CIBTech Journal of Zoology ISSN: 2319–3883
Online, International Journal, Available at http://www.cibtech.org/cjz.htm
2023 Vol.12, pp.236-247/Naresh et al.
***Research Article** (Open Access)*

ANTIOXIDANT PROPERTIES OF CHITOSAN SILVER NANOPARTICLES (AgNPs) FROM *PORTUNUS PELAGICUS* AND *PORTUNUS SANGUINOLENTUS* SHELL WASTE

Naresh Mugada*, Pavan Kumar Kommuri and Ramesh Babu Kondamudi
Department of Marine Living Resources, Andhra University, Visakhapatnam 533003, India
**Author for Correspondence*

ABSTRACT
Chitin was extracted from the cuttlebone of *Portunus pelagicus* and *Portunus sanguinolentus* and chitosan was prepared through deacetylation. The percentage of dry weight chitosan recovered from dried crab shell chitin powder from *Portunus pelagicus* and *P. sanguinolentus* was 33.19±1.48 and 43.08±1.47, respectively. The percentage of DPPH radical scavenging activities of Chitosan silver nanoparticles at 100 mg/ml concentration from *Portunus pelagicus* was 81.45±0.85d, *Portunus sanguinolentus* with 79.49±1.00d and for commercial sample 70.68±1.03d. Based on the potential antioxidant activity, scavenging ability on hydroxyl radicals and chelating abilities on ferrous ions, the chitosan from the *Portunus* species may not only be used as a potent natural antioxidant but also as a possible food quality enhancer ingredient in the pharmaceutical industry.

Keywords: *Chitosan, Silver nanoparticles, Antioxidant activity, Pearson's correlation.*

INTRODUCTION
Crustacean shell is composed of 30–40% proteins, 30–50% mineral salts, and 13–42% of chitin occurring in α-, β- and γ-forms. In the processing of shrimps for human consumption, between 40 and 50% of the total mass is waste and 40% of this waste is chitin. The utilization of shellfish waste has thus been able to solve environmental problems, being an alternative waste disposal method (Muthu *et al.,* 2021). To date, the major source of industrial chitin comes from wastes of marine food production, mainly crustacean shells, e.g. shrimp, crab or krill shells. About 40% of the waste is chitin, incrusted with calcium carbonate and astaxanthin, and containing meat and a small amount of lipid residues (Arbia *et al.,* 2013).
On Earth, chitosan is the second most abundant polysaccharide next to cellulose. As a natural renewable resource, chitosan has a number of unique properties such as antimicrobial activity, non-toxicity and biodegradability, which attract scientific and industrial interest in the fields such as biotechnology, pharmaceuticals, wastewater treatment, cosmetics, agriculture, food science and textiles (Li *et al.,* 1997)**.** In recent years, the development of a novel protocol for the synthesis of metallic nanoparticles with appropriate morphologies and sizes has been attracting the attention of researchers in the field of nanotechnology and biotechnology (Fafal *et al.,* 2017). Silver nanoparticles (AgNPs) have been studied for disease management in aquaculture due to their antifungal, antibacterial, and antiviral characteristics. Nonetheless, while such approaches are still in the works, they demonstrate the promise and applicability of AgNPs in the aquaculture business (Camacho-Jiménez *et al.,* 2020)**.** Antioxidants, also known as anti-oxidants or anti-oxidation substances, work by binding to damaging oxidants and reducing their destructive activity. These can also aid in the recovery of cells that have already been damaged. Any atom, collection of atoms, or molecule with one unpaired electron in an outer orbit is referred to as a free radical. Free radicals are produced by both internal and external sources (Kumar *et al.,* 2017). India has long been one of the world's leading producers of marine crustaceans. Apart from freshwater shrimp and mud crabs, the majority of India's crustacean catch is made up entirely of marine species. Andhra Pradesh's landings increased by 34% in 2019 compared to 2018, accounting for 7.2 percent of all landings in India. Crab landings in India averaged 0.32 lakh tonnes per year in 2019-2020 (CMFRI, 2020). Crabs are one of the most diverse groups of crustaceans. Crabs, both fresh and marine, are high in a variety of nutrients that are essential for human health. Crab processing also generates a

lot of liquid and solid waste, which causes disposal and landfill issues. To mitigate the negative environmental effects, these underutilised but commercially valuable discards or by-products must be recycled and reused. Furthermore, crab processing produces a considerable number of by-products and waste (Nanda *et al.,* 2021)**.**

In the present study, an attempt has been made to develop an effective process for the synthesis of Ag-NPs chitosan with amplified antioxidant properties. The physicochemical properties of as-prepared chitosan nanoparticles were analyzed and discussed in detail.

MATERIALS AND METHODS

Sample preparation

Crab shells (*Portunus pelagicus* and *Portunus sanguinolentus*) were obtained from fishing harbour, Visakhapatnam. Samples were kept chilled in ice during transportation to the laboratory. The crab shells were completely separated from the crab waste in the laboratory, cleaned, placed in polyethylene bags and stored at −20° C until used. The crab shells were dried in the oven at 70° C for 24 hours or longer until they were completely dried. The dried shells were ground using a mortar and pestle and sieved. Dried ground shell was placed in glass bottles and stored at room temperature until used. Commercial chitosan (catalogue number 27391) was obtained from the Sisco Research Laboratories Private Limited, India. All chemicals used were analytical grade purchased from Merck.

Extraction of chitosan

The extraction of chitosan from the shells of *Portunus pelagicus* and *Portunus sanguinolentus* includes the following steps.

