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FREE RADICAL SCAVENGING ACTIVITY OF DIFFERENT EXTRACTS OF *GRACILARIA FERGUSONII*

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ABSTRACT

The present study was made on antioxidant activity was performed by hydroxyl and DPPH radical scavenging methods for different organic solvent extracts of marine red algae *Gracilaria fergusonii J. Agardh.* In this study, scavenging activity was observed in different concentration (100, 250, 500,750, 1000 *µg/ml)* of three different solvent extracts like methanol, chloroform and water. The performances of scavenging activity of above extracts were comparing with standard ascorbic acid. An IC_{50}value of methanol, chloroform and aqueous extract of hydroxyl radical were recorded at 940.28 µg/ml, 490.24 µg/ml and 924.65 µg/ml respectively. In contrast, theIC_{50} values of DPPH radical were recorded at 755.14 µg/ml, 852.5µg/ml and 878.84µg/ml respectively. The IC_{50} value of standard ascorbic acid of hydroxyl and DPPH radical were recorded at 68.24µg/ml and 486.99µg/ml.

KEYWORDS

Antioxidant activity, Hydroxyl radical, DPPH radical, *Gracilaria fergusonii* and Organic solvent extracts.

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INTRODUCTON

Macroscopic thalloid algae are the major renewable resources of marine eco system. These marine algae are having rich source of bioactive secondary products. Nowadays seaweeds are used as dietary food supplements in daily life and it regulates the human health[1]. In human system at end of metabolism the consumable O_2 capable the potential to damage the cell molecules. They are called “Free Radicals”. They are capable of attacking the healthy cells of the body, consuming them to lose their structure and function.

Antioxidants to protect the cells and organ system of the body against reactive oxygen species (ROS), human have evolved a highly sophisticated and complex antioxidant protection system that functions interactively to neutralize free radicals. Cellular defenses against ROS include enzymes such as catalase which degrades hydrogen peroxide into water and oxygen and antioxidants such as tocopherol and ascorbic acid neutralize them. Free radical scavengers are antioxidants which can provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species (ROS) and subsequent lipid production, protein damage and DNA strand breaking[2]. The present study was made to evaluate the radical scavenging activity such as hydroxyl and DPPH by methanol, chloroform and aqueous extract of red algae *Gracilaria fergusonii* from Manapad coast, Tamil Nadu, India.

MATERIAL 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.

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 the *in-vitro* assays DPPH and hydroxyl radical scavenging methods. Vitamin C standard was dissolved in methanol and diluted quantitatively to obtain a concentration of 100µg ml^{-1}.

DPPH radical scavenging assay

The free radical scavenging activity of the fractions was measured *in vitro* by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay[3]. About 0.3 mM solution of DPPH in 100% ethanol was prepared and 1 ml of this solution was added to 3 ml of fraction dissolved in ethanol at different concentrations (100-1000 µg/ml). The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using a spectrophotometer. The % of scavenging activity at different concentrations was calculated using the following equation and the IC50 value of the fractions was compared with that of ascorbic acid, which was used as the standard.

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

Where A is the absorbance of control (DPPH solution without the sample), B is the absorbance of DPPH solution in the presence 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.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was determined by a slightly modified method of the 2-deoxyribose oxidation method[4]. Hydroxyl radical was generated from H_2O_2 by Fenton reaction in the presence of $FeSO_4$• $7H_2O$. A reaction mixture contain with each 0.2 ml of 10 mM $FeSO_4$. $7H_2O$, 10 mM EDTA and 10 mM 2-deoxyribose was mixed with 0.2 ml of the extract solution and 0.1 M phosphate buffer (pH 7.4) was added into the reaction mixture until the total volume reached to 1.8 ml. Then 0.2 ml of 10 mM H_2O_2 was finally added to the reaction mixture and incubated at 37^{0}C for 4 h. After incubation, each 1 ml of 2.8% TCA (trichloroacetic acid) and 1.0% TBA (thiobarbituric acid) were added. Then, the mixture was placed in a boiling water bath for 10 min. The absorbance was measured at 532 nm.

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

RESULTS AND DISCUSSION

In the present study, the antioxidant properties such as DPPH radical and hydroxyl radical scavenging activity of several concentration ranging from 100 to 1000 *µg/ml* of methanol, chloroform and aqueous extract of red algae *G. fergusonii* was determined.

