Sujeetha M *et al*. / *International Journal of Phytopharmacology*. 6(2), 2015, 111-113.

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

ANTIOXIDANT PROPERTY OF SOME EXTRACTS DERIVED FROM THE MUD CRAB, *SCYLLA SERRATA*

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ABSTRACT

Antioxidants play a vital role in various health benefits and control number of health disorders. The natural antioxidants have received attention in the recent years. These antioxidants are abundantly present as bioactive compounds or they can be extracted as bioactive peptides. Marine organisms are also the best resources for identification and extraction of bioactive peptides. The mud crab, *Scylla serrata* which has great economic and commercial values is considered for the present study. This crab contains bioactive peptides that have important biological activities and one such is antioxidant activity. The soluble Chitosan, hemolymph and muscle extracts of the crab, *Scylla serrata* were tested for free radical scavenging activity by DPPH and ABTS assays. The present study has confirmed that the muscle extract of the crab, *Scylla serrata* has maximum antioxidant activity.

Key words: Bioactive peptides, Antioxidants, DPPH assay and ABTS assay.

INTRODUCTION

Marine organisms are rich sources of structurally diverse bioactive compounds with valuable nutraceutical, pharmaceutical and cosmoceuticcal potential (Dai-Hung Ngo *et al.*, 2011). The human body is constantly subjected to physiological imbalances and exposure to extrinsic toxic substances that perturb normal function leading to various health condition (Na Young Yoon *et al.*, 2011). Antioxidative property derived from the natural sources has a positive effect on human health as they can protect the human body against damages by reactive oxygen species (ROS), which attack macromolecules such as membrane lipids, proteins and DNA that lead to many health disorders such as cancer, diabetes mellitus, neuro degenerative and anti inflammatory diseases with severe tissue injuries. Oxidative stress caused by free radicals and reactive oxygen species contributes to oxidation of bio molecules and cellular damage (Zhimei *et al.*, 2007). In recent years, there has been increasing interest in finding natural antioxidant, since they can protect progress of many chronic diseases (Chibuike *et al.*, 2012). Plant and animal food protein sources of antioxidant peptides include seaweeds, pea, soy, fish, quinoa, flaxseed, milk casein, whey and egg (Kajal *et al.*, 2013). Bioactive peptides derived from marine sources proved to have antioxidant and antimicrobial properties (Pitchiah 2013). Dietary consumption of antioxidants can supplement the endogenous enzymatic and non enzymatic antioxidant system against oxidative stress. The radical quenching activities of food antioxidants are due to the ability of the antioxidants to participate in single electron transfer reaction (Na Young Yoon *et al.*, 2011)., thus abundance of peptidic amino acid residues that can transfer electrons to the free radicals at physiological pH can contribute to enhanced anti oxidative property. The antioxidant activity of Chitosan and its derivatives has attracted the most attention. Chitin is the second most abundant biopolymer on earth after cellulose and it is one of the most abundant polysaccharides. In the food industry soluble Chitosan (Rangika *et al.*, 2011) and Chitosan Oligosaccharides have been used as dietary food additives and functional factors for their food beneficial effects as well as drug carriers. In food and pharmaceutical industries, many synthetic commercial

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antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butyhydroquinone (TBHQ) and propyl gallate (PG) have been used to retard the oxidation and peroxidation process. However, the use of these synthetic antioxidants must be under strict regulation due to potential health hazards (Dai-Hung Ngo *et al.,* 2011). Hence, the search for natural antioxidants as safe alternatives is important in the food industry. There has been 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) (Sang-Bo Kim *et al.,* 2014).

The present study aims at the objective of testing the efficacy of some extracts from the mud crab, *Scylla serrata* to scavenge the free radicals. The antioxidant property of some extracts of the mud crab, *Scylla serrata* was investigated *in vitro* by determining 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis[3-ethylbenzthiazoline-6-sulfonic acid] (ABTS+) radical scavenging activities.

MATERIALS AND METHODS

The mud crab, *Scylla serrata* was bought from Kasimedu local market in Chennai. *S. serrata* inhabits burrows in the mangrove habitat and these species are found in the coastal waters of India.

Preparation of the samples

Extraction of Chitin and Chitosan

The crab shells were washed and cleaned thoroughly to remove foreign materials. The shells were grinded to fine particles. Chitin and Chitosan was extracted using the traditional process (Abdulwadud *et al.,* 2013). The demineralization process was done by using hydrochloric acid. The filtrate was soaked in 5% NaOH (w/v) in the ratio of 1:10 and kept for 24 hrs at room temperature for deproteinization. The powdered form of Chitin obtained from the crab shell was deacetylated with 42 .3% NaOH and the filtrate was dried to obtain Chitosan. The soluble Chitosan was used for antioxidant assay.