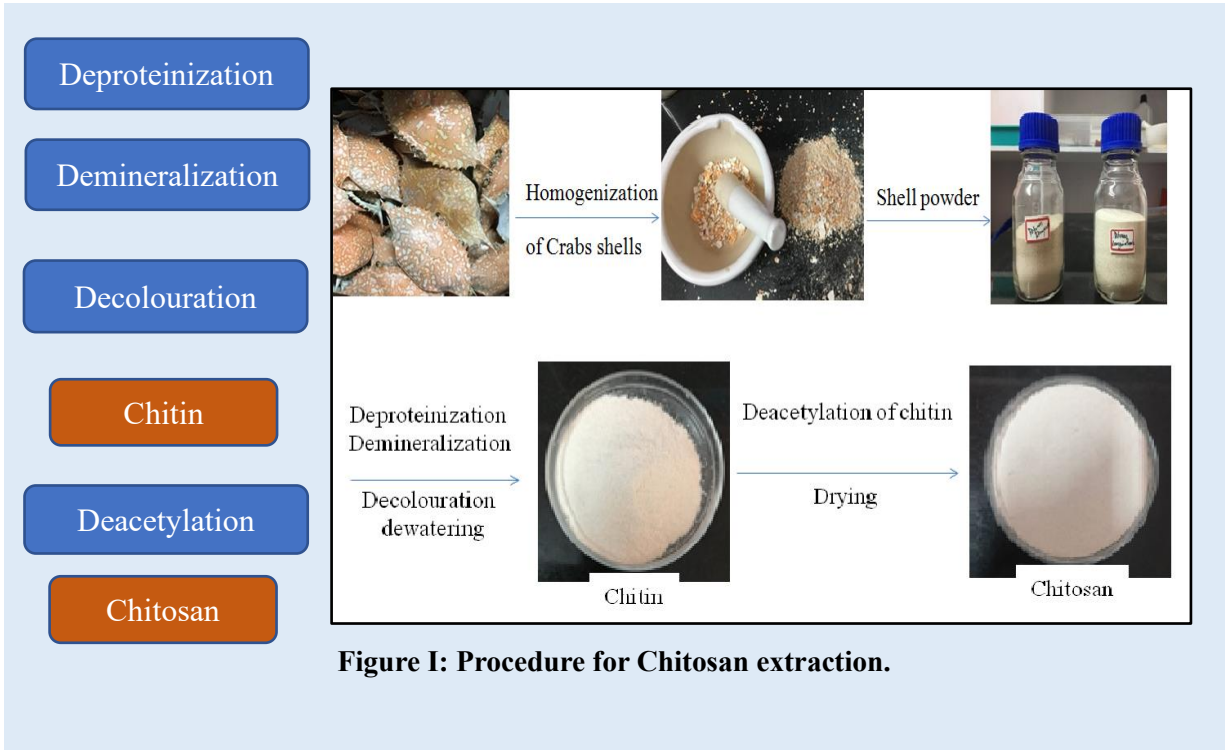

Figure I: Procedure for Chitosan extraction.

Deproteinization

The shells were deproteinized using a high concentration of sodium hydroxide (NaOH) (2M). The dried shells to solution ratio is 1:10. (w:v). The protein was then heated and agitated for 2 hours at 80° Celsius. The mixture was then filtered and rinsed with distilled water for 30 minutes to achieve pH neutrality (pH-7). Finally, we dried shell powder in an oven at 80° Celsius for 12 hours (Shahidi and Synowiecki, 1991).

Demineralization

The demineralization of chitin was carried out in accordance with the approach of Al Sagheer *et al.,* (2009), with insignificant modifications. The deproteinized crab shells were demineralized using 2.5 % (w/v) hydrochloric acid (HCl) at room temperature (20° C) for 6 hours to remove the mineral content, with a ground shell to solution ratio of 1:10 (w:v). The samples were then vacuum-filtered and rinsed with distilled water for 30 minutes until pH neutral (pH-7). The demineralized shells were dried in an oven at 60° C for 24 hours.

Discoloration and dewatering

Decolorizing was accomplished by treating the samples with acetone for 10 minutes, drying them for 2 hours at room temperature, and then removing the resultant residues. To obtain crab chitin, the decolorized shells were washed with distilled water, cleaned, filtered, and dried in an oven at 60° C for 24 hours (Al Sagheer *et al.,* 2009).

Deacetylation of chitin

The chitin was subsequently deacetylated using the Yen *et al.,* (2009) technique. At 105° C for 2 hours, the chitin was treated with 40 % (w/w) aqueous sodium hydroxide (NaOH) with a chitin-to-solution ratio of 1:10 (w:v). To extract chitosan, the chitin was filtered through filter paper and rinsed with deionized water until pH neutral (pH 7). The chitosan was then dried in an oven at 60° C for 24 hours.

Biosynthesis of chitosan silver nanoparticles

The synthesis of chitosan silver nanoparticle was carried out by dissolving, 5 gm of chitosan from the species such as *Portunus pelagicus, Portunus sanguinolentus* and commercial chitosan in 250 ml of 1% acetic acid followed by before boiling for 30 minutes with continual stirring. This was then filtered to obtain a clear solution. Approximately 7.5 mL of freshly made 0.1 M AgNO3 was added to a 1% acetic acid-containing chitosan solution, followed by 100 ml of 1 M NaOH. The solution was stirred for 10 hours at 90° C. The colour of the solution altered from colourless to light yellow and then to yellowish brown, indicating the production of AgNPs (Sanpui *et al.,* 2011).

