

Short Communication

Antioxidant activity of horn-eyed ghost crab (*Ocypode brevicornis*) from foreshore estate, southeast coast of India

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In recent decades, the shift from synthetic to natural food antioxidants has fostered interest in finding the sources and the screening of inexpensive raw materials for consumption. Exogenous antioxidants are those which are derived from plants and animal sources through diet. Ghost crab *Ocypode brevicornis* is a species of ghost crab which has a box-shaped carapace and is called the sand scavenger. They are an important biodiversity species native to the Indian Ocean. They are usually found in the intertidal zones. The crab is loaded with high antioxidant properties, which could necessarily replace the expensive synthetic ones. Hence, the objective of the study was to determine the antioxidant potential in the muscle tissues of ghost crabs. The samples were dissected for performing various non-enzymatic antioxidant assays. The data obtained were represented in graphs and table, which show the higher antioxidant activity of DPPH – Radical Scavenging Assay with IC 50 value of 12.76 µg/mL and in Deoxyribose degradation assay with IC 50 value of 15.93 µg/mL. The findings of the study implicate that ghost crabs can be consumed as habitual seafood and play an important role as a bioindicator for human-induced disturbances.

Keywords: Antioxidant activity, Crustacean, Ghost crab, IC 50, Muscle tissues, Seafood

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Introduction

Crustaceans (subphylum Crustacea) are the largest group of organisms belonging to phylum Arthropoda, and they include crabs, shrimps, lobster, barnacles, and woodlice. They show an enormous diversity ranging from small planktonic to giant forms being

omnivorous and few others being cannibalistic, hold an important niche in the environment, contributing a vital role in the food web and thereby improving the complexity and functioning of the ecosystem^{1,2}. Seafood consisting of crustaceans has been recommended for its health-promoting excellence. Amidst them, crabs rank third due to their demand as a high-flavoured delicacy and compatibility with meat along with the nutritional benefits of having high protein content with the addition of vitamins, minerals, etc., and therapeutic properties in curing asthma and chronic fever³⁻⁶.

The biggest invertebrate found along the ocean coast is *Ocypode*, a genus of Ghost crab that is found in large quantities all over the world digging burrows, feeding, and mating in the intertidal zone of sandy beaches. Notably, their bodies resemble boxes with thick, elongated eye stalks, one claw larger than the other and capable of camouflaging to blend with the sand. It is crucial to explain that they play an important role as mesopredators and scavengers in the littoral food web, preying on eggs and young ones of turtles and organic matter that gets washed ashore, provoking their significant purpose as bio-indicator species⁷. Being exposed to various stress and pollution, the immune system of crustaceans has evolved various defence mechanisms by production of antioxidants important in protecting the cells⁸ (Fig. 1).

The cellular defence mechanism contains enzymatic antioxidants that are produced within the body to catalyse the conversion of harmful reactive species into less harmful substances and non-enzymatic antioxidants primarily obtained from the diet that directly scavenge free radicals together, being exclusively gender and seasonally-dependent^{9,10}. Antioxidant reactions are vital for moulting to increase metabolic activity and



Fig. 1 — Ghost crab *Ocypode brevicornis*

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oxygen uptake in the cells. Oxidative stress occurs when the free radicals produced exceed their removal due to oxygen consumption in cells, which results from the accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and leads to structural alterations of DNA, RNA and proteins¹¹⁻¹³. In addition to organism defences, the intake of dietary antioxidants is essential for biological systems^{14,15}.

In foods, antioxidants are supplemented to minimise changes in flavour and nutritional value to make it marketable. The discovery of antioxidants derived from natural sources is considered highly crucial, as it serves as a major substitute for synthetic antioxidants that pose a risk to human health^{16,17}. High doses and long-term intake of synthetic antioxidants cause DNA damage, skin allergies, gastrointestinal problems and also increased the risk of cancer¹⁸⁻²⁰. Seafood is loaded with vital nutrients, such as essential vitamins and minerals, in addition to its superior protein content. One such seafood diet is crab meat, which is preferred less over shrimp and fish due to a lack of knowledge about them. Hence, the current study attempts to investigate the antioxidant properties in the muscle tissues of *Ocypode brevicornis* (Horn-eyed ghost crab), whose presence is vital for the Chennai coast²¹.

