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Antioxidant Activity of astaxanthin isolated from *Charybdis Edwardsii* (Leene and Buitendijk ,1949) from Pazhayar, Southeast Coast of India

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ABSTRACT
Astaxanthin extracted from Red Crab *Charybdis edwardsii* was assayed for antioxidant activity viz., total antioxidant, reducing power, hydrogen peroxide radical scavenging assay, and DPPH free radicals scavenging activity. Final concentration of astaxanthin was quantified to be 49.05 µg/g using the standard astaxanthin. The antioxidant activity was found to be 60%, hydrogen peroxide scavenging activity showed 89% inhibition, whereas 1,1-diphenyl-2-picrylhydrazl (DPPH) activity showed 86% of scavenging activity. Present investigation proves that the astaxanthin from *C. edwardsii* acts as a promising antioxidant source, which can be used in food, pharmacological and aquaculture sectors.

Keywords: *Charybdis edwardsii, astaxanthin, antioxidant, DPPH*

INTRODUCTION
The agricultural and food processing sectors face the problems of disposing waste products such as exoskeleton of crustaceans, thereby converting the waste material into the economic by products and it is common in the shellfish industries with the waste represents greater than 80 % of landings. Thus, it has been estimated that solid waste from the crab industries is now in excess of a million kg/year in USA (Meyers *et al*., 1971). The processing of crustaceans such as shrimps and crabs generates large quantities of wastes in the form of head and body shells. These account for approximately 35–45% of the whole crustaceans weight (Khumallambam *et al*., 2011). The recovery of these valuable components from the waste would not only improve the economy for crustacean processors, but would minimize the pollution of crustacean wastes (Kuo *et al*.,1976). One possible use of this waste is as a source of carotenoids, protein and lipid for the diet of Salmonids raised in aquaculture. While the protein and lipids can be supplied from other sources, there is no source of the carotenoids astaxanthin other than from dietary crustacean. Carotenoids are a group of fat-soluble pigments occurring widely in nature. The aquatic animals such as crustaceans are known to contain various carotenoids and are considered as one of the important sources of natural carotenoids (Matsuno, 2001).There is currently considerable interest in the role of carotenoids in delaying or preventing degenerative diseases such as atherosclerosis, cancer, aging (Halliwell, 1997; Rice-Evans and Burdon, 1994; Mathews-Roth, 1991) and eye diseases (Pratt, 1999; Kirschfeld, 1982). The antioxidant activity of astaxanthin has been reported to be 10 times stronger than that of other carotenoids, namely, zeaxanthin, lutein, canthaxanthin, and *â*-carotene (Miki, 1991). Astaxanthin has been associated with reduced risk of diseases such as age-related macular degeneration and ischemic diseases, effects attributed to its potent antioxidant activity (Tso and Lam, 1996). Astaxanthin is a substance best known for giving the pinkish-red colour to the flesh of salmonids (salmons and trouts), as well as shrimps, lobsters and crayfishes (Koller *et al*., 2014). Besides the colorant properties, astaxanthin displays a central role for the immune-system of these fishes and positively impacts their fertility (Koller *et al.,* 2014). It is claimed to possess as much as 10 times the antioxidant potential of other carotenoids such as β-carotene, canthaxantin, zeaxanthin and lutein; and 100 times more that α-tocopherol. Among the deep-water

crab resources, the most promising is that of *Charybdis edwardsii* which gave a catch rate of 3500 kg/h in try net hauls carried out off Mangalore and Ponnani (Silas, 1969). The aim of the present study is to evaluate the antioxidant activity of astaxanthin extracted from *Charybdis edwardsii.*

MATERIALS AND METHODS

Collection

The study animal *Charybdis edwardsii* was sourced from Pazhayar fish landing centre of (Lat 11º 21'22" N; long 79º 50'55" E) South east coast of India, Average weight of crab ranged from25 to 36 grams. Crabs were washed in tap water to remove debris and soil then stored in deep freezer until further analysis.

Extraction

Astaxanthin content was determined according to the modified method of Tolasa *et al*. (2005) and Metusalach *et al*. (1997). Dried crab sample (30 g) was extracted thrice, with 50ml of acetone using incubating shaker for 48 hrs at room temperature. After extraction,the samples were centrifuged at 4000g at 4°C for 5 min. To separate the waterinsoluble compounds, the acetone extracts of the sampleswere transferred to 40 ml of petroleum ether in a 250 mlseparating funnel. Then, 100 ml of distilled water containing 0.5% (w/v) sodium chloride was added to the mixture. After continuous shaking by hand, approximately 20 minutes later, the petroleum ether layer was separated and transferredinto a 50 ml volumetric flask.

