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SCREENING OF ANTI-OXIDANT ACTIVITY OF METHANOLIC EXTRACT OF *GRACILARIA FERGUSONII* J.AG. (RED SEAWEED) IN HARE ISLAND, THOOTHUKUDI, TAMIL NADU, INDIA

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Abstract:

In the present study, anti-oxidant activity of methanol extract of Gracilaria fergusonii J.Ag. collected from Hare Island, Thoothukudi in the south east coast of Tamil Nadu, India was screened. Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical and a reducing power by Cupric Reducing Antioxidant Capacity (CUPRAC) assay. The percentage of scavenging activity of DPPH by methanol extract at 100µg, 200µg, 300µg, 400µg and 500µg were 31.35, 45.23, 61.76, 79.54 and 82.93% respectively. At a concentration of 100µg, 200µg, 300µg, 400µg and 500µg of methanol extract, the absorbance were 0.295, 0.302, 0.328, 0.367 and 0.384 respectively. These results similar to those obtained from the DPPH assay in which 500µg showed the highest total anti-oxidant capacity, followed by 400µg, 300µg and 200µg, and lastly 100µg. The results showed that both DPPH scavenging activity and absorbance were increased when the concentration of methanol extract was also increased. Vitamin C, a strong anti-oxidant was also used as control, and the anti-oxidant potential was compared to all tested samples.

Keywords: *Seaweeds, Gracilaria fergusonii, anti-oxidant, methanolic extract, Hare Island.*

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INTRODUCTION:

The anti-oxidant activity of several naturally occurring compounds have been known for decades. Recently, many types of seaweed have been considered as source of reactive oxygen species inhibitors. They can be used as food additives and can also provide protection against tissue oxidation [1]. The present investigation has also proved that seaweed Polyphenols possess anti-oxidant activity to scavenge free radicals. Dietary natural anti-oxidants are reported to help in preventing aging and other diseases. There are some evidences that seaweeds contain compounds with a relatively high anti-oxidant and anti-proliferative activity. Seaweeds are low in fat but contain vitamins and bioactive compounds like terpenoids, sulfated polysaccharides and polyphenolic compounds, the latter being a potential natural anti-oxidant not found in land plants [2]. Anti-oxidant compounds scavenge free radicals such as peroxide, hydro peroxide or lipid peroxyl and thus reduce the level of oxidative stress and slow or prevent the development of complications associated with oxidative stress related diseases [3]. Many synthetic anti-oxidants have shown toxic and mutagenic effects, which have shifted attention towards naturally occurring anti-oxidants. A great number of naturally occurring substances like seaweeds have been recognized to have anti-oxidant abilities [4]. Hence the present study was conducted to screen the anti-oxidant activity of the selected red seaweed *Gracilaria fergusonii* J.Ag.

MATERIALS AND METHODS:

Collection of Plant Materials

The collection of *Gracilaria fergusonii* J.Ag. was made during the low tidal and subtidal regions (up to 1m depth) by hand picking from Hare Island, Thoothukudi in the south east coast of Tamil Nadu, India. The collected materials were washed thoroughly with marine water in the field itself to remove the epiphytes and sediment particles. Then the samples were packed separately in polythene bags in wet conditions and brought to the laboratory, then thoroughly washed in tap water followed by distilled water to remove the salt on the surface of the thalli. They were stored in 5% formalin solution. For drying, washed specimens were placed on blotting paper and spread out at room temperature in the shade. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use 30g powdered samples were packed in Soxhlet apparatus and extracted with chloroform for 8h separately [5].

Antioxidant activity

DPPH Free Radical Scavenging Assay

Methanol extract of *Gracilaria fergusonii* J.Ag. was analyzed for the antioxidant activity based on the scavenging activity of the 1,1-diphenyl2-picryl hydrazyl (DPPH) free radical using the method of Mensor *et al.* [6]. DPPH is a stable free radical and acts as a scavenger for other radicals. Rate reduction of a chemical reaction using DPPH is a useful indicator of the radical state of a reaction. Methanol extract were prepared in triplicates at different concentrations (100-500µg/ml) and transferred into 1ml of 0.3mM methanolic DPPH solution (Sigma Aldrich). Samples were left to stand for 30 minutes in the light and the absorbance was measured at 517nm, zeroing the spectrophotometer with a methanol blank. The DPPH radical had a dark violet colour solution, and once neutralized, became pale yellow allowing visual monitoring of the radical reaction. Ascorbic acid was used as a positive control from Sigma was also used for a comparison. The percentage of inhibition was calculated using the following equation:

Inhibition Percentage = 1 – Absorbance of Sample – Absorbance of Blank / Absorbance of Control X 100

