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ANTIOXIDANT PROPERTY OF THE CRUDE PEPTIDE EXTRACTS OF A FRESH WATER CRAB *Oziotelphusa senex senex*

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ABSTRACT

Crustaceans have been recognized as rich sources of bioactive compounds with valuable nutraceutical and pharmaceutical potentials. Fresh water crab Oziotelphusa senex senex is the abundantly existing crab with unknown health benefits Cellular damage caused by reactive oxygen species has been implicated in several diseases; hence antioxidants have significant importance in human health Antioxidants play an important role as health protecting factor.Scientific evidences suggests that antioxidants reduce the risk for chronic diseases including cancer and heart diseases .So the.the antioxidant activity of the hemolymph from the fresh water Crab *Oziotelphusa senex senex was*. evaluated .Hemolymph of *Oziotelphusa senex senex* a fresh water crab, were subjected for its antioxidant activity using DPPH,ABTS and hydrogen peroxide scavenging assays. In antioxidant assay, the percentage of 2, 2-diphenyl-1-picrylhydrazyl scavenging activity was recorded as 57.5%. ABTS scavenging activity (68%) hydrogen peroxide scavenging (64%).The antioxidant activity of the hemolymph increased in a concentration dependent manner. Hence, the present study revealed that the hemolymph from the fresh water crab *Oziotelphusa senex senex* can be used as an accessible source of natural antioxidants with consequent health benefits.

KEYWORDS: Crab, *Oziotelphusa senex senex,* Hemolymph, DPPH ,ABTS.

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INTRODUCTION

Antioxidants may have a positive effect on human health since they can protect thehumanbody against deterioration by free radicals and reactive oxygen species (ROS), including singlet oxygen, hydrogen peroxide, superoxide anion, and hydroxyl radicals. ROS and free radicals attack macromolecules such as DNA, proteins and lipids, leading to many health disorders including inflammatory, aging, diabetes, neurodegenerative, cardiovascular and cancer diseases.[1-2] Organisms possess several defense mechanisms to control the level of ROS.[3]When such defense mechanisms become unbalanced, antioxidant supplement can be used to reduce the oxidative damage. Repairing such damages by naturally occurring substances mainly by supplementation of food having antioxidant property is becoming one of the most acceptable modes of modern therapy.[4] Recently, the use of natural antioxidants available in food and other biological substances has attracted significant interest due to their presumed safety, nutritional and therapeutic values.[5-7] Antioxidant activities of bioactive peptides are mainly due to the presence of hydrophobic amino acids, some aromatic amino acids and histidine[8] the use of these synthetic antioxidants must be under strict regulation due to potential health hazards.[9] Hence, the search for natural antioxidants as safe alternatives is important in the food industry. There has beens a lot of research on obtaining these natural antioxidants like antioxidant and cholinesterase inhibitory activities of the organic solvent extracts of shrimp by-products using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2´-Azino-bis[3-ethylbenzothiazoline-6-sulfonic acid] (ABTS) .[10] Freshwater crabs are an important component of the fauna of limnic environments .[11] Crabs, among other invertebrates are considered as an essential shell fishery product.[12] About 1300 species of freshwater crabs, distributed throughout the tropics and subtropics regions .[13]They are the best sources for food products including protein source for aquatic lives as well as for human. The nutritional quality of the crab proteins is very favourable when compared with other poultry animals. The haemolymph proteins of crustaceans are unique in composition, as they do not contain immunoglobulin or albumin like proteins and the protein composition varies in relation to physiological and functional state of the animal. The relative contributions of haemocyte phenoloxidase and hemocyanin in the standard physiological ratio at which they occur in haemolymph have been investigated in the crab, *Cancer magister*.[14] .The circulating hemolymph in crustaceans contains biologically active substances such as complement, lectins, clotting factors and antimicrobial peptides.[15] Hemolymph of crab has two components, hemolymph plasma and hemocytes, which play major roles in their immune mechanism. The literature on antioxidant property of the freshwater crab, *Oziotelphusa senex senex* was scanty Hence, the present is focused on to identify the antioxidant activity of the hemolymph from the crab *Oziotelphusa senex senex*.