ANTIOXIDANT ACTIVITY

Assay of DPPH radical scavenging activity

The DPPH scavenging activity of chitosan silver nanoparticles from Portunus pelagicus, Portunus sanguinolentus, and commercial chitosan was measured using the technique by Esmaeili & Sonboli (2010)**.** 0.5 ml of 0.1 mM DPPH ethanol solution and 0.48 ml of buffer were added to the 20 µl of each sample. As a control, buffer and DPPH were utilized without the chitosan silver nanoparticle. The mixture was vortexed rapidly for one minute before incubating at room temperature in the dark for 30 minutes. Following that, the sample's absorbance was measured against a blank using a spectrophotometer at 517 nm. The percentages of scavenging activity were used to express the results. The following equation was used to calculate the capability to scavenge the DPPH radical.

$$\text{DPPH Scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of Test}}{\text{Absorbance of control}} \times 100$$

Assay of ABTS radical scavenging activity

The assay for radical scavenging activity of 2, 2'-azino-bis (3- ethylbenzothiazoline-6-sulfonic acid (ABTS) was adapted from Re. *et al.,* (1999). The stock solution contained ABTS at a concentration of 7 mM and potassium persulfate at a concentration of 2.4 mM. Before use, the reaction mixture was allowed to stand at room temperature for 12-16 hours in the dark. When the ABTS solution was diluted with methanol, the absorbance at 734 nm was 0.70±0.01. 1 ml of chitosan silver nanoparticle extracts were mixed with 1.2 ml of ABTS and 1.8 ml of ethanol. After a 7-minute incubation period, absorbance was measured at 734 nm. The results were reported as a percentage of total scavenging activity.

$$\text{ABTS Scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Assay of Hydrogen Peroxide radical scavenging activity

The ability of chitosan silver nanoparticles to scavenge hydrogen peroxide (H_2O_2) was measured using the method developed by (Patel *et al.,* 2010). In phosphate buffer, a 40 mM solution of H_2O_2 was produced (pH

7.4). The chitosan silver nanoparticle samples were dissolved in 0.1M phosphate buffer (pH 7.4). To 10 µl of each sample, 0.6 ml H_2O_2 was added, and the final volume was brought up to 3 ml with phosphate buffer. The absorbance of H_2O_2 at 230 nm was measured 10 minutes later against a blank solution containing the phosphate buffer but no H_2O_2. After 40 minutes, the absorbance value (at 230 nm) of the reaction mixture was measured. The following formula was used to calculate the proportion of H_2O_2 scavenging by chitosan silver nanoparticles and standard compounds. The results were expressed as a percentage of scavenging activity.

$$\text{Hydrogen peroxide scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of Test}}{\text{Absorbance of control}} \times 100$$

Assay of Superoxide radical Scavenging Activity

The method developed by Sudha *et al.,* (2017) was used to assess the superoxide scavenging activity of test samples and commercial chitosan silver nanoparticles. In separate test tubes, 0.02 ml of 10, 25, 50, 75, and 100 mg/ml concentrated chitosan silver nanoparticles of Portunus pelagicus, Portunus sanguinolentus test samples, and commercial chitosan were taken. A test tube was filled with 0.2 ml of sample, 0.2 ml of EDTA, 0.1 ml of NBT, 0.05 ml of riboflavin, and 2.64 ml of phosphate buffer. Instead of the chitosan silver nanoparticle sample, a control tube was made with DMSO. All tubes were vortexed, and absorbance at 560 nm was recorded at zero time. For 30 minutes, the tubes were lit with a fluorescent bulb. At 560 nm, absorbance was measured once more. The difference in absorbance was used to calculate superoxide scavenging activity. The results were reported as a percentage of total scavenging activity.

$$\text{Superoxide scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Assay of Ferric Reducing Antioxidant Power

The ferric ion reduction ability (FRAP) of chitosan silver nanoparticles was measured using the technique of Benzie & Strain (1996). The FRAP reagent was made by combining 300 mM sodium acetate buffer (pH 3.6), 10.0 mM (tripyridyl triazine) TPTZ solution, and 20.0 mM $FeCl_3.6H_2O$ solution in a volume ratio of 10:1:1. 3 ml of FRAP reagent was added to each 0.5 ml sample, and the reaction mixture was incubated at 37° C for 30 minutes. The rise in absorbance at 593 nm was measured. For the standard calibration graph, fresh $FeSO_4$ working solutions (0.1-1.5mM) were employed. The antioxidant capacity was determined using the linear calibration curve based on the ability of the sample to reduce ferric ions.

Assay of Total antioxidant activity

The total antioxidant capacity (TAC) of chitosan silver nanoparticles from *Portunus pelagicus, Portunus sanguinolentus*, and commercial chitosan was measured spectrophotometrically using the phosphomolybdenum test (Prieto *et al.,* 1999). In sealed test tubes, 0.3 ml of chitosan silver nanoparticle samples in methanol were combined with 2.7 ml phosphomolybdenum reagent (28 mM sodium phosphate and 4 mM ammonium molybdate in 0.6 M sulfuric acid). Incubation lasted 90 minutes in a 95° C water bath. After cooling to room temperature, the absorbance of the solutions was measured against a blank with a spectrophotometer at 695 nm (0.3 ml methanol without chitosan silver nanoparticle extract). The standard calibration graph was created using 0.2-2 ml ascorbic acid.

STATISTICAL ANALYSIS

The results were expressed as mean ± standard deviation of three individual replicates and the data was assessed by one-way analysis of variance (ANOVA) and followed by the Duncan post hatch test of significance and correlation analysis by using Statistical Package for the Social Sciences (SPSS) 28.0 software (IBM/SPSS, Chicago, USA).

