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Antioxidant Activities of Chitin and Chitosan from Marine Lobster *Panulirus Homarus* from The South East Coast of India

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ABSTRACT:

The present paper discusses the antioxidant potentials of chitin and chitosan from the marine lobster *Panulirus homarus* collected from Indian Ocean at the southern part of India. The antioxidant properties were observed through DPPH, Total polyphenolic compound, total antioxidant assay, Reducing power assay and H_2O_2 assays. The results indicated that Biopolymer chitin and chitosan exhibited highest antioxidant properties. Chitin and chitosan of lobster *Panulirus homarus* are valuable marine biopolymers showed potent antioxidant effects.

Keyword: *Chitin, Chitosan, DPPH, H_2O_2*

INTRODUCTION

Lobsters are active hunters, feeding on a variety of animals, including crab, shellfish, marine worms, starfish, sea urchins and fish. When outside their burrows, juveniles are prey for many fish species. Larval and post-larval lobsters are prone to predation by crabs and finfish species. Lobsters become less vulnerable to predation as they grow, except during molding periods when they shed their hard outer shell [1]. High demand for spiny lobsters in South-East Asian countries has generated considerable interest in capture as well as culture of these species. *Palinurid* lobsters contribute to 40.7% of world catch of lobsters. The world's largest producers of *Palinurid* lobsters are Australia, New Zealand, Cuba, Brazil, South Africa, United States, India and Mexico [2]. Lobster shell derives from a sophisticated structure of chitin and calcium carbonate. N-Acetyl glucosamine units form long chains of chitin twelve to eighteen chitin chains join to form a chitin fibril and the fibrils are covered with a thin layer of proteins and arrange parallel to each other in the epithelium [3]. According to previous studies Lobsters are processed for their meat and contribute to the economy, but leave behind a large volume of shells. Most commonly, crustacean waste is dumped in the sea, soil or landfills. A significant amount of bioactive compounds was recovered from the waste shells of crustaceans as value added products [4].Chitin is extracted from crustacean shell through two major steps. Deproteination takes place at diluted alkaline conditions while demineralization occurs at diluted acidic conditions. Chitin converted into chitosan through the deacetylation at very strong alkaline conditions with high temperature. The conversion of chitin to chitosan can be carried out effectively at milder condition by enzymatic method to remove the acetyl groups from chitin structure [5]. Previous studies states that chitin exits as a naturally partially deacetylated form depending on the source it is very difficult to clearly distinguish between chitin and chitosan. Therefore, the term chitin and chitosan can be used interchangeably. Usually, the term chitosan is used when there is more than a 50% degree of deacetylation [6]. The crab shell extracts of *Liagore .rubromaculata* showed antioxidant property and reduced the free radical damages. The total antioxidant potential of soft shelled crab exhibited maximum antioxidant potential of 49% and minimum effect of 32% was recorded in hard shelled crab. In the reducing power assay the maximum reducing ability of 59% was noticed in soft shelled crab. The lease reducing capability of 42% was recorded in hard shelled crab [7]. DPPH is also considered as a good kinetic model for peroxylradicals. Antioxdant activity is fundamental property and much important for life. Many of the biological functions such as anti-mutagenicity, anti-carcinogenicity and anti-aging, among others originate from this property.Previous reports suggests that protein from *L. rubromaculata* crab and Hemolymph exhibited DPPH scavenging activity. Since the effect of antioxidants on DPPH radical scavenging was due to their hydrogen donating ability [8].

Earlier studies indicated that Chitosan, a biopolymer of glucosamine derived from chitin is a biopolymer with antioxidant properties. It is well known for its cholesterol lowering effect. The chitosan oligosaccharides reduced the plasma glucose level in diabetic animals. Chitin and chitosan are of great importance owing to their relatively high percentage of nitrogen compared to synthetically substituted cellulose [9]. Marine invertebrates rely solely on innate immune mechanisms that include both humoral and cellular responses. Humoral immunity in marine invertebrates is characterized by antimicrobial agents present in the blood cells and plasma. Cellular immunity in marine invertebrates is based on cell defense reactions, including encapsulation, nodule formation, and phagocytosis [10].

MATERIALS AND METHODS

Sample collection

The Spiny lobsters were collected from a local fish landing center at Chinnamuttam, Kanyakumari, and South India and brought to the laboratory. The spiny lobsters were identified as *Panulirus homarus.* The shells were washed and dried under sun light. The samples were weighed and packed into the airtight containers.

Extraction of chitin and chitosan

The lobster shells obtained were washed thoroughly with distilled water and dried in an oven to constant weight at a temperature of 35° C. Then 100g shells of *Panulirus homarus* was taken for the extraction process. The extraction method was based on the standardized protocol [11].

