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Chitosan extracted from *Portunus sanguinolentus* (three-spot swimming crab) shells: its physico-chemical and biological potentials

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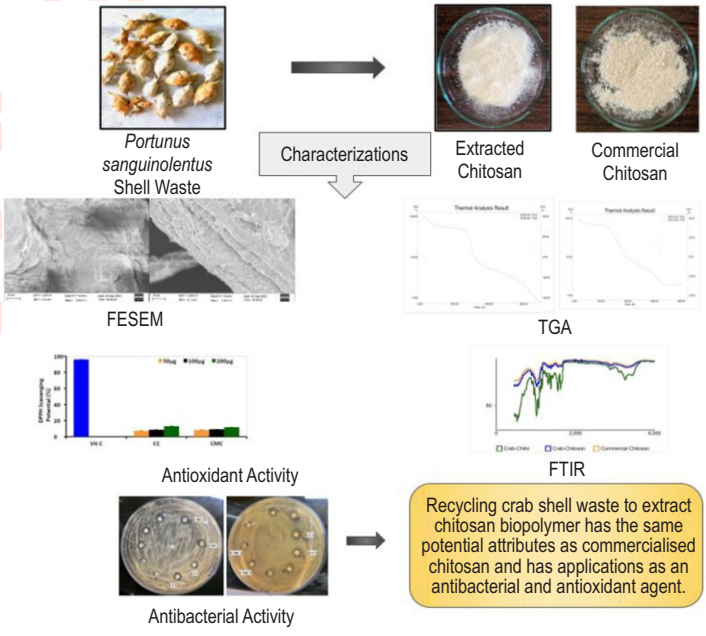
Abstract

Aim: To summarize the extraction, physio-chemical, antibacterial and antioxidant properties of extracted chitosan from the exoskeleton of three-spot swimming crab, *Portunus sanguinolentus* as compared to commercial chitosan.

Methodology: Chitosan biopolymer was extracted through demineralisation, deproteinization and deacetylation. The physico-chemical characterization of the extracted chitosan was carried out using Fourier Transforms Infrared Spectroscopy (FTIR), Field Emission Scanning Electron Microscopy (FESEM), X-Ray Diffraction (XRD), Thermogravimetric analysis (TGA) and Colorimetric analysis. Antibacterial activity using different concentrations of chitosan against *Escherichia coli* and *Aeromonas hydrophila* were tested by disc-diffusion method, while antioxidant activity was estimated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and H_2O_2 scavenging assay.



Results: Crab chitosan (CC) showed the maximal zone of inhibition (17.00±0.50 and 15.16±0.577 mm), while commercial chitosan (CMC) showed 12.67±0.577 and 14.17±0.288 mm) against *E. coli* and *A. hydrophila* respectively. The scavenging potential of extracted chitosan ranged from 14 to 17% at different concentrations (0.05 to 0.2 mg ml^{-1}). Crab chitosan efficiency to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals varied from 7.2 to 12.98% at different concentrations (0.05 to 0.2 mg ml^{-1}).

Interpretation: Overall, the extracted chitosan exhibited properties to commercial chitosan, suggesting that it could be utilized as a dietary supplement in the fishery sector to protect against bacterial infections. This circular economy approach could significantly benefit sustainable waste management and environmental health.

Key words: Antibacterial, Biopolymer, Chitosan, Crab, *Portunus sanguinolentus*

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Introduction

Decapod waste denotes the residual materials that are generated during manufacturing or consumption of crustaceans, primarily because of technological or commercial limitations. Without appropriate management, such waste poses environmental risks. As both population expansion and waste generation surge, estimates indicate that the seafood processing industry alone produces 6–8 million tonnes of shell waste each year (Chakravarty and Edwards, 2022). Unfortunately, recycling of decapod waste is not a common practice and most of it is dumped without any prior processing. However, decapod waste can be a valuable source of raw materials for the production of biocompounds. The annual production of crustacean waste is in the millions of tonnes, highlighting the need for effective processing and management methods that produce high-quality byproducts while minimizing negative environmental impacts. A study by Santos *et al.* (2020) demonstrated the potential of crustacean waste for the production of valuable products, further emphasizing the importance of sustainable management of these resources. Chitin, the precursor of chitosan and the second most prevalent natural biomaterial on Earth, is primarily derived from crustaceans' exoskeletons, as well as from fungi to a limited extent (Jiménez-Gómez and Cecilia, 2020).

The cuticles of various crustaceans, primarily crabs and shrimp, are the primary sources of raw material for chitin synthesis (Mohan *et al.,* 2019). Crab shells are particularly advantageous as a potential material with significant economic benefits since they contain proteins, minerals, and chitin that can be deacetylated to synthesize chitosan. Chitin is a polysaccharide with linear structure consisting of a dual unit of monomer, N-acetyl-2-amino-2-deoxy-d-glucose (N-acetyl-d-glucosamine) and (2-amino-2-deoxy-d-glucose) d-glucosamine which are joined together by β-(1–4) glycosidic linkage (Xavier and Chattopadhyay, 2023). Since chitin deacetylates into chitosan, there is a difference in composition between the two-monomer units primarily responsible for the variations present between chitin and chitosan. In contrast to chitin, which has a higher proportion of N-acetyl-2-amino-2-deoxy-d-glucose monomers, chitosan has a higher concentration of 2-amino-2-deoxy-d-glucose monomers in its primary structure. Because of this difference in composition, chitosan is soluble in aqueous acidic medium as the amino groups are protonated, but chitin is less soluble in acidic solvents (Peter *et al.,* 2021).

By modifying several functional groups mainly including the amino, hydroxyl, and/or acetyl groups, various derivatives with different properties can be synthesized. These properties include biodegradability, biocompatibility, solubility in mild acidic medium, non-toxicity, non-carcinogenicity, non-immunogenicity, and more, making them a promising green alternative to commercially available synthetic counterparts (Paulino *et al.,* 2006). The properties of chitin and chitosan are significantly influenced by the source, degree of deacetylation, protein concentration, and extraction techniques used. The physical and chemical characteristics of chitin and chitosan, such as the level of acetylation, solubility, viscosity, and molecular weight, will differ based on the preparation method. These desirable characteristics have motivated numerous researchers to develop effective, environmentally friendly, and relatively easy-to-process materials that can replace conventional, ecologically harmful substances in biomedical, environmental, and energy-related applications (Sarbon *et al.,* 2015). Chitosan, the deacetylated form of chitin, is a biopolymer that has been extensively studied for its various biological activities such as antimicrobial, antioxidant, anticancer and anti-inflammatory (Kim, 2018). Chitosan, a biopolymer derived from the shells of crustaceans, has been investigated for its potential antimicrobial and antioxidant properties. Several studies have demonstrated the effectiveness of chitosan against various bacterial strains, making it a promising alternative to synthetic antibacterial agents.

