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IN VITRO ANTIOXIDANT EVALUATION OF *GRACILARIA FERGUSONII* USING VARIOUS SOLVENT EXTRACTS

Jenifer P. and C. P. Balakrishnan*

Department of Botany, Aditanar College of Arts and Science, Virapandianpatnam, Tiruchendur - 628 216, Tamil Nadu, India.

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***Correspondence for Author**
Dr. C. P. Balakrishnan
Department of Botany, Aditanar College of Arts and Science, Virapandianpatnam, Tiruchendur - 628 216, Tamil Nadu, India.

ABSTRACT

The present study was made on *in vitro* evaluation of red algae *G. fergusonii* was determined by different solvent extract at different concentration (100, 250, 500, 750, 1000 µg ml^{-1}) by NO and H_2O_2 radical scavenging activity was compare with standard ascorbic acid. IC_{50} values of NO radical scavenging activity of methanol, chloroform and aqueous extract were reported at 690.77, 222.77 and 1058.65 µg/ml respectively. IC_{50} values of H_2O_2 radical scavenging activity of methanol, chloroform and aqueous extract were reported at 983.76, 266.23 and 1525 µg/ml respectively. In the mean time the IC_{50} value of standard ascorbic acid was recorded for NO and H_2O_2 are 163.05µg/ml and 855.38µg/ml respectively. An IC_{50} values was found that chloroform extract is more effective in NO and H_2O_2 free radical scavenging than that of methanol and aqueous extracts.

KEYWORDS: *G. fergusonii*, Free Radicals, Nitric Oxide (NO), Hydrogen Peroxide (H_2O_2).

INTRODUCTION

Seaweeds as natural sources with a high degree of bioavailability of trace elements are strongly advised for fast grown children and pregnant women. In contrast to their use as a source of food, marine algae are widely used in the life science as the source of compounds with diverse structural forms and biological activities.[1,2,3,4] Seaweeds are considered to be a rich source of antioxidants.[5,6,7,8,9] An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is an essential biological process of energy production in many living organisms. It is a chemical reaction that transfers electron from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which

start chain reactions that damage cells. Antioxidants are the substances that inhibit oxidation and are capable of counteracting the damaging effects of oxidation in body tissue. They prevent damage caused by free radicals.

Free radicals are reactive oxygen species (ROS) or reactive nitrogen species (RNS) generated in the body during normal metabolic activities or by environmental conditions.[10] The most common ROS include superoxide anion (O_2), hydrogen peroxide (H_2O_2), peroxyl radicals (HO_2) and reactive hydroxyl (OH) radicals. RNS includes nitric oxide (NO) and peroxynitrite anion (ONOO-).[11,12] Free radicals, the partially reduced metabolites of oxygen and nitrogen, are highly toxic and reactive. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. Marine algae are one of the natural resources in the marine ecosystem. They contain various biologically active compounds which have been used as source of food, feed and medicine. The present study deals with the *in-vitro* evaluation of scavenging free radicals of marine red algae *G. fergusonii* J. Agardh by NO and H_2O_2 radical scavenging method of various solvent like methanol, chloroform and aqueous extract in different concentration with compare to standard ascorbic acid.

MATERIALS AND METHODS

Collection and preparation of seaweeds

Marine red algae *Gracilaria fergusonii* J. Agardh was collected from Manapad coast of Tamil Nadu, India (8.3775°N; 78.0522°E) at low tide. Specimen was washed thoroughly in seawater to remove extraneous matter such as epiphytes and sand. After collection, fresh samples were taken into plastic jar and brought back to the laboratory immediately. Samples were washed by tape water for several times, then gently brushed and rinsed with distilled water and then dried at room temperature. The dried seaweed powder was stored in refrigerator for further uses.

Antioxidant activities

Preparation of the extracts and standard

10g of powdered sample was subjected to extract with methanol using Soxhlet extractor for six hours and the extraction was repeated twice. Similar process was done by chloroform and distilled water. The extracts were then concentrated to dryness under reduced pressure and controlled temperature (40-50^{o}C). The resultant residues were kept in a refrigerator for further use.

Weighed quantities of methanol, chloroform and water residues were dissolved in respective solvents. The stock solutions were serially diluted with respective solvents to get lower concentrations (1000, 750, 500, 250, 100 µg ml^{-1}). Each concentration was prepared in triplicate. These were subjected to *in-vitro* assay of NO and H_2O_2 radical scavenging methods.