MATERIALS AND METHODS

Sample collection

Fresh water Crab (*Oziotelphusa senex senex)* were collected from the paddy fields of Kundratur, Thiruvallur district,Tamilnadu,India.Healthy Male crabs of uniform size, and free from disease were used for experimental purpose and each crab was subjected to single bleed collection .The weight of the collected crabs ranged between 50-100g.The crabs were acclimatized for a week in the laboratory.

Collection of hemolymph

Haemolymph of *Oziotelphusa senex senex* was collected aseptically from the base of one of the second walking legs using a sterile syringe.To avoid haemocyte degranulation and coagulation, the hemolymph was collected along with ice-cold citrate EDTA buffer (510m M NaCl ; 0.1M glucose; 30mM trisodium citrate; 20mM citric acid; 10mM EDTA, pH 4.6)[16] as anticoagulant. Haemolymph was centrifuged at 2000 rpm for 15 min at 4°C.Supernatant was collected by aspiration and stored at 4°C until use.

Protein Estimation Assay

The amount of protein was measured according to Lowry's (1951) method with different concentrations of Bovine serum albumin (BSA) as a standard.[17].The concentration was calculated in response to the absorbance at 650 nm in a spectrophotometer.

ANTIOXIDANT ASSAY

DPPH Free Radical Scavenging Assay

The free-radical scavenging activity of *Oziotelphusa senex senex* haemolymph extract was measured by decrease in absorbance of methanolic solution of DPPH.[18]A stock solution of DPPH was prepared in methanol and 5 ml of this stock solution was added to 1 ml of the hemolymph extract solution at different concentration of 100-500 µg/ml. After 30 min, absorbance was measured at 517 nm.Scavenging activity was expressed as the percentage inhibition.

Scavenging ability (%) = [A517 of control-_A517 of sample) /_A517 of control] X100.

ABTS radical scavenging activity

ABTS assay was performed according to standard methods.[19].The stock solution was prepared by mixing equal volumes of 7 mM ABTS solution and 2.45 mM potassium persulfate solution followed by incubation for 12 h at room temperature in dark to yield a dark-colored solution containing ABTS radicals. Working solution was prepared freshly before each assay by mixing of stock solution to 50% methanol for an initial absorbance of about 0.700 (± 0.02) at 745 nm, with temperature control set at 30°C.Free radical scavenging activity was assessed by mixing 300 µl of hemolymph extracts with 3.0 ml of ABTS working standard.The decrease in absorbance was measured exactly 1 min after mixing the solution, the final absorbance was noted up to 6 min. Data for each assay was recorded Ascorbic acid was used as positive controls.The scavenging activity was estimated based on the percentage of ABTS radicals scavenged by the following formula:

% Scavenging = [(A0 − As)/A0] × 100
A0 - absorption of control, AS - absorption of tested solution.

Hydrogen Peroxide Radical Scavenging Assay

The scavenging capacity for hydrogen peroxide was measured according to the standard method.[20] A solution of hydrogen peroxide (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically at 230nm 0.1 ml of various fractions (100-500µg/ml)), ascorbic acid was transferred into the test tubes and their volumes were made up to 0.4 ml with 50 mM phosphate buffer (pH7.4) . After addition of 0.6 ml hydrogen peroxide solution, tubes were vortexed and absorbance of the hydrogen peroxide at 230 nm was determined after 10 min, against a blank. 50 mM phosphate buffer without hydrogen peroxide was used as blank. Hydrogen peroxide scavenging ability was calculated by the formula:

% Scavenging = (1 − Ae/Ao) × 100
where Ao is the absorbance without sample, and Ae is absorbance with sample.