Chitosan has also been shown to exhibit antioxidant activity, which may have applications in preventing oxidative damage in biological systems. *Portunus sanguinolentus* chitosan has been found to possess both antioxidant and antibacterial properties (Kaya *et al.,* 2016). Its antioxidant activity is mainly due to the presence of amino and hydroxyl groups, which act as free radical scavengers and help prevent oxidative damage to cells and tissues. Crab chitosan has also been shown to have antibacterial activity against a variety of pathogenic bacteria, including *Escherichia coli* and *Staphylococcus aureus.* This antibacterial activity is believed to be due to the ability of chitosan to disrupt the bacterial cell membrane and inhibit cell growth. Crab chitosan, derived from the shells of crustaceans, has been found to possess antioxidant activity. This is attributed to the presence of reactive amino and hydroxyl groups in the polymer's structure, which can scavenge free radicals and prevent their harmful effects (Zhang *et al.,* 2020).

The degree of deacetylation and molecular weight of chitosan have been reported to influence its antioxidant activity. The natural origin and biocompatibility of crab chitosan make it an attractive alternative to synthetic antioxidants, which may have adverse health effects. These properties make crab chitosan a promising candidate for various applications in the biomedical, pharmaceutical, and food industries. *Portunus sanguinolentus* waste, specifically collected from Balasore, Odisha can be repurposed into large scale production of chitosan, hence sustainable crustacean waste management providing insights of the dual beneficiary scheme, which includes the extraction of chitosan from sea food waste and an effective waste management process. The present study, therefore, aims to explore the potential of utilizing blue swimming crab waste for the production of chitosan, characterizing its physico-chemical composition, investigating its antibacterial activity, and evaluating its antioxidant capacity. The study also includes a comparison of the properties of the synthesized chitosan with those of commercially available chitosan.

Materials and Methods

Sample collection and pretreatment: *P. sanguinolentus* (three-spot swimming crab) shells were collected from the Balasore,

Odisha fish market and brought to the laboratory of P.G Dept. of Zoology, KKS Women's College. The crab shells were cleaned with tap water, sun-dried until they reached a stable weight until no moisture is retained, and then crushed into a fine powder which was sieved to remove any large particles. The powdered raw samples were stored in airtight containers at 4°C until further use. Commercial chitosan whose source is also decapod was obtained from Sigma-Aldrich Company Ltd., UK.

Chemical extraction of chitin and chitosan: The samples were demineralized in an acidic medium (1N HCl) using a magnetic stirrer (REMI) for 1 hr at room temperature in order to get rid of mineral components like calcium carbonate and calcium phosphate. Following the demineralization step, deproteinization was carried out in an alkaline environment to dissociate the peptide bonds by using 15% NaOH for 6 hr at 80°C under constant stirring. This step is performed to remove possible pigments or odour from the samples using a bleaching agent like 4% NaOCl to obtain colourless chitin. Deacetylating chitin is the principal method used to produce chitosan in most cases. The process of deacetylation involves the substitution of reactive and hydrophilic amino groups for the hydrophobic acetyl ones. Alkali deacetylation is more often employed than acid deacetylation because of its increased effectiveness in chemical hydrolysis, which deacetylates chitin using either acids or alkalis for the extraction of chitosan. Here, a 50% NaOH solution was employed for 6 hr at 110°C while being stirred continuously.

Characterization

Yield and Moisture Content: Chitin and chitosan yield was calculated by dividing the weight of chitosan to the weight of synthesized chitin before deacetylation (Özbay *et al.,* 2012). Moisture content was determined by drying 0.1– 0.5 g sample at 100°C for 24 hr. Percentage of moisture (%) was calculated as follows: Moisture (%) = (Weight of petri dish and sample before drying - Weight of petri dish and sample after drying) / Sample weight (AOAC, 2006).

Field Emission Scanning Electron Microscopy (FESEM): Imaging was done using Sigma 300, Carl Zeiss, Germany at magnifications of 2500 and 5000X (Mukheem *et al.,* 2022).

Fourier Transform Infrared (FTIR) Spectroscopy: Using a Frontier Spectrometer from PerkinElmer with a UATR Two connection, the infrared spectral analysis was done. The spectra were taken with a resolution of 4 cm^{-1} across the wavelength range of 450 to 4000 cm^{-1} (Madhu *et al.,* 2022).

X-ray Diffraction (XRD): X-ray diffraction pattern was recorded in order to assess the crystallinity of chitosan. Data were recorded with a scanning angle with a range from 5° to 40° with 2° min^{-1} scan rate.

Thermogravimetric Analysis (TGA): Thermogravimetric analysis (TGA) was conducted using a Shimadzu DTG-60, Japan. Under a nitrogen atmosphere, the sample was heated from 30 to 600°C @ of 10°C min^{-1} (30 ml min^{-1}) of (Felix *et al.,* 2022).

Colorimetric Analysis: The color of solid particles or in solution was measured using the HunterLab EasyMatch QC instrument with a dual-beam xenon flash lamp to confirm the deacetylation of chitin to chitosan. The color was determined using a three-dimensional scale (Lab*).

Biological Properties

Antibacterial Activity: The antibacterial activity of both extracted crab chitosan and commercial chitosan was evaluated using the Disc Diffusion method, as described by Das *et al.* (2005), against selected fish pathogenic bacteria including *E. coli* (ATCC-25922) and *A. hydrophila* (ATCC-7966). The bacteria were cultured in Nutrient Broth (NB) from Hi-media. Different concentrations of chitosan were tested for their antibacterial activity. For the antibacterial screening, Muller Hinton Agar plates (Hi-media) were prepared and specific concentrations of bacteria (*E. coli* ATCC-25922 and *A. hydrophila* ATCC-7966) were swabbed on the plates. Sterile paper discs of 6 mm diameter (Hi-Media) were then soaked in the solutions of CMC1 (100 $\mu g^{-1} ml^{-1}$), CMC2 (500$\mu g^{-1} ml^{-1}$), CC1 (100 $\mu g^{-1} ml^{-1}$), and CC2 (500 $\mu g^{-1} ml^{-1}$). A sterile disc was soaked in acetic acid served as a control treatment. The plates were incubated at 37°C for 24 hr, and the zone of inhibition was measured (in mm) to determine the antibacterial activity of both commercial chitosan and extracted crab chitosan.