NO scavenging method

Sodium nitroprusside in aqueous solution at physiological pH, spontaneously produce nitric oxide, which reacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction.[13] Greiss Illosvoy reagent was slightly modified using napthylethylene diamine dihydrochloride (0.1%) instead of 1-napthylamine (5%).[14] Scavengers of nitric oxide compete with oxygen and reduce the production of nitric oxide.[15] The reaction mixture (3ml) containing Sodium nitroprusside (10mM, 2ml), phosphate buffer saline (pH 7.4, 0.01M, 0.5ml) and extract (methanol, chloroform and water, 100-1000µg ml^{-1}) or standard solution (0.5ml) was incubated at 25^{o}C for 150 min. After incubation, 0.5ml of the reaction mixture containing nitrite was pipetted and mixed with 1ml of sulphanil acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5min for completing diazotization. Then, 1ml of napthylethylene diamine dihydrochloride (0.1%) was added, mixed and allowed to stand for 30 min in diffused light. The absorbance of the pink colored chromophore was measured at 540nm against the corresponding blank solutions in an Elico double beam UV-VIS spectrophotometer. The percentage of NO scavenging by the different concentrations of the algae sample and the standard was calculated using the following formula

$$\text{Scavenging activity (\%)} = [(A - B)/A] \times 100.$$

Where A is the absorbance of control, B is the absorbance of the sample (extract/ascorbic acid).

A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was calculated by using linear regression analysis of MS Excel.

H_2O_2 scavenging method

The ability of the methanol, chloroform and water extracts of the algae sample to scavenge hydrogen peroxide was determined by the modified method of Dehpour *et al*., 2009[16] as discussed.[17] Solution of hydrogen peroxide (40Mm) was prepared in phosphate buffer saline

(pH 7.4). 1 ml of different concentrations of the extract (100 to 1000 µg/ml) was added to 2 ml of hydrogen peroxide solution in PBS and absorbance measured at 560nm using UV spectrophotometer against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extract and standard compound was calculated using the given formula.

Scavenging activity (%) = [(A – B)/A] x 100.

Where A is the absorbance of control (extract and PBS without H_2O_2), B is the absorbance of the sample (extract/ascorbic acid and PBS with H_2O_2).

A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was calculated by using linear regression analysis of MS Excel.

Calculation of IC_{50}

IC_{50} value was calculated by using the formula to determine the regression equation Y= mx + c in MS Office excel version 2007.

RESULTS AND DISCUSSION

Free radicals are highly reactive molecules containing one or more unpaired electrons; they donate or take electrons from other molecules in an attempt to pair their elections and generate a more stable species. In the present study, the free radical scavenging activity in different concentration (100, 250, 500, 750, 1000 µg/ml) of different solvent extracts of *G. fergusonii* has been carried out by methods of H_2O_2 radical and NO radical. All results were compared with standard ascorbic acid.

NO radical scavenging activity

The percentage inhibition and IC_{50} values of NO radical scavenging activity of methanol extracts of *G. fergusonii* presented in Table 1. The IC50 values of methanol extract of *G. fergusonii* and ascorbic acid were found to be at 690.77µg/ml and 163.05µg/ml respectively. The % inhibition and IC_{50} values of nitric oxide radical scavenging activity of chloroform extracts of *G. fergusonii* presented in Table 2. The IC_{50} values of the chloroform extract of *G. fergusonii* and ascorbic acid were found to be at 222.77µg/ml and 163.05µg/ml respectively. The Percentage inhibition and IC_{50} values of NO radical scavenging activity of aqueous extracts of *G. fergusonii* presented in Table 3. The IC_{50} values of the aqueous extract of *G. fergusonii* and ascorbic acid were found to be 1058.65µg/ml and 163.05µg/ml respectively.

Nitric oxide is a free radical that is generated when sodium nitroprusside reacts with oxygen to form nitrite, induces the inflammatory response and its toxicity multiplies if it reacts with O_2 radicals to form peroxynitrite.[18]

Table 1: % inhibition and IC_{50} values of *in-vitro* NO radical by methanol extract of *G. fergusonii* and Ascorbic acid.

