WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 6, 1505-1518. Research Article ISSN 2277– 7105

SCREENING OF PHYTOCHEMICAL AND IN VITRO ANTIOXIDANT EFFICACY ON SELECTED RED SEAWEED (*ACANTHOPHORA SPECIFERA*) COLLECTED FROM GULF OF MANNAR, TAMILNADU, INDIA

[1*]R.Rajesh Kumar and [2]K.Jeyaprakash

[1]Research Scholar, Department of Biochemistry, Rajah Serfoji Govt. College (Autonomous), Thanjavur-613005, Tamilnadu, India.

[2]Head, PG and Research of Department of Biochemistry, Rajah Serfoji Govt. College (Autonomous), Thanjavur-613005, Tamilnadu, India.

Article Received on
29 March 2015,

Revised on 22 April 2015,
Accepted on 13 May 2015

***Correspondence for Author**
R. Rajesh Kumar
Research Scholar, Department of Biochemistry, Rajah Serfoji Govt. College (Autonomous), Thanjavur-613005, Tamilnadu, India.

ABSTRACT

Acanthophora Specifera is a species of marine red seaweed and in the family Rhodomelaceae. They were freshly collected from Mandapam Coastal Area, Rameswaram Tamilnadu, India and rinsed in seawater and packed in aseptic bags for further proceedings to laboratory. Seaweeds are potential renewable resources in the marine environment. It has been used as antioxidant and antimutagen. Hence, the present study was carried out to exhibit the preliminary phytochemical screening and invitro antioxidant properties of various extracts of *Acanthophora specifera*. Methanol, ethanol and water extracts were prepared for further analysis. Out of these three extracts, methanolic extract showed the maximum phytoconstituents. The results of the present study revealed the presence of tannin, saponin, flavonoids, steroids, terpenoids, alkaloids, aminoacid, polyphenol, anthroquinone, glycosides. Our study also demonstrated the good invitro antioxidant efficacy of methanolic extract of *Acanthophora specifera*. Therefore, We concluded that *Acanthophora specifera* may be used as rich source of phytoconstituents and natural antioxidants.

KEYWORDS: Red Seaweed, Phytochemical screening, In vitro antioxidant, *Acanthophora specifera*, Efficacy.

INTRODUCTION

Seaweeds are potential renewable resources in the marine environment. It is generated enormous amount of bioactive compounds with immense medicinal potential. Nowadays, the uses of antibiotics have increased due to infections.[1]

The first investigation antibiotic activity carried out by Pratt *et al*., (1944).[2] Since algae have been used in traditional medicine for a long time and also some algae have bacteriostatic, bactericidal, antifungal, anti viral and anti tumor activity, they have been extensively studied by several researchers. Seaweed is rich in antioxidants such as carotenoids, pigments, polyphenols, enzymes. Seaweeds are the most excellent source of Vitamin A, B1, B12, C, D and E.[3]

The mineral nutrient present in seaweeds are diverse and the main elements being magnesium, sodium, potassium and calcium. The chemical composition of seaweeds varies with species, habitat, maturity and environmental conditions.[4]

Among the different compounds with functional properties, antioxidants are the most widely studied. Antioxidants are the substances, which can defend serious human diseases including melanoma, cardiac disorders, diabetes, cancer, inflammatory that explain their potential use in increasing shelf life of food and as medicine.[5]

Free radical induced oxidation is one of the major reasons in deterioration of nutritional quality and other physical attributes of food items under storage. Previous studies in animal models and cell culture have suggested that seaweed phytochemicals have the potential to inhibit progression of carcinoma formation.[6]

Although thousands of bioactive compounds have been discovered, the need for novel therapeutic compounds is still urgent in concern of number of new diseases and resistant strains of micro organisms. Therefore, the present study was carried out to demonstrate the preliminary phytochemical screening and in vitro antioxidant properties of various extracts of *Acanthophora specifera*.