Materials and Methods

Sample collection and preparation of tissue homogenate

Ghost crab, *O. brevicornis* was identified based on morphological characters and geographical location^{22,23}. The crab samples were collected from Pattinapakkam beach, also known as Foreshore estate in Chennai, Tamil Nadu, India, along the Bay of Bengal hosting a crowded seafood market in the country and extends up to 437m (1,434 ft) with its coordinates 13°02'N, 80°27'E (Fig. 2). The live

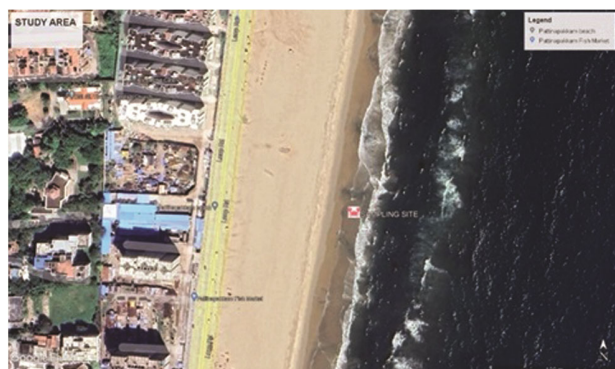


Fig. 2 — Map showing the study area

samples were collected from fishermen by handpicking method along the seashore and transported in ice box to the laboratory for further analysis. A total of 40 crabs were collected, with length and width ranging from 4 to 5 cm and weights of 40 to 80 g, respectively. The crabs were sacrificed by spiking process²⁴. Then, the muscle tissue was dissected and centrifuged using a buffer solution to perform non-enzymatic antioxidant assays.

Antioxidant analysis

DPPH Radical scavenging assay

Modified diphenyl-picrylhydrazyl (DPPH) radical assay was carried out using 95% methanol as blank and ascorbic acid as standard and incubated for 30 minutes in the dark. Using a spectrophotometer, the produced purple colour was measured at 515nm²⁵. The percentage of inhibition was calculated by

$$\% I = (C - E) / C \times 100$$

where % I=% of Inhibition, C=Absorbance of control (methanol), and E=Absorbance of extract (crab muscle tissue).

Nitric oxide radical inhibition assay

Using Griess reagent, the reaction mixture containing the extract, sodium nitroprusside, phosphate buffer, and Dimethyl Sulfoxide (DMSO) was incubated at 25°C for 150 minutes, followed by which sulphanilic acid reagent and N-(1-Naphthyl) ethylene diamine (NEED) was added and allowed to stand in diffused light for 30 minutes. A pink-colored chromophore was formed. The absorbance was measured at 540 nm¹¹.

Ferric-reducing antioxidant power assay (FRAP)

To 10 mg/mL of extract, equal volumes of 0.2 M phosphate buffer and 1% ferricyanide were added and incubated at 50°C for 20 minutes. Post incubation, TCA was added and centrifuged at 1000 rpm for 10 minutes. The upper layer was thoroughly mixed with deionised water and ferric chloride. The absorbance was measured at 700 nm, where a higher absorbance indicates a higher reducing power²⁶.

Superoxide radical scavenging activity by alkaline DMSO method

The superoxide radical is generated by the addition of sodium hydroxide to air-saturated DMSO. The generated superoxide remains stable in solution and reduces nitro blue tetrazolium (NBT) into formazan dye at room temperature, which can be measured at

Table 1 — Non-enzymatic antioxidant activity in muscle tissues of *Ocypode brevicornis*

S. No	Activity*	IC 50 ($\mu\text{g/mL}$) \pm SE	R ²
1	Superoxide Radical Scavenging Activity by Alkaline DMSO	65.24 \pm 0.052	0.9589
2	DPPH – Radical Scavenging Assay	12.76 \pm 0.031	0.9881
3	Nitric Oxide – Radical inhibition Assay	62.64 \pm 0.023	0.9606
4	Ferric Reducing Antioxidant Power Assay (FRAP)	57.14 \pm 0.029	0.9985
5	Vitamin C	63.14 \pm 0.027	0.9871
6	Deoxyribose Degradation Assay	15.93 \pm 0.078	0.9977

*All data in antioxidants analysis were expressed in IC 50 value \pm SE of samples

560 nm. The absorbance in the sample was measured at 560 nm²⁷.