S. No	Concentration (µg/ml)	% of activity (±SEM)*	
		Sample (Methanol extract)	Standard (Ascorbic acid)
1	100	22.29±0.056	45.67±0.071
2	250	39.18±0.049	53.68±0.082
3	500	51.30±0.053	59.74±0.090
4	750	53.03±0.026	61.26±0.165
5	1000	55.63±0.056	65.80±0.085
		IC_{50} value =690.77	IC_{50} value =163.05

*values are expressed as mean ± standard deviation (n=3).

Table 2: % inhibition and IC_{50} values of *in-vitro* NO radical by chloroform extract of *G. fergusonii* and Ascorbic acid.

S. No	Concentration (µg/ml)	% of activity (±SEM)*	
		Sample (Chloroform extract)	Standard (Ascorbic acid)
1	100	44.81±0.005	45.67±0.071
2	250	51.73±0.066	53.68±0.082
3	500	59.30±0.044	59.74±0.090
4	750	63.20±0.024	61.26±0.165
5	1000	68.61±0.007	65.80±0.085
		IC_{50} value =222.77	IC_{50} value =163.05

*values are expressed as mean ± standard deviation (n=3).

Table 3: % inhibition and IC_{50} values of *in-vitro* NO radical by aqueous extract of *G. fergusonii* and Ascorbic acid.

S. No	Concentration (µg/ml)	% of activity (±SEM)*	
		Sample (Aqueous extract)	Standard (Ascorbic acid)
1	100	17.75±0.017	45.67±0.071
2	250	24.68±0.034	53.68±0.082
3	500	29.65±0.042	59.74±0.090
4	750	44.58±0.034	61.26±0.165
5	1000	45.45±0.027	65.80±0.085
		IC_{50} value =1058.65	IC_{50} value =163.05

*values are expressed as mean ± standard deviation (n=3).

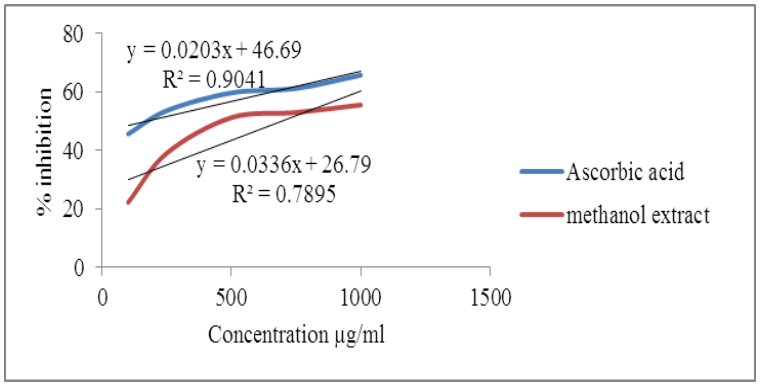

Fig 1: % inhibition of NO radical by methanol extract and ascorbic acid.

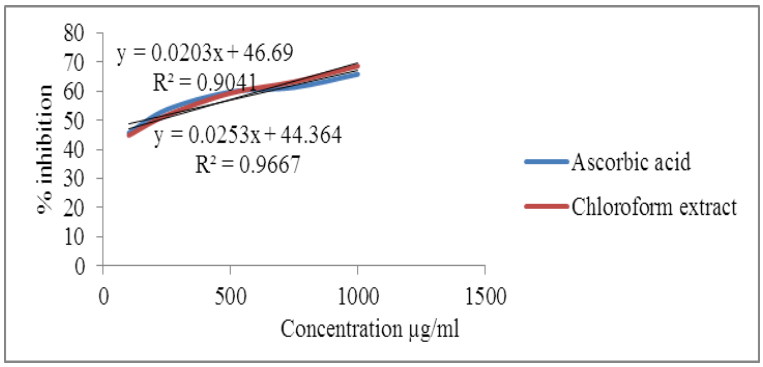

Fig 2: % inhibition of NO radical by chloroform extract and ascorbic acid.