RESULTS

Extraction of chitosan

The physicochemical parameters of chitosan isolated from test crab species were compared to commercial chitosan. The results were all expressed as a mean with a standard deviation. To determine the significance of the investigated parameters, one-way analysis of variance (ANOVA) is utilised. In the analysis, a 'P' value of ≤ 0.05 was deemed a significant difference. The resulting chitin was white powder and odourless, whereas

the chitosan was odourless, crystalline, and white in colour. The dry weight of chitosan derived from dried shell chitin powder was used to compute the yield. The percentage of dry weight chitosan recovered from dried crab shell chitin powder from *Portunus pelagicus* and *P. sanguinolentus* was 33.19±1.48 and 43.08±1.47, respectively. In comparison to *Portunus sanguinolentus*, there is a substantial difference in chitosan levels, and yield is lower in *Portunus pelagicus*.

According to our observations and findings, the tested crab carapaces are a good and dependable alternative source of chitin and chitosan. Because of the availability and cheap cost of the source, the high yield obtained justifies the potential of *Portunus pelagicus* and *Portunus sanguinolentus* crab shells chitosan utilisation as an economic source for the synthesis of chitosan on an industrial scale. The yield obtained, however, is further influenced by the loss of sample mass/weight caused by the excessive removal of acetyl groups from the polymer during deacetylation (*i.e.* the conversion of chitin to chitosan) (Sarbon *et al.,* 2015).

ANTIOXIDANT ACTIVITY

DPPH radical scavenging activity

The percentage of DPPH radical scavenging activities of Chitosan silver nanoparticles at 100 mg/ml concentration from *Portunus pelagicus* was 81.45±0.85^{d}, *Portunus sanguinolentus* with 79.49±1.00^{d} and for commercial sample 70.68±1.03^{d}. Half maximal effective concentration (EC_{50}) values for above three samples were 26.09, 27.31 and 44.76 molar units respectively.

Table 1: Inhibition percentages of DPPH radical scavenging activities of tested samples.

S/N	Conc. (mg/ml)	*Portunus pelagicus*	*Portunus sanguinolentus*	Commercial Chitosan
1	10	69.87±1.00^{a}	68.74±1.08^{a}	62.76±1.77^{a}
2	25	76.44±1.14^{b}	74.56±0.68^{b}	65.41±1.22^{b}
3	50	78.64±0.74^{bc}	76.69±0.77^{c}	66.35±0.96^{bc}
4	75	79.44±0.76^{c}	77.47±1.12^{c}	68.13±0.66^{c}
5	100	81.45±0.85^{d}	79.49±1.00^{d}	70.68±1.03^{d}
6	EC_{50}	26.09 molar units	27.31 molar units	4.76 molar units

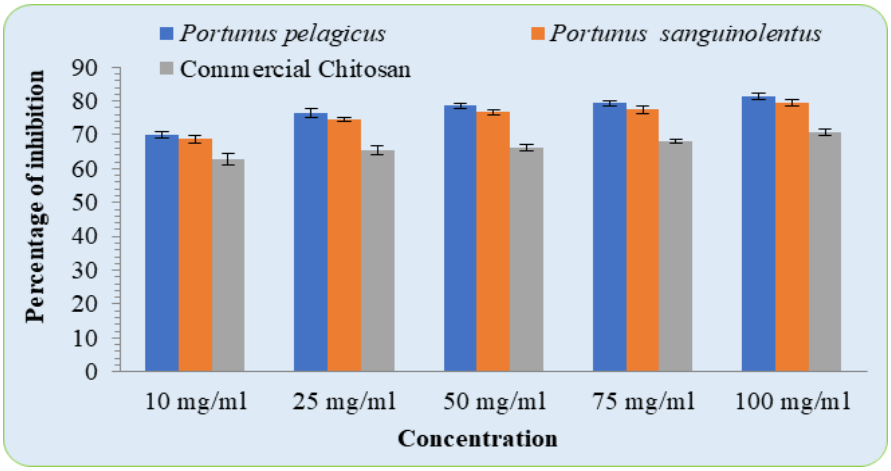

Figure 1: DPPH activities of chitosan silver nanoparticles of *Portunus pelagicus*, *Portunus sanguinolentus* and commercial chitosan.

ABTS radical scavenging activity

The percentage of ABTS radical scavenging activities of Chitosan silver nanoparticles of *Portunus pelagicus, Portunus sanguinolentus* and for commercial chitosan nanoparticles at 100 mg/ml were 93.52±0.59^{d}, 95.7±0.86^{d} and 89.64±1.23^{e} respectively. Half maximal effective concentration (EC_{50}) values for above three samples were 30.41, 30.33 and 54.28 molar units in that order.

Table 2: Inhibition percentages of ABTS radical scavenging activities of tested samples.

S/N	Conc. (mg/ml)	*Portunus pelagicus*	*Portunus sanguinolentus*	Commercial Chitosan
1	10	81.95±1.04^{a}	82.65±0.97^{a}	64.81±1.14^{a}
2	25	85.52±0.90^{b}	88.74±1.18^{b}	69.78±1.02^{b}
3	50	92.69±1.31^{c}	92.6±1.16^{c}	74.59±1.10^{c}
4	75	91.55±0.82^{cd}	91.82±0.98^{c}	80.77±1.05^{d}
5	100	93.52±0.59^{d}	95.7±0.86^{d}	89.64±1.23^{e}
6	EC50	30.41 molar units	30.33 molar units	54.28 molar units

