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Chitosan Nanoparticles revealing their chemical characterization – Evaluation of Antioxidant and Antibacterial studies

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Abstract

Chitosan is a biopolymer categories derived from the exoskeleton of crustaceans, has received much interest due to its biocompatibility, biodegradability, and functional properties. This study investigates the extraction and characterization of *Placuna placenta* chitosan, commonly known as the windowpane oyster, and shows its resistance antioxidant and antibacterial activities. Chitosan was extracted through a series of demineralization, deproteinization, and deacetylation processes the synthesis of a high-quality biopolymer. The structural features of chitosan were analyzed by UV-Vis Spectrometer, FTIR analysis. Antioxidant activity was evaluated by DPPH radical scavenging and reducing power assays, which showed greater free radical inhibition compared to standard antioxidants. Additionally, using agar well diffusion, the antibacterial activity of chitosan was assessed against a selection of human clinical pathogens, including *Salmonella typhi, Klebsiella pneumoniae, Proteus mirabilis, Escherichia coli, Streptococcus pyogenes, Staphylococcus aureus, Vibrio parahaemolyticus, Salmonella paratyphi, Vibrio cholerae,* and *Klebsiella oxytoca*. MBC and MIC were also assessed in this investigation, and the chitosan demonstrated a satisfactory antibacterial activity result.In study shows that the *Placuna placenta* is a cost-effective, non-toxic source of material possesses with antibacterial activity as well as antioxidant properties.

Keywords: Chitosan, *Placuna placenta*, Bioactivity, DSC, SEM, FTIR

1. Introduction

Bivalve molluscs of the *Placuna placenta* (*P. placenta*) species are known for their window pane shells. The windowpane oyster mostly inhabit in smooth muddy bottoms with particle sizes below 0.125 mm. It flourishes in shallow bays (up to 5 m deep) that are protected from severe wave action. The salinity of this species has a distribution range of 10-34 percent. Typically, the convex side of the oysters lies flat on the substratum [1]. In Goa, *P. placenta* is harvested for human food in other locations, it is not now consumed. It should be noted that this oyster is cultivated in the Philippines for its flesh, which is used to make a variety of regional specialties [2]. The lime mineral content was derived from the shell finds extensive commercial use, particularly in the cement industry. Chitin is a mucopolysaccharide and it is naturally available one that is richly occurring in insect cuticles, crustacean shells, the cell walls of some fungi and bacteria. Chitin is deacetylated in the presence of alkali to yield chitosan, the water solubility of which can be attained by the formation of salts with various acids through the amino group of the D-glucosamine unit [3–5]. The structural organization of chitin is made up of residues of 2-acetamido-2-deoxy-(14)- glycopranose (N-acetyl-D-glucosamine) as compared to chitosan, which consists of 2-amino-2-deoxy glycopranose residues (D-glucosamine units). The exoskeleton of crustaceans is the most important source of biomass in the production of chitin, and about 1,560 million tons are available as industrial sources of chitin in the whole world because this source is based on the basis of marine sources [6]. Crustacean

