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Original Article

ANTIOXIDANT PROPERTIES *CLADOPHORA SOCIALIS* GREEN ALGAE OF SEAWEEDS COLLECTED FROM RAMESWARAM IN INDIA

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ABSTRACT

Objective: *Cladophora Socialis* (*CS)* green algae are one of the most common seaweed water filamentous green algae reported to three different extracts (Hexane, Ethyl acetate and Acetone) have antioxidant activities.

Methods: The evaluation of antioxidant properties was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis 3ethylbenzthiazoline-6-sulphonic acid (ABTS), ferric reducing antioxidant power (FRAP), nitric oxide scavenging assay (NO), reducing power, hydroxyl, superoxide radical scavenging (SOD), hydrogen peroxide radical assay, the metal chelating activity as well as phosphomolypdenum assay.

Results: Among the tested CS, the maximum antioxidant activity was recorded in the extract of CS. Whereas Acetone extract of green algae showed good antioxidant potential.

Conclusion: This study suggests that green algae Acetone extracts contain different potential antioxidant compounds capable to scavenge different types of free radicals.

Keywords: *Cladophora Socialis*, Green algae, Free radical scavenging, Antioxidant activity, Phosphomolypdenum assay

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INTRODUCTION

Marine organisms are potential sources of bioactive secondary metabolites with the potential for use in the development of new pharmaceutical agents and many of these substances have been demonstrated to possess interesting biological activities [1-4]. Marine algae were reported to produce a wide variety of bioactive secondary metabolites as antimicrobial, antifeedant, antihelmintic and cytotoxic agents and the bioactive substances included alkaloids, polyketides, cyclic peptides, polysaccharide, phlorotannins, diterpenoids, sterols, quinones, lipids and glycerols and marine macro-algae are considered as the actual producers of some bioactive compounds with high activity [5, 6]. Hence they have drawn great attention recently [7-10]. Filamentous fig. 1 shows the alga *Cladophora* is a common inhabits of freshwater locations.

Fig. 1: ***Cladophora socialis***

It is called blanket weed in some places, not an inappropriate name when in late summer, dense floating rafts of *Cladophora* can be found both at the pond's edge and in the open water, buoyed up with the oxygen generated by its own photosynthesis. *Cladophora* is capable of branching and seems to produce little or no mucilaginous secretion. This, and the fact that salts tend to crystallize on the filaments of older specimens, gives it a rougher, grittier feel than other filamentous algae. It is also more readily colonized by

epiphytic diatoms and other algae and provides a protected foraging environment for the smaller pond creatures such as protozoa, worms, small crustaceans and insect larvae [11, 12]. There are various reports on the chemical constituents of the green algae *Cladophora* species in different parts of the world [13-16]. Also several of chemical investigations on *Cladophora* species yielding antioxidant, antimicrobial and anti-cancer substances were reported [17, 18]. Therefore, the present investigation was attempted to study the antioxidant properties of three different crude extracts of marine diatom green algae *Cladophora Socialis*.

MATERIALS AND METHODS

Chemicals

DPPH, ABTs and FRAP were obtained from Sigma Aldrich (Steinheim, Germany). Hexane, Ethyl acetate and Acetone were of HPLC grade (Lab-Scan, Dublin, Ireland). All the other reagents were of analytical grade and obtained from Merck (Darmstadt, Germany).

Algal materials

Cladophora Socialis green algae were collected from the Rameswaram area on January 5th, 2022. The freshly collected seaweeds were washed with clean seawater to remove salt, epiphytes and sand attached to the surfaces of the samples and transported to the laboratory. The samples were carefully rinsed with tap water, wiped with a paper towel. For *CS the* stipes and hapteres were removed and the new and old parts of the blades were separated. The samples were lyophilized for 72 h, pulverized into powder and stored at 80 °C prior to extraction.

Preparation of sample extract

5 g of *CS* powdered seaweed was extracted overnight with 100 ml of three different extracts (Hexane, Ethyl acetate and Acetone) at room temperature and centrifuged at 2800 rpm for 10 min. The supernatant was collected in a separate bottle after passing through a filter paper and the residue was re-extracted three times under the same conditions as mentioned above. The combined extracts were freeze-dried. These extracts were kept at 80 °C until analysis. The freeze-dried extracts were redissolved used for the analysis.