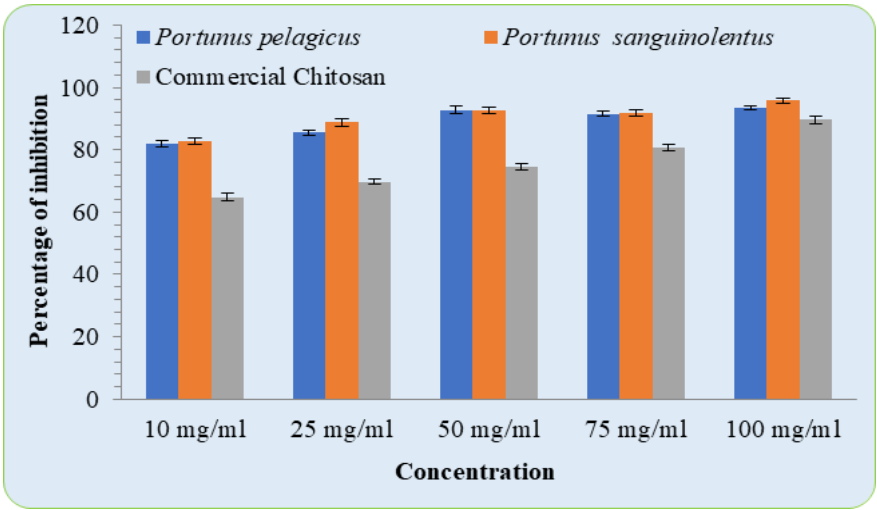

Figure 2: ABTS activities of chitosan silver nanoparticles of *Portunus pelagicus*, *Portunus sanguinolentus* and commercial chitosan.

Hydrogen peroxide radical scavenging activity

Remarkable hydrogen peroxide radical scavenging activities were observed with tested samples. The percentage of activities of Chitosan silver nanoparticles of *Portunus pelagicus*, *Portunus sanguinolentus* and for commercial nanoparticles at 100 mg/ml were 98.43±0.61c, 98.4±0.55b and 90.67±1c respectively. 31.10, 37.49, and 23.64 molar units were half maximal effective concentration (EC_{50}) values for above three samples in that order.

Table 3: Inhibition percentages of Hydrogen peroxide radical scavenging activities.

S/N	Conc. (mg/ml)	*Portunus pelagicus*	*Portunus sanguinolentus*	Commercial Chitosan
1	10	93.06±0.97^{a}	95.6±1.08^{a}	81.23±1.15^{a}
2	25	95.36±0.45^{b}	96.3±0.9^{a}	87.4±2.15^{b}
3	50	97.26±0.41^{c}	96.5±1.05^{a}	87.9±2.76^{bc}
4	75	98.26±0.66^{c}	98.1±0.65^{b}	90±2.11^{c}
5	100	98.43±0.61^{c}	98.4±0.55^{b}	90.67±1^{c}
6	EC50	31.10 molar units	37.49 molar units	23.64 molar units

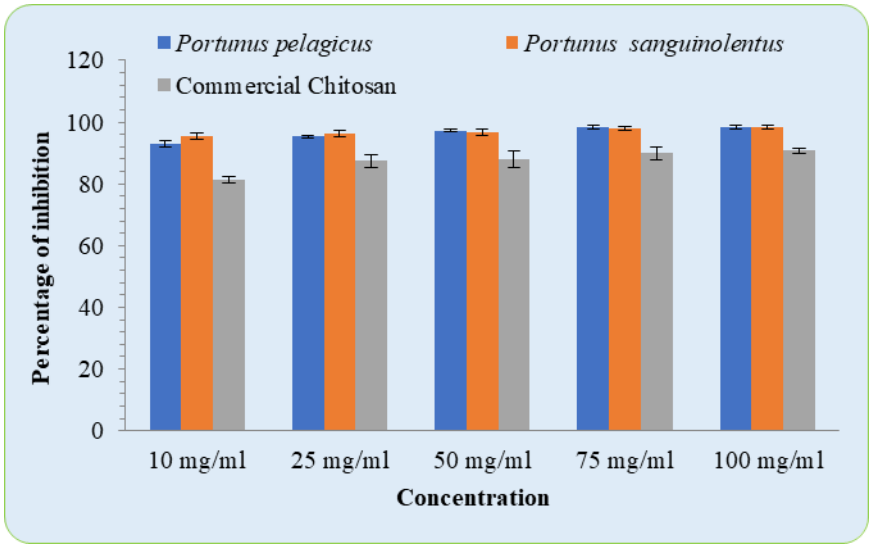

Figure 3: Hydrogen peroxide radical scavenging activities of chitosan silver nanoparticles of *Portunus pelagicus, Portunus sanguinolentus* and commercial chitosan.

Superoxide radical scavenging activity

The percentage of scavenging activities at 100 mg/ml and half maximal effective concentration (EC_{50}) values of chitosan silver nanoparticles of *Portunus pelagicus* were 67.62±1.2e and 48.73 molar units, for *Portunus sanguinolentus* were 72.69±1.86e and 54.91 molar units and in favor of commercial chitosan silver nanoparticles were 61.71±1.05e and 40.57 molar units precedingly.

Table 4: Inhibition percentages of Superoxide radical scavenging activities.

S/N	Conc. (mg/ml)	*Portunus pelagicus*	*Portunus sanguinolentus*	Commercial Chitosan
1	10	50.72±1.00^{a}	55.61±0.82^{a}	32.12±1.11^{a}
2	25	53.57±1.04^{b}	57.98±1.15^{b}	40.51±0.99^{b}
3	50	58.88±0.98^{c}	61.84±0.94^{c}	48.37±1.98^{c}
4	75	63.65±0.86^{d}	69.8±1.16^{d}	57.04±1.67^{d}
5	100	67.62±1.2^{e}	72.69±1.86^{e}	61.71±1.05^{e}
6	EC50	48.73 molar units	54.91 molar units	40.57 molar units

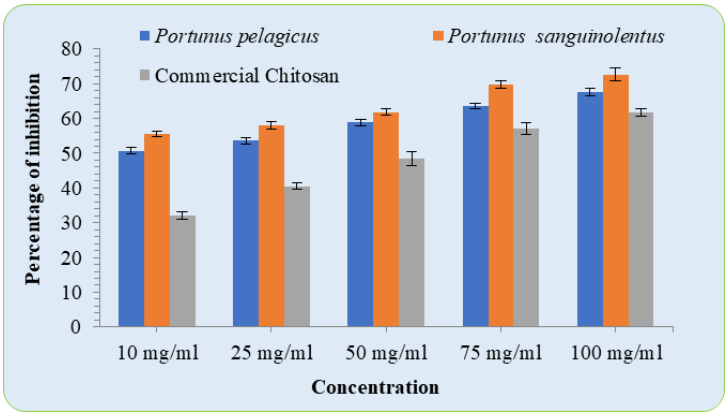

Figure 4: Superoxide radical scavenging activities of chitosan silver nanoparticles of *Portunus pelagicus, Portunus sanguinolentus* and commercial chitosan.