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shell waste usually consists of protein (20 to 40 percent), calcium and magnesium salts, and primarily carbonates and phosphates (30 to 60 percent), chitin (20 30 percent), and lipids (0 to 14 percent), and varies with species and seasonal conditions [7]. Crustaceans contain high levels of the most common chitin, α-chitin, and squid skeletons contain about 40% chitin, most of which is β-chitin, which has a different structure [8]. The backbones of chitin, cellulose and chitosan are very similar. Notably, the Polysaccharides isolated from the cuttle bone of *Sepia aculeata* and S. *brevimana* were found to possess antibacterial and antifungal properties [9]. Chitosan is significant in the digestive tract where it is involved in drug delivery, wound healing, as well as fats and heavy metal removal. Nevertheless, it is not easily digested and has limited absorption, poor solubility, and low transfection efficiency. Moreover, chitosan has a wide range of biological pharmacological characteristics, such as bacteriostatic, immunomodulatory, antioxidant, anti-tumor activities, anti-human immunodeficiency virus, anticoagulant, antiallergy, anti-diabetes, and anti-inflammation, anti-Alzheimer's and adipogenesis inhibitory properties [10-13]. The nanoparticle type of chitosan improves all of these properties by increasing surface area, solubility, and bioavailability, extending its applicability across several domain [14]. According to previous studies, chitosan nanoparticles have been created as medication carriers. Insulin-loaded chitosan nanoparticles may improve intestinal function, insulin absorption, and bioavailability [15].The effectiveness of gene transfer in cells has been improved by the use of these nanoparticles as gene carriers. Chitosan microspheres have been employed to transport drugs to the stomach [16]. Antibacterial medications like metronidazole and amoxicillin can be released under controlled conditions into the gastrointestinal tract with the help of reacetylated chitosan microspheres. In agriculture, chitosan nanoparticles work as pesticide and fertiliser transporters, increasing agrochemical efficiency while lowering environmental effect through controlled release mechanisms. It improves crop yield by encouraging plant growth, increasing disease resistance, and improving soil quality, making it a viable and environmentally acceptable alternative to contemporary agricultural procedures. In the food industry, they serve as natural preservatives, increasing shelf life by suppressing microbiological development. Chitosan has a diverse range of antimicrobial action against filamentous fungi, bacteria, yeast, and even viruses. *Salmonella choleraesuis, Salmonella tyhphimurium, Pseudomonas aeruginosa, Escherichia coli and Streptococcus mutans* have all been tested for their antibacterial properties against ChNP [17]. It has been found to be more effective against gram-positive bacteria than gram negative bacteria with some research studies suggesting that gram-negative bacteria are more vulnerable. Additionally, *Aspergillus niger, Fusarium solani, Rhizoctonia solani, Collectotrichum gloeosporioides, and Candida albicans* have all been shown to be susceptible to the antifungal effects of CHNP [14]. Reactive oxygen species (ROS) are formed in normal respiration, metabolism, xenobiotic autoxidation, and in response to physiological stress linked to various diseases [18, 19]. ROS accumulation can exceed the defense mechanisms of the body resulting in oxidative stress, although ROS production is important to maintain cellular homeostasis. This state is able to stimulate the effect of certain signaling pathways, cause cellular injury and eventually leads to disease progression or cell death. Oxidative stress has a close relationship with various health conditions such as diabetes, cancer, atherosclerosis, arthritis, respiratory diseases, and chronic inflammatory diseases. These correlations also show that the biological systems might not necessarily have adequate resources to counter these challenges. According to Halliwell and Gutteridge (2007), ROS especially the free radicals may be highly toxic to cells and tissues causing a significant rise of cytotoxicity [19]. Organisms generate their

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own antioxidants (produced internally or acquired externally) to fight oxidative damage. The most important protective factors are endogenous ones, urate, glutathione, and vitamins C and E. Nevertheless, oxidative stress can deplete these defenses, and in some circumstances, some of these vitamins can serve as prooxidants with pro-inflammatory or oxidizing characteristics [20]. Because oral supplementation does not always result in a significant increase in the level of free radical scavengers in human, other antioxidant agents are essential to provide effective protection [21]. Synthetic antioxidants are frequently used to retard oxidation in the pharmaceutical sector but their toxicity is of concern to health. As a result, the interest in natural antioxidants (especially plant-derived) has increased in the recent past [22]. There is a lot of commercial potential in the marine organisms which are a rich source of wide variety of structurally diverse bioactive compounds. Some of these are essential minerals and vitamins, proteins, bioactive peptides, natural pigments, polyunsaturated fatty acids, and sulfated polysaccharides [23]. Some well-known algal antioxidant compounds are *carotenoid*(*a-carotene, b-carotene*, *fucoxanthin, astaxanthin*), evergreen (*mycosporin-like* amino acid, such as *mycosporin-glycine*), *gallate, phlorotannin (*such as *phloroglucinol*), and tocopherol. Park et al made a comparison between partially deacetylated hetero-chitosans and chitosan with high level of deacetylation where the highly deacetylated sample showed good scavenging ability against DPPH, hydroxyl, carbon-centered, and superoxide radicals. These results add further evidence to the high antioxidant potential of chitosan, even at in vitro radical scavenging mechanisms. In the latter research article, *Placuna placenta* chitosan was synthesized through a green approach using nontoxic and completely biodegradable chemicals [23],[24]. It was been examined in regard to its antibacterial activity against *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Salmonella choleraesuis*. DPPH radical scavenging ability assay , Superoxide anion scavenging activity, Hydroxy radical Scavenging activity were determined as in vitro assays to determine antioxidant capacity.