DPPH (1, 1-diphenyl-2-picryl hydrazyl) radical scavenging assay

The radical scavenging activity of *CS* three different extracts (Hexane, Ethyl acetate and Acetone) against DPPH● was determined spectrophotometrically in a dark room by the method [19]. DPPH● is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH● reacts with an antioxidant compound that can donate hydrogen and gets reduced. The change in colour (from deep violet to blue) was measured. The intensity of the yellow colour developed was depends on the amount and nature of radical scavenger present in the sample. 1 ml of various concentrations *CS* extract was taken, 1 ml of DPPH was added and this made up to 3 ml with water. The blue colour developed was read at 517 nm and AA was used as a standard.

$$\% \text{ Scavenging activity} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

ABTS^{+}(2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid assay)

The total antioxidant activity of the samples was measured by ABTS•$^{+}$radical cation decolorization assay according to the method [20]. ABTS•$^{+}$was produced by reacting 7 mmol ABTS^{+}aqueous solution with 2.4 mmol potassium persulfate in the dark for 12-16 h at room temperature. The radical was stable in this form for more than two days when stored in the dark at room temperature. Then, 2 ml of diluted ABTS•$^{+}$solution was added to the sample varying concentrations of *CS* three different (Hexane, Ethyl acetate and Acetone) extract. The blank contained water in place of *CS* extract. After 30 min of incubation at room temperature, the absorbance was recorded at 734 nm and compared with standard AA. Percentage of inhibition was calculated.

$$\% \text{ Scavenging} = \frac{\text{Control OD - Test OD}}{\text{Control OD}} \times 100$$

FRAP (ferric reducing antioxidant power assay)

The FRAP procedure described by Benzie and Strain was used. The principle of this method is based on the reduction of a ferric-tripyridyl triazine complex to its ferrous-colored form in the presence of antioxidants [21-23]. Aliquots of 100 µl *CS* extract were mixed with 3 ml FRAP reagent and the absorbance of the reaction mixture at 593 nm was measured spectrophotometrically after incubation at 37 °C for 10 min. For the construction of the calibration curve five concentrations of $FeSO_4$ $7H_2O$ (5 to 50 µmol/l) were used and the absorbencies were measured as the sample solution. The values were expressed as the concentration of antioxidants having a ferric-reducing ability equivalent to that of 1 mmol $FeSO_4$. AA was used as the reference standard.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was determined according to the method [24]. Nitric oxide generated from sodium nitroprusside in an aqueous solution at physiological pH interacted with oxygen to produce nitrite ions, which can be estimated by using the Griess reagent. Scavenging of nitric-oxide act against oxygen, leading to reduced production In brief, 3.0 ml of 10 mmol sodium nitroprusside in phosphate-buffered saline was mixed with different concentrations of the *CS* extract and incubated at 25 °C for 150 min. 0.5 ml of the incubated solution was taken and mixed with 0.5 ml of Griess reagent. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1-naphthylethylenediamine dihydrochloride was measured at 546 nm. AA was used as the reference standard.

Superoxide anion radical scavenging assay

Measurement of superoxide anion scavenging activity based on the method [25]. 0.1 ml of *CS* extract was mixed with 1 ml of NBT and 1 ml of NADH solution. This mixture was incubated at 25 °C for 5 min. A control was performed with the reagent mixture but without the sample. Absorbance was measured spectrophotometrically at 560 nm. AA was used as the reference standard.

Hydroxyl radical scavenging activity

The scavenging activity of the *CS* extract on hydroxyl radical was measured according to the method [26]. Various concentrations (200-1000 µg/ml) of *Cladophora Socialis* green algae three different extracts were added with 1.0 ml of Ferrous ammonium sulphate - EDTA solution, 0.5 ml of EDTA solution (0.018%), and 1.0 ml of dimethyl sulphoxide (DMSO). The reaction was initiated by adding 0.5 ml of AA and incubated at 80–90 °C for 15 min in a water bath. After incubation, the reaction was terminated by the addition of 1.0 ml of ice-cold TCA. 3 ml of Nash reagent was added and left at room temperature for 15 min. The reaction mixture without a sample was used as control. The intensity of the color formed was measured spectrophotometrically at 412 nm against a reagent blank. AA was used as the reference standard.