MATERIALS AND METHODS

Collection of Seaweeds

Acanthophora specifera were collected from Gulf of Mannar, Rameswaram, Tamilnadu, India. The collected samples were cleaned well with sea water to remove all the extraneous

matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in sterile bags. Then the samples were washed with tap water and distilled water and spread in the dark room for drying, after which the dried samples were powdered and subsequently stored at 4°C.

Preparation of extract

25gms of the powder of *Acanthophora specifera* were transferred into three different conical flask (250ml).The conical flask containing 100ml of three different solvents viz. Ethanol, Methanol and water. The conical flask containing *Acanthophora specifera* powder and solvent was shaked it well for 48 hours by free hand. After 3 days, the extracts were filtered using Whatmann filter paper No.1. and was transferred into china dish. The supernatant was completely removed by keeping the china dish over a boiling water bath at 45^{o}C.The obtained extracts were stored at 4°C in air tight bottle until further use.

Qualitative Preliminary Phytochemicals Screening

Chemical tests were carried out on the various of Acanthophora specifera extract using standard procedures to identify the phytochemicals namely Anthraquinones, Alkaloids, Carbohydrates, Aminoacids, Polyphenols, Tannins, Phlobatannins, Saponins, Flavonoids, Terpenoids, Triterpenoids, Glycosides following the methodology of Harborne (1973).[7]

Quantitative determination of Total Flavonoids and Total Phenols

Determination of Total Phenols

Total phenols were estimated by the method of Edeoga *et al*., (2005).[8]

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5 ml of the extract was pipetted into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The sample was made up to mark and left to react for 30 min for colour development. This was measured at 505 nm.

Determination of Total Flavonoids

Flavonoids were determined by the method of Bohm and Kocipai-Abyazan (1994).[9]

10 g of the sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm).

The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

IN VITRO ANTIOXIDANT ACTIVITY OF *ACANTHOPHORA SPECIFERA*

Preparation of extract

Different concentrations of *Acanthophora specifera* **(**20, 40, 60 and 80 µg/ml) were chosen for *in vitro* antioxidant activity. L-Ascorbic acid was used as the standard.

DPPH Radical Scavenging Assay

DPPH radical-scavenging activity was determined by the method of Shimada, *et al.*, (1992).[10] Briefly, a 2 ml aliquot of DPPH methanol solution (25µg/ml) was added to 0.5 ml sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity.

$$\text{Radical scavenging activity (\%)} = 100 - \left(\frac{A_C - A_S}{A_C} \right) X\ 100$$

Where A_C = control is the absorbance and A_S = sample is the absorbance of reaction mixture (in the presence of sample).

Hydroxyl Radical Scavenging Assay

The scavenging activity for hydroxyl radicals was measured with Fenton reaction by the method of Yu *et al.*, (2004).[11] Reaction mixture contained 60µl of 1.0mM $FeCl_3$, 90µl of 1mM 1,10- phenanthroline, 2.4 ml of 0.2M phosphate buffer (Ph 7.8), 150µl of 0.17M H_2O_2, and 1.5 ml of extract at various concentrations. Adding H_2O_2 started the reaction. After incubation at room temperature for 5 min, the absorbance of the mixture at 560nm was measured with a spectrophotometer. The hydroxyl radical scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = ((A_0 - A_1) / A_0 \times 100)$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

Superoxide Anion Scavenging Assay

The superoxide anion radicals scavenging activity was measured by the method of Liu *et al*., (1997).[12] In this experiment, the superoxide anion was generated in 3 ml of Tris-HCl buffer (100 mM, pH 7.4) containing 0.75 ml of NBT (300 µM) solution, 0.75 ml of NADH (936 µM) solution and 0.3 ml of different concentrations of the extract. The reaction was initiated by adding 0.75 ml of PMS (120 µM) to the mixture. After 5 min of incubation at room temperature, the absorbance at 560 nm was measured in spectrophotometer. The superoxide anion scavenging activity was calculated according to the following equation

$$\% \text{ Inhibition} = ((A_0\text{-}A_1) / A_0 \times 100)$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