2. Preparation and Characterization of the Chitosan

2.1 Material and methods

Species of marine bivalves, namely *Placuna placenta* (windowpane oyster) were collected from the coastal region district of Ramanathapuram (Tamil Nadu). The specimens were identified using the usual methodical reference after being brought to the lab in plastic bags. The species placenta was cleaned with tap water and allowed to dry naturally. A powder chitin was made from the dried *Placuna placenta* in preparation for further extraction.

2.2 Extraction of chitin and chitosan from P. placenta

Chitin was isolated from *P. placenta* using Takiguchi's technique [5]. 20gm of shell powder was demineralized with 300ml of 2N HCL over 24 hours with steady stirring and then filtered. The distilled water was used to wash the filtrate once more, and the process was repeated until the pH of the wash liquid was neutral. The weight of chitosan was then measured after the filtered sample was dried once more in a Hoover drier. 300 cc of 1N NaOH was used to deproteinized the sample, which was stirred continuously for 24 hours at 80°C. The sample was cleaned with distilled water before fresh NaOH was added, and NaOH was changed periodically. These samples were filtered, cleaned, and dried after a 24-hour period. Additionally, the sample's weight was noted.

2.3 Preparation of the chitosan

A 1% (v/v) acetic acid solution was used to dissolve 2% (w/v) chitin(*P. placenta*) to generate chitosan solution. To achieve full dissolution, the solution was constantly agitated for a whole day at room temperature.

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1M NaOH was used to add in a chitosan solution to stable the pH of 5.5. The solution was filtered to exclude any undissolved particles. In addition, a 0.1% (w/v) sodium tripolyphosphate (TPP) solution was produced in deionized water [21]. The TPP solution was added dropwise to the chitosan solution to create the nanoparticles while being magnetically stirred at room temperature. The chitosan/TPP ratio was kept at 5:1. Ionic cross-linking occurred when TPP was introduced to chitosan, causing the positive amino groups in chitosan to bond with the negative phosphorus groups in TPP, forming nanoparticles. To make sure the reaction was finished, the liquid was agitated for an additional thirty minutes.

2.4 Collection and Purification

The nanoparticle solution was rotated for 30 minutes at 12,000 rpm in a centrifuge in order to extract the nanoparticles. To get rid of any remaining TPP, the nanoparticles were rinsed three times with deionized water after discarding the clear liquid on top [19]. The chitosan nanoparticles were then freeze-dried and kept for later usage at -20 °C. The Bright white pellets was collected in a Eppendorf tube and kept it for the further characterization techniques.

2.5 Characterization of the chitosan

2.5.1 UV-vis spectrophotometer

The synthesized ChNPs were initially characterized using a UV-vis spectrophotometer (UV-1800, Shimadzu, Japan) at 200-800 nm Absorbance. 10 mM Solution was utilized as a blank. Absorbance reading were noted.

2.5.2 FTIR

FTIR analysis which is used for characterizing the chemical composition of chitosan. The chitosan was analysed using a Bio-Rad FT-IR-40 (USA) [25]. Each 10mg sample was crushed after being added with 100 mg of dry potassium bromide (KBr) to form a salt disc with a diameter of 10 mm for further spectrum analysis. Spectra were obtained within the wave numbers range of cm^{-1}.

2.5.3 Statistical Analysis

The experimental data was analysed using one-way ANOVA and Dunnett's multiple comparison (GraphPad Prism version 5.0, GraphPad Software, USA) at the 0.05 level to detect mean differences.

2.6 Antibacterial Activity

2.6.1 Bacterial strain

Staphylococcus aureus,Klebsiella oxytoca Salmonella typhi, Vibrio cholerae, Escherichia coli, Salmonella paratyphi,Vibrio parahaemolyticus Proteus mirabilis and *Streptococcus pyogenes* are the ten human clinical pathogens has been chosen for this study. These Bacterial species were obtained from the SIMATS University, Tamil Nadu.

2.6.2 Preparation of Inoculum

Test tubes were filled with nutrient broth, which was then autoclaved for 15 minutes at 15 lbs pressure (26). The nutrition broth was sterile, and each bacterial strain was introduced individually. The combination was then incubated at 37°C for 24 hours.

