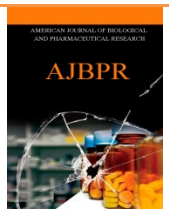
e-ISSN - 2348-2184
Print ISSN - 2348-2176

AMERICAN JOURNAL OF BIOLOGICAL AND PHARMACEUTICAL RESEARCH

Journal homepage: www.mcmed.us/journal/ajbpr



IN VITRO ANTI-OXIDANT ACTIVITY OF FUCOIDAN EXTRACTED FROM *PADINA DISTROMATICA* HAUCK (BROWN SEAWEED) FROM HARE ISLAND, THOOTHUKUDI, TAMIL NADU, INDIA.

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Article Info

Received 19/10/2014
Revised 26/10/2014
Accepted 05/11/2014

Key words: -Brown seaweed, *Padina distromatica,* Fucoidan, Anti-oxidant, DPPH, CUPRAC.

ABSTRACT

The aim of the present study was to assess the antioxidant activity of fucoidan extracted from *Padina distromatica* Hauck in Hare Island, Thoothukudi, Tamil Nadu, India. Free radical scavenging activity was evaluated using 1,1-diphenyl2-picryl hydrazyl (DPPH) free radical method and a reducing power by Cupric Reducing Antioxidant Capacity (CUPRAC) assay. The percentage of scavenging activity of DPPH by fucoidan at 100µg, 200µg, 300µg, 400µg and 500µg were 40.43, 59.68, 68.47, 87.32 and 94.33%, respectively. At a concentration of 100µg, 200µg, 300µg, 400µg and 500µg of fucoidan, the absorbance were 0.302, 0.317, 0.369, 0.395 and 0.427 respectively. These results similar to those obtained from the DPPH assay in which 500µg showed the highest total antioxidant 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 fucoidan 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.

INTRODUCTION

Seaweeds are used by coastal populations for thousands of years owing to the high nutritional values [1-2]. However, the industrialization of seaweeds does not necessarily need the consumption. Medical and pharmaceutical industries are also interested since seaweeds are rich in active molecules [3-4]. Indeed, the therapeutic potentials of certain substances are extremely promising, especially used as pharmacological actions [5-6]. Besides, the use of fucoidans extracted from brown seaweeds allows fighting against the formation and growth of malignant tumors [7-8].

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Numerous studies have investigated the biological activities of algae extracts [9]. Seaweeds in shallow water habitats can be exposed to a combination of ultraviolet light and air that readily leads to the formation of free radicals and other Reactive Oxygen Species (ROS). Despite the exposure to harmful ROS, healthy seaweeds lack oxidative damage in their structural components (*i.e.*, fatty acids) and resist oxidation during storage, indicating the presence of protective antioxidant defense systems in their cells [10-11]. By donating an electron, antioxidants neutralize free radicals that would otherwise oxidize biomolecules leading to cell death and tissue damage [12-13]. Accordingly, interest in the search for natural antioxidants from seaweeds has been increasing in recent years. The overall aim of this type of research is discovery of compounds and or extracts that can counteract free radical induced and other oxidative stress processes,

and in so doing decrease the incidence of human diseases directly related to these processes [14].

Antioxidant activity has been reported in numerous genera of seaweeds including *Gracilaria*, *Halymenia*, *Hydroclathrus*, *Polysiphonia* and *Turbinaria*. Natural antioxidants from algae are known to play an important role against various diseases and aging processes [15]. The detected antioxidant compounds in algae from these genera and others have potential anti-aging, dietary, anti-inflammatory, antibacterial, antifungal, cytotoxic, anti-malarial, anti-proliferative, and anticancer properties [16]. Fucoidan is a natural polysaccharide made essentially of sulphated L-fucose residues. Also known as sulphated fucan present only in brown seaweeds. Fucoidan is present in the cell walls of brown seaweeds including *Undaria, Sargassum, Turbinaria, Padina* etc [17-18]. Though many studies on identifying the structural properties of fucoidan have been carried out, the structure still remains uncertain due to the absence of strict regularity and the numerous components that make up fucoidan as a whole [19]. Most fucoidans have very complex chemical composition and only little regularity in the structural components is known present [20]. Fucoidan largely contains sulphated L-fucose residues. Hence fucose is the primary sugar in fucoidan. Sulphate groups also represent a large component of fucoidan and the biological activity of fucoidan is strongly related to its sulphate content [21]. Besides fucose and sulphate, other monosaccharides (glucose, mannose, galactose, xylose, etc), uronic acids, and even protein are present in detectable amounts. All these compounds have increased the difficulty in structural elucidation of fucoidan [22]. Hence the present research was aimed to isolate the fucoidan from *Padina distromatica* Hauck in Hare Island, Thoothukudi, Tamil Nadu, India and screen for anti-oxidant potential.