Nitric Oxide Radical Scavenging Assay

Nitric oxide radical scavenging activity was determined according to the method reported by Garrat (1964).[13] Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. 2 ml of 10mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations and the mixture incubated at 25^{o}C for 150 min. From the incubated mixture 0.5 ml was taken out and added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1.0 ml naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = ((A_0\text{-}A_1) / A_0 \times 100)$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

Fe^{2+} Chelating Assay

The chelating activity of the extracts for ferrous ions Fe2+ was measured according to the method of Dinis *et al*., (1994).[14] To 0.5 ml of extract, 1.6 ml of deionized water and 0.05 ml of $FeCl_2$ (2mM) was added. After 30 s, 0.1 ml ferrozine (5mM) was added. Ferrozine reacted

with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 min at room temperature, the absorbance of the Fe^{2+}-Ferrozine complex was measured at 562 nm. The chelating activity of the extract for Fe^{2+} was calculated as.

$$\text{Chelating rate (\%)} = (A_0 - A_1) / A_0 \times 100$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

Reducing Power Assay

The Fe^{3+} reducing power of the extract was determined by the method of Oyaizu (1986).[15] The extract (0.75 ml) at various concentrations was mixed with 0.75 ml of phosphate buffer (0.2M, pH 6.6) and 0.75 ml of potassium hexacyanoferrate ($K_3Fe(CN)_6$) (1%, w/v), followed by incubating at 50°C in a water bath for 20 min. The reaction was stopped by adding 0.75 ml of trichloroacetic acid (TCA) solution (10%) and then centrifuged at 800g for 10 min. 1.5 ml of the supernatant was mixed with 1.5 ml of distilled water and 0.1 ml of ferric chloride ($FeCl_3$) solution (0.1%, w/v) for 10 min. The absorbance at 700 nm was measured as the reducing power. Higher absorbance of the reaction mixture indicated greater reducing power.

RESULTS

Qualitative Preliminary Phytochemical Screening

The phytochemicals present in the methanolic, ethanolic, and water extract of Acanthophora specifera were identified and presented in the Table.1.Our results indicates the presence of more phytochemicals in the methanolic extract as compared to ethanolic and water extracts. Hence, the methanolic extracts were selected for the further study.

Table .1- Phytochemical screening of different extracts of *Acanthophora specifera*

S. No.	Secondary Metabolites	Extracts		
		Ethanol	Methanol	Water
1	Tannin	+	+	+
2	Phlobatannins	+	+	+
3	Saponin	+	+	+
4	Flavonoids	++	++	+
5	Steroids	+	+	+
6	Terpenoids	+	+++	+
7	Triterpenoids	+	+	+
8	Alkaloids	+	++	+
9	Carbohydrate	-	-	+
10	Amino acid	+	++	+

11	Anthroquinone	+	++	+
12	Polyphenol	++	+++	--
13	Glycoside	+	+	+

(+)Presence (-) Absence (+++) High concentrations

Quantitative determination of Total Flavonoids and Total Phenols

The total phenolic content and flavonoids contents present in the methanolic extract of Acanthophora specifera were given in Table.2 and found to contains 217.59mg/g (total phenols) and 124.87 mg/g (total flavonoids).

Table 2.Quantitative Analysis of *Acanthophora specifera*

S.No	Phytoconstituents	Results (mg/g)
1	Total Phenols	217.59
2	Total Flavonoids	124.87