FRAP radical scavenging activity

In favor of ferric reducing ability power (FRAP), the percentage of activities at 100 mg/ml the outcome observed as 95.92±0.98b, 95.16±1.53c and 92.31±0.9d for chitosan silver nanoparticles of *Portunus pelagicus, Portunus sanguinolentus* and commercial chitosan respectively, in the same order the half maximal effective concentration (EC_{50}) values were 38.51, 30.89 and 37.39 molar units.

Table 5: Inhibition percentages of FRAP radical scavenging activities.

S/N	Conc. (mg/ml)	*Portunus pelagicus*	*Portunus sanguinolentus*	Commercial Chitosan
1	10	87.78±0.94^{a}	87.14±1.53^{a}	83.38±1.28^{a}
2	25	88.32±0.76^{a}	90.52±0.79^{b}	86.16±0.73^{b}
3	50	94.58±0.90^{b}	92.77±1.98^{bc}	89.18±1.31^{c}
4	75	94.63±1.39^{b}	94.39±1.55^{c}	90.29±1.43^{cd}
5	100	95.92±0.98^{b}	95.16±1.53^{c}	92.31±0.9^{d}
6	EC50	38.51 molar units	30.89 molar units	37.39 molar units

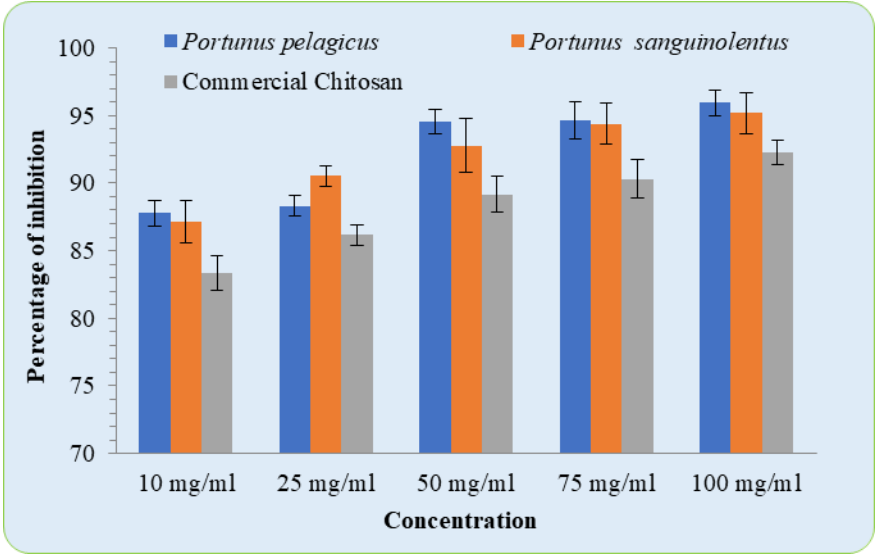

Figure 5: FRAP activities of chitosan silver nanoparticles of *Portunus pelagicus*, *Portunus sanguinolentus* and commercial chitosan.

Total antioxidant radical scavenging activity

Total antioxidant activities of the samples were estimated with the help of ascorbic acid calibration cure. All the end results were expressed in percentage of inhibition by converting ascorbic equivalents. The percentage of activities at 100 mg/ml, chitosan silver nanoparticles of *Portunus pelagicus, Portunus sanguinolentus* and commercial chitosan were 96.56±1.29e, 86.16±0.48e and 92.30±0.90d respectively. The half maximal effective concentration (EC_{50}) values were 32.89, 37.58 and 37.3 molar units in that order.

Table 6: Inhibition percentages of Total antioxidant radical scavenging activity.

S/N	Conc. (mg/ml)	*Portunus pelagicus*	*Portunus sanguinolentus*	Commercial Chitosan
1	10	51.28±1.09^{a}	45.12±0.40^{a}	83.37±1.28^{a}
2	25	71.10±0.78^{b}	51.07±0.87^{b}	86.16±0.73^{b}
3	50	78.44±1.78^{c}	67.33±0.74^{c}	89.18±1.30^{c}
4	75	91.00±0.92^{d}	75.72±0.54^{d}	90.29±1.43^{c}
5	100	96.56±1.29^{e}	86.16±0.48^{e}	92.30±0.90^{d}
6	EC50	32.89 molar units	37.58 molar units	37.3 molar units

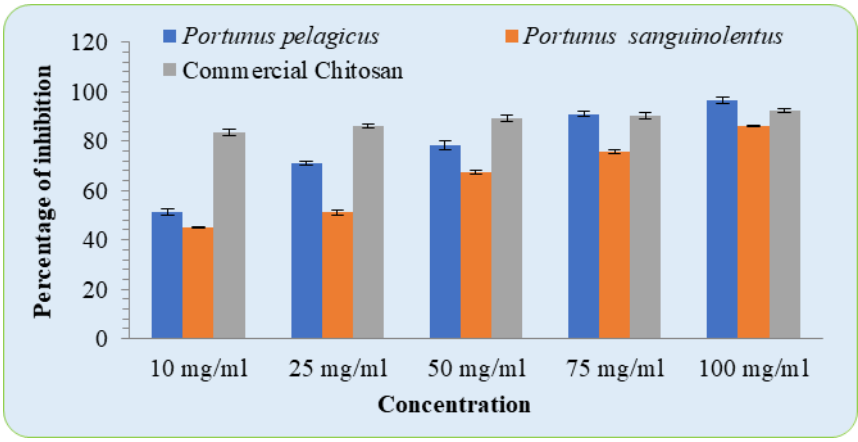

Figure 6*:* Total antioxidant radical scavenging activity of chitosan silver nanoparticles of *Portunus pelagicus*, *Portunus sanguinolentus* and commercial chitosan.

