

RESEARCH PAPER

Assessment of the Antibacterial and Antioxidant Efficacy of Nano-Chitosan Surface Derived from Fish Scale Waste

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ABSTRACT

The biodegradability, biocompatibility, and low toxicity of chitosan make it an attractive polymer for use in drug delivery systems like nanoparticles. A Study Using XRD, FTIR, and SEM for Characterization. The observation of extremely wide peaks at $2\theta = (10^\circ, 20^\circ, 26^\circ)$ in X-ray diffraction studies of both pure and local extracted chitosan verifies the degree of crystallinity and structural conformance of the produced compound by means of the conformity of the angle values. The antimicrobial activity has been studied against different pathogenic bacterial isolates. By the well diffusion method, the highest inhibition zones were showed 30.5 mm, 26.2 mm, 23 mm against *Klebsilla pneumoniae*, *Salmonella typhi* and *Staphylococcus aureus* respectively, But, *Candida albicans* was appeared sensitivity with 17 mm at same concentration 30mg/mL of local extract chitosan compared with standard product. Antioxidant Activity have been studied free radical scavenging activities by ascorbic acid, Chitosan extract and stander chitosan, Chitosan extract showed radical scavenging (4 -16) % with Concentration (1- 2) mg/ml. Conclusion the antimicrobial activity against different microbial activity chitosan (well diffusion method), at (30 mg/mL) the bacteria appear different inhibition zone with high result *Klebsiella Pneumoniae*, and at (30 mg/mL) high Inhibition zone more than all antibiotic. The highest activity of local extracted chitosan against *klebsiella pneumoniae*. That indigenous extract may be utilized as an affordable antibacterial and antioxidant and can be made from fish and sold in an environmentally responsible manner.

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INTRODUCTION

Since antibiotics are still the primary means of controlling germs, combating bacterial resistance is one of the issues of the modern world. Chitosan is a versatile polymer that has been used in drug delivery system as nanoparticles because of

biodegrade-able, biocompatible, and low toxicity [1]. It accounts as a polymer of glucosamine obtained from chitin by alkaline deacetylation, (removal of COCH_3 acetyl groups from the original structure of chitin) with alkali [2,3]. The shells of these crustaceans are first removed and then

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ground into powder. Chitosan also occurs naturally in some microorganisms such as fungi and yeast [4]. The structural factors, including molecular weight, degree of deacetylation, derivative form, concentration, and source, significantly influence the material's qualities, [5-7]. The antimicrobial properties of compounds derived from chitosan have been studied extensively [8]. Chitosan was obtained from shrimp shell waste by deproteinization, demineralization, decolorization and deacetylation processes. Chitosan with derivatives exhibited a variety of physicochemical and biological properties resulting in numerous applications in areas ranging from waste water treatment to agrochemical, environmental and industrial uses [9,10]. In addition, with absence allergenicity, its bioactivity makes it a very attractive substance for various applications as a biomaterial in the pharmaceutical and medical fields [11,12]. Due to its biological properties, chitosan has gained attention in the medical, food, and agricultural sectors [13]. In biomedical applications, it is used for chemical encapsulation, controlled drug release, and environmental remediation. In biomedical applications, as material for chemicals encapsulation and controlled release and in environmental remediation [14]. They have already been recognized as a promising drug carrier and have recently become a new area of interest in the field of biomedical applications [15]. Chitosan is an eco-friendly, multipurpose substance that is essential to antimicrobial applications. Moreover, chitosan has been investigated in conjunction with antibiotics to combat bacteria that form biofilms, *Acinetobacter baumannii* strains are a common pathogen that can cause severe nosocomial infections acquired in hospitals, particularly in intensive care units. These infections can include bacteremia, pneumonia, and urinary tract infections [16]. Another proposed mechanism is the binding of chitosan with microbial DNA, which leads to the inhibition of the mRNA and protein synthesis via the penetration of chitosan into the nuclei of the microorganisms [17]. In this mechanism, chitosan molecules are believed to penetrate the bacterial cell wall composed of multilayered, cross-linked murein to reach the plasma membrane, and reach the plasma membrane. Furthermore, low-molecular-weight chitosan may exhibit superior antibacterial activity compared to high-molecular-weight chitosan [18]. Nonetheless, the antibacterial

