPH is a highly stable nitrogen centered free radical. The indication of color changes from violet to yellow upon reduction by either the process of hydrogen or electron-donation. The bioactive substances which are able to perform this reduction can be considered as antioxidant and therefore radical scavenges[47]). The corresponding bioactive extract usually measured from the bleaching of violet colored methanol solution of DPPH through the ability of hydrogen atoms or electron donation. The screening of methanol extract of three Sargassum species (S. horneri, S. macrocarpum & S. siliquastrum), reported for the highest antioxidant activities [48]). The methanolic extract of S. siliquastrum showed higher antioxidant activity [14]. The methanolic extracts of C.clavulatum, E.intestinalis and G.edulis showed higher antioxidant activity due to the high content of the lipid soluble total chlorophylls especially Chl-a and related compounds [49], [50], to the high content of total carotenoids [51], [52], [53]. Although H2O2 itself is not very reactive, it may convert into more reactive species such as singlet oxygen and hydroxyl radicals. Therefore, it is very important to remove H2O2 for the protection of living organisms. Addition of H2O2 to in human cells in culture can lead to transition metal ion-dependent HO- DNA mediated oxidative damage. [54]. The expression of antioxidant activity is thought to be concomitant with the development of reductions, as these species are known to be free radical chain terminators [55]. The reductive capacity of the various algal extracts could indicate their potential as antioxidants. The presence of redacting antioxidants in the interested tested samples would be result in the reduction of Fe²⁺/ ferricyanide complex to the ferrous form measuring the formation of Perls Prussian blue at 700nm [56]. In our study, the behavior of the different algal extracts fractions in relation to the radial scavenging and antioxidant activities was tested independently. Thus, significant different between the extracts and the fractions were found exhibiting the highest antioxidant activity and free radical and superoxide radical scavenging activity. The antioxidant activity of the extract might be due to its effective free radical scavenging activity as well as its high reductive capability determined in the study. Additional studies are needed to characterize the bioactive compounds responsible for the observed activities. Therefore, the algal species can be used as an accessible source of natural antioxidants and a possible food supplement.

5. CONCLUSION

The present study the methanolic and acetone extracts of Gracilaria edulis, Centerocerous clavulatum and Enteromorpha intestinalis has showed maximum and potent scavenging effect with lower concentration level. The study concluded that the methanol and acetone is suitable organic solvent antioxidant metabolite extraction also the studied seaweeds is an alternate source for natural antioxidants instead of commercial antioxidant in drug industries.

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