DPPH radical scavenging activity

The % inhibition and IC_{50} value of DPPH radical scavenging activity of different concentration (100, 250, 500, 750, 1000 *µg/ml)* of methanol, chloroform and aqueous extracts of *G. fergusonii* are presented in Table No.1. With this method it was possible to determine the anti-radical power of an antioxidant by measuring of a decrease in the absorbance of DPPH at 517 nm. Result a colour change from purple to yellow, the absorbance decreased when the DPPH was scavenged by an antioxidant through donation of hydrogen to form a stable DPPH molecule. The DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) a stable free radical to decolorize in the presence of antioxidants[5]. The IC_{50} value of the methanol, chloroform and aqueous extracts were recorded at 755.14 µg/ml, 852.5µg/ml and *878.84*µg/ml respectively. Whereas, IC50 value of the reference ascorbic acid was 486.*99*µg/ml. A lower IC_{50} value indicates greater antioxidant activity. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule[6].

The DPPH radical has been widely used to test the potential of compounds as free radical scavengers of hydrogen donors and to investigate the antioxidant activity of plant extracts[6,7]. As the concentration of seaweed extract increased, the DPPH radical scavenging activity also increased. Decreased concentration of DPPH radical due to the scavenging ability of the soluble constituents in the extract of seaweed and the standard ascorbic acid as a reference compound presented the highest activity at all concentrations. This shows that seaweed possess hydrogen donating capabilities and acts as an antioxidant. Figure No.1, 2 and 3 shows the DPPH radical scavenging activity of methanol, chloroform and aqueous extract of *G. fergusonii* compared with ascorbic acid. Selvaraju *et al.,* (2011)[8] reported that the brown seaweed Sargassum wightii possessed higher antioxidant content. The reducing power property indicated the Padina sp and Eisenis cottanii extracts shows the consist of higher antioxidant compounds in the seaweeds which are involved in electron donors and will be able to reduced the oxidative strees from lipid per oxidation process[9]. From the result methanol extract was more effective in DPPH radical scavenging activity than that of other two extracts.

Hydroxyl radical scavenging activity

The % inhibition of Hydroxyl radical scavenging activity of different concentration (100, 250, 500, 750, 1000 *µg/ml)* methanol, chloroform and aqueous extracts of *G. fergusonii* are presented in Table No.2. The hydroxyl radical is said to be detrimental and initiates auto-oxidation, polymerization and fragmentation of biological molecules[10,11]. The identification of compounds that have excellent hydroxyl scavenging activity would be significant for some diseases caused by oxidative stress. It has been demonstrated that plants contain many natural antioxidants compounds which have been identified as hydroxyl radical scavengers[12]. The IC_{50} value of the methanol, chloroform and aqueous extracts were recorded at 940.28 µg/ml, 490.24 µg/ml and 924.65 µg/ml respectively. Whereas IC50 value of the standard ascorbic acid was 68.24 µg/ml. In figure 4, 5 and 6shows the hydroxyl radical scavenging activity of methanol, chloroform and aqueous extract of *G. fergusonii* was compared with ascorbic acid. From this observation, chloroform extract was more effective in hydroxyl radical scavenging activity than that of other two extracts.

Table No.1: Data of DPPH radical scavenging activity of *G. fergusonii*

S.No	Sample	Concentration *(µg/ml)*	Absorbance (λ 517 nm)*		% Inhibition	IC_{50} *(µg/ml)*
			Control	Sample/Standard		
1	Standard (Ascorbic acid)	100	0.582	0.466±0.002	19.93	486.99
		250		0.363±0.002	37.62	
		500		0.258±0.001	55.67	
		750		0.156±0.004	73.19	
		1000		0.153±0.004	73.71	
2	Methanolic extract	100	0.582	0.420±0.036	27.83	737.02
		250		0.397±0.039	31.79	
		500		0.379±0.011	34.88	
		750		0.262±0.008	54.98	
		1000		0.236±0.019	59.45	
3	Chloroform extract	100	0.582	0.495±0.016	14.95	838.97
		250		0.451±0.017	22.51	
		500		0.349±0.038	40.03	
		750		0.312±0.046	46.39	
		1000		0.263±0.066	54.81	
4	Water extract	100	0.582	0.554±0.068	4.81	867.99
		250		0.495±0.029	14.95	
		500		0.398±0.012	31.62	
		750		0.324±0.044	44.32	
		1000		0.258±0.018	55.67	