DISCUSSION

The foremost part of the prospective study is the extraction and characterisation of chitosan from Portunus pelagicus and Portunus sanguinolentus crab shells. Chitosan extraction necessitates strong chemical treatments. The majority of the crab shell is polysaccharide chitin, with just trace amounts of proteins and minerals. Deproteinization removes proteins, whereas demineralization removes carbon and other salts. Based on the increased yield of pure quality chitosan, the current method of chitosan extraction outperforms other available methods. The procedures of deproteinization and demineralization were done twice. This contributes to a higher production of chitin from the shells. The final deacetylation of chitin at room temperature for two days resulted in a longer reaction time and a higher yield of chitosan.

Santos *et al*., (2020) rediscovered that chitosan is a cationic polymer derived from the deacetylation of chitin, which is prevalent in crustacean, insect, arthropod exoskeletons, and mollusc exoskeletons. The chemical extraction method for extracting chitin includes the phases of deproteinization, demineralization, and discolouration. Chitin must be deacetylated in order to produce chitosan.

Anand Kumar Keshari *et al.,* (2020) disclosed that the DPPH, Hydrogen peroxide, hydroxyl radicals, superoxide scavenging methods confirmed the silver nanoparticles has antioxidant, hydrogen peroxide, hydroxyl radicals and superoxide scavenging activities. These properties of silver nanoparticles occur due to the presence of functional groups on the surface of silver nanoparticles.

Studies in humans on Chi-Ag-NPs are very sparse, previously Dara *et al.,* (2020) worked out on Chi-Ag-NPs composites, which exhibited antioxidant properties as exposed by radical scavenging and reducing power assays. The DPPH solution which has purple colour turns to yellow on addition of Chi-Ag-NPs, which specifies the scavenging capability of free radicals and existence of antioxidant activity. Furthermore, these composites have ability to reduce the Fe3+ to ferrous form. This Fe2+ complex can be examined by determining the Perl's Prussian blue at 700 nm. The DPPH and FRAP activities of the Chi-Ag-NPs was concentration dependent. These activities increased with increase in concentration.

The ABTS method is based on generating a cation radical ABTS•+ formed by emitting one electron from the nitrogen atom. The use of ABTS•+ radical has the advantage over the DPPH radical as it can be used in both aqueous and organic media. ABTS method is also useful in studying the effect of pH on the antioxidant activity of various compounds (Bedlovičová *et al.,* 2020). Our results clearly and strongly disclose that the chi-Ag-NPs had strong antioxidant capacity, this can be confirmed by the outcomes of the different methods applied for the evaluation and along with the preceding statements of previous research.

The antioxidant potential of chitosan has yet to be fully realised; there has been little research in the area of enhancing chitosan's antioxidant capacity through nanoparticles, and a fundamental understanding of chitosan's antioxidant activity is severely missing. Furthermore, the antioxidant activity of chitosan is demonstrated by its scavenging ability on DPPH, ABTS, Hydrogen peroxide, and superoxide radicals, as

well as its chelating ability on ferrous ions and overall antioxidant activity. It is obvious from the findings of this study that chitosan derived from the crab shells of *Portunus pelagicus* and *Portunus sanguinolentus* could be employed as another potential natural source of antioxidant, food supplement, or medicinal ingredient.

With increasing concentrations, the antioxidant activities of the extracted chitosan from *Portunus pelagicus* and *Portunus sanguinolentus* increased. When compared to commercial chitosan, the extracted chitosan had a high hydrogen peroxide radical scavenging activity but a low superoxide scavenging activity. In scavenging H_2O_2 from *Portunus pelagicus*, the produced AgNPs had a robust antioxidant activity of about 98.43%. Chitosan silver nanoparticles from *Portunus pelagicus* and *Portunus sanguinolentus* crabs had nearly identical hydrogen peroxide radical scavenging capabilities, however commercial nanoparticles silver nanoparticles were superior to the other investigated samples based on EC_{50} values. *Portunus sanguinolentus* Chitosan silver nanoparticles have higher ABTS radical scavenging activity than commercial nanoparticles.

Chitosan silver nanoparticles from *Portunus pelagicus* and *Portunus sanguinolentus* crabs had nearly identical hydrogen peroxide radical scavenging capabilities, however commercial nanoparticles silver nanoparticles were superior to other evaluated materials based on EC_{50} values. The results were backed up by the fact that chitosan silver nanoparticles from *Portunus pelagicus* had significant FRAP activity, while chitosan silver nanoparticles from *Portunus sanguinolentus* had the highest EC_{50} of the three samples. Three samples had half maximal effective concentrations (EC_{50}) of Total antioxidant activity of 32.89, 37.58, and 37.3 molar units, respectively. The results showed that a *Portunus pelagicus* chitosan silver nanoparticle was the best test sample. Duncan's multiple range test, which yields significance levels for the difference between any pair of means, regardless of whether a significant F occurred from a first analysis of variance, was used to evaluate all of the data. The best way to describe linear relationships is to utilize Pearson correlation.