Hemolymph

The hemolymph from the crab was collected with suitable anticoagulant .The supernatant obtained after centrifuge was used for the *in vitro* assays within 16h. (Samuthirapandian *et al.,* 2010).

Muscle extract

About 2g of body muscle was taken from the crab, *Scylla serrata* and homogenised with animal lysis buffer. After centrifuge, the supernatant was extracted and kept at 20˚C for the *in vitro* assays (Wenjie *et al.,* 2006).

Antioxidant assays

The soluble Chitosan, hemolymph, and muscle extract of crab, *Scylla serrata* were considered for following antioxidant assays.

DPPH assay

The DPPH radical-scavenging activity was measured using standard method of Blois (1958) with slight modification. Two ml of $6x10^{-5}$ M methanolic solution of DPPH was added to 50 µl of the samples (soluble Chitosan, hemolymph and muscle extracts of crab, *Scylla serrata*). The decrease in absorbance at 515 nm was recorded in a spectrophotometer after sixteen minutes of incubation at room temperature. The scavenging effect was represented as the percentage inhibition of DPPH radical by using the formula

$$IP= [(A_C-A_A/A_C)]x 100$$

Where A_C is the absorbance of the control and A_A is the absorbance of the antioxidants at t = 16 mins.

ABTS aasay

The ABTS+ radical scavenging activity was determined using standard method of Re *et al.,* (1999) with slight modification. For the present study the test samples (soluble Chitosan, hemolymph and muscle extracts of crab, *Scylla serrata*) and the ABTS solution was diluted with absolute ethanol to an absorbance of 0. 700 at 734 nm and equilibrated at 30 ^{o} C and reagent blank reading was taken (A_o) After addition of 2.0 ml. of diluted ABTS solution to 20 µl. of samples, the absorbance reading was taken at 30^{o} C exactly 6 mins after initial mixing. The percentage inhibition of absorbance at 734 nm was calculated using the formula:

$$IP= [(A_C-A_A/A_C)]x 100$$

Where A_C is the absorbance of the control and $A_A$is the absorbance of the antioxidant at t = 6 min.

RESULTS AND DISCUSSION

Reactive oxygen species (ROS) including superoxide (O_2-), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO) are generated by respiration and normal metabolism. Their production in the body causes various diseases such as neurodegenerative diseases (e.g., AD), cancer, rheumatoid arthritis, and atherosclerosis through cell or DNA damage (Sang-Bo Kim *et al.,* 2014). There has been an increasing interest in utilization of marine products and novel bioprocessing technologies for isolation of some bioactive substances with antioxidative property from marine food products to be used as functional foods and nutraceutical (Dai-Hung Ngo *et al.,* 2011). The *invitro* assays like DPPH and ABTS assays is the principal mode to measure the radical scavenging activity (Delia *et al.,* 2010). The antioxidant activity (AA) was determined for each sample as shown in Table: 1, the extracts of mud crab *Scylla serrata* showed a potent radical scavenging activity.

Table 1. Determination of Antioxidant activity of extracts of crab, *Scylla serrata*

S. No	Sample	DPPH Assay		ABTS Assay	
		Absorbance at 515 nm	Percentage of Inhibition	Absorbance at 734 nm	Percentage of inhibition
1	Chitosan	0.409	35%	0.424	41%
2	Hemolymph	0.51	19%	0.62	33%
3	Muscle	0.323	49%	0.281	61%
4	Control	0.63	-	0.715	-

The absorbance values obtained by DPPH assay at 515 nm are 0.409, 0.575 and 0.323 for soluble Chitosan, hemolymph and muscle extract respectively. The absorbance values obtained by ABTS assay at 734 nm for soluble Chitosan, hemolymph and muscle extract are 0.424, 0.62 and 0.281 respectively. The absorbance value is inversely proportional to the percentage of antioxidant activity. Therefore the muscle extract of crab, *Scylla serrata* has maximum potential of radical scavenging activity. The formation of peptides and amino acids during hydrolysis of protein extracts of crustacean during fermentation is expected to be responsible for the antioxidant activity (Nakkarike *et al.*, 2008). Thus in the present study the extracts derived from crab, *Scylla serrata* containing low molecular weight proteins is responsible for scavenging free radicals.

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