2.6.3 Agar well diffusion method

Agar well diffusion method were performed to determine the antimicrobial activity of chitosan with the principle devised by Sharifi et al. Using fresh bacterial cultures (24 h), bacteria with adjusted growth were inoculated into the agar wells. There was uniform distribution of 10^7 CFU/ ml on plates of nutrient agar with sterily cotton swabs [25]. Aseptically, 5 mm diameter wells were prepared aseptically with a sterile cork borer. A stock solution of 10 mg/ml chitosan in 10mM EDTA was prepared and 25, 50, 75 and 100 ug/ml was added to test wells labeled accordingly. There were also negative and positive controls prepared containing 10% EDTA (10 mM) and tetracycline (1 mg/ml in 10 mM EDTA) respectively. The plates were then kept at 37°C, 24 hrs, after which the antimicrobial activity was

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determined by by measuring the diameter of the inhibition zones around each well.

2.7 Antioxidant Activity Assay

2.7.1 DPPH Radical scavenging ability assay

DPPH radical scavenging activity was determined according to the procedure used by Shimada et al. (23). In short, 0.1 mM solution of DPPH was prepared in absolute methanol. To this 1 ml DPPH solution was added with 4 ml sample solution (dissolved in 40 percent methanol) with the concentration in the range of 0.125 to 2 mg/ml. The mixture was transferred into a brand-new 1.5 mL centrifuge tube, agitated well, and allowed to sit at room temperature for 15 minutes in the dark. The absorbance value, which was inversely related to the compounds' capacity to scavenge free radicals, was measured using a spectrophotometer at 517 nm (25).As reference antioxidants were butylated hydroxytoluene (BHT) and L-ascorbic acid. The scavenging activity was expressed as a percentage:

2.7.2 Superoxide anion scavenging activity

Zhang et al.'s modified approach was used to test the superoxide radical scavenging activity (25,26). Superoxide anion radicals were produced in Tris-HCl buffer in this assay (16 mM, pH 8.0, 4.5 ml) that contained 0.5 ml Nitro blue tetrazolium (NBT, 300 µM), 0.5 ml NADH (468 mM), and different doses of the polysaccharide samples ranging between 0.125 and 2 mg/ml. It was started to reaction with the addition of 0.5 ml of Phenazine Methosulfate (PMS, 60 1.TMO) to this mix. The absorbance was measured later on at 560 nm against a blank after 5 minutes of incubation at the room temperature. A lower result of absorbance showed increased superoxide radical scavenging activity. As positive controls, BHT and L-ascorbic acid were used. They estimated the amount of scavenging activity as follows:

Scavenging impact (%) = $[(A0 - A1)/A0]\times 100$

Where A_0 is the absorbance of the control and A_s is the absorbance of the sample

2.7.3 Hydroxyl radical scavenging activity

Chitosan hydroxyl radical scavenging activity was assessed on the basis of the Fenton reaction (Fe2+ + H2O2 + Fe 3+ + OH-, OH+), and the inferred values were re-stated as a percentage inhibition. Hydroxyl radicals are very reactive, exhibit short diffusion distances, and can cause extensive cellular decay, thus, they must be treated with extreme caution. Hydroxyl radicals were produced by a procedure which is outlined by *Smirnoff and Cumbes* (27).The following ingredients were used such as 3 ml sodium phosphate buffer (150 mM, pH 7.4), 10 mM FeSO4 7H2O, 10 mM EDTA, 2 mM sodium salicylate, 200 l of 30% H 2O2, and various polysaccharide concentrations (0.125-2 mg/ml). Stock solutions of H 2 O 2 were omitted in the control group in favor of sodium phosphate buffer (28 to 30). Then absorbance was noted at 510 nm by using spectrophotometer after incubating at 37 o C for 1 hour. Using the following formula, the hydroxyl radical scavenging activity was calculated:

Scavenging impact (%) = $[(A0 - A1)/A0]\times 100$

Where A_0 is the absorbance of the control and A_s is the absorbance of the sample

3. Result and Discussion

The yield of chitosan by *Placuna placenta* (windowpane oyster) differs significantly with the method of extraction used. This is normally done through sequential demineralization using hydrochloric acid, deproteinization using sodium hydroxide and finishing deacetylation in concentrated sodium hydroxide to transform chitin to chitosan. This is the first research where the physicochemical properties of the chitosan obtained particularly in *P. placenta* shells are assessed. Chitin and chitosan yields have been reported to vary across species, including when belonging to same taxonomic group. In this study, it was