Values were expressed as Mean ± SD for triplicates

***IN VITRO* ANTIOXIDANT ACTIVITY**

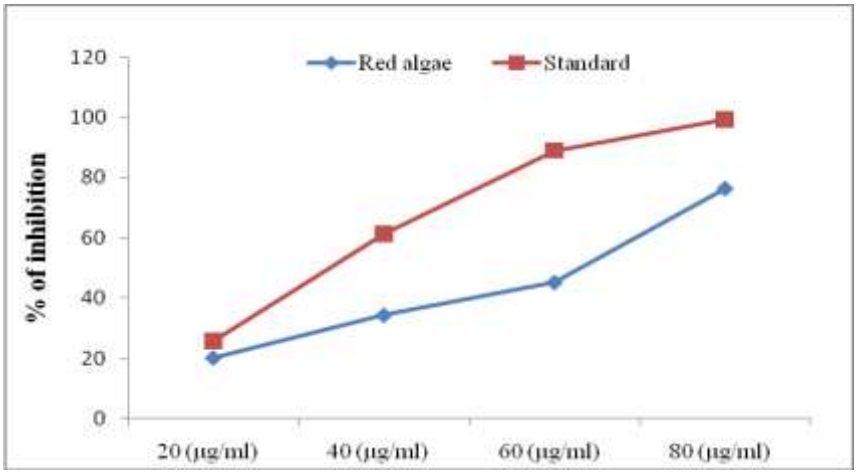
DPPH Free Radical Scavenging Activity

DPPH free radical-scavenging activity of methanolic extract of *Acanthophora specifera* was given in Table.3&Figure.1. The present study revealed 76.31% of inhibition at 80µg/ml concentration of sample as compare to standard ascorbic acid.

Table 3- Percentage of DPPH Radical Scavenging Activity of mehanolic extract of *Acanthophora specifera (Red algae)* at different concentrations

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
Red algae	20.15±1.41	34.30±2.40	45.18±3.16	76.31±5.34	56.75
Standard (Ascorbic acid)	25.6±2.04	61.26±4.90	88.98±7.11	99.34±7.94	35.03

Values were expressed as Mean ± SD for triplicates



Values were expressed as Mean ± SD for triplicates.

Fig.1- Percentage of DPPH Radical Scavenging Activity of mehanolic extract of *Acanthophora specifera (Red algae)* at different concentrations

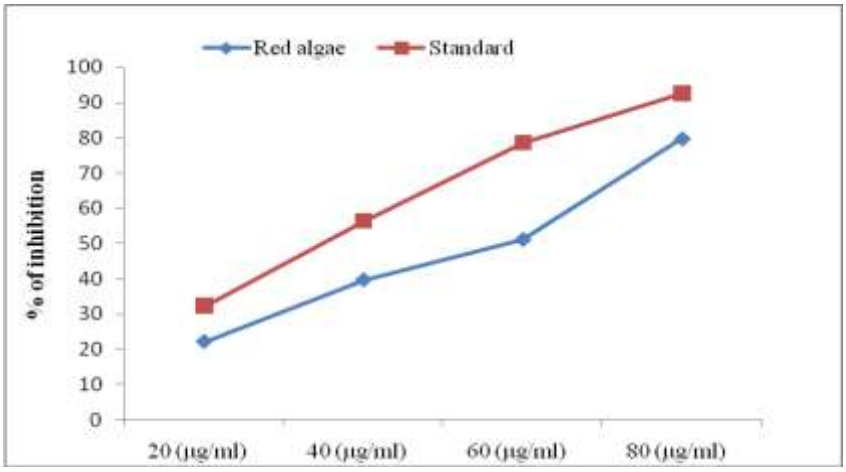
Hydroxy Radical Scavenging Activity

Hydroxy radical scavenging activity of methanolic extract of *Acanthophora specifera* was given in Table.4&Figure.2. The present study revealed 79.81% of inhibition at 80µg/ml concentration of sample as compare to standard ascorbic acid.

Table 4- Percentage of Hydroxy radical scavenging activity of mehanolic extract of *Acanthophora specifera (Red algae)* at different concentrations

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
Red algae	22.14±1.54	39.72±2.78	51.18±3.58	79.81±5.58	48.29
Standard (Ascorbic acid)	32.21± 2.51	56.45± 4.40	78.65±6.13	92.75±7.2	35.14

Values were expressed as Mean ± SD for triplicates



Values were expressed as Mean ± SD for triplicates

Fig. 2- Percentage of Hydroxy radical scavenging activity of mehanolic extract of *Acanthophora specifera (Red algae)* at different concentrations