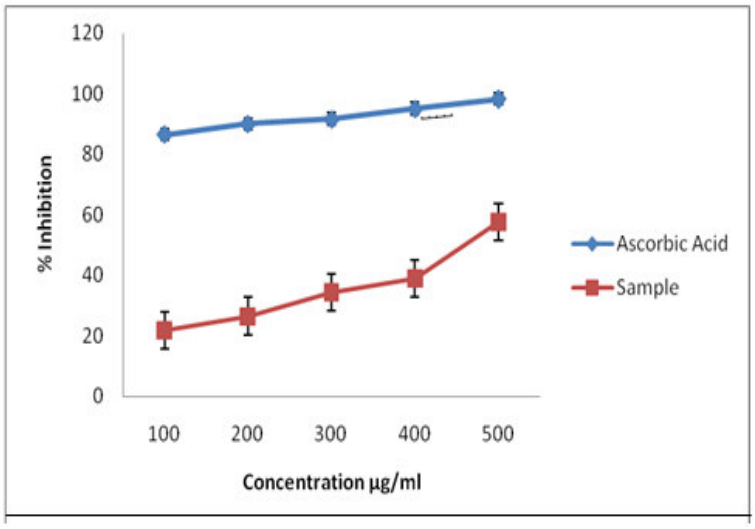
RESULTS

When hemolymph of *Oziotelphusa senex senex* was assayed using different methods, the following data was observed.

DPPH assay

The free radical scavenging activity of protein from crab *Oziotelphusa senex senex* haemolymph was assessed by DPPH assay.The potential decrease in concentration of DPPH radical was due to scavenging property of hemolymph of *Oziotelphusa senex senex* and showed significant free radical scavenging activity of 56.5 % at 500 µg/ml.(Figure 1).

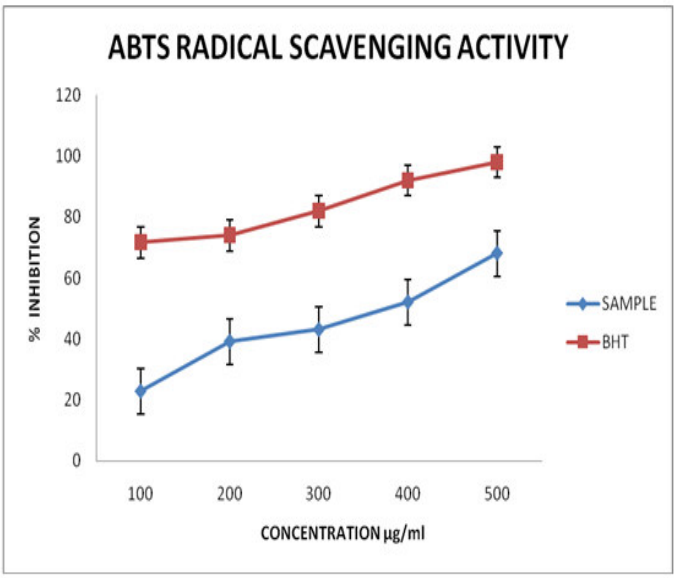
Figure 1
Free radical scavenging effect of the sample by DPPH



ABTS Assay

As depicted in Figure 2, the maximum scavenging ability of the hemolymph extracts from crab *Oziotelphusa senex senex* was 68 % at 500 µg/mL .The graph showed significant decrease in the concentration of ABTS radical due to the scavenging ability of the extract.