Formula:

$$\% \text{ HRSA} = \text{from } [(A0 - A1)/A0] \text{ X}100$$

Where A0 is the absorbance of the control and A1 is the absorbance of the extract/standard.

Scavenging activity against hydrogen peroxide

The scavenging capacity of *CS* extracts on hydrogen peroxide was determined according to the method [27]. Test tubes were prepared with 2.0 ml of various extracts (200-1000 µg/ml) and a solution of H_2O_2 (1.2 ml, 40 mmol) in phosphate buffer (pH 7.4). A blank solution was prepared in the same way but without H_2O_2. After incubation of the mixture during 10 min, the absorbance was recorded at 230 nm. AA was used as the reference standard. The scavenging activity was calculated using the following

$$\% \text{ Scavenging activity} = [(Ac–At)/Ac] \ 100$$

Where

Ac absorbance of the control

Reducing ability assay

The reducing power of *CS* extract was evaluated according to the method [28]. Different amounts of the extracts (200-1000 µg/ml) were suspended in distilled water and mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6), and 2.5 ml of 1% $K_3Fe(CN)_6$. The mixture was incubated at 50 °C for 20 min; 2.5 ml of 10% TCA was added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increase in absorbance of the reaction mixture indicated the ability of reducing power. AA was used as standard.

Metal chelating activity

Ferrozine quantitatively chelates with $Fe^{2+}$to form a red-colored complex. But in the presence of other cheating agents, the formation of ferrozine-Fe^{2+}complex is disrupted and hence the intensity of red color also decreases. The chelating activity of a compound to compete with ferrozine for the ferrous ions can be evidenced by the reduction in the color. Metal chelating activity is one of the significant antioxidant mechanisms as it reduces the concentration of the catalyzing transition metal in lipid peroxidation [29, 30]. In this assay, 1 ml of ferric chloride (2 mmol; diluted 20 times) is mixed with different dilutions of the *CS* extract (1 ml). The reaction is initiated by the addition of 1 ml of ferrozine (5 mmol; diluted 20 times). The absorbance is measured at 562 nm after 10 min. The positive controls that can be used in this assay are EDTA, and citric acid. The ability of the sample to chelate ferrous ions canfrom the following equation:

$$\text{Chelating effect (\%)} = (A0 - A1/A0) \times 100$$

Where; A0 is the absorbance of control, A1 is the absorbance in the presence of the sample

Phosphomolybdenum complex method

In the phosphomolybdenum complex method [31, 32], the reduction of Mo (VI) to Mo (V) is detected at 695 nm by a spectrophotometer due to the formation of green phosphate Mo (V) compounds at acidic pH. For the total antioxidant capacity assay, 0.1 ml of *CS* extract is mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mmol sodium phosphate and 4 mmol ammonium molybdate) in eppendorff tube. The tubes are then caped and incubated at 95 °C for 90 min in a thermal block. After incubation, the reaction mixture is cooled to room temperature and the absorbance is measured at 695 nm against the reagent blank. AA may be used as the standard antioxidant.

Statistical analysis

All the assays were carried out in triplicate. Experimental results are expressed as mean±standard deviation. The results were analyzed using a one-way analysis of variance and the group means were compared using Duncan's multiple range tests using SPSS version 16.

RESULTS AND DISCUSSION

Free radical DPPH in the emergence of such lifestyle diseases such as atherosclerosis, heart attack, stroke, cancer, diabetes, senile cataracts and accelerated aging. These enzymes catalyze the production of large amounts of reactive oxygen [33]. The extracts of *CS* (fig. 2) showed good antioxidant activity and effective scavenging of the DPPH radical. The percentage of inhibition *CS* three different extract (Hexane, Ethyl acetate and Acetone), at the concentration of 1000 µg/ml). Antioxidant activity of the genus Cladophora agree with results obtained by reported that the extracts of *CS* also exhibited relatively high DPPH radical scavenging activities [34-37].