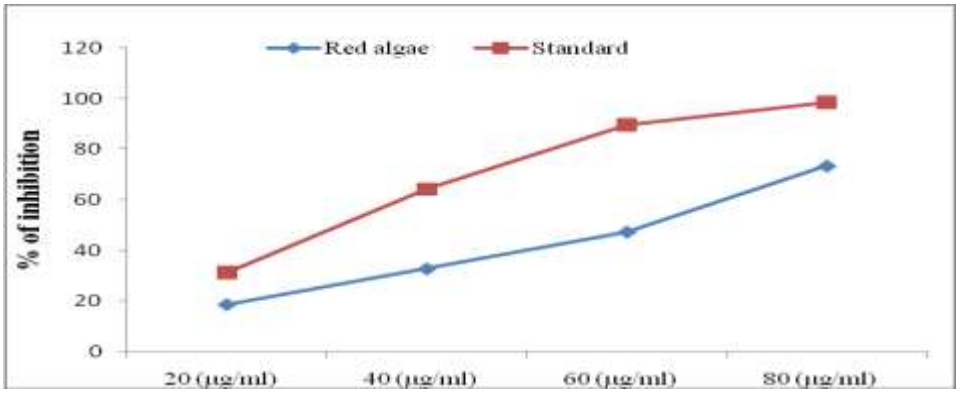
Superoxide Anion Scavenging Activity

Superoxide anion scavenging activity of methanolic extract of *Acanthophora specifera* was given in Table.5&Figure.3. The present study revealed 73.41% of inhibition at 80µg/ml concentration of sample as compare to standard ascorbic acid.

Table 5- Percentage of Superoxide Radical Scavenging Activity of methanolic extract of *Acanthophora specifera (Red algae)* at different concentrations

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
Red algae	18.57±1.29	32.72±2.29	47.31±3.31	73.41±5.13	57.84
Standard (Ascorbic acid)	31.25 ± 2.50	64.23 ± 5.13	89.54 ± 7.16	98.51 ± 7.88	31.62

Values were expressed as Mean ± SD for triplicates



Values were expressed as Mean ± SD for triplicates

Fig. 3- Percentage of Superoxide Radical Scavenging Activity of methanolic extract of *Acanthophora specifera (Red algae)* at different concentrations

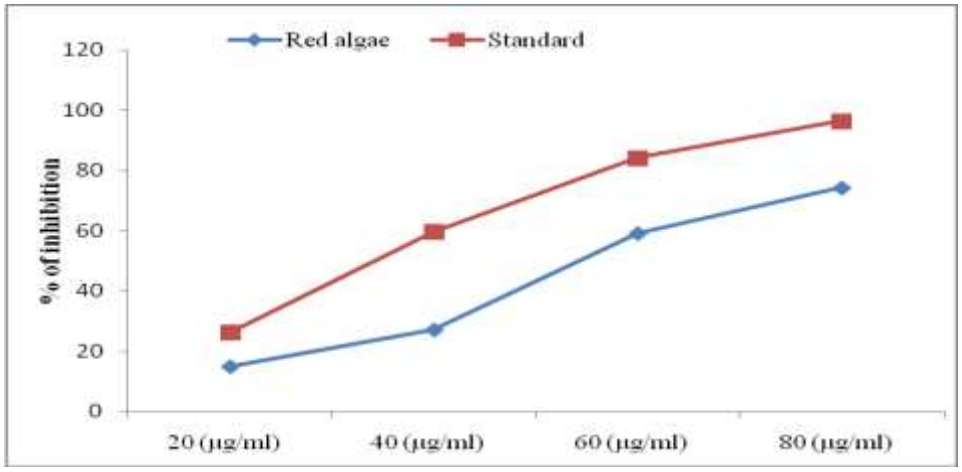
Nitric oxide Scavenging Activity

Nitric oxide scavenging activity of methanolic extract of *Acanthophora specifera* was given in Table.6&Figure.4. The present study revealed 74.31% of inhibition at 80µg/ml concentration of sample as compare to standard ascorbic acid.