Vitamin C

Vitamin C was determined by adding the sample with Trichloroacetic acid, 2,4- dinitrophenyl hydrazine (DNPH) and thiourea with the concentration of 1 mg/mL and incubated at room temperature for 3 hours. Post incubation, sulfuric acid was added, and the absorbance was measured at 530 nm²⁸.

Deoxyribose degradation assay

To the extract of concentration 10 mg/mL, distilled DMSO was introduced and incubated for 30 minutes at 37°C. After incubation, ice-cold trichloroacetic acid and thiobarbituric acid in 0.25 N HCl were added and kept in a boiling water bath for 30 minutes and the absorbance was measured at 532 nm spectrophotometrically²⁹.

Results and Discussion

Organisms activate their antioxidant defence system using enzymes and proteins that act as enzymatic and non-enzymatic antioxidants. They neutralise the ROS by preventing cellular damage. The non-enzymatic antioxidant assay performed in Ghost Crab showed higher antioxidant activity. The results are expressed in terms of IC 50 value. It is the concentration of the sample necessary to cause 50% inhibition obtained by interpolation from linear regression analysis³⁰. A lower IC50 value is associated with a higher radical scavenging activity³¹. The table shows considerably higher antioxidant activity in DPPH – Radical Scavenging Assay and Deoxyribose Degradation Assay in the ghost crab. The creation of dietary antioxidants from aquatic resources to counteract oxidative stress may greatly benefit from an understanding of the antioxidant activity in ghost crab species (Table 1). Amazingly, several aquatic organisms can survive in most polluted areas because of the antioxidant defence system that gives protection through the response of

scavenging free radicals³². The antioxidant properties of mud crabs have been reported to protect the cells against free radicals¹⁶.

The non-enzymatic antioxidant activity test performed in Ghost crab *Ocypode brevicornis* showed an alkaline DMSO assay test with inhibition of about 65%, nitric oxide radical inhibition assay with inhibition activity of 62% and Vitamin C Ascorbic acid assay showing 63% activity level. The results were promising and showed higher antioxidant properties in them. Because of their biological characteristics, antioxidant peptides from marine products have been employed in diets and for therapeutic purposes³³ (Fig. 3). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) build up as a result of cells using up oxygen, which causes oxidative stress¹².

The majority of biological systems naturally contain oxygen, which exacerbates the harm caused by ionising radiation⁸. Free radicals and hydroxyl radicals can damage biomolecules present in crab cells, including proteins, lipids, DNA, and RNA, by ionising radiation and lipid peroxidation³⁴. DNA damage, particularly double-strand breaks, is difficult for cells to repair. Numerous illnesses may be linked to oxidative stress via protein modification, membrane damage, protein tyrosine nitration, and RNA oxidation¹³, including cancer, atherosclerosis, diabetes, and liver cirrhosis³⁵. Antioxidants are added to food to reduce alterations in flavour, colour, aroma, and nutritional content. They have the ability to shield the body against the damaging effects of free radicals and degenerative illnesses.

Several lipid antioxidants can exert prooxidant effects toward other molecules under certain circumstances, and so antioxidants for food and therapeutic use must be characterised carefully¹⁶. Hence, Non-enzymatic antioxidants are used to serve the purpose. Since oxidation is a key issue influencing food quality, the incorporation of antioxidants into packaging materials has gained popularity. Although