Antioxidant activity:

a) 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The ability to scavenge DPPH radical by chitin and chitosan was estimated by the method [12]. 0.1 mg product in 1ml of acetic acid solution was dissolved. The samples were taken as 20, 40, 60, 80µl was mixed with 2960 µl of DPPH (0.1 Mm DPPH in ethanol) with vigorous shaking. The reaction mixture was stored in the dark at room temperature for 20 min and the absorbance was measured against blank samples at 517 nm. The scavenging activity was calculated by the following equation: Scavenging activity (%): (Absorbance Blank – Absorbance sample/ Absorbance Blank) *100

b) Total polyphenolic compound

Phenolic contents of crude extracts were estimated by the method [13]. Briefly 20, 40, 60, 80µl aliquot of sample (Chitin and chitosan) was mixed with 2.0 ml of 2% Na2CO3 separately and allowed to stand for 2 minutes at room temperature. After incubation, 100 µl of 50% Folin ciocalteou's phenol reagent was added and the reaction mixture was mixed thoroughly and allowed to stand for 30 minutes at the room temperature in the dark. Absorbance of all the sample solutions was measured at 720 nm using spectrophotometer. Polyphenolic contents are expressed as quercetin equivalent per gram.

c) Total antioxidant activity

Total antioxidant activity was measured using the method [14]. 7.45 ml of 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) were mixed together in 250 ml of distilled water and labeled as a Total Antioxidant Capacity (TAC) reagent.20, 40, 60,80µl of extract was dissolved in 3 ml of TAC reagent separately. Blank was maintained with distilled water replacing the TAC reagent. Absorbance of all sample mixtures was measured at 695 nm. Total antioxidant activity is expressed as the number of equivalents of Gallic acid.

d) Reducing power

Reducing power of different crude extract was determined by the method [15]. 20, 40, 60, 80 µl of samples (chitin and chitosan) were mixed with 2.5 ml of phosphate buffer (0.2 M, pH-6.6) and 2.5 ml of potassium ferric cyanide (1%) separately. 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 layer, 2.5 ml solution was mixed with 2.5 ml of distilled water at 0.5 ml of ferric chloride (0.1%). Absorbance of all the solution was measured at 700 nm. Increased absorbance is indicated increased reducing power.

e) Hydrogen peroxide radical scavenging assay

Scavenge hydrogen peroxide was determined from chitin and chitosan. Hydrogen peroxide (10 mM) solution was prepared in the phosphate buffer saline (0.1 M, PH-7.4). 20, 40, 60, 80 µl (0.1mg/1ml) of the extract was rapidly mixed with 2 ml of hydrogen peroxide solution. The absorbance was measured at 230 nm in the UV spectrophotometer (Shimazdu, UV-160) against a blank (without hydrogen peroxide) after 10 minutes of incubation at 37°C. The percentage of scavenging of hydrogen peroxide was calculated using the following formula. % Scavenging (H_2O_2) = $A_0 - A_1 / A_0 * 100$

Where A0-Absorbance of control, A1-Absorbance of sample

RESULTS AND DISCUSSION

The present investigation deals with the studies of antioxidant properties of chitin and chitosan obtained from the marine lobster *Panulirus homarus*. The antioxidant activity of chitin and chitosan extract from marine lobster crude extract were observed using DPPH method. The absorbance was measured at 517 nm. Figure 1 shows the comparative analysis of the marine lobster of chitin and chitosan crude extract using DPPH method. Maximum activity was seen in the chitosan that chitin. Total Phenolic compound method was measured at 720 nm. Maximum activity was seen in chitosan that chitin. Figure 2 shows the comparative analysis of chitin and chitosan of the lobster. Figure 3 shows the comparative analysis of the marine lobster from chitin and chitosan crude extract using Total Antioxidant Assay method. The absorbance was measured at 695 nm. Figure 4 shows the comparative analysis of the marine lobster from chitin and chitosan crude extract using reducing power assay method. The absorbance was measured at 700 nm. Figure 5 shows the comparative analysis of the marine lobster from chitin and chitosan crude extract using $H_2 O_2$ Assay method. The absorbance was measured at 535 nm. Spiny lobsters (Palinuridae) are one of the most commercially important groups of decapod crustaceans that are usually inhabitants of hard substrates associated with coral reefs, rocky shores and boulder-strewn bottoms. *Panulirus homarus* which has a wide distribution in the Indo-West Pacific region is the most dominant species along the southwest and southeast coasts of India.*P. homarus* is having three recognized sub-species. They are *P.-homarus-homarus, P. homarus megasculptus* and *P. homarus rubellus*[16].