activity of the chitosan coating may be impacted by temperature and the surrounding matrix. It has been discovered that when it comes to chitosan's antibacterial activity, molds and yeasts have a higher susceptibility than bacteria [19]. The transmission electron micrograph of *Escherichia coli* showed that normal cells were surrounded by compact surface cell membranes in the absence of chitosan derivative, with no internal components released and no noticeable surface ruptures. Chitosan inhibits the growth of microorganisms by chelating essential metals and nutrients [20]. On the cell surface, chitosan can form an oxygen barrier that can inhibit the growth of aerobic bacteria or form a polymer membrane that keeps nutrients out of the cell [21]. Also showed that bacteria might be killed by chitosan binding to teichoic acids and subsequent membrane lipid extraction. One possible explanation for cell death is a series of the mentioned chemical events that take place at the same time [22]. The synthesis of bacterial toxins and microbial growth may be inhibited by chitosan's binding of bacterial trace metals [23]. A variety of pathogens, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, are responsible for foodborne disease outbreaks [24]. For gram-positive bacteria, it is thought that chitosan with a high molecular mass may form films around the cell that prevent nutrient absorption, whereas gram-negative bacteria are more susceptible to chitosan with a low molecular mass, which disrupts their metabolism [25]. Chitosan's broad-spectrum antimicrobial activity makes it effective against both Gram-positive and Gram-negative bacteria. It demonstrated that chitosan's electrostatic interactions with bacterial cell walls significantly promote biofilm breakdown and restrict Bacterial development [26].

In the present study, chitosan has been prepared from fish shells using a simple and environmentally eco-friendly method. Their potential as promising biomaterials will also be discussed. Also, the biological activity of the compound was studied to show the biological effect on various types of bacteria and stud the antioxidant efficacy.

MATERIALS AND METHODS

Fish shells were obtained in fresh conditions from a local fish market and thoroughly washed with tap water, desiccated at room temperature and subjected to size reduction followed by air-

dried, and add Hydrochloric acid HCl (analytical reagents, Rankem), glacial acetic acid 100% (Merck) and sodium hydroxides NaOH pellets (Rankem) were purchased from Rankem and Merck chemicals. Commercial chitosan (86% de-acetylated) was purchased from India Sea Foods, Kerala in India.

Extraction of local chitosan

Demineralization: The powdered Fish shells were demineralized with 1 M HCl at room temperature (10g of Fish shells powder in 100 ml of water) at solid-to-solvent ratio 1:10 under continuous stirring for 2 h, then washed several times with distilled water to neutralize excess acid. After filtering, deproteinization particles were obtained. Demineralized powder was treated with 15% NaOH solution at room temperature for 24 hours. After which they are washed with distilled water and filtered. **Decolorization:** Deprotonated sample was bleached in 15% sodium hypochloride at a solid to-solvent ratio of 1:10 room temperature for 15 min, and subsequently washed and dried for 12 hours. **Deacetylation:** the last process was carried out by adding 50 % NaOH at room temperature for 3 days and filtered in order to retain chitosan. The samples were then left uncovered and oven dried, then collect the chitosan product.

Antimicrobial Assay

Eight bacterial isolates were collected from patients in different departments of Baghdad Teaching Hospital for testing. and Microbiology Laboratory used BD phoenix cultivations to examine these specimens, which comprised urine, stool, nose, throat, blood, sputum, wound. Both gram positive and gram negative. The antimicrobial action was study by screened against some bacterial, after 24 hours of incubation at 37 °C and the diameters of zone of inhibition (mm) appeared around each disc of chitosan containing the test compound were measured accurately, we used disc diffusion method, we compare the local extract of chitosan with Antibiotic disc (CEFTAZIDIME, CEFOTAXIME, AMPICILLIN, TICARA+CLAUV, CEFOXITIN, VANCOMYCIN).

Statistical Analysis

Statistical Packages for the Social Sciences (SPSS) (2016) was used to analyses the impact of variables on the study's variables. In this study, the Chi-square test was employed to find a considerable difference between percentages (0.05 and 0.01 probability).