CUPRAC Assay

The CUPRAC (Cupric Reducing Antioxidant Capacity) method was also applied for the determination of anti-oxidant activity of methanol extract of *Gracilaria fergusonii* J.Ag. Copper chloride ($CuCl_2$) solution (0.01M) was prepared by dissolving 0.426g $CuCl_2$ in water and diluting the solution to 250ml. Ammonium acetate (NH_4Ac) buffer (pH 7, 1.0M) was made by dissolving 19.27g of NH_4Ac in water, and diluting this solution to 250ml. Neocuproine (Nc) solution (0.075M) was prepared fresh by dissolving 0.039g Nc in 96% ethanol and diluting to 25ml with ethanol. Methanol extract was prepared in triplicates at different concentrations (100-500µg/ml) and added into a solution containing 1ml $CuCl_2$, 1ml NH_4Ac, 1ml neocuproine and 0.1ml water. Test samples were incubated for 10 minutes at room temperature and the final absorbance was measured at 450nm, zeroing the spectrophotometer with water blank [7].

RESULTS AND DISCUSSION:

DPPH Free Radical Scavenging Assay

Crude methanol extract of *Gracilaria fergusonii* J.Ag. at various concentrations (100-500µg) were tested for anti-oxidant activity via the DPPH and CUPRAC assays. The experimental results are illustrated in Tables, where methanol extract was established to possess anti-oxidant activity. Vitamin C was used as a positive control for the DPPH assay. Anti-oxidant activity was determined by assaying the

reduction of DPPH radicals. The inhibition percentage of all tested samples showed a concentration dependent pattern as shown in Table 1. The percentages of anti-oxidant property of the methanol extracts at concentrations ranging from 100-500µg, however, were lower than vitamin C. Vitamin C had over 90% scavenging activity at a concentration of 100µg, whereas the tested methanol extract required a concentration of 500µg to reach a similar percentage. The percentage of scavenging activity of DPPH by methanol extract of *Gracilaria fergusonii* J.Ag. at 100µg, 200µg, 300µg, 400µg and 500µg were 31.35, 45.23, 61.76, 79.54 and 82.93% respectively. Among the various concentration of methanol extract used, 500µg methanol extract of *Gracilaria fergusonii* J.Ag. had the strongest scavenging ability while 100µg methanol extract of *Gracilaria fergusonii* J.Ag. had the lowest. The results showed that the scavenging activity was increased when the concentration of methanol extract was also increased. Vitamin C, a strong anti-oxidant was also used as control, and the anti-oxidant potential was compared to all tested samples.

CUPRAC Assay

Table 2 showed the reducing power of methanol extract of *Gracilaria fergusonii* J.Ag. on copper ions using the CUPRAC assay. Higher absorbance readings showed higher reducing ability of the samples. All samples exhibited the ability of reducing coppers ions from Cu(II) to Cu(I) in a concentration dependent manner. 500µg methanol extract showed the highest reducing activity when compared to the other concentration of methanol extract. At a concentration of 100µg, 200µg, 300µg, 400µg and 500µg, the absorbance were 0.295, 0.302, 0.328, 0.367 and 0.384 respectively. These results similar to those obtained from the DPPH assay in which 500µg showed the highest total antioxidant capacity (TAC), followed by 400µg, 300µg and 200µg, and lastly 100µg. The results showed that the absorbance and anti-oxidant activity was increased when the concentration of methanol extract was also increased. Vitamin C, a strong anti-oxidant was also used as

Table.1: Scavenging effects on DPPH free radical by various concentrations of methanol extract of *Gracilaria fergusonii* J.Ag. and Vitamin C

Concentration (µg)	Percentage of anti-oxidant effect on DPPH	
	Vitamin C	Methanol Extract
100	90.69±2.11	31.35±0.14
200	93.24±1.43	45.23±0.23
300	99.57±2.99	61.76±0.17
400	99.92±1.76	79.54±0.34
500	99.98±2.34	82.93±0.16

Table.2: CUPRAC assay by various concentrations of methanol extract of *Gracilaria fergusonii* J.Ag. and Vitamin C

Concentration	Wave length (nm)	ABSORBANCE(nm)	
		Vitamin C	Methanol Extract
100µg	450	0.318±0.002	0.295±0.018
200µg	450	0.356±0.001	0.302±0.009
300µg	450	0.415±0.001	0.328±0.011
400µg	450	0.446±0.003	0.367±0.020
500µg	450	0.569±0.002	0.384±0.016

control, and the anti-oxidant potential was compared to all tested samples.

CONCLUSION:

From the present study, it was concluded that the methanolic extract of *Gracilaria fergusonii* J.Ag., an important red seaweed (Rhodophyceae) showed significant anti-oxidant potential which is evident by the results received. Among the various concentrations of methanolic extracts studied, 500µg had the highest effect than other concentrations such as 400, 300, 200 and 100µg. However further studies required to identify the exact mechanism of action and the structure of the secondary metabolites which is responsible for anti-oxidant activity for the development as potent anti-oxidant drug.

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