(*Values are expressed as mean ± SEM (Standard Error Mean) n=3)

Table No.2: Data of Hydroxyl radical scavenging activity of *G. fergusonii*

S.No	Sample	Concentration *(µg/ml)*	Absorbance (λ 532 nm)*		% Inhibition	IC_{50} *(µg/ml)*
			Control	Sample/ Standard		
1	Standard (Ascorbic acid)	100	0.465	0.254±0.009	45.38	68.24
		250		0.198±0.004	57.42	
		500		0.153±0.076	67.09	
		750		0.139±0.005	70.11	
		1000		0.133±0.039	71.39	
2	Methanolic extract	100	0.465	0.371±0.012	20.22	940.28
		250		0.348±0.011	25.16	
		500		0.314±0.005	32.47	
		750		0.253±0.008	45.59	
		1000		0.225±0.013	51.61	
3	Chloroform extract	100	0.465	0.275±0.064	40.86	490.24
		250		0.272±0.045	41.50	
		500		0.254±0.026	45.38	
		750		0.173±0.009	62.79	
		1000		0.168±0.018	63.87	
4	Water Extract	100	0.465	0.400±0.055	13.98	924.65
		250		0.369±0.065	20.65	
		500		0.308±0.027	33.76	
		750		0.272±0.024	41.51	
		1000		0.218±0.006	53.12	

(*Values are expressed as mean ± SEM (Standard Error Mean) n=3)

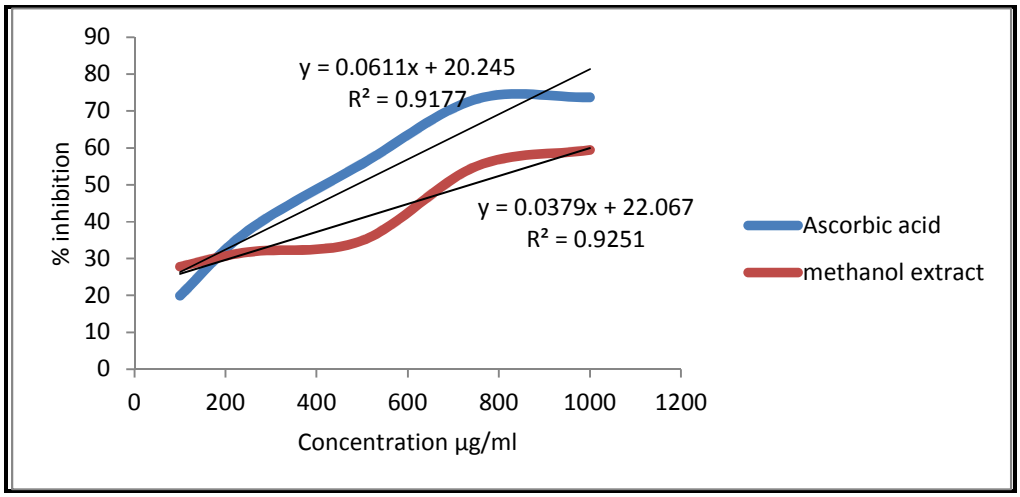

Figure No.1: % inhibition of DPPH radical scavenging activity of the methanol extract of *G. fergusonii* and ascorbic acid