As a result, the produced nanoparticles could be used as free radical scavengers in the treatment of a variety of ailments. Furthermore, AgNPs may be advantageous in the development of newer and more potent antioxidants. From these findings, we may deduce that nanoparticle manufacturing could be a key to solving our many health problems.

CONCLUSION

In the future, trash disposal will have to meet increasingly stringent environmental regulations, necessitating the recovery of usable byproducts from garbage. Integral utilisation of renewable resources is undeniably a laudable goal, and new reclamation technologies are on the way. To address some of the difficulties of pollution and disposal costs, there is great potential for transforming waste rubbish into value-added products. Chitosan is becoming increasingly popular, and the potential of recovering this valuable resource from discarded marine shells is a fascinating issue in and of itself. Chitosan-based nanotechnologies are undeniably popular and important in today's world. The majority of current research efforts are concentrated in the biomedical field, with culinary applications garnering very little attention.

ACKNOWLEDGEMENTS

The authors acknowledge the financially supported by University Grant Commission-Rajiv Gandhi National Fellowship, New Delhi, India. The authors wish to thank the Department of Marine living Resources, College of Science and Technology, Andhra University, Visakhapatnam for providing the facilities to carry out this work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Muthu M, Gopal J, Chun S, Devadoss AJ, Hasan N, Sivanesan I (2021). Crustacean waste-derived chitosan: Antioxidant properties and future perspective. *Antioxidants*, **10**(2) 228.

Arbia W, Arbia L, Adour L, Amrane A (2013). Chitin extraction from crustacean shells using biological methods–a review. *Food Technology and Biotechnology*, **51**(1) 12-25.

Li QT, Dun ET, Grandmaison EW, Goosen MFA (1997). Applications of Chitin and Chitosan, in Applications and properties of chitosan, M.F.A. Goosen, *Editor. CRC Press.*

Fafal TU, Taştan PE, Tüzün BS, Ozyazici M, Kivcak B (2017). Synthesis, characterization and studies on antioxidant activity of silver nanoparticles using *Asphodelus aestivus* Brot. aerial part extract. *South African Journal of Botany*, **112** 346-53.

Camacho-Jiménez L, Álvarez-Sánchez AR, Mejía-Ruíz CH (2020). Silver nanoparticles (AgNPs) as antimicrobials in marine shrimp farming: A review. *Aquaculture Reports*, **18** 100512.

Kumar S, Sharma S, Vasudeva N (2017). Review on antioxidants and evaluation procedures. *Chinese Journal of Integrative Medicine*, **6** 1-2.

CMFRI (2020). Marine Fish Landings in India, 2019.

Nanda PK, Das AK, Dandapat P, Dhar P, Bandyopadhyay S, Dib AL, Lorenzo JM, Gagaoua M (2021). Nutritional aspects, flavour profile and health benefits of crab meat based novel food products and valorisation of processing waste to wealth: A review. *Trends in Food Science and Technology*, **112** 252-67.

Shahidi F, Synowiecki J (1991). Isolation and characterization of nutrients and value-added products from snow crab (*Chionoecetes opilio*) and shrimp (*Pandalus borealis*) processing discards. *Journal of Agricultural and Food Chemistry*, **39**(8) 1527-32.

Al Sagheer FA, Al-Sughayer MA, Muslim S, Elsabee MZ (2009). Extraction and characterization of chitin and chitosan from marine sources in Arabian Gulf. *Carbohydrate Polymers*, **77**(2) 410-9.

Yen MT, Yang JH, Mau JL (2009). Physicochemical characterization of chitin and chitosan from crab shells. *Carbohydrate Polymers*, **75**(1) 15-21.

Sanpui P, Chattopadhyay A, Ghosh SS (2011). Induction of apoptosis in cancer cells at low silver nanoparticle concentrations using chitosan nanocarrier. *ACS Applied Materials and Interfaces,* **3**(2) 218-28.

Esmaeili MA, Sonboli A (2010). Antioxidant, free radical scavenging activities of Salvia brachyantha and its protective effect against oxidative cardiac cell injury. *Food and Chemical Toxicology,* **48**(3) 846-53.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine,* **26**(9-10) 1231-7.

Patel A, Patel A, Patel NM (2010). Determination of polyphenols and free radical scavenging activity of *Tephrosia purpurea* linn leaves (Leguminosae). *Pharmacognosy Research*, **2**(3) 152.

Sudha A, Jeyakanthan J, Srinivasan P (2017). Green synthesis of silver nanoparticles using *Lippia nodiflora* aerial extract and evaluation of their antioxidant, antibacterial and cytotoxic effects. *Resource-Efficient Technologies*, **3**(4) 506-15.

Benzie IF, Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, **239**(1) 70-6.

Prieto P, Pineda M, Aguilar M (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, **269**(2) 337-41.

Sarbon NM, Sandanamsamy S, Kamaruzaman SF, Ahmad F (2015). Chitosan extracted from mud crab (*Scylla olivicea*) shells: physicochemical and antioxidant properties. Journal of Food Science and Technology, **52** 4266-75.

Santos VP, Marques NS, Maia PC, Lima MA, Franco LD, Campos-Takaki GM (2020). Seafood waste as attractive source of chitin and chitosan production and their applications. *International Journal of Molecular Sciences*, **21**(12) 4290.

Keshari AK, Srivastava R, Singh P, Yadav VB, Nath G (2020). Antioxidant and antibacterial activity of silver nanoparticles synthesized by *Cestrum nocturnum*. *Journal of Ayurveda and Integrative Medicine*, **11**(1) 37-44.

Bedlovičová Z, Strapáč I, Baláž M, Salayová A (2020). A brief overview on antioxidant activity determination of silver nanoparticles. *Molecules*, **25**(14) 3191.