Antioxidant Assay: The antioxidant activity of extracted crab chitosan and commercial chitosan was assessed following two methods: 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method (Blois, 1958) and H_2O_2 scavenging assay, performing the procedure outlined by Mukhopadhyay *et al.* (2016).

Statistical Analysis: Results are shown as mean ± standard deviation (SD), and Student's t-test was performed to determine significant differences between the mean values. The threshold for statistical significance was set at $P < 0.05$.

Results and Discussion

Chemical extraction process for chitosan typically involves several steps, like acidic demineralization, alkaline deproteinization, bleaching with a chemical agent, and alkaline deacetylation. The resultant chitin and chitosan's physico-chemical characteristics are significantly influenced by each of these processes. However, this conventional extraction method also generates chemical waste, which necessitates neutralization and purification of wastewater (Ali *et al.,* 2018; Küçükgülmez, 2018). In this study, chitosan was extracted from crab chitin using acidic and basic treatments Fig. 1., resulting in a yield of 43.85% of chitin and 61.5% purified chitosan. Although this method also involves chemical treatments, the use of crab

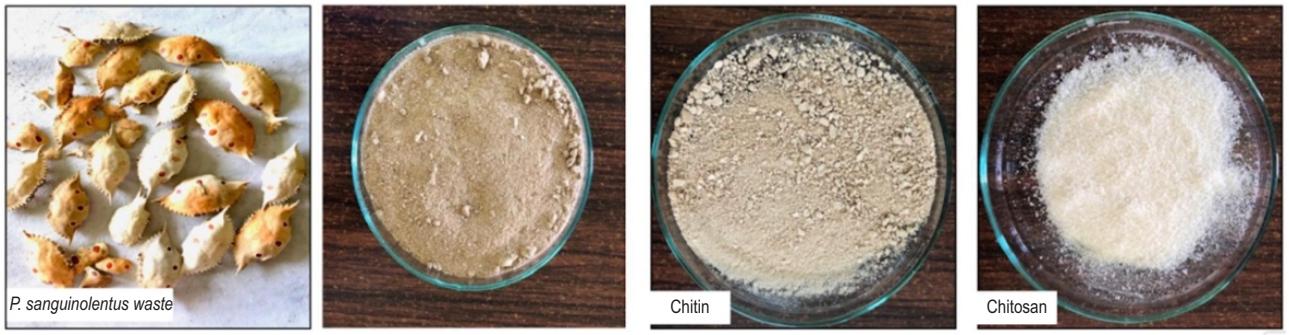

Fig. 1: Utilisation of three-spot swimming crab shells (*Portunus sanguinolentus*) to obtain chitin and chitosan

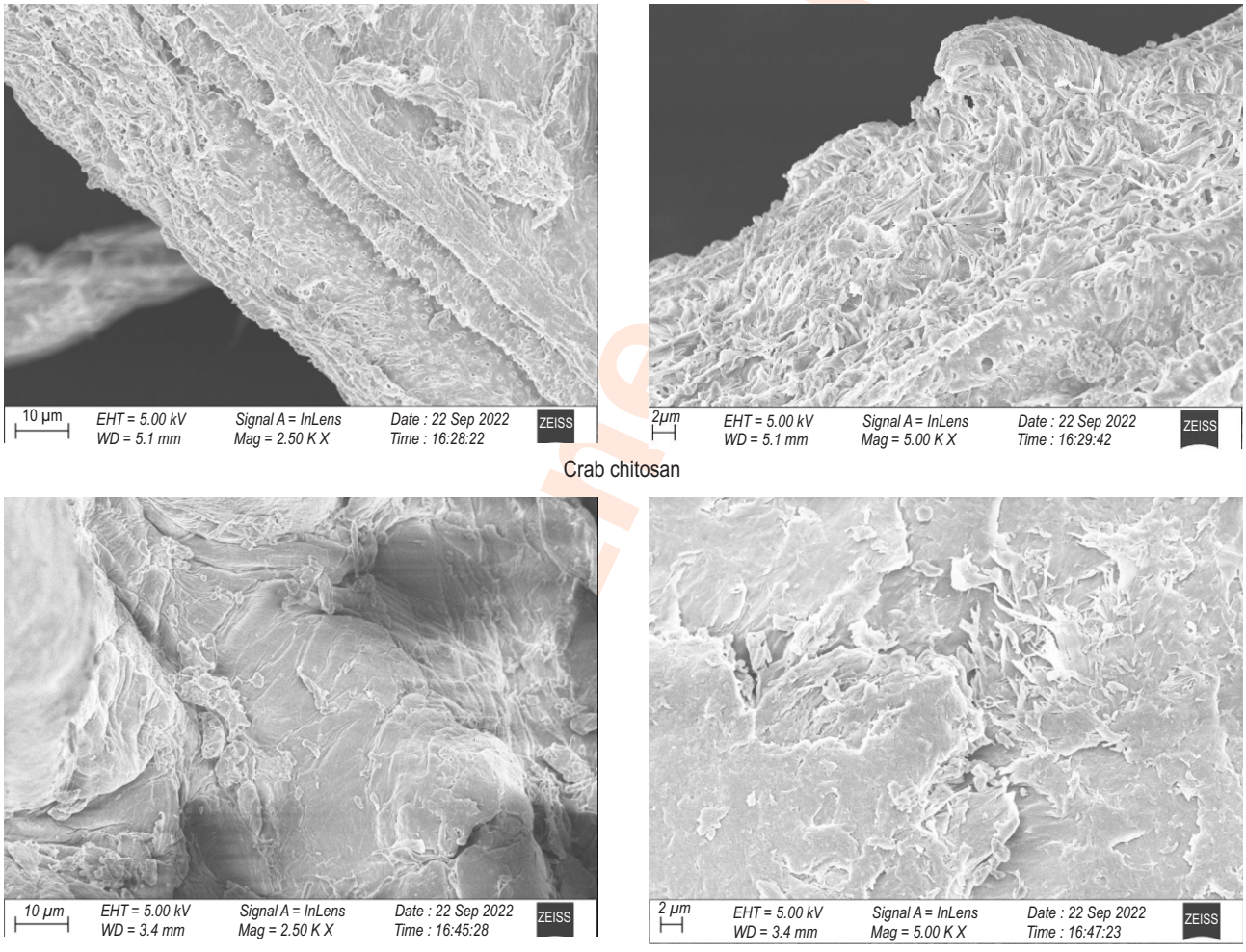

Fig. 2: FESEM micrographs of crab chitosan and commercial chitosan.