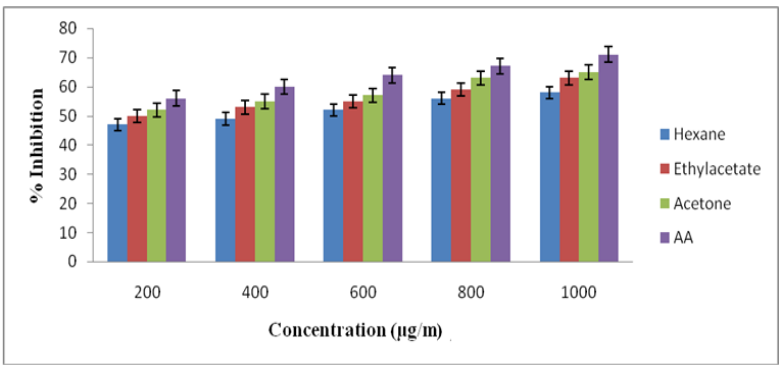

Fig. 2: Effect of hexanel, ethyl acetate and acetone marine algae seaweeds extracts of *Cladophora Socialis* on DPPH assay, Each value is expressed as mean±standard deviation (n=3)

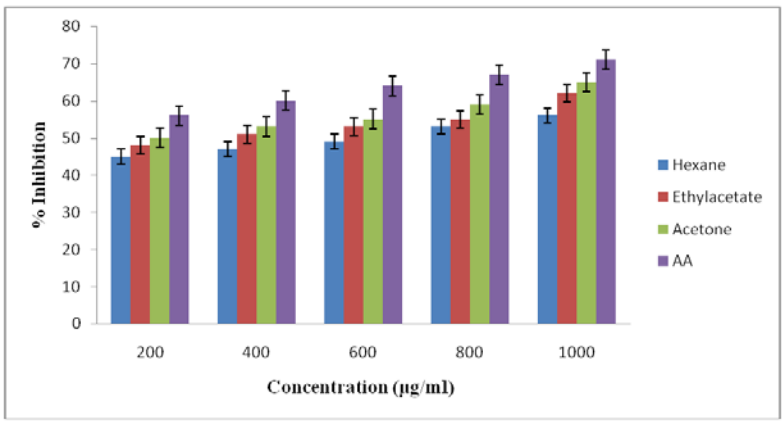

Fig. 3: Effect of hexanel, ethyl acetate and acetone marine algae seaweeds extracts of *Cladophora Socialis* on ABTS assay, Each value is expressed as mean±standard deviation (n=3)

In the present study, *CS* showed a maximum % of inhibition at 1000 µg/ml concentration and these are slightly lower than that of the standard AA, (fig. 3). The results of the present study indicate that the extract of green seaweed exhibited higher ABTS radical activity. The results indicated that acetone extract has a significant effect on the scavenging of ABTS radicals. However, the limitations of ABTS assay, such as the capability of a sample to react with ABTS radical rather than to inhibit the oxidative process and the slow reaction of many phenolics necessitate a compatible evaluation of antioxidant activity using other assays as well [38].

The antioxidant activity of the three different extracts (Hexane, Ethyl acetate and Acetone) determined by FRAP assay varied, as seen in fig. 4. The reducing powers were found to be higher in acetone extract when compared to AA. At the concentration of *CS* 50% of FRAP generated by incubation was scavenged. Ferric-reducing power is an important indicator of the antioxidant potential of a compound or an extract. The ability to reduce ferric ions indicates that the antioxidant compounds are electron donors and could reduce the oxidized intermediate of lipid peroxidation processes, thus acting as primary and secondary antioxidants [39-41].

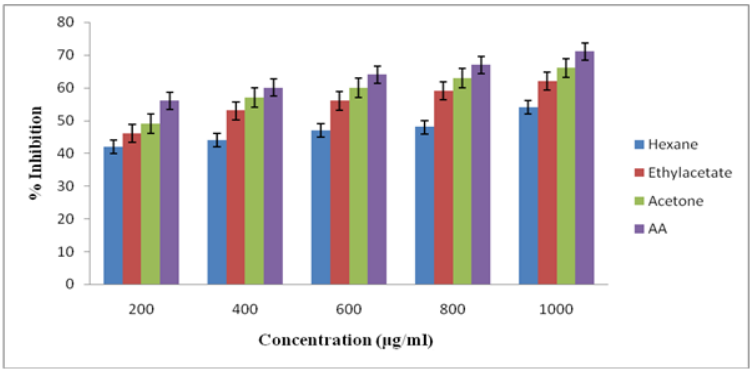

Fig. 4: Effect of hexanel, ethyl acetate and acetone marine algae seaweeds extracts of *Cladophora Socialis* on FRAP assay, Each value is expressed as mean±standard deviation (n=3)