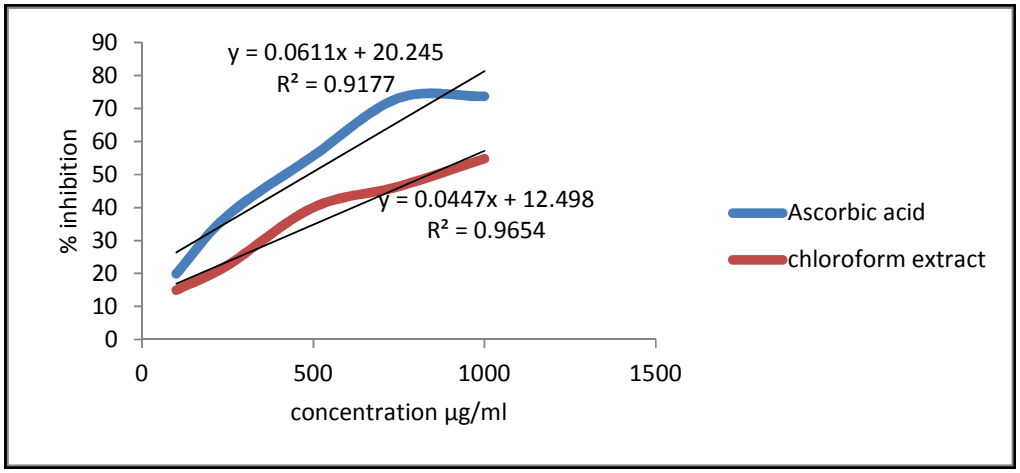

Figure No.2: % inhibition of DPPH radical scavenging activity of the chloroform extract of *G. fergusonii* and ascorbic acid

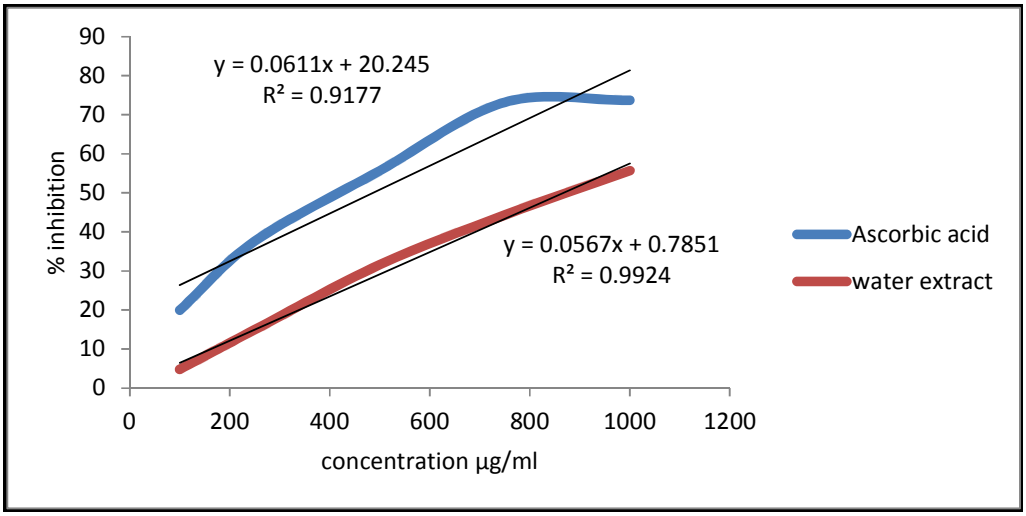

Figure No.3: % inhibition of DPPH radical scavenging activity of the aqueous extract of *G. fergusonii* and ascorbic acid

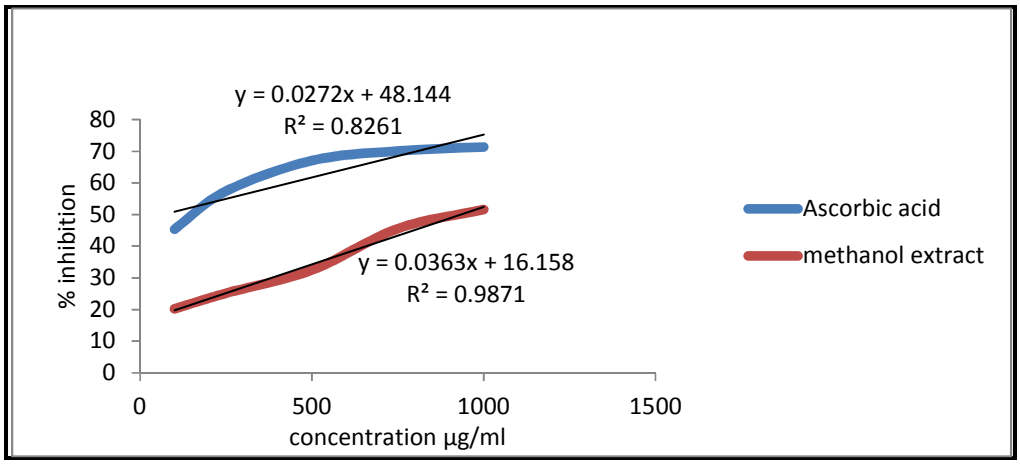

Figure No.4: % inhibition of Hydroxyl radical scavenging activity of the methanolic extract of *G.fergusonii* and ascorbic acid