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observed that the percentage of chitosan yield of *P. placenta* shells is 18.8 percent [26],[27].To compare, *Penaeus monodon* and metapenaeus stebbingi carapaces were reported to produce 14.6% and 17.5% chitosan respectively, which is smaller than the value of *P. placenta* used in this study [28],[29]. On the contrary, chitosan yields far better in the squid, *Sepioteuthis lessoniana*, with a value as high as 57.14 percent [30],[31]. On the same note, the *D. singhalensis* pen produced 85 percent chitosan and 37.65 percent chitin whereas Sepia pharaonic raw cuttlebone yielded 27.76 percent sulfated chitosan. Chitin extracted in this source has been partially deacetylated to give 39.45 percent chitosan. The total recovery of sulfated chitin made of chitosan (approximately 83%) was in line with yields reported in the analysis of *D. scortum* shells (84.03%) and higher than the yields of commercially produced chitosan made of shrimp and crab shells. Polysaccharides isolated in shoots of Phyllostachys edulis gave total sugar percentages of 88.4%, 81.7%, and 93.9% under conditions of water, sulfuric acid, and sodium hydroxide respectively. Further, Palpandi et al. have documented that the operculum and shell of snail *Nerita crepidularia* have 23.91 % and 35.43 %, respectively of chitin [29],[32]. The Odote et al. documented the chitosan yield of prawns, crabs, lobsters and insect larvae with 75.1%, 74.6%, 74.3% and 66.7% respectively. Pasiyazpazham Ramasamy et al. (2014) demonstrate that *S. kobiensis* averagely produced 43.77, which was above the level of cuttlefish S. *inermis* and S. *prashadi* and below the squid *S. lessoniana* and *D. sibogae* [33]. Generally, the yield of chitosan produced under the *P. placenta* shells is quite low in comparison to other mollusks and crustaceans, a fact predicted by the form of calcium carbonate that comprises its shell.

3.1 UV- spectrometer

The formation of chitosan NPs was confirmed by the LABMAN Spectrophotometer UV absorption spectrum. The UV spectrum of the chNP obtained showed a strong absorption peak edge at 339 nm (Fig. 1a).

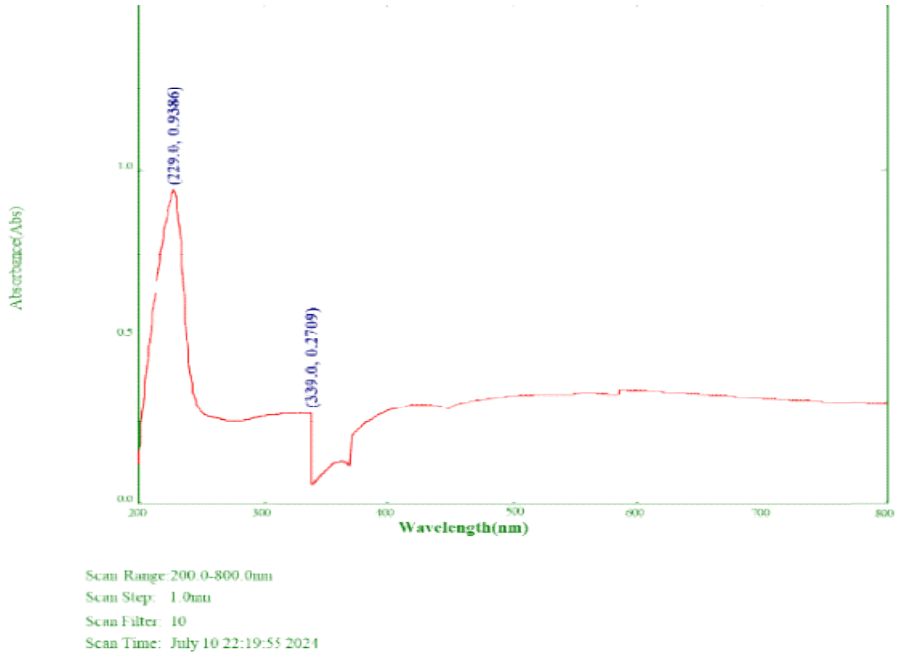

Fig. 1: U-V spectrum of Chitosan isolated from the *P. placenta* Chitosan Nanoparticles