shells as a raw material for chitosan extraction presents a more sustainable approach to chitin utilization, as crab shells are readily available and abundant waste product from the seafood industry. The moisture content was found to be 1.56% due to the hygroscopic nature of chitosan (Szymańska and Winnicka, 2015). The FESEM images of crab chitosan and commercial chitosan were examined in the current study, and it was observed that during N-deacetylation of crab chitosan, a distinctly arranged microfibrillar

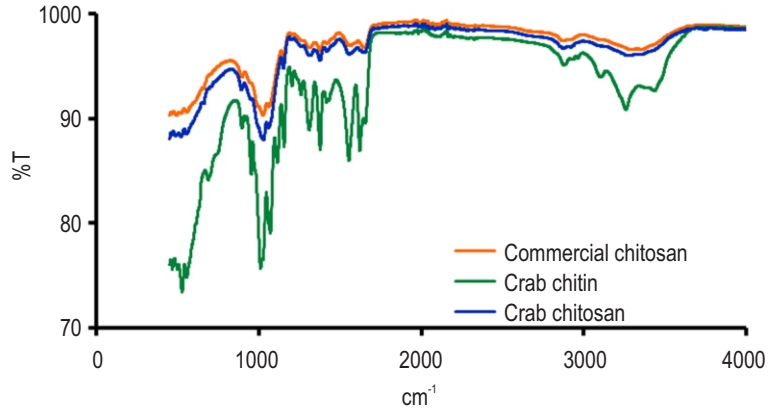

Fig. 3: FTIR spectra of crab chitosan and commercial chitosan.

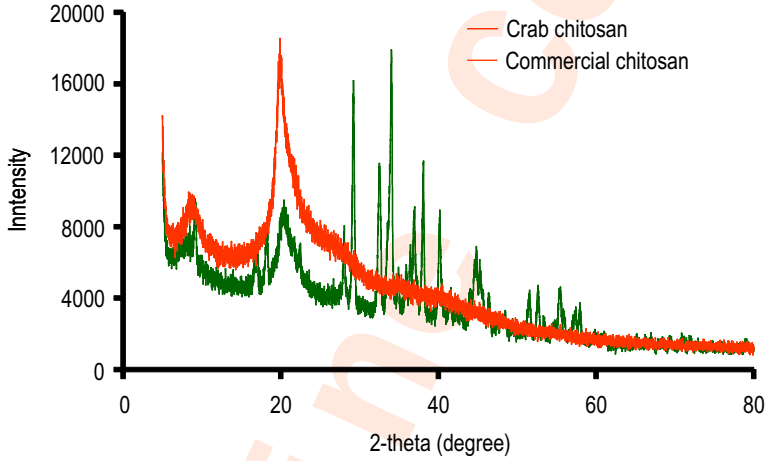

Fig. 4: XRD pattern of commercial chitosan and crab chitosan (2θ values).

crystalline structure was formed (Fig. 2). The microfibrillar crystalline structure observed in crab chitosan is thought to be due to high degree of deacetylation (DD) of chitosan extracted from *P. sanguinolentus* exoskeleton, which results in the formation of crystalline regions in chitosan. The arrangement of crystalline regions in a microfibrillar structure is likely due to the unique structure of chitin in the exoskeleton of crustaceans, which has a layered, fibrous structure.

The current study observed that the extracted crab chitosan had multiple layers of flakes, and in some areas, a porous nature could be seen. In contrast, commercial chitosan appeared to have a microfibrillar structure, but upon higher magnification, layers of crumbling flakes were observed. This is consistent with the findings reported by many researchers (Yen *et al.,* 2009; Kumari *et al.,* 2016). The observed differences in the morphology of crab chitosan and commercial chitosan may be due to differences in the source and preparation of chitosan. The observed crumbling flakes in commercial chitosan may be due to the breaking down of chitosan structure during processing. In both instances, the chitosan had a rod-like structure with the bulk of the rod-like structures appearing in groups and assembled, while others were fragmented and small in size. This indicates that the chitosan in the acid had not completely dissolved (Hisham *et al.,* 2021). The presence of fragmented and small-sized chitosan rods in the acid may be attributed to incomplete dissolution of chitosan due to insufficient acid concentration, stirring rate, or time. These fragmented chitosan rods may have implications for the physico-chemical and biological properties of chitosan, as they may have different surface areas and interactions with biological systems compared to intact chitosan rods. The morphology of chitosan can have implications for its physico-chemical and biological properties, such as its surface area, porosity, and interactions with the biological systems (Aranaz *et al.,* 2021). The surface characteristics of chitosan were examined using FTIR. Various functional groups were assigned to the peak