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

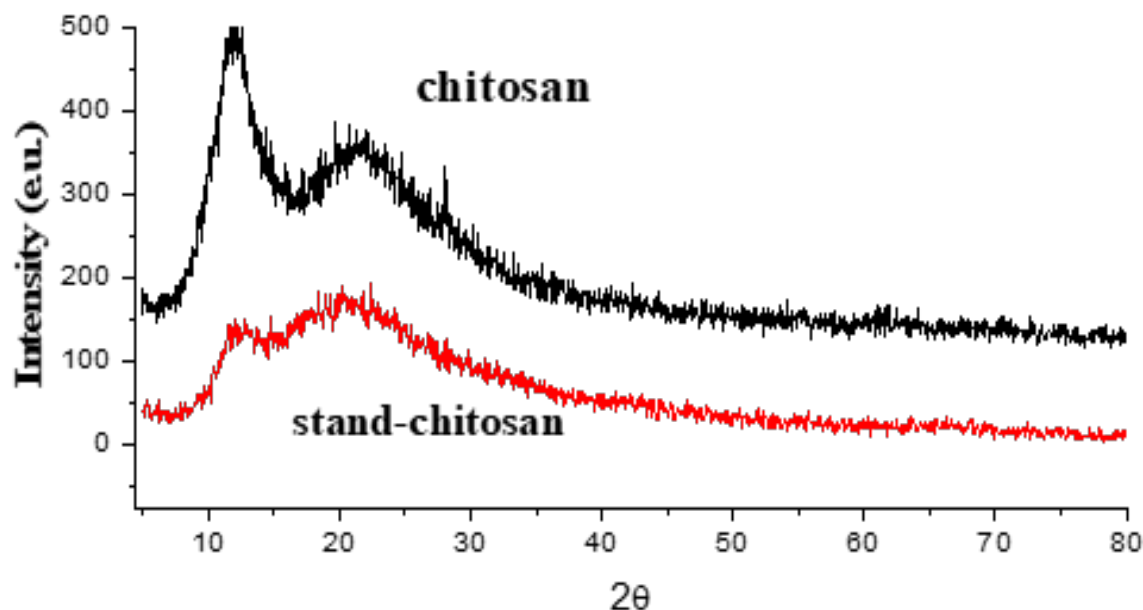


Fig. 1. XRD spectra of Chitosan.

χ^2 : Chi-square, Σ : Summation, O: Observed No.,
E: Expected No.

Note: 0.05 *Significant ($P \leq 0.05$).

0.01 **Highly Significant ($P \leq 0.01$). NS
insignificant.

Characterization

Morphological characterization determination of dried Chitosan was performed by (FTIR, XRD, SEM) the structure of the Chitosan was characterized by the X-ray diffraction (XRD) technique using a Shimadzu diffractometer model XRD -6000, employing Cu K α (0.154 nm) radiation. X-ray diffraction (XRD) pattern of chitosan sample was recorded using X-ray diffract meter operating at a voltage of 40 kV and a current of 30 mA with Cu Ka radiation ($k = 1.54060 \text{ \AA}$). It was recorded in the 2 θ range at room temperature. Mixing methods and equipment info in long blocks (FTIR) spectra of local extracted chitosan were recorded by using Shimadzu 8201 PC. The spectral region between 4000 and 400 cm^{-1} was scanned and KBr disc method was used for recording (SEM) images that were obtained using Tescan VegIII. The morphological structure of local extracted chitosan was observed under a scanning electron microscope (SEM). The SEM images were recorded using JEOL (Modell-6390) scanning electron microscopy operated at an applied voltage of 10 KV.

Antioxidant Activity

I follow the guideline according (NAG) 2024. Antioxidant Activity (Evaluation of DPPH Free Radical Scavenging Activity). DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity It is generally considered that the inhibition of lipid peroxidation by an antioxidant can be explained by various mechanisms. One is the free radical-scavenging activity where DPPH is a stable free radical with a maximum absorbance at 517 nm in ethanol. When DPPH encounters a proton-donating constituent such as an antioxidant, the radical would be scavenged and the absorbance is decrees. According this principle, expressed as its ability in scavenging the DPPH radical the antioxidant activity of the substance [27]. By the action of nitrogen on the C-2 position of the chitosan, Chitosan excludes various free radicals. Due to the reaction between the free radicals and the residual free amino group to form stable macromolecule, radicals the scavenging activity of chitosan or forming ammonium groups from the amino groups by absorbing hydrogen ions from the solution and then reacting with radicals through an additional reaction [28].