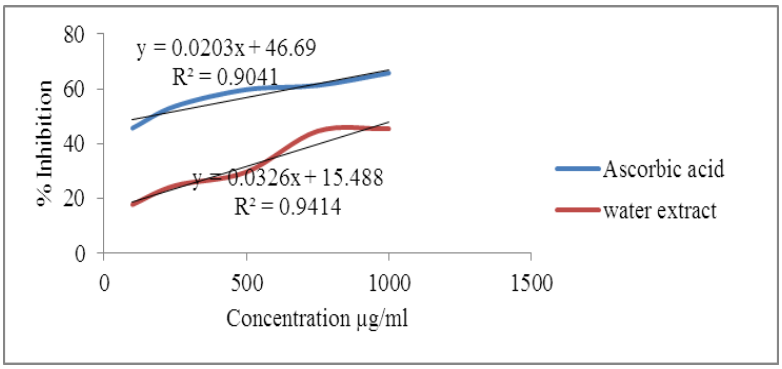

Fig 3: % inhibition of NO radical by aqueous extract and ascorbic acid.

Nitric oxide is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling and inhibition of platelet aggregation and regulation of cell mediated toxicity.[19] Constant production of nitric oxide radical results in direct tissue toxicity and septic shock associated vascular collapse. Conditions such as multiple sclerosis, arthritis, juvenile diabetes and ulcerative colitis show involvement of its chronic level expression.[20] Figure 1, 2 and 3 shows the percentage of NO radical scavenging activity of methanol, chloroform and aqueous extract of *G. fergusonii* compared with ascorbic acid.

H_2O_2 radical scavenging activity

The % inhibition and IC_{50} values of hydrogen peroxide radical scavenging activity of methanol extracts of *G. fergusonii* presented in Table 4. The IC_{50} values of the methanol extract of *G. fergusonii* and ascorbic acid were found to be 983.76µg/ml and 855.38µg/ml respectively. The Percentage inhibition and IC_{50} values of hydrogen peroxide radical scavenging activity of chloroform extracts of *G.fergusonii* presented in Table 5. The IC_{50} values of the chloroform extract of *G. fergusonii* and ascorbic acid were found to be 266.23µg/ml and 855.38µg/ml respectively.

The % inhibition and IC_{50} values of hydrogen peroxide radical scavenging activity of aqueous extracts of *G. fergusonii* presented in Table 6. The IC_{50} values of the aqueous extract of *G. fergusonii* and ascorbic acid were found to be 1525µg/ml and 855.38µg/ml respectively. The cytotoxic effect of free radicals is deleterious to mammalian cells and mediates the pathogenesis of many chronic diseases, but it is responsible for killing of pathogens by activated macrophages in the immune system.[21] Seaweeds contain different varieties of inorganic and organic substances which can be used for human health for examples polyphenols, carotenoids and tocopherols, terpenes, ascorbic acid, alkaloids.[22] H_2O_2 itself is not very reactive; it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important throughout food systems.[23]

The antioxidants have main roles in scavenging the free radicals, maintaining the cell integrity, slow down ageing and prevent the development of complications associated with oxidative stress- related diseases and cancer.[24] Figure 4, 5 and 6 shows the % of H_2O_2 radical scavenging activity of methanol, chloroform and aqueous extract of *G. fergusonii* compared with standard ascorbic acid.

Table 4: % inhibition and IC_{50} values of in-vitro H_2O_2 radical by Methanol extract of *G. fergusonii* and Ascorbic acid.

S. No	Concentration (µg/ml)	% of activity (±SEM)*	
		Sample (Methanol extract)	Standard (Ascorbic acid)
1	100	5.27±0.014	17.23±0.009
2	250	15.63±0.016	25.03±0.025
3	500	25.52±0.006	31.33±0.019
4	750	38.63±0.086	48.54±0.009
5	1000	50.75±0.064	55.30±0.016
		IC_{50} value =983.76	IC_{50} value =855.38

*values are expressed as mean ± standard deviation (n=3).

Table 5: % inhibition and IC_{50} values of in-vitro H_2O_2 radical by Chloroform extract of *G. fergusonii* and Ascorbic acid.

S. No	Concentration (µg/ml)	% of activity (±SEM)*	
		Sample (Chloroform extract)	Standard (Ascorbic acid)
1	100	46.06±0.004	17.23±0.009
2	250	51.26±0.003	25.03±0.025
3	500	55.00±0.009	31.33±0.019
4	750	59.70±0.005	48.54±0.009
5	1000	76.50±0.006	55.30±0.016
		IC_{50} value =266.23	IC_{50} value =855.38

*values are expressed as mean ± standard deviation (n=3).