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3.2 FTIR

FTIR studies of CNP were carried out to analyse the nanoparticles' structures and features. FT-IR spectrum of CNP shows the major peak lies between 3372.773 cm^{-1} and 451.889 cm^{-1}. Simultaneously, the FT-IR spectrum of the chitosan extracted from the shell of *P. placenta* exhibits a characteristic peak (Fig. 2). A wide and depth intense peak observed at 3372.773 cm^{-1} is related to O-H stretching vibrations, typical of the hydroxyl (OH) groups usually present in alcohols and phenols. The band also overlaps with N-H stretching, indicating the hydrogen bonding with a chitosan backbone. The strong band at 1061.595 cm^{-1} is due to C-O stretching vibration, which implies the presence of ether or alcohol functions in glucosamine units of chitosan. A unique absorption is seen at 796.643 cm-1 that relates to C-H out-of-plane bending, in alkenes or aromatic structures, confirming the semi-crystalline structure of the polysaccharide [34],[35]. A band at 417.092 cm^{-1} in the lower frequency region is assigned to C-Br or C-Cl stretching indicating trace halogenated residues which could be due to extraction or purification procedures. The presence of CCl functional groups or other halogenated functionalities is further supported by a closely related band at 451.889 cm^{-1}.. Subapradha *et al. (2013)* reported that the FT-IR spectrum of chitosan from S.*lessosiana* gladius which shoed the intense peak range at 3426.27cm^{-1}. Seedevi *et al* (2016) Studies shows that FT-IR spectrum of Sulfated polysaccharides are synthesized from the *Graticularia corticate* band exhibiting major peaks at 3404.96 cm-1, corresponding to strong– OH signals, and at 2924.36 cm^{-}1, which is attributed to the stretch vibration of -CH. polysaccharides isolated and purified from *Diaphragma juglandis fructus* [36].The vibration of the hydroxyl band group caused the greatest peaks at 3383 cm-1, the sulfated group caused the peak at 1238 cm-1, and C-H stretching caused the peak at 2939 cm-1.

3.3 XRD

X-ray diffraction (XRD) was used to study the crystallographic characteristics of the chitosan material obtained in the marine environment (Fig. 3). The resulting diffraction pattern showed prominent two peaks at 20 =

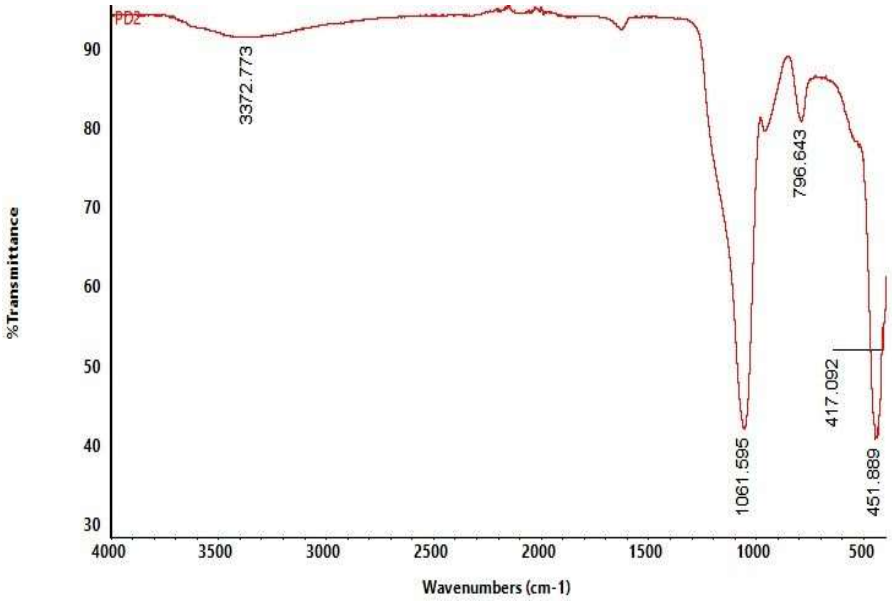

Fig. 2: FT-IR spectrum of chitosan nanoparticles

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23.07 and 33.36 o, d -spacing = 3.85 and 2.68 Angstrom at the corresponding values, respectively. The sharpness of the peaks exhibiting a narrow Full Width at Half Maximum (FWHM) (0.10 o) and relative intensities of 100% and 12.1% pointing to the semi-crystalline structure [28]. The strong peak at 23.07 is indicative of (hkl) Facet often linked to the inter molecular hydrogen bonding of chitosan indicating a partially crystalline structure based on correlation of the organized polymer chains. Weaker peak at 33.36 o and its d-spacing, 2.68 o could be due to the remaining mineral material or secondary regions that are crystallized into the matrix.