The results showed that *CS* had scavenging activity and this value is comparably lower than that of the standard AA, (fig. 5). The IC_{50} values of three different extracts (Hexane, Ethyl acetate and Acetone) of *CS*. It was also found that the IC_{50} value of the algal extracts was lower than that of AA. The suppression of nitric oxide release may be partially attributed to direct scavenging by the extracts of *CS*, which decrease the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro*. Recently, seaweed extracts and fractions have been considered to be a rich source of antioxidants and different types of antioxidants have been isolated from various species of seaweeds [42-47]. The potential antioxidant compounds were identified as some pigments (fucoxanthin, astaxanthin, carotenoid e. g.) and polyphenols (phenolic acid, flavonoid, tannins e. g.), which are widely distributed in seaweeds and are known to exhibit higher antioxidative activities, which have been reported through various methods of reactive oxygen species scavenging activity and the inhibition of lipid peroxidation [48-50].

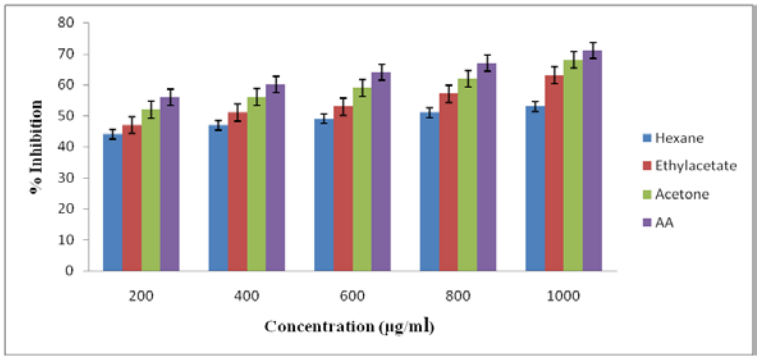

Fig. 5: Effect of hexane, ethyl acetate and Acetone marine algae seaweeds extracts of *Cladophora Socialis* on nitric oxide assay, Each value is expressed as mean±standard deviation (n=3)

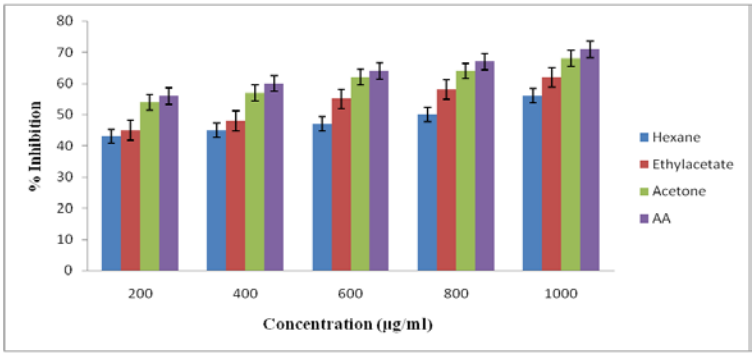

Fig. 6: Effect of hexane, ethyl acetate and acetone marine algae seaweeds extracts of *Cladophora Socialis* on reducing power assay, Each value is expressed as mean±standard deviation (n=3)

Reducing capacity is considered as a significant indicator of the potential antioxidant activity of a compound or sample. The presence of reductants (i.e. antioxidants) causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, by measuring the formation of Perl's Prussian blue at 655 nm, the amount of Fe^{2+}can be monitored [51]. Higher absorbance indicated higher reducing power. Fig. 6 elucidated the reductive capabilities of *CS* compared to AA. The reducing power of *CS* was increased with quantity of sample *CS* could reduce the most Fe^{3+}ions, which had a lesser reductive activity than the standard of AA. The IC value of *CS* and higher than AA, respectively.

Hydroxyl radical are the major active oxygen causing lipid peroxidation in enormous biological damage. The highly reactive hydroxyl radical can cause oxidative damage to DNA, lipid and protein [32]. In this study, *CS* was found to scavenge significantly and in a dose-dependent manner and may protect the DNA, protein and lipid from damage. The results for hydroxyl scavenging assay are shown in fig. 7. The concentrations for 50% inhibition was found to be *CS* and greater than AA, respectively.