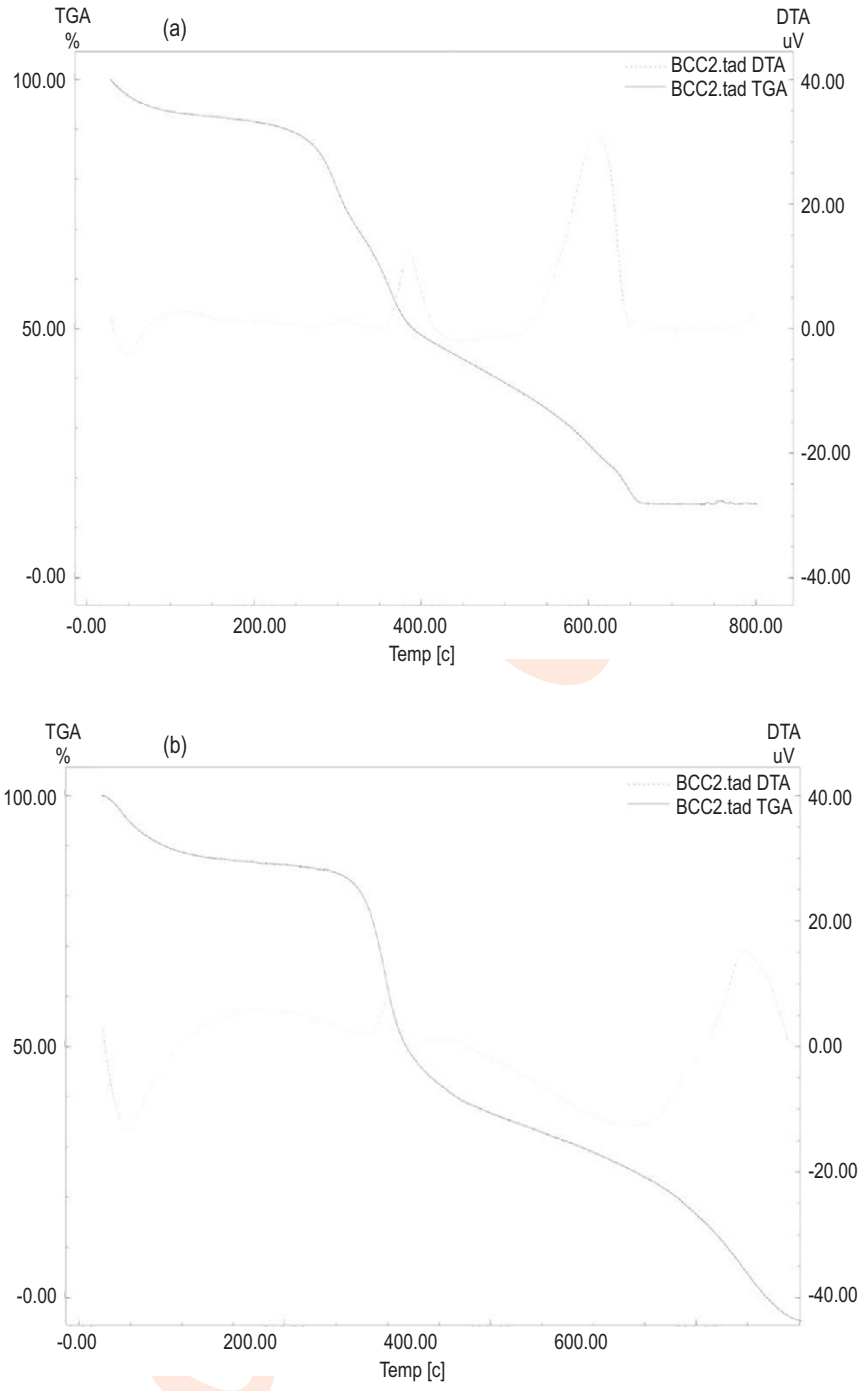

Fig. 5: TGA thermograms of crab chitosan (a) and TGA thermograms of commercial chitosan (b).

according to their respective wave numbers. According to the FTIR spectra analysis presented in Fig. 3., the absorption peaks observed in the region of 3288.97 and 3291 cm^{-1} for extracted crab chitosan and commercial chitosan, respectively, correspond to the vibrating and aliphatic-OH and-NH stretching vibration of free amino groups (Kumari *et al.,* 2016). Additionally, the peak at 1647.14 and 1647.21 cm^{-1} represents CO stretching from the acetyl group, and the peak at 1024.43 and 1024.34 cm^{-1} indicates the stretching vibration of glucosamine. These observations are consistent with previous studies (Si Trung and Bao, 2015). Furthermore, an absorption peak at 892.82 and 892.5 cm^{-1} corresponds to the ring stretching for ß-1-4 glycosidic linkage

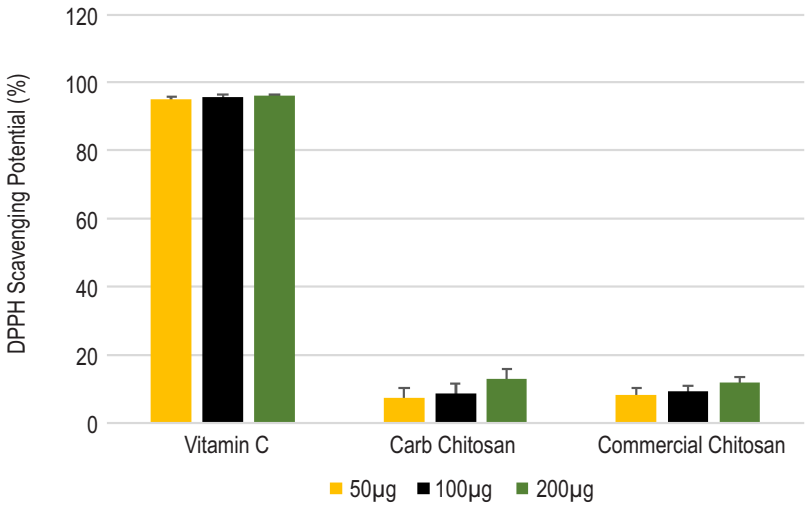

Fig. 6: DPPH scavenging potential of crab chitosan and commercial chitosan. Values are mean of three replicates ±S.D.

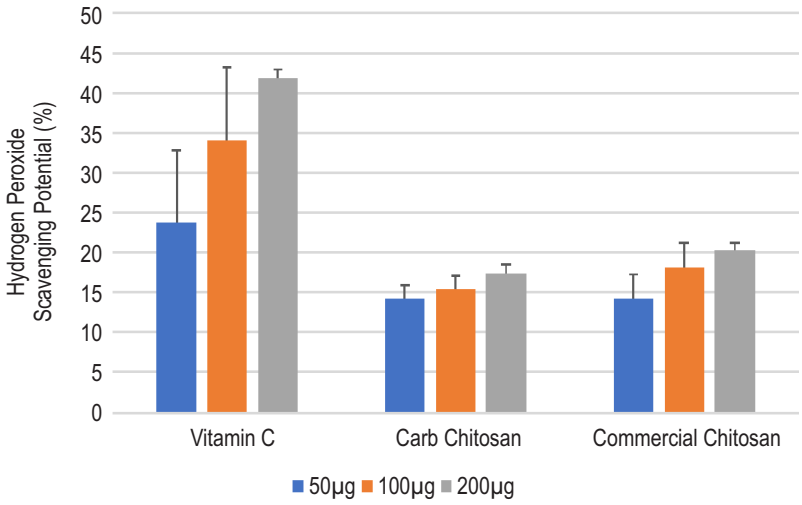

Fig. 7: Hydrogen peroxide scavenging potential (%) of crab chitosan and commercial chitosan. Values are mean of three replicates ±S.D.