Table 6- Percentage of Nitric oxide scavenging activity of mehanolic extract of *Acanthophora specifera* (*Red algae)* at different concentrations

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
Red algae	14.95 ±1.04	27.15 ±1.90	59.18 ±2.74	74.31 ±3.10	55.83
Standard (Ascorbic acid)	26.21 ± 2.04	59.62± 4.65	84.23 ± 6.56	96.45 ± 7.52	46.63

Values were expressed as Mean ± SD for triplicates



Values were expressed as Mean ± SD for triplicates

Fig 4- Percentage of Nitric oxide scavenging activity of mehanolic extract of *Acanthophora specifera* (*Red algae)* at different concentrations

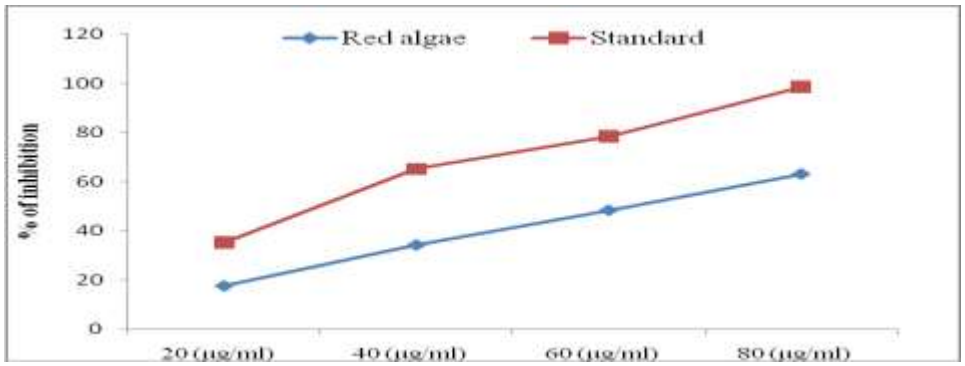
Fe^{2+} Chelating Activity

Fe^{2+} chelating activity of methanolic extract of *Acanthophora specifera* was given in Table.7&Figure .5. The present study revealed 63.18% of inhibition at 80µg/ml concentration of sample as compare to standard ascorbic acid.

Table 7- Percentage of Iron chelating activity of mehanolic extract of *Acanthophora specifera(Red algae)* at different concentrations

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
Red algae	17.57±1.22	34.27±2.59	48.41±3.38	63.18±4.42	61.86
Standard (Ascorbic acid)	35.23 ± 2.81	65.21 ± 5.28	78.51± 6.28	98.65 ± 7.89	30.96

Values were expressed as Mean ± SD for triplicates



Values were expressed as Mean ± SD for triplicates

Fig 5- Percentage of Iron chelating activity of mehanolic extract of *Acanthophora specifera (Red algae)* at different concentrations

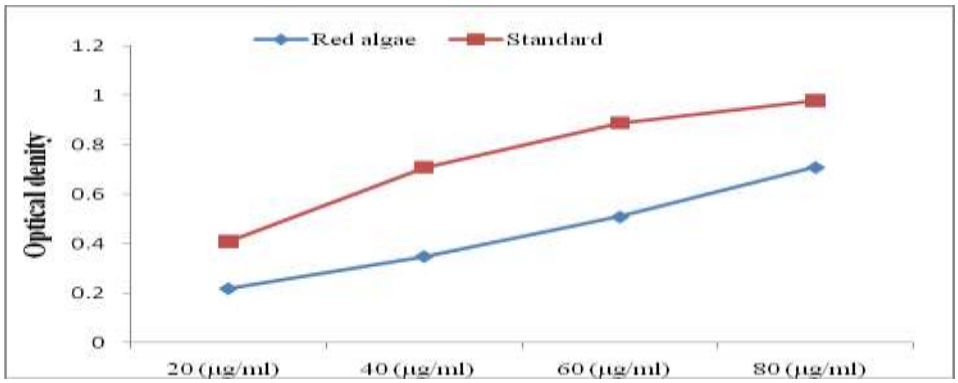
Reducing Power Activity

Reducing power activity of methanolic extract of *Acanthophora specifera* was given in Table.8&Figure .6. The present study revealed 0.71% of inhibition at 80µg/ml concentration of sample as compare to standard ascorbic acid.