Superoxide scavenging activity of *CS* exhibited a maximum of inhibition at the concentration of 1000 µg/ml, which is equal with the standard AA fig. 8. The IC_{50} value of three extracts of *CS* and it was higher than that of standard AA. The results of the present investigation are in agreement with those who investigated the antioxidant activities of different seaweeds in their studies and reported *Laminaria digitata* and *Himanthalia elongata* exhibited the most valuable antioxidant activities [52].

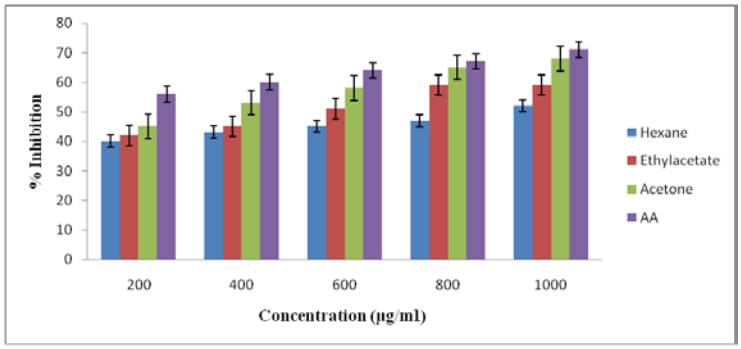

Fig. 7: Effect of hexane, ethyl acetate and acetone marine algae seaweeds extracts of *Cladophora Socialis* on hydroxyl radical assay, Each value is expressed as mean±standard deviation (n=3)

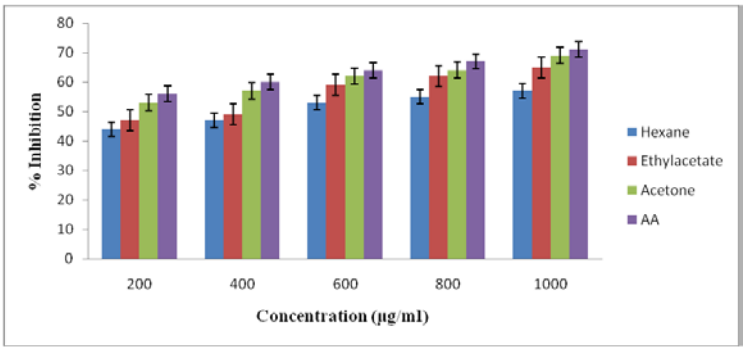

Fig. 8: Effect of hexane, ethyl acetate and acetone marine algae seaweeds extracts of *Cladophora Socialis* on superoxide anion assay, Each value is expressed as mean±standard deviation (n=3)

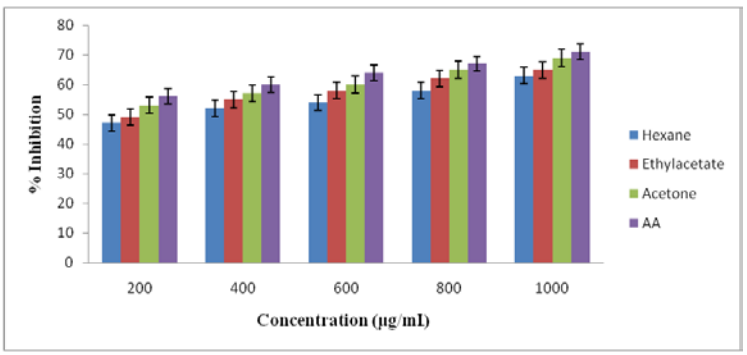

Fig. 9: Effect of hexane, ethyl acetate and acetone marine algae seaweeds extracts of *Cladophora Socialis* on hydrogen peroxide radical assay, Each value is expressed as mean±standard deviation (n=3)

Many species of seaweed possess the scavenging ability of hydrogen peroxide [53]. It can cross membranes and may slowly oxidize a number of compounds. Hydrogen peroxide itself is not very reactive, but sometimes it can be toxic to cells because of the rise in the hydroxyl radicals in the cells. The H_2O_2 radical scavenging assay was also performed with the three different extracts of the seaweed samples. Fig. 9 shows the results, *CS* showed concentration-dependent activity and the H_2O_2 scavenging effect at a concentration. This activity was comparable to the scavenging effect at the concentration of AA.