(Kumirska *et al.,* 2010). The presence of these peaks in both extracted crab chitosan and commercial chitosan indicates the presence of similar chemical structures in both chitosan samples. The comparable peak frequencies observed for both extracted crab chitosan and commercial chitosan suggest that the extraction process used in this study did not significantly alter the chemical structure of the chitosan. However, it should be noted that the Degree of Deacetylation and molecular weight of chitosan could also influence the FTIR spectra (Arantes *et al.,* 2015). The XRD pattern comparison of commercial chitosan and chitosan prepared from crab crustacean waste is shown in Fig. 4. Peaks obtained at 2θ values were commercial chitosan exhibiting three strong peaks at 5.01°, 8.91°, and 19.93° whereas the remaining were weak peaks. However, a total of ten peaks were observed in the isolated chitosan powder, where two strong peaks were at 29.23° and 34.05°, while others were weak peaks.

The TGA thermograms of the extracted crab chitosan and commercial chitosan (Fig. 5a, b) displayed an initial weight loss (1st stage) occurring between 30-250°C, representing a loss of 10% and 15%, respectively. This weight loss can be attributed to the evaporation of moisture present in the samples. This was followed by a transitional weight loss (2nd stage) between 250-400°C, which accounted for 40% weight loss in crab chitosan. This stage is attributed to the thermal and oxide decomposition of chitosan, involving the vaporization and elimination of volatile

Table 1: Color characteristics of commercial chitosan and crab chitosan

Sample	L*	a*	b*
Commercial chitosan	87.32	0.80	17.36
Crab chitosan	73.08	3.00	16.95

L* indicates lightness, a* is the red/green coordinate, and b* is the yellow/blue coordinate; [a]WI (whiteness index) = 100 – [(100 – L*)2 + a*2 + b*2]1/2. and [b]Each value is expressed as mean of three replicates ± S.E. Means with different letters within columns are significantly different (P < 0.05).

Table 2: Antibacterial activity of different chitosan against selected fish pathogens

Chitosan Type/Concentration	*E. coli* (ATCC-25922)	*A. hydrophila* (ATCC-7966)
	Zone of Inhibition (mm)	
Crab chitosan1 (100 $\mu g^{-1} ml^{-1}$)	17.00±0.50	15.16±0.577
Crab chitosan2 (500 $\mu g^{-1} ml^{-1}$)	16.16±0.577	14.5±0.50
Commercial chitosan1 (100 $\mu g^{-1} ml^{-1}$)	12.67±0.577	14.17±0.288
Commercial chitosan2 (500 $\mu g^{-1} ml^{-1}$)	11.83±0.288	12.83±0.288

compounds such as CO, CO_2, solvents, low-molecular-weight molecules, and functional groups (Acosta-Ferreira *et al.,* 2020). In the final weight loss (3rd stage) between 400-800°C, the weight loss was reported to be 33% and 35% for crab chitosan and commercial chitosan, respectively. It is noteworthy that the major thermal degradation for commercial chitosan was noticed at 250 to 400°C contributing to a weight loss of 47%, indicating weaker thermal stability than crab chitosan in high-temperature areas. The apparent weight losses at various phases are attributed to the breakage of side chain groups of amino acid residues and dissociation of peptide bonds.

The perusal data presented in Table 1., showed that the Commercial chitosan had higher L* and WI values, making it appear lighter and whiter compared to purified crab chitosan. On the other hand, crab chitosan had a higher a* value (3.0), indicating more redness, while commercial chitosan had a lower a* value (0.80). In terms of b* value, commercial chitosan has a higher yellowness value (17.36) compared to purified crab chitosan (16.95). These results suggest that crab chitosan has a slightly red and noticeably yellow colour compared to commercial chitosan. Several factors affect the colour of chitosan, including the source of chitin, method of extraction, and processing conditions.

In this case, the difference in color between the commercial chitosan and purified crab chitosan could be due to the difference in their sources and extraction methods. commercial chitosan is usually extracted from shrimp or crab shells through industrial processes that involve bleaching and deacetylation, which could result in a whiter and lighter appearance. On the other hand, the purified crab chitosan was obtained through a chemical extraction process that may not have undergone extensive bleaching or deacetylation, which could contribute to its slightly red and noticeably yellow color. The differences in color between the two-chitosan samples could also have an impact on their applications (Wani, Masoodi and Akhter, 2021). It has been established that chitosan possess antibacterial properties against various types of microorganisms, making it a highly valuable characteristic for potential therapeutic applications (Confederate *et al.,* 2021).

The study investigated the antibacterial activity of both chitosan types against certain fish pathogens, as shown in Table 2. Results indicated that both crab chitosan and commercial chitosan of different concentrations exhibited zones of inhibition against *E. coli* (ATCC-25922) and *A. hydrophila* (ATCC-7966), with the maximum zone of inhibition (17.00±0.50 and 15.16±0.577 mm) observed in crab chitosan against *E. coli* (ATCC-25922) and *A. hydrophila* (ATCC-7966), respectively. The reason for reduced zone of inhibition in higher concentration is that at higher concentrations, protonated chitosan may coat the cell surface and stop intracellular component leakage whereas lower concentrations of chitosan bind to the negatively charged cell surface, disrupt the cell membrane, and ultimately kill the cell by causing intracellular components to leak out. These findings provide a novel approach to enhance the antibacterial activity against *A. hydrophila* and *E. coli.* Chitosan's antimicrobial effects are primarily attributed to its ability to bind to cell DNA through protonated amino groups, preventing the microbial RNA production (Younes *et al.,* 2014; Yilmaz, 2019). Another potential mechanism by which chitosan exerts its antibacterial activity is through acting as a chelating agent and producing toxins that ultimately prevent microbial development (Divya *et al.,* 2017). The antibacterial activity of chitosan extracted from *P. sanguinolentus* exoskeleton was found to be superior to that of commercial chitosan against Gram-negative bacteria which might be due to the higher Degree of Deacetylation value and molecular weight of chitosan extracted from *P. sanguinolentus* exoskeleton. Chitosan's antioxidant