Table 6: % inhibition and IC_{50} values of in-vitro H_2O_2 radical by aqueous extract of *G. fergusonii* and Ascorbic acid.

S. No	Concentration (µg/ml)	% of activity (±SEM)*	
		Sample (Aqueous extract)	Standard (Ascorbic acid)
1	100	21.52±0.0105	17.23±0.009
2	250	29.10±0.0166	25.03±0.025
3	500	34.04±0.0135	31.33±0.019
4	750	35.26±0.0101	48.54±0.009
5	1000	39.77±0.0340	55.30±0.016
		IC_{50} value =1525	IC_{50} value =855.38

*values are expressed as mean ± standard deviation (n=3).

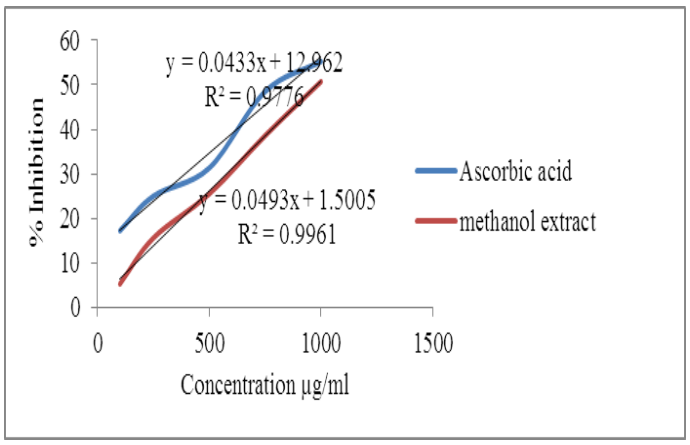

Fig 4: % inhibition of H_2O_2 radical by methanol extract and ascorbic acid.

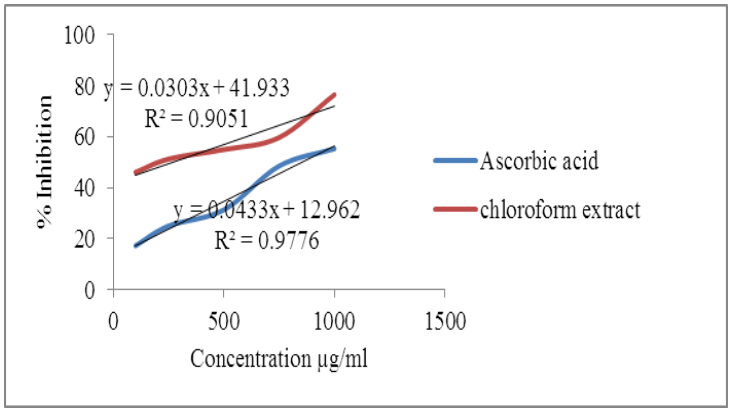

Fig 5: % inhibition of H_2O_2 radical by chloroform extract and ascorbic acid.

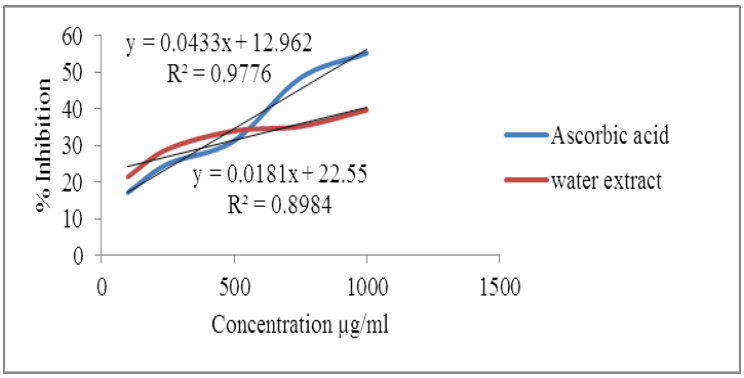

Fig 6: % inhibition of H_2O_2 radical by aqueous extract and ascorbic acid.

CONCLUSION

The result obtained in the present study concluded that the chloroform extract of *G. fergusonii* was more effective in NO and H_2O_2 radical scavenging activity when compared to the other methanol and aqueous extracts. It shows significant and natural sources of antioxidant. On the basis of this results, further isolation of bioactive compounds would assist to acertain its potency and safety as a lead candidate of antioxidant for pharmaceutical uses.

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