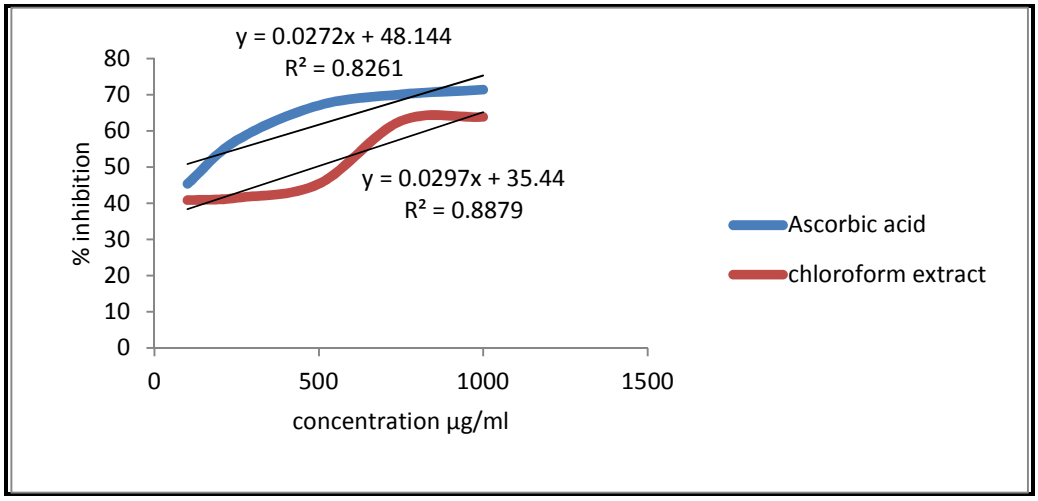

Figure No.5: % inhibition of Hydroxyl radical scavenging activity of the chloroform extract of *G.fergusonii* and ascorbic acid

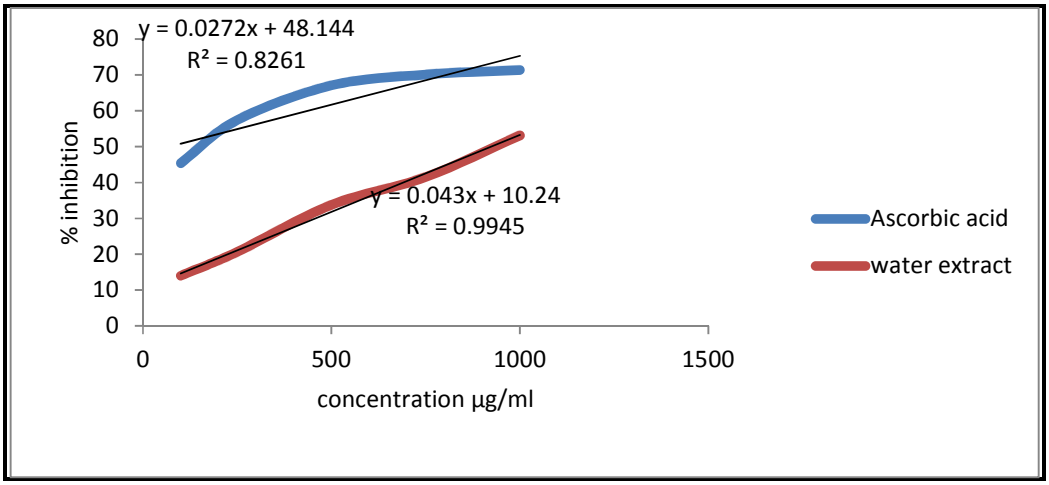

Figure No.6: % inhibition of Hydroxyl radical scavenging activity of the aqueous extract of *G.fergusonii* and ascorbic acid

CONCLUSION

Renewable macroalgae are available in the vast marine ecosystem that contains natural antioxidative compounds. These compounds have biological activities against several human diseases. Seaweeds are grown under varying physicochemical conditions, which contain more secondary metabolites and natural ant oxidative compounds. In recent, most of the food and pharmaceutical products contain synthetic antioxidants that are vulnerable in human system. Thus, fast growing renewable seaweeds have the capacity to prevent the free radicals and which are alternative for synthetic antioxidants.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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