Table 8- Reducing power of mehanolic extract of *Acanthophora specifera (Red algae)* at different concentrations

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)
Red algae	0.22±0.008	0.35±0.021	0.59±0.040	0.71±0.049
Standard (Ascorbic acid)	0.41± 0.03	0.71 ± 0.05	0.89± 0.07	0.98 ± 0.08

Values were expressed as Mean ± SD for triplicates



Values were expressed as Mean ± SD for triplicates

Fig 6- Reducing power of mehanolic extract of *Acanthophora specifera (Red algae)* at different concentrations

DISCUSSION

The present study revealed the presence of important phytochemicals such as tannin, saponin, flavonoids, steroids, terpenoids, alkaloids, aminoacid, anthraquinone, poly phenol, glycoside in methanolic extract of *Acanthophora specifera*. Our study also demonstrated in vitro antioxidant property of *Acanthophora specifera* in respect to DPPH radical scavenging assay, Hydroxyl radical scavenging assay, Superoxide anion scavenging assay, Nitric oxide scavenging assay, Fe^{2+} chelating assay, Reducing power assay due to the presence of flavonoids and phenols. It was suggests that the plant might have an antioxidant, anti allergic, anti inflammatory, anti microbial, anti cancer activity.[16]

The presence of tannins shows that the plant is astringent as documented and suggested that it might have antiviral and antibacterial activities and can aid in wound healing and burns. Saponins and glycoside are also very important classes of secondary metabolites as some are cardio active and used in treatment of heart conditions.[17]

DPPH radicals react with suitable reducing agents and then electrons become paired off and the solution loses colour stoichiometrically with the number of electrons taken up. Such reactivity has been widely used to test the ability of compounds of red algae extracts to act as free radical scavengers. Though the extracts showed good DPPH scavenging activity but it was less effective than standard ascorbic acid. This activity is due to the presence of phenolic and flavonoids components in the plant extracts.[18]

In ferric reducing antioxidant power assay, a yellow colour of the test solution changes to various shades of green and blue is depending upon the reducing power of each compound. The presence of radicals causes the conversion of the ferricyanide complex used in this method to the ferrous form. Therefore by measuring the formation of pearls Prussian blue spectroscopically, a higher absorbance indicates a higher reducing power.[19]

Hydrogen peroxide is a biologically relevant, non radical oxidizing species may be formed in tissues through oxidative processes. Hydrogen peroxide which in turn generate hydroxyl radicals (**.**OH) resulting in initiation and propagation of lipid peroxidation. The ability of the extracts to quench (**.**OH) seems to be directly related to the prevention of the lipid peroxidation and appears to be moderate scavenger of active reactive oxygen species.[20]

Superoxide anion radical is generated by four electron reduction of molecular oxygen into water. This radical also formed in aerobic cells due to electron leakage from the electron transport chain super oxides are generated from molecular oxygen of oxidative enzymes and as well as non enzymatic reactions such as auto oxidation by catecholamines.[21]

Nitric oxide is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation, antimicrobial and antitumor activities.[22]

CONCLUSION

Our present investigation have demonstrated the phytochemical and in vitro antioxidant efficacy of *Acanthophora specifera* (Red seaweed) collected from the Mandapam Coastal Area, Rameswaram, Tamilnadu, India. The findings of the present study revealed the use of *Acanthophora specifera* as a natural antioxidant.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest. The research received no specific grant from any funding agency in the public, community, or non-for profit sectors.

ACKNOWLEDGEMENT

The authors are thankful to Dr.S.Velavan, Associate professor, Dept of Biochemistry, Maruthu pandiyar Arts and Science College, Thanjavur, Tamilnadu, India for his timely help s support.