MATERIALS AND METHODS

Collection of Plant Materials

The collection of *Padina distromatica* Hauck (Figure 1) was made during the low tidal and subtidal regions (up to 1m depth) by hand picking from Hare island, Thoothukudi (Lat 8° 48'N; Long 78° 11'E) located 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.

Extraction of Fucoidan

Fucoidan was extracted by Rioux method [20]. Dried seaweed was mixed with 1% (w/v) $CaCl_2$ solution (1:30 ratio) and then stirred for 4 hours at 85℃ at 455 ± 5rpm using a stirrer RZR1 (Caframo Ltd. Canada). The supernatant was separated by centrifugation (16,887g, 20 min), and vacuum filtration on Whatman No. 4 filter. The filtered liquid was mixed with 2 volumes of 95% ethanol and 1 volume of 2% (w/v) NaCl and then stirred for 1 hour at room temperature for alcoholic precipitation of fucoidan. This solution was kept at -20ºC for 48 hours. The pellet containing fucoidan was recovered by centrifugation (16,887 g, 12 min). Then, it was resolubilized in 100 ml of fresh deionized water and dialyzed for 48 hr by using membrane of 15 KDa (Sigma, USA) to remove minor constituents and solvents. Fucoidan was recovered by freeze drying and preserved at -20℃ in a sealed tube to keep away from humidity.

Antioxidant activity

DPPH Free Radical Scavenging Assay

Fucoidans extracted from *Padina distromatica* Hauck were 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. [23]. 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. Fucoidan samples 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 and commercial fucoidan from Sigma was also used for a comparison. The percentage of inhibition was calculated using the following equation:

$$\text{Inhibition Percentage} = \frac{1 - \text{Absorbance of Sample} - \text{Absorbance of Blank}}{\text{Absorbance of Control}} \times 100$$

CUPRAC Assay

The CUPRAC (Cupric Reducing Antioxidant Capacity) method was also applied for the determination of anti-oxidant activity of fucoidan extracted from *Padina distromatica* Hauck. 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. Fucoidan samples were 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 [24].

RESULTS AND DISCUSSION

Fucoidan is a stored sulfated polysaccharides of brown seaweeds and is composed mainly of α-(1-2) or α-(1-3) linked L-fucose residues [18-19]. The precise structure of the fucoidans from *Fucus vesiculosus* and *Ascophyllum nodosum* [20] remains uncertain although the main repeating unit has been confirmed (Figure 2).

Most fucoidans have very complex chemical composition and only little regularity in the structural components is known present [21]. Fucoidan largely contains sulphated L-fucose residues. Hence fucose is the primary sugar in fucoidan. Sulphate groups also represent a large component of fucoidan and the biological activities of fucoidan are strongly related to its sulphate content [22]. Besides fucose and sulphate, other monosaccharides (glucose, mannose, galactose, xylose, etc), uronic acids, and even protein are present in detectable amounts. All these compounds have increased the difficulty in structural elucidation of fucoidan [23].

Antioxidant activity

DPPH Free Radical Scavenging Assay

Crude fucoidan of *Padina distromatica* Hauck at various concentrations (100-500µg) were tested for antioxidant activity via the DPPH and CUPRAC assays. The experimental results are presented in Tables and Figures, where fucoidan were established to possess antioxidant activity. Vitamin C was used as a positive control for the DPPH assay. Antioxidant 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 and Figure 3. The percentages of anti-oxidant property of the fucoidans 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 fucoidan required a concentration of 500µg to reach a similar percentage. The percentage of scavenging activity of DPPH by fucoidan extracted from *Padina distromatica* Hauck at 100µg, 200µg, 300µg, 400µg and 500µg were 40.43, 59.68, 68.47, 87.32 and 94.33%, respectively. Among the various concentration of fucoidan used, 500µg fucoidan of *Padina distromatica* Hauck had the strongest scavenging ability while 100µg fucoidan of *Padina distromatica* Hauck had the lowest. The results showed that the scavenging activity was increased when the concentration of fucoidan 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 and Figure 4 showed the reducing power of fucoidan samples on copper ions using the CUPRAC assay. Higher absorbance readings indicated 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 fucoidan showed the highest reducing activity when compared to the other concentration of fucoidan.