activity may be related to its ability to bind molecules and scavenge hydroxyl radicals. Chitosan can bind metal ions or donate hydrogen or a pair of electrons to scavenge free radicals. To evaluate the ability of crab chitosan and commercial chitosan to scavenge free radicals, this study measured their ability to reduce the stable radical DPPH. In this assay, an antioxidant must donate a hydrogen atom to the DPPH radical to convert it to a non-radical reduced state. The reduction in the purple color of DPPH corresponds to the number of electrons it accepts, and this reduction can be measured by a decrease in absorbance at a specific wavelength (Molyneux, 2004). The ability of chitosan to scavenge radicals was evaluated by measuring the total DPPH scavenging capacity of crab chitosan and commercial chitosan at varied concentrations (50 µg, 100 µg, and 200 µg), as shown in Fig. 6. The decrease in color intensity is directly proportional to the inhibition of DPPH, indicating the antioxidant capacity of chitosan.

The findings of this study suggest that on increasing the concentrations of chitosan resulted in a decrease in DPPH activity, thereby reducing its antioxidant capacity. The maximum inhibition was observed at 200 µg for both crab chitosan and commercial chitosan, demonstrating that both chitosan were equally effective in their radical-scavenging activity. However, it should be noted that the antioxidant activity of Vitamin C/ascorbic acid is primarily due to its ability to donate electrons from the second and third carbon. In previous studies it has been reported that ascorbic acid using DPPH radical scavenging consistently found superior antioxidant activity compared to chitosan (Yen *et al.,* 2008; Prabu and Natarajan, 2012). The capacity of chitosan to scavenge H_2O_2 was assessed at various concentrations, and the resulting oxidative damage on deoxyribose was plotted in Fig. 7. At the highest concentration (200 µg), commercial chitosan demonstrated 20.25% inhibition, while crab chitosan showed 17.47% inhibition. In contrast, the standard ascorbic acid exhibited 42% inhibition, indicating better scavenging activity against H_2O_2 induced oxidative damage. The study indicates that the reducing power of chitosan increased with increasing concentration.

In conclusion, this study highlights the potential of chitosan extracted from *P. sanguinolentus* exoskeleton as a cost-effective and efficient source of chitosan with superior physicochemical and biological properties compared to commercial chitosan. The results of this investigation might have an impact on the development of novel antimicrobial and antioxidant agents in the future. Because of this, it can be claimed that chitosan is effectively synthesized from crab shells for use in the biomedical, pharmaceutical, and food industries, among other things.

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Authors' contribution: J. Pradhan: Conceptualization, investigation, formal analysis of data and drafting of manuscript; **B. Baisakhi:** Performed experiment, investigation, formal analysis of the data and drafting of manuscript; **B.K. Das:** Conceptualization and critical revision of the manuscript; **K. Jena and S. Ananta:** Free radical scavenging potential, data analysis and interpretation of results. **D. Mohanty:** Performed experiment and investigation.

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Ethical approval: This research did not involve experiments with human or animal participants.

Conflict of interest: The authors declare that there are no conflicts of interest.

Data availability: All data generated and analyzed are included in this research article.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology.*

References

Acosta-Ferreira, S., O.S. Castillo, J.T. Madera-Santana, D.A. Mendoza-García, C.A. Núñez-Colín, C. Grijalva-Verdugo, A.G. Villa-Lerma, A.T. Morales-Vargas and J.R. Rodríguez-Núñez: Production and physicochemical characterization of chitosan for the harvesting of wild microalgae consortia. *Biotechnol. Rep.,* **28**, e00554 (2020).

Ali, M.E.A., M.M.S. Aboelfadl, A.M. Selim, H.F. Khalil and G.M. Elkady: Chitosan nanoparticles extracted from shrimp shells, application for removal of Fe (II) and Mn (II) from aqueous phases. *Sep. Sci. Technol.,* **53**, 2870–2881 (2018).

Association of Official Agricultural Chemists (AOAC): Moisture of meat. Official methods of analysis of AOAC International. 18th Edn., Method 950.46, Gaitherburg M.D. (2006)

Aranaz, I., A.R. Alcántara, M.C. Civera, C. Arias, B. Elorza, A. Heras Caballero and N. Acosta, Chitosan: an overview of its properties and applications. *Polymers,* **13**, 3256 (2021).

Arantes, M.K., C.L. Kugelmeier, L. Cardozo Filho, M. R. Monteiro, C.R. Oliveira and H. J. Alves: Influence of the drying route on the depolymerization and properties of chitosan. *Polym. Eng. Sci.,* **55**, 1969-1976 (2015).

Blois, M.S.: Antioxidant determinations by the use of a stable free radical. *Nature*, **181**, 1199-1200 (1958).

Confederate, L.G., C.G. Tuchilus, M. Dragan, M. Sha'at and O.M.

Dragostin: Preparation and antimicrobial activity of chitosan and its derivatives: A concise review. *Molecules,* **26**, 3694 (2021).

Das, B.K., J. Pradhan, P.Pattnaik, B.R.,Samantaray and S.K.,Samal.: Production of antibacterials from the freshwater alga *Euglena viridis* (Ehren). *World J. Microbiol. Biotechnol.*, **21**, 45–50 (2005)

Divya, K., S. Vijayan, T.K. George and M.S. Jisha: Antimicrobial properties of chitosan nanoparticles: Mode of action and factors affecting activity. *Fibers Polym.,* **18**, 221-230 (2017).

Felix, C.B., W.H. Chen, A.T. Ubando, Y.K. Park, K.Y.A. Lin, A. Pugazhendhi and C.D. Dong: A comprehensive review of thermogravimetric analysis in lignocellulosic and algal biomass gasification. *Chem. Eng. J.,* **445**, 136730 (2022).

Hisham, F., M.M. Akmal, F.B. Ahmad and K. Ahmad: Facile extraction of chitin and chitosan from shrimp shell. *Mater. Today*: *Proceedings,* **42**, 2369-2373 (2021).