Ethical approval

The study was approved by Al Rafidain University College, Baghdad- Iraq (The third session /REC 8 on 29/10/2024). This was done following the ethical standards of the 1964 Helsinki Declaration

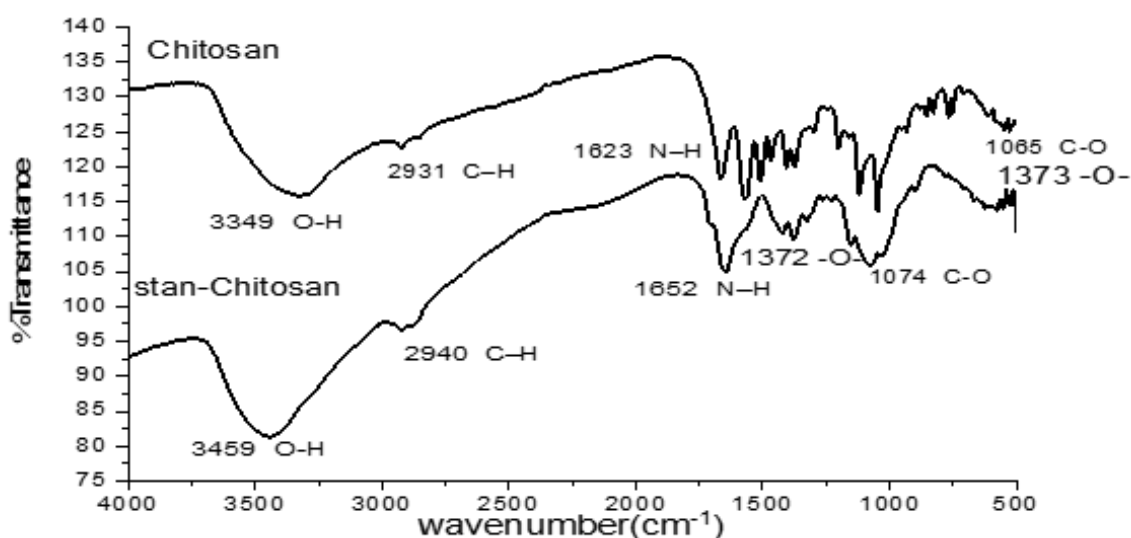


Fig. 2. FTIR spectra of Chitosan.

and its later comparable ethical standards or amendments, as well as the ethical standards of the national and/or the institutional research committee. The maneuver was explained, and written consent was taken from all couples before starting the study. This work included pathogenic bacteria collected from patients.

RESULTS AND DISCUSSION

XRD diffraction, the X-ray diffraction patterns of Chitosan are given in the Fig. 1 XRD analysis helps in describing the physical properties of samples in terms of crystalline structure and in addition it also assesses the compatibility of each component material present in the blended films [29]. The Figure shows the X-ray diffractogram details and patterns of pure chitosan, the X-ray diffractogram of chitosan (Fig. 1) had a semi crystalline nature with two main diffraction peaks at around $2\theta=10^\circ$ and 20° . The semi crystalline nature of pure chitosan was confirmed from the appearance of the diffraction peak centered at diffraction angle $2\theta=10^\circ$ and sharp diffraction peak at 20° which are indicative of high degree of crystalline morphology [30]. The chitosan molecule easily forms the crystalline regions and this may be due to the presence of plenty of -OH and $-NH_2$ groups in the chitosan structure, which could form stronger inter and intramolecular hydrogen bonds [31]. Fig.

2 illustrates the FTIR spectra of the manufactured chitosan in compared to standard chitosan. Which were recorded in the middle infrared (4000 cm^{-1} to 500 cm^{-1}). The main characteristic peaks of shell fish chitosan is at 3349 cm^{-1} bands of (-OH stretch), 2931 cm^{-1} (C-H stretch), 1581 cm^{-1} for (N-H bend), 1373 cm^{-1} (bridge O stretch), and 1065 cm^{-1} (C-O stretch). Whereas the main corresponding peaks of standard chitosan were at 3459 , 2940 , 1652 and 1595 , 1372 and 1074 cm^{-1} respectively [32,33]. Fig. 3 show the morphology, it was found to be in the form of flakes and with rough surfaces, it referred chitosan non homogenous and non-smooth facade with straps and shrinkage. These surface features of prepared chitosan agreed with the literature [34].

Antimicrobial Assay

Eight bacterial were collected from patients in different departments Baghdad Teaching Hospital for testing. The Microbiology Laboratory used were tested using BD Phoenix Device cultivations to examine these specimens, which comprised urine, stool, nose, throat, blood, sputum, wound, abscess, and pleura samples. The antimicrobial action was investigated by screened against some bacterial, after 24 hours of incubation at 37°C and the diameters of zone of inhibition (mm) formed around each disc containing the test compound