Quantification of astaxanthin

The extracted astaxanthin is re dissolved in 3 ml of acetone and read @ 468 nm.

$$\text{AST } (\mu g/g) = \frac{A468nm \times V \text{ extract} \times \text{Dilution factor}}{0.2 \times W \text{ sample}}$$

Where, A is absorbance, V is volume of extract, 0.2 is theA468 of 1 µg/g of standard astaxanthin and W is weight of sample in grams. (Simpson and Haard, 1985).

Total antioxidant activity

Total Antioxidant Capacity (TAC) reagent was prepared by mixing of 7.45 ml sulphuric acid (0.6 mM solution), 0.9942 g of sodium sulphate (28 mM solution) and 1.2359 g of ammonium molybdate (4 mM solution) in 250 ml of distilled water. 300 µl of isolated astaxanthin from the crab was dissolved in 3 ml of TAC reagent. Distilled water was used as blank. Absorbance of all sample mixtures was measured at 695 nm. Gallic acid was used as standard to determine totalantioxidant activity (Khan *et al*., 2009).

Reducing power

1.0 ml of isolated astaxanthin from the crab was mixed with 2.5 ml of phosphate buffer (0.2 M, pH-6.6) and 2.5 ml of potassium ferric cyanide (1%). Reaction mixture was kept in a water bath at 50°C for 20 minutes. After incubation, 2.5 ml of Trichloroacetic acid (10%) was added and centrifuged at 650 rpm for 10 minutes. From the supernatant, 2.5 ml solution was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (0.1%). Absorbance of the solution was measured at 700 nm after every 10 minutes intervals up to 30 minutes. Increased absorbance indicated increased reducing power (Govindarajan *et al*., 2003).

Hydrogen peroxide radical scavenging assay

Hydrogen peroxide (10 mM) solution was prepared in the phosphate buffer saline (0.1 M, PH-7.4). 1 ml (0.25 mg) of the isolated astaxanthin from the crab was rapidly mixed with 2 ml of hydrogen peroxide solution. The samples were incubated at 37°Cfor 10 min and the absorbance was measured at 230 nm against a blank (without hydrogen peroxide; Govindarajan *et al*., 2003). The percentage of scavenging of hydrogen peroxide was calculated using the following formula:

% Scavenging = [Absorbance of control - Absorbance of test sample/Absorbance of control] ×100

Where blank – phosphate buffer; control – hydrogen per oxide without astaxanthin; test astaxanthin with hydrogen peroxide.

1, 1-diphenyl-2-picrylhydrazl (DPPH) free radicals scavenging activity

1 ml of test solution was mixed with 1 ml of 0.1 mM DPPH in methanol. The mixture was incubated in dark place for 30 min at 25°C. After 30 minutes, absorbance wasrecorded at 517 nm. (Shimada *et al*., 1992)The percentage of DPPH free radicals scavenging activity was calculated by following equation:

% Scavenging = [Absorbance of control - Absorbance of test sample/Absorbance of control] ×100

Where blank – methanol; control - DPPH in methanol without astaxanthin; test – astaxanthin with DPPH in methanol.

RESULTS AND DISCUSSION

Quantification of astaxanthin

The concentration of astaxanthin obtained from *C. edwardsii* was found to be 49.05 µg/g. The present investigation was correlated with the earlier findings of Renata Aline dos Santos da Fonseca *et al.*(2011) and (Shahidi and Synowiecki, 1990).

Antioxidant activity of astaxanthin standard and isolated astaxanthin

Antioxidant properties of astaxanthin was evaluated since, astaxanthin possess the properties of free radical scavenging, anti-aging, anti-cancer, anti-inflammatory etc. In the present investigation, total antioxidant property of astaxanthin was evaluated the antioxidant potential of 68 % was found in astaxanthin standard, 60 % was found in *C. edwardsii* but it is lower than that of gallic acid the standard drug i.e. 75 % (Fig. 1). Sudhakar *et al.*(2011) recorded the total antioxidant activity ranged from 28.52 % to 80.26 % in *P. Sanguinolentus* crab shell chitosan sample. The present test was also correlated with the study of Soundarapandian et *al.*(2014) in which the total antioxidant activity was found to be 32 to 49% in soft and hard shelled crabs of *C. Lucifera* and 86 % for Gallic acid.