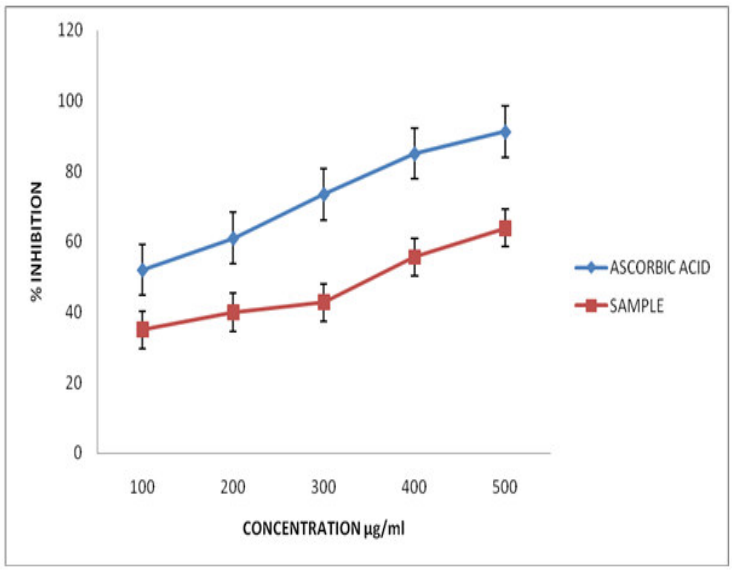
Figure 2
ABTS scavenging effect of the sample



H_2O_2 Scavenging activity

The scavenging effect of hydroxyl radical was investigated and the results were shown in percentage of inhibition (Figure 3). The result showed that the hemolymph of *Oziotelphusa senex senex* had significant hydrogen peroxide scavenging activity of 64 %.

Figure 3
Hydrogen peroxide scavenging effect of the sample



DISCUSSION

The hemolymph of decapods has received considerable attention by a number of crustacean physiologists because of the wide range of variability observed in its constituents. Crustacean haemolymph proteins vary with nutritional state and bioactivity. In the recent years, great attention has been paid to study the bioactivity of natural products due to their potential pharmacological utilization. Crabs are the wonderful resource of bioactive proteins with awide range of antimicrobial and antioxidant properties which is highly supported in the haemolymph study of *Charybdis lucifera*,[21] *Liagore Rubromaculata*[22] and haemolymph of *penaeid shrimp*.[23]Antioxidants protect against oxidative damage of cells.[24]In the present study, haemolymph of *O.senex senex* was investigated for antioxidant activity .The DPPH scavenging activity of the extract was found to be 58% compared with standard Ascorbic acid at 98%.The present result showed that a significant decrease in the concentration of DPPH dical due to scavenging ability of the hemolymph .The present study was compared with the hemolymph of Spider crab *Doclea cravis*,[25] where they reported the scavenging effect of hemolymph extract on the DPPH radical was 90.21 %, 89.13% at two different concentrations of 50 and 100 mg ml. The peroxidase substrate 2,2'-azino-bis(3-ethylbenzthiazoline -6- sulphonic acid) (ABTS), forming a relatively stable radical (ABTS•) upon one-electron

oxidation, has become a popular substrate for estimation of total antioxidant capacity. ABTS assay has showed the antioxidant property of the hemolymph with 68% inhibition and the H_2O_2 scavenging activity of 64% was observed at various concentrations.This shows that hemolymph protein can be a good antioxidant for removing hydrogen peroxide free radicals. Similar results were observed with the haemolymph of some crustaceans using DPPH assay, ABTS and Hydrogen peroxide assay .[26-27] Antioxidant activity has been reported earlier in the haemolymph of the Grapsid crab *Grapsus strigosus,*[28]Spider crab (*Doclea Cravis)*,*Portunus sanguinolentus*,*Callinectus sapidus*,*Paralithodes brevipes* [29.]

CONCLUSION

In the present study, antioxidant activities of the crude haemolymph of *Oziotelphusa senex senex* was investigated.The peptides were found to possess radical scavenging and antioxidant activities, as determined by scavenging effect on the DPPH, ABTS and Hydrogen peroxide .In this study,it can be concluded that the haemolymph of *Oziotelphusa senex senex* was found to be a promising source of highly potent antioxidant agent and can be used as an accessible source of natural antioxidants with consequent health benefits.The antioxidant assays done so far will serve as a baseline data for further studies that may confirm the hypothesis that brachyuran crabs, hemolymph are indeed potential source of novel compounds with biological potential.Future work could focus on the isolation, purification and characterization of the bioactive components. Further purification of the active compounds is necessary in order to identify their chemical nature and to evaluate their potency as novel drug.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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