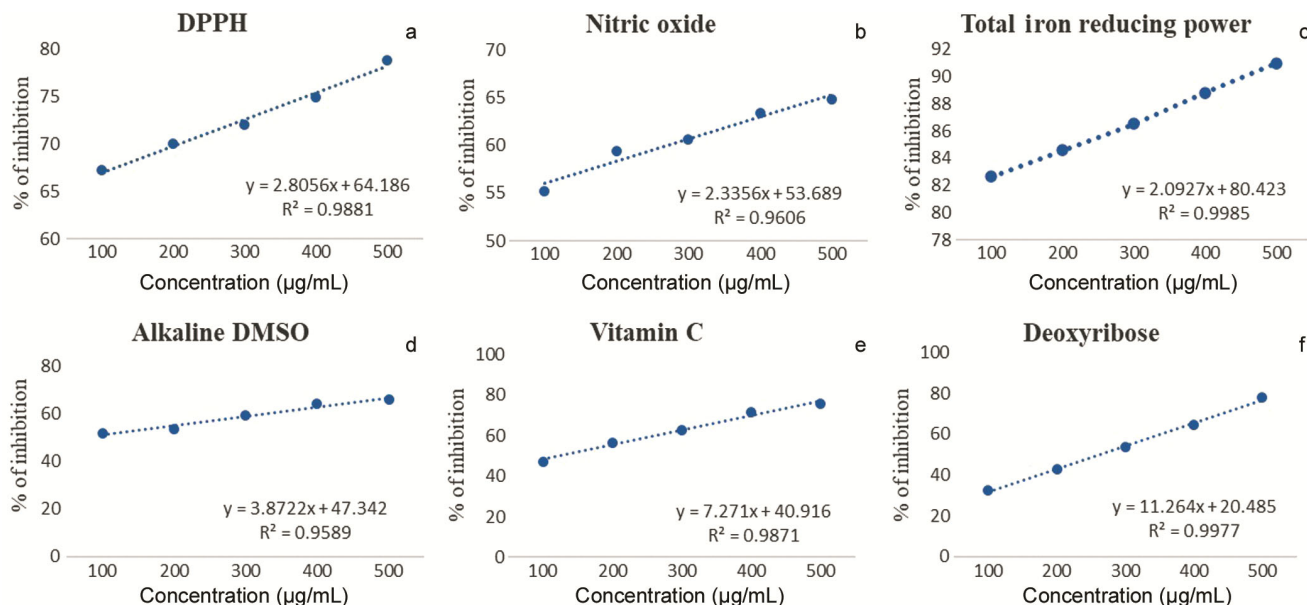


Fig. 3 — Antioxidant activity of Ghost Crab *Ocypode brevicornis* a) DPPH; b) Nitric oxide; c) Total iron reducing power; d) Alkaline DMSO; e) Vitamin C; and f) Deoxyribose

synthetic antioxidants, which are being used in industry are stable and effective, their usage is restricted in many nations because they are not entirely safe for human health³⁶. At the moment, butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT) are the antioxidants most commonly utilised in active packaging¹⁷. Over the years, significant areas of research have been employed on the natural antioxidants to extract bioactive molecules from marine organisms for their applications in nutraceuticals and therapeutics as a substitute for synthetic antioxidants, thereby reducing the ill effects on human health³⁷. However, natural antioxidants are easily available, eco-friendly, and cost-effective as they are a valuable alternative to synthetic ones under regulatory limits³⁸.

In terms of the biology and management of fisheries, constant observation of natural stocks is essential for sustainable ecosystem management. It is believed that these studies ought to be conducted to keep an eye on the region's crab population. The results of this investigation will also serve as a valuable database for upcoming research on this type of crab. The findings indicated that although though the Ghost Crab, *Ocypode brevicornis*, is smaller than expected, it possesses a significant level of antioxidant properties compared to *Scylla olivacea*, which makes it a valuable source of marine bioactive mixtures¹⁶. Thus, more investigation into the extraction and purification of bioactive substances is

required to unlock this marine organism's hidden potential.

Conclusion

Naturally available sources contain countless ingredients that can serve as health-promoting agents. Intake of seafood is one of the nutritionally superior animal protein sources accessible to man. Coastal people are ingrained by consuming seafood, but the importance of ghost crabs goes unnoticed. Nutritional information and antioxidant status of the Ghost crab showed a significant nutrient content and higher antioxidant properties. Ghost crab, as a potent source with a strong antioxidant profile over commonly consumed shrimps and fishes, could provide superior benefits to enhance the body's oxidative stress defence. The study reveals that the crab is an ideal diet loaded with natural antioxidants, placing it as a promising source in the food and pharmaceutical industries. It may also help to prevent any deficiency and illness by enhancing the scavenging activity in the body. Nevertheless, it is highly recommended that more studies be conducted on the bioactive compounds from the purified tissue extract of ghost crabs. The results of this study are expected to be used as a reference to provide insights to maximise the sustainable use of naturally available marine resources and understand the consumption of crab meat as a routine diet to enhance the antioxidant capability of the body.

Conflict of interest

The authors declare no conflict of interest.

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