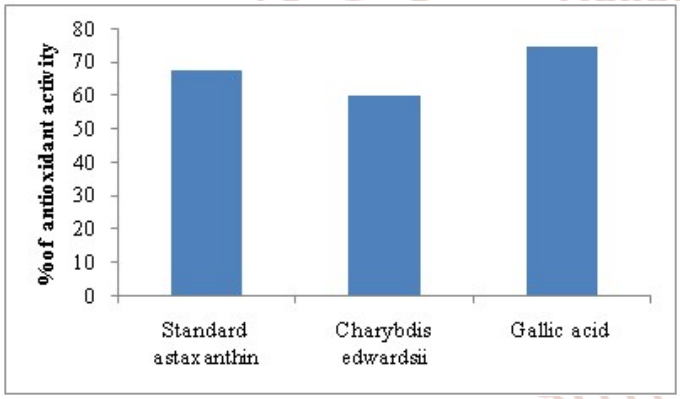

Figure1. Percentage of total antioxidant activity

Reducing power assay

The present investigation revealed the reducing effect of astaxanthin. Astaxanthin possess the ability to donate an electron to free radicals which leads to neutralization of the radicals. Reducing power was evaluated using ferric ions. There was increase in Optical Density for every 10mins in both the standard and the test samples. Due to increase in Optical Density, the result reveals the good reducing property.In reducing power assay, standard drug ascorbic acid was dominating agent that shows the maximum reducing ability i.e 0.06 for 10mins and 0.09 for 30 minutes, which is followed by astaxanthin standard that shows 0.05 for 10 mins and 0.08 for 30 minutes. The reducing ability of Red crab (*C.edwardsii*) was0.04 for 10 mins and 0.07 for30 mins. Thus, the result correlates astaxanthin as good reducing agents. Similar result was reported by Yen *et al.* (2014) and Soundarapandian *et al*. (2014).

Hydrogen peroxide radical scavenging assay

Hydrogen peroxide is a standard free radical compound which enhances the lipid peroxidase acitivity. During the present study, the ability of astaxanthin to scavenge hydrogen peroxidewas assayed. The maximum activity of 89% inhibition was seen in ascorbic acid followed by standard astaxanthin of 68 % inhibition. The scavenging activity of 67 % (Fig. 2) was seen in *C. Edwardsii* (Red crab), these results further proves that astaxanthin has a good antioxidant property. The present study was corelated with the study of Soundarapandian *et al*. (2014).

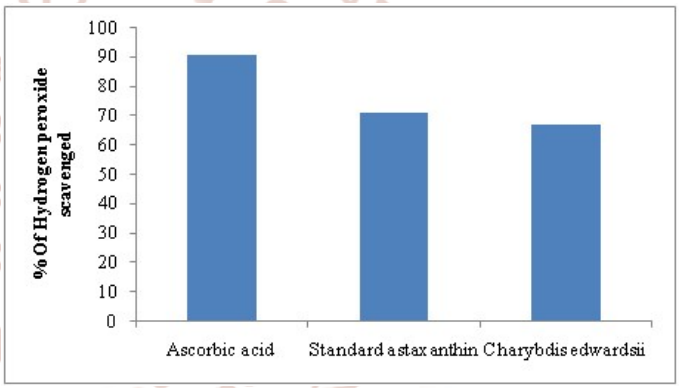

Figure2. Percentage of hydrogen peroxide scavenging assay

Scavanging ability on 1, 1- diphenyl-2-picrylhydrazl radicals (DPPH) assay

DPPH is one of the stable free radical used for the assay of scavenging capacity of astaxanthin. The DPPH activity of standard Gallic acid was found to be 86%. Standard astaxanthin had 79 %, where as *C. edwardsii* (Red Crab) possessed72 % (Fig. 3).

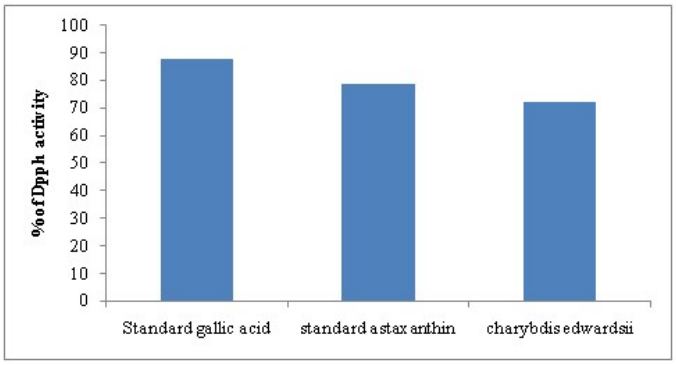

Figure3. Percentage of 1, 1- diphenyl-2-picrylhydrazl radicals (DPPH) activity

Hence the results proved both Standard astaxanthin and astaxanthin extracted from *Charybdis edwardsii* possessed a good DPPH activity which predicted that DPPH would have picked up the electron in the presence of a free radical scavenger which is reflected as the percentage of DPPH activity. Similar result was reported by Yen et al.(2014) as it showed 82.9% scavenging activity. From the study, it has been proved that *Charybdis edwardsii* acts as a potent source of natural antioxidants, which can be used in the food and pharmaceutical sectors.

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