Table 1. Scavenging effects on DPPH free radical by various concentrations of fucoidan extracted from *Padina distromatica* Hauck and Vitamin C

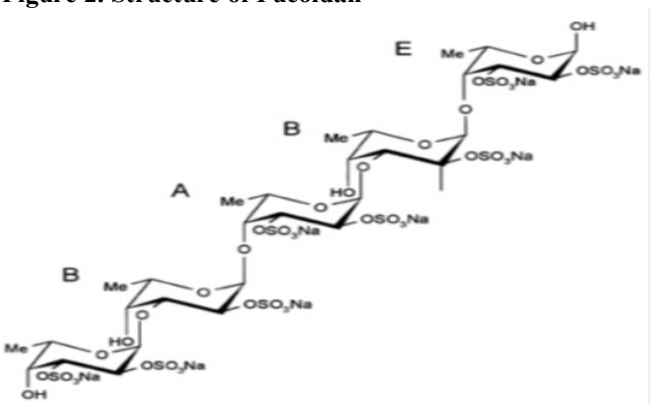
Concentration	Percentage of anti-oxidant effect on DPPH	
	Vitamin C	Fucoidan
100µg	90.69±2.11	40.43±2.80
200µg	93.24±1.43	59.68±1.46
300µg	99.57±2.99	68.47±2.51
400µg	99.92±1.76	87.32±2.62
500µg	99.98±2.34	94.33±2.23

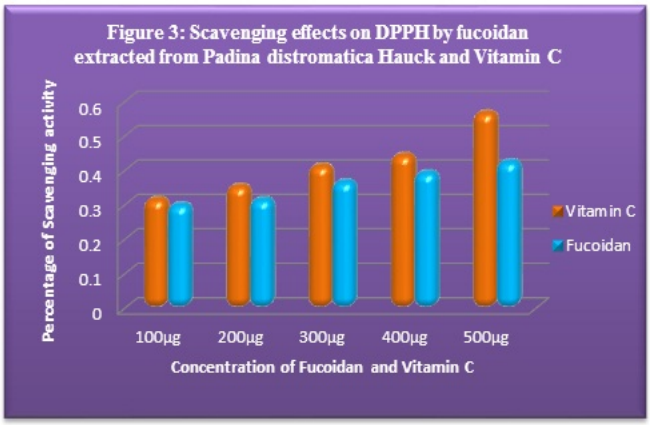
Table 2. CUPRAC assay by various concentrations of fucoidan extracted from *Padina distromatica* Hauck and Vitamin C

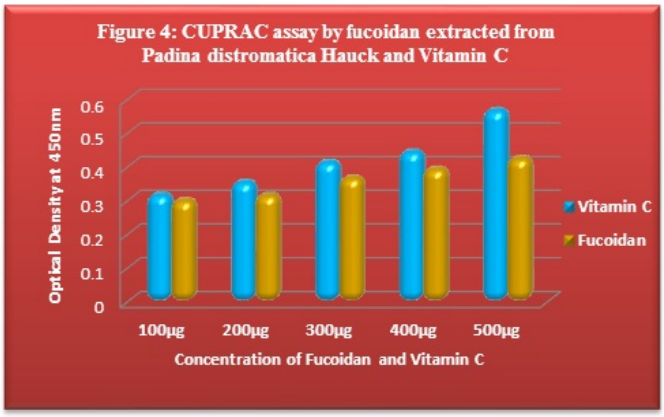
Concentration	Wave length (nm)	Absorbance (nm)	
		Vitamin C	Fucoidan
100µg	450	0.318±0.002	0.302±0.003
200µg	450	0.356±0.001	0.317±0.031
300µg	450	0.415±0.001	0.369±0.027
400µg	450	0.446±0.003	0.395±0.005
500µg	450	0.569±0.002	0.427±0.002

Figure 1. Natural Habit of *Padina distromatica* Hauck

Figure 2. Structure of Fucoidan







At a concentration of 100µg, 200µg, 300µg, 400µg and 500µg, the absorbance were 0.302, 0.317, 0.369, 0.395 and 0.427 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 fucoidan 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.

Research on fucoidan has so far been carried out in Japan, Korea, France, Australia, China, and the United States. Studies have indicated that fucoidan is non-toxic, non-allergenic, and has no negative effects on the human body once consumed [25]. This statement is further supported by the fact that nutraceutical and food supplements containing fucoidan have been marketed for a number of years with no known adverse effects [26]. No toxicological changes were observed when rats were orally administered with up to 1000mg/Kg body weight per day of fucoidan for 28 days, but when the dose was increased to 2000 mg/Kg body weight per day of fucoidan, the plasma ALT level, a biomarker of liver injury was increased indicating that the consumption of fucoidan up to 1000mg/Kg body weight per day was safe in rodents [27].

CONCLUSION

Fucoidan is known to exhibit a wide variety of biological activities. Among them are anticoagulant, antioxidant, antiviral, antithrombic, and anticancer activities [22]. Many researchers have targeted the anticoagulant, anticancer, and antioxidant activities of fucoidan as being the most important activities in fucoidan. The effectiveness of these activities is related to the chemical composition of fucoidan [8]. Seaweed polysaccharides are usually heterogeneous and branched, it may contain monosaccharide components with acetyl groups and the amount of sulfation is irregular [17].

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