3.4 Antibacterial activity of chitosan extracted from P. placenta

Bivalve, *Placuna placenta* (windowpane oyster) exhibited antibacterial functions toward 8 out of 10 of its strains in clinical isolates (Fig. 4 and Table 1). *P. placenta* showed maximun inhibitionzone of 20mm at a concentration of 100µg/ml (12 mm and 14 mm, respectively). The biggest inhibition was 75 µg/ml against *Salmonella paratyphi* (12 mm), whereas S. aureus showed the most minimal inhibition (11 mm). *P. placenta* elicited the highest (11 mm) inhibition zone against *S. paratyphi* or the lowest zone (9 mm) against Vibrio parahaemolyticus when tested at 50 µg/ml (38). At 25 µg/ml the *S. paratyphi* and *V. parahaemolyticus* inhibition zones were 11

Fig. 4: Antibacterial activity of the Chitosan from *P. placenta*

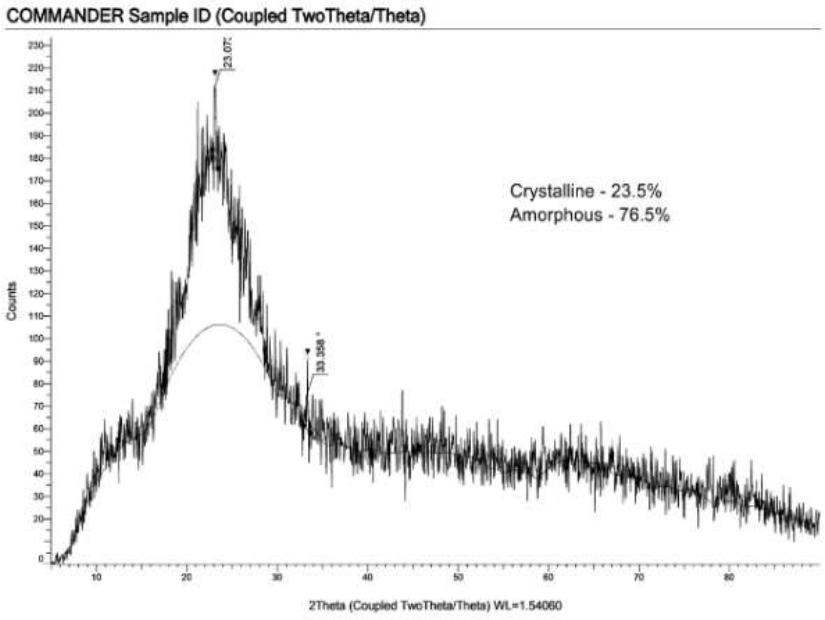

Fig. 3: XRD of chitosan nanoparticles

Chitosan Nanoparticles

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Table 1: Antibacterial activity of the Chitosan from *P. placenta*

S. No	Name of the strains	Zone of inhibition (mm)					
		25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	+ve	-ve
1	*S. aureus*	9	9	11	12	17	-
2	*K. pneumonia*	11	11	13	14	24	-
3	*K. oxytoca*	10	10	12	16	23	-
4	*V. parahaemolyticus*	8	9	10	11	22	-
5	*E. coli*	-	-	9	11	20	-
6	*S. typhi*	12	14	15	17	27	-
7	*S. paratyphi*	11	11	12	16	26	-
8	*V. cholera*	-	-	9	11	24	-
9	*P. mirabilis*	9	9	11	12	24	-
10	*S. pyogenes*	10	11	13	17	25	-

and 8 mm respectively. Tetracycline served as a positive control and gave an inhibition zone of 20 mm (Streptococcus pyogenes), 22 mm (Salmonella typhi), 26 mm (Staphylococcus aureus), 20 mm (K. pneumoniae), 21 mm (Vibrio cholerae), 22 mm (V. parahaemolyticus) and 23 mm (Escherichia coli, *S.paratyphi*, *Proteus mirabilis)* shows 20mm. The chitosan concentration was directly proportional to antibacterial efficacy. Similarly in other studies *Seedevi et al.* documented that polysaccharide extracts of gladius of *Sepioteuthis lessoniana* yielded 20 mm inhibition zone against *S. paratyphi* [41]. Likewise, Vairamani et al. revealed that *Sepiella inermis* polysaccharides at a concentration of 100 µg/ml had a 12 mm inhibition halo against *K. pneumoniae*. Wang et al. (2018) observed that GCP polysaccharide of *Chaetomium globosum* had maximum inhibition zone of 16.2 60.56 mm against E. coli, and 30.3 60.56 mm against S. aureus were recorded. Moreover, the antibacterial potential of sulfated polysaccharides in *Gracilaria corticata* was reported by Seedevi et al. (2016). In 100µg/ml concentration shows a maximum inhibition zone against *K. oxytoca* (19mm), and lowest inhibition zone of 12mm was found against *V. cholera.*