Jiménez-Gómez, C.P. and J.A. Cecilia: Chitosan: A natural biopolymer with a wide and varied range of applications. *Molecules,* **25**, 3981 (2020).

Kaya, M., F. Dudakli, M. Asan-Ozusaglam, Y.S. Cakmak, T. Baran, A. Mentes and S. Erdogan: Porous and nanofiber α-chitosan obtained from blue crab (*Callinectes sapidus*) tested for antimicrobial and antioxidant activities. *LWT - Food Sci. Technol.,* **65**, 1109-1117 (2016).

Kumirska, J., M. Czerwicka, Z. Kaczyński, A. Bychowska, K. Brzozowski, J. Thöming and P. Stepnowski: Application of spectroscopic methods for structural analysis of chitin and chitosan. *Mar. Drugs.,* **8**, 1567–1636 (2010).

Küçükgülmez, A.: Extraction of chitin from crayfish (*Astacus leptodactylus*) shell waste. *Alint. Zir. Bilim. Derg.,* **33**, 99–104 (2018).

Kumari, S., P. Rath and A.S. Kumar: Chitosan from shrimp shell (*Crangon crangon*) and fish scales (*Labeo rohita*): Extraction and characterization. *Afr. J. Biotechnol.,* **15**, 1258-1268 (2016).

Kim, S.: Competitive biological activities of chitosan and its derivatives: antimicrobial, antioxidant, anticancer and anti-inflammatory activities. *Int. J. Poly. Sci.,* **2018**, Article ID 1708172 (2018).

Madhu, S., Y. Devarajan, M. Balasubramanian and M.P. Raj: Synthesis and characterization of nano chitosan obtained using different seafood waste. *Mater. Lett.,* **329**, 133195 (2022).

Mohan, K., S. Ravichandran, T. Muralisankar, V. Uthayakumar, R. Chandirasekar, P. Seedevi, R.G. Abirami and D.K. Rajan: Application of marine-derived polysaccharides as immunostimulants in aquaculture. A review of current knowledge and further perspectives. *Fish Shellf. Immunol.,* **86**, 1177-93 (2019).

Molyneux, P.: The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. Sci. Technol.,* **26**, 211-219 (2004).

Mukheem, A., S. Shahabuddin, N. Akbar, I. Ahmad, K. Sudesh and N. Sridewi: Development of biocompatible polyhydroxyalkanoate/ chitosan-tungsten disulphide nanocomposite for antibacterial and biological applications. *Polymers,* **14**, 2224 (2022).

Mukhopadhyay, D., P. Dasgupta, D.S. Roy, S. Palchoudhuri, I. Chatterjee, S. Ali and S.G. Dastidar: A sensitive *in vitro* spectrophotometric hydrogen peroxide scavenging assay using 1, 10-phenanthroline. *Free Radic. Antioxid.,* **6**, 124-132 (2016).

Özbay, T., Ö. Başţürk and M.A. Sungur: The efficiency of chitin and chitosan of shell waste of Mantis shrimp (*Squilla* sp.), squid (*Sepia* sp.) and blue crab (*Callinectes sapidus*, Rathbun, 1896). *Yunus Res. Bullet.,* **12**, 13–19 (2012).

Paulino, A.T., J.I. Simionato, J.C. Garcia and J. Nozaki: Characterization of chitosan and chitin produced from silkworm chrysalides. *Carbohydr. Polym.,* **64**, 98-103 (2006).

Peter, S., N. Lyczko, D. Gopakumar, H.J. Maria, A. Nzihou and S. Thomas: Chitin and chitosan based composites for energy and environmental applications: A review. *Waste Biom. Valorizat.,* **12**, 4777-804 (2021).

Prabu, K. and E. Natarajan: *In vitro* antimicrobial and antioxidant activity of chitosan isolated from *Podophthalmus vigil. J. Appl. Pharm. Sci.,* **2**, 075-082 (2012).

Santos, V.P., N.S. Marques, P.C. Maia, M.A.B.D. Lima, L.D.O. Franco, and G.M.D. Campos-Takaki: Seafood waste as an attractive source of chitin and chitosan production and their applications. *Int. J. Mol. Sci.,* **21**, 4290 (2020).

Sarbon, N.M., S. Sandanamsamy, S.F. Kamaruzaman and F. Ahmad: Chitosan extracted from mud crab (*Scylla olivicea*) shells: physicochemical and antioxidant properties. *J. Food Sci. Techno.,* **52**, 4266-4275 (2015).

Si Trung, T. and H.N.D. Bao: Physicochemical properties and antioxidant activity of chitin and chitosan prepared from pacific white shrimp waste. *Int. J. Carbohydr. Chem.,* **2015**, 1-6 (2015).

Szymańska, E. and K. Winnicka: Stability of chitosan—a challenge for pharmaceutical and biomedical applications. *Mar. Dru.,* **13**, 1819-1846 (2015).

Wani, T. A., F.A. Masoodi and R. Akhter: Preparation and characterization of chitosan flake and chitosan nanopowder gels: A comparative study of rheological, thermal and morphological perspectives. *LWT- Food Sci. Technol.,* **148**, 111771 (2021).

Xavier, K.M. and K. Chattopadhyay: Bioactive and functional nutraceuticals from shrimp processing wastes. In: Advances in Fish Processing Technologies. 1st Edn., Chapter: 15, Taylor and Francis. pp. 321-336 (2023).

Yen, M.T., J.H. Yang and J.L. Mau: Antioxidant properties of chitosan from crab shells. *Carbohydr. Polym.,* **74**, 840–844 (2008).

Yilmaz Atay, H.: Antibacterial activity of chitosan-based systems. Functional chitosan: Drug delivery and biomedical applications, **6**, 457-489 (2019).

Younes, I., S. Hajji, V. Frachet, M. Rinaudo, K. Jellouli and M. Nasri: Chitin extraction from shrimp shell using enzymatic treatment. Antitumor, antioxidant and antimicrobial activities of chitosan. *Int. J. Biol. Macromol.,* **69**, 489-498 (2014).

Zhang, J., Y. Fu, P. Yang, X. Liu, Y. Li and Z. Gu: ROS scavenging biopolymers for anti-inflammatory diseases: classification and formulation. *Adv. Mater. Interfaces*, **7**, 2000632 (2020).