REFERENCES

1. Skulberg, O.M. Micoalgal as a source of bioactive molecules experience from Cyanophyte research. *Journal of applied Phycology*, 2000; 12: 341-348.
2. Pratt, R., Daniel, T.C., Eier, J.B., Gunnison, J.B., Kumler, W.D., Oneto, J.F., Strait, L.A., Spoehr, H.A., Hardin, G.J., milner, H.W., Smith, and Strain, H.H. Chlorellin. An antibacterial substance from *Chrolella*. Science., 1944; 99: 351-352.
3. Justo GZ, Silva MR, Queiroz MLS, Effects of green algae *Chlorella vulgaris* on the response of the host hematopoietic system to intraperitoneal ehrlich ascited tumor transplantation in mice. *Immunopharm. Immunotoxicol.*, 2001; 23: 199-131.
4. Burtin P, Nutritional value of seaweeds, electronic journal of environmental, Agricultural and food chemistry, 2003; 2(4): 498-503.
5. Peter KJ, Amsler CD, Amsler MO, Mc Clintock JB, Dunbar RB and Baker BJ, Acomparitive analysis of macroalgae from the western Antartic peninsula, Phycologia, 2005; 44: 453-463.
6. Duan XJ, Zhang WW, Li XM and Wang BG, Evaluation of antioxidant property of extract and fractions obtained from red algae, *Polysiphonie urcelata*, Food chemistry, 2006; 95: 37-43.
7. Harborne JB . *Phytochemical methods*, London. Chapman and Hall, Ltd., 1973; 49-188.
8. Edeoga H.O., D.E. Okwu and B.O Mbaebie. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology., 2005; 4(7): 685- 688.

9. Boham BA, Kocipai-Abyazan R . Flavonoids and condensed tannins from leaves of Hawaiian vaccinium vaticulatum and V. calycinium. Pacific Sci., 1974; 48: 458-463.
10. Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. Antioxidative properties of *xanthum* on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry, 1992; 40: 945–948.
11. Yu W., Zhao Y., and Shu B.. The radical scavenging activities of *radix puerariae* isoflavonoids: A chemiluminescence study. *Food Chem.*, 2004; **86:** 525-529.
12. Liu F., Ooi C.V.E., and.Chang S. T. Free radical scavenging activity of mushroom polysaccharide extracts. *Life Sci.*, 1997; 60: 763-771.
13. Garrat D.C. *The quantitative analysis of drugs.* Japan: Chapman and Hall., 1964; **3**: 456-458.
14. Dinis TCP. Madeira V. M. C., and Almeidam L. M.. Action of phenolic derivates (acetoaminophen, salycilate, and 5- aminosalycilate) as inhibitors of membrane lipid peroxidation and peroxyl radicals scavengers.*Ach. Biochem. and Biophy.*1994; 315: 161-169 .
15. Oyaizu M. Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucose amine.*Jap. J. Nutr.*, 1986; 44: 307-315.
16. Chowdhury, N.Y., Islam, W., Biological activities of isolated compounds from *Vitex negundo* leaf. *Journal of Bio-Science.*, 2010; 18: 53-59.
17. Oloyode, O. I. Chemical profile of unripe pulp of Carica papaya. Pakistan Journal of Nutrition, 2005; 4(6): 379-381.
18. Murali Krishna.T, Meena.G, Kavya.T, In Vitro Determination of Antioxidant and Anti-Bacterial activities of *Vitex negundo* Linn. Int J Pharm BioSci, 2013; 4(1): (P) 121 – 127.
19. Park Ys, Heo Bg, Ham Ks, Kang Sg, GorinsteinS.Analytical Determination of Bioactive Compounds as an indication of fruit quality. AOAC Int., 2012; 95(6): 1725-1732.
20. Oboh G, Akinyemi AJ, Ademiluyi AO.Antioxidant and inhibitory effect of red ginger and white ginger on (Fe2+) induced lipid peroxidation in rat brain in vitro. Exp oxicol Pathol., 2012; 64(1-2): 31-36.
21. Mustafa AG, Singh IN, Wang J, Carrico KM, Mitochondrial protection after traumatic brain injury by scavenging lipid peroxyl radicals. J Neurochem. 2010;14(1):271-280.
22. Lata H, and Ahuija GK, Role of free radicals in health and diseases. Ind J Physio & Allied Sci, 2003; 57: 124-132.