Previous studies reported that chitosan from shrimp significantly reduced serum free fatty acids and malondialdehyde concentrations, elevate superoxide dismutases, and display catalase and glutathione peroxidase activities, the latter being the major antioxidant enzymes in the body. This indicates that chitosan regulated the antioxidant enzyme activities and reduced lipid peroxidation. The present investigation showed that the chitin and chitosan in the Lobster *Panulirus homarus* exhibited DPPH scavenging activity. The reducing power ability of the chitin and chitosan greatly depends on the presence of reductones which could have shown antioxidant potential by breaking the free radical chain by donating a hydrogen atom. The result of the present study reveals that the strongest H2O2 scavenging activity was observed for chitosan at various concentrations when compared to scavenger of hydrogen peroxide [17]. Previous studies states that the scavenging activities of chitin and chitosan increased with the from 1 to 2% (w/v). The antioxidant property of chitin was less when compared to the chitosan. The total phenolic content for the crab shell extracted of chitin and chitosan showed the antioxidant activity contents. The maximum activity of (TPC) was found in the marine crab chitin than the chitosan [18]. The present study demonstrated a significant correlation between the total polyphenolic compound activity and the DPPH radical scavenging activity. The reducing power ability of the chitin and chitosan of the lobster *Panulirus homarus* have determination coefficients. Antioxdant activity is fundamental property and much important for life. Free radicals produced by radiation, chemical reactions and several redox reactions of various compounds could contribute to protein oxidation, DNA damage, lipid peroxidation in living tissues and cells. It was also observed that the hydrogen peroxide can cross cell membrane and may oxidize a number of compounds. Previous study determines the crab shell chitin and chitosan act in including antioxidant activity. It is a diffusible free radical which plays as an effective molecule in diverse than boiological systems including antimicrobial and anticancer activities [19].

Earlier studies stated that the reducing power assay indicates the presence of antioxidant constitutents which could reduce the ability. Hence the shrimp shell extracted of chitin and chitosan exhibited the antioxidant acitivity and shown the effect. In the present work it is determined that the extracts of chitin and

chitosan at the different concentration of 20µl, 40µl, 60µl, and 80µl exhibited antioxidant activities. It is confirmed through means of antioxidant assays like DPPH, Total polyphenolic compound, total antioxidant, Reducing power assay and H_2O_2. [20].

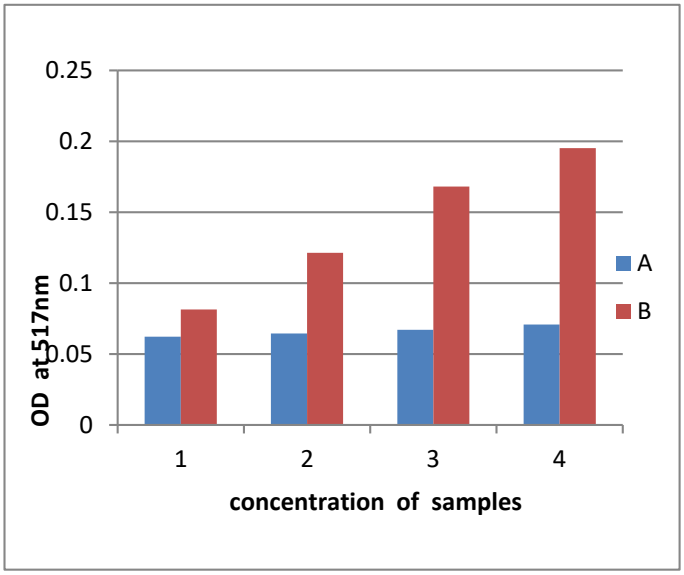

Fig 1: Comparative analysis of Chitin and Chitosan in *Panulirus homarus using* DPPH assay