The metal iron chelating ability of seaweeds may be attributed to the presence of endogenous chelating agents, mainly phenolics, because certain phenolic compounds have properly oriented functional groups, which can chelate metal ions [54], Ferrozine can quantitively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted, with the result that the red color of the complex is decreased. Fig. 10 shows the metal chelating activity of *CS* when comparable to the scavenging effect at the concentration of AA.

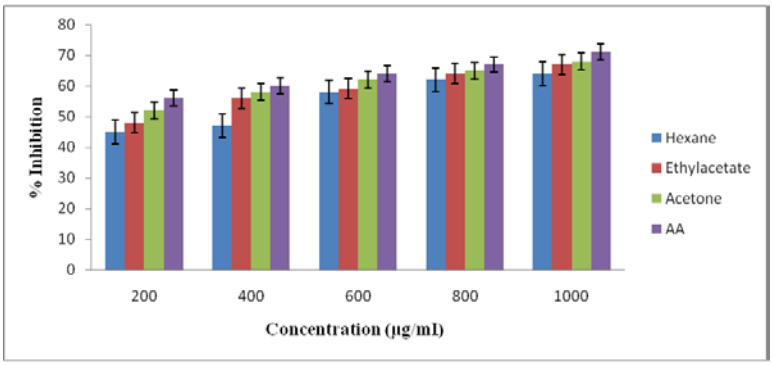

Fig. 10: Effect of hexane, ethyl acetate and acetone marine algae seaweeds extracts of *Cladophora* socialis on metal chelating activity assay, Each value is expressed as mean±standard deviation (n=3)

Phosphomolybdenum assay, which is a quantitative method to evaluate water-soluble and fat-soluble antioxidant capacity (total antioxidant capacity), the extract demonstrated high electron-donating capacity showing its ability to act as chain terminators, transforming relative free radical species into more stable non-reactive products [55]. This revealed increased phosphomolybdenum reduction of *CS* to the quantity of the sample. Fig. 11 shows the IC 50 value of *CS* higher than when compared to standard AA.

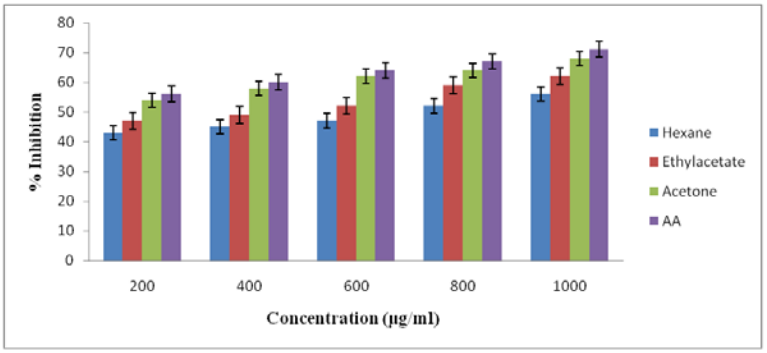

Fig. 11: Effect of hexane, ethyl acetate and acetone marine algae seaweeds extracts of *Cladophora Socialis* on phosphomolypdenum assay, Each value is expressed as mean±standard deviation (n=3)

CONCLUSION

In the present study, the *Cladophora Socialis* marine macroalgae three different extracts (Hexane, Ethyl acetate and Acetone) of seaweeds at varying concentrations were shown as a potential DPPH, ABTS, FRAP, nitric oxide, reducing power, hydroxyl, superoxide radical scavenging, hydrogen peroxide radical assay, the metal chelating activity as well as phosphomolypdenum assay. Some extracts showed higher antioxidant activity when compared to commercial antioxidants. From the present study, it can be concluded that the solvent extracts of marine macroalgae exhibit good antioxidant activity. The acetone extract showed a good result when compared to the AA. The results shown here indicate that the seaweed extracts can be a good source of natural antioxidants. Further investigation is needed to isolate and identify the specific class of compound that is responsible for the antioxidant activity.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare that they don't have any conflict of interest.

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