3.5 Antioxidant

DPPH radical scavenging assay can be described as one of the most popular antioxidant indexing techniques because it enables rapid screening- of many samples and sufficiently sensitive to identify bioactive compounds in low amounts. The assay monitors the reduction in absorbance of the DPPH radical after the reduction by antioxidants, donating hydrogen atoms to quench DPPH. DPPH, hydroxyl and superoxide radical scavenging assessments showed that their antioxidant activities increased with higher doses of crude, fractionated and purified polysaccharides [37]. Chitosan derived crude extract of *Placuna placenta* shells had 7.19 and 27.21 of DPPH-scavenging activity at 0.125 g/ml and 2 g/ml respectively [38]. Sulfated polysaccharides that were purified proved more active, with 13.78% at 0.125 g/ml and 38.57% at 2 g/ml. Comparatively, normal antioxidants L-ascorbic acid and BHT had scavenging rates of 38.32% versus 41.32% at 0.125 g/ml and 78.80 percent versus 82.67 percent at 2 g/ml, respectively [39]. Inhibition of hydroxyl radical (OH) was also considered as concentration-dependent [40],[41],[42],. To illustrate, purified polysaccharides of the *Monostroma oxyspermum* were capable of exhibiting scavenging activity of 22.29-68.19% between 10-160 ug/ml concentrations [43]. Sulfate content is a critical contributor on scavenging efficiency and differences between the species of algae in the antioxidant capacity tends to correlate with such variations in molecule weight and structures. Special focus is given to superoxide radicals due to their indirect effect on lipid oxidation by indirectly producing

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hydrogen peroxide (H 2 O 2) that further catalyzes the formation of hydroxyl radical precursors [36]. The crude fraction of polysaccharides of *Gracilaria debilis* inhibited superoxide radicals by 5.38 percent at 0.1 g/ml and 23.56 percent at 0.6 g/ml, fractionated extracts recorded better inhibition with 9.51 percent and 43.68 percent respectively at the same concentrations, whereas purified extracts were 12.78 percent and 52.23 percent respectively. When compared, L-ascorbic acid and BHT it was found that they obtained 35.2 and 40.95 percent of inhibition at the conc of 0.1 g/ml, whereas 72.32 and 78.43 percent at the conc value of 0.6 g/ml. Hydroxyl radicals are the most reactive ones and may cause a lot of damage to adjacent biomolecules that led to aging, cancer, and other degenerative disorders (42). These radicals have been demonstrated to be stabilised by polysaccharides, and this aspect limits the damage caused to cells. *P. placenta* chitosan exhibited inhibition rate of 6.68 and 34.56 percent to hydroxyl radical scavenging at 0.5 and 3 micro gram/ml as compared to L-ascorbic acid 35.22 and 73.95 percent at same concentrations and BHT 40.42 and 78.78 percent respectively. The fractionated form of chitosan was 9.18 and 46.77 per cent at 0.5 mg/ml and higher concentrations, compared with purified sulfated polysaccharides 56.32 per cent. In comparison, 0.5 g/ml of L-ascorbic acid and BHT had shown 35.2 percent and 40.42 percent inhibition respectively with 72.95 percent and 78.78 percent at 3 g/ml. In general, the concentration of the scavenging ability of polysaccharide hydroxyl radicals was proportionally dependent on concentration, which could be explained by Fenton reaction mechanism [44].

4. Conclusion

Chitosan is extracted from the window pane oyster shell *P. placenta* structural and functional groups was characterized by Uv-Spectrometry, FT-IR and XRD. Chitosan is a non-toxic, and cost-effective substance with significant antibacterial and antioxidant properties. Hence the studies indicate that bivalve chitosan has high antioxidant activity and might be employed in the food and pharmaceutical industries.

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