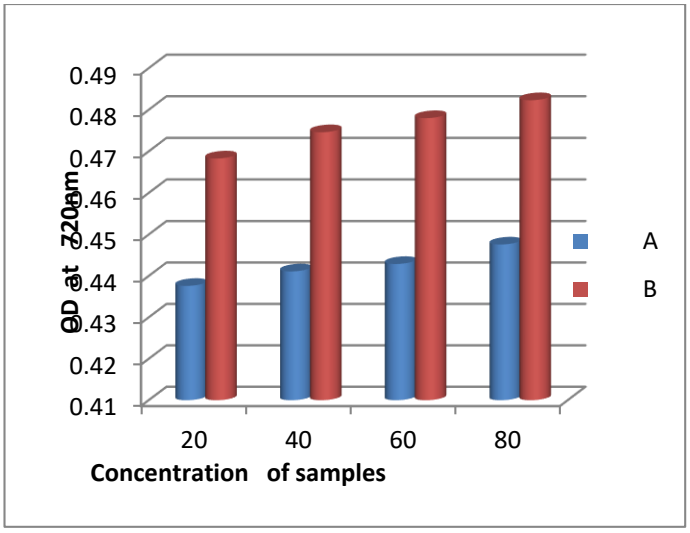

Fig2: Comparative analysis of Total Polyphenolic Compound in chitin and chitosan of Lobster *Panulirus homarus*

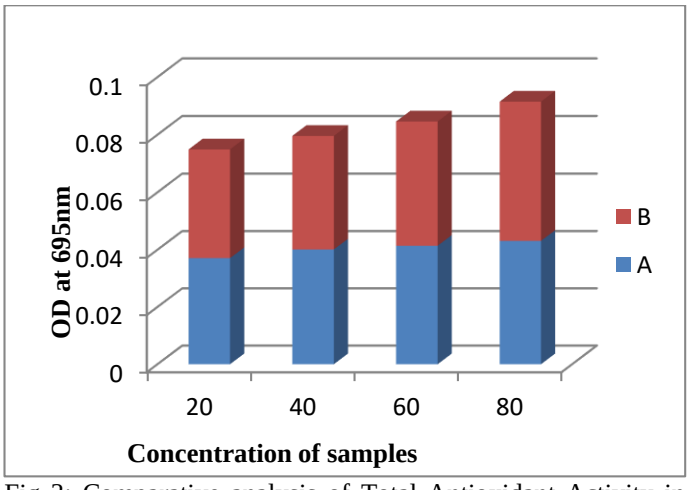

Fig 3: Comparative analysis of Total Antioxidant Activity in chitin and chitosan of Lobster *Panulirus homarus*

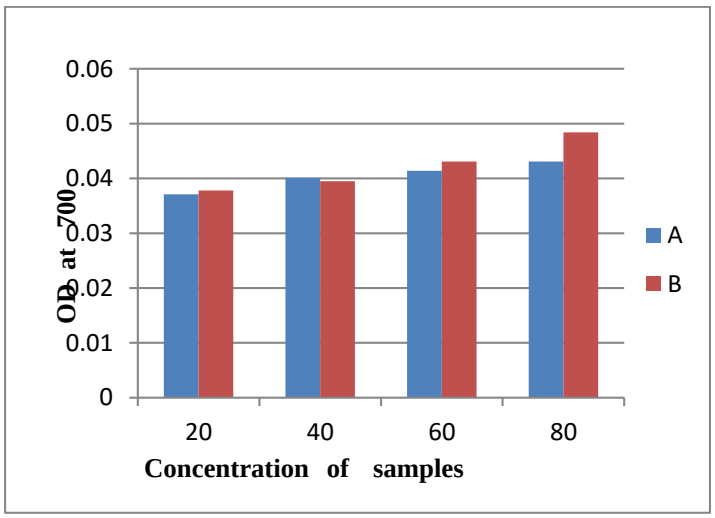

Fig 4: Comparative analysis of Reducing Power in chitin and chitosan of Lobster *Panulirus homarus*

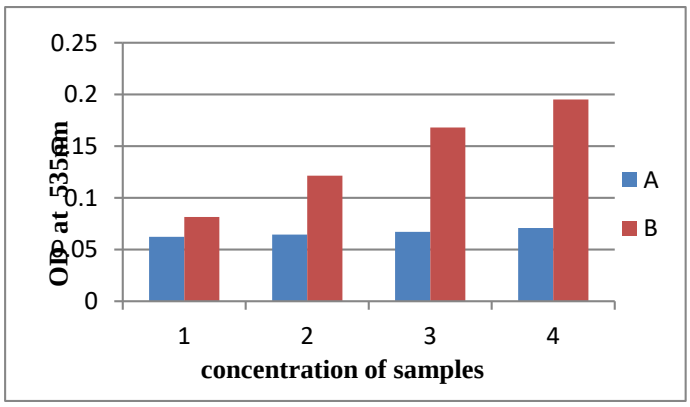

Fig 5: Comparative analysis of H_2O_2 Assay in chitin and chitosan of Lobster *Panulirus homarus*

CONCLUSION

We conclude that the Biopolymers of chitin and chitosan obtained from the Lobster *Panulirus homarus* act as antioxidant agents. They have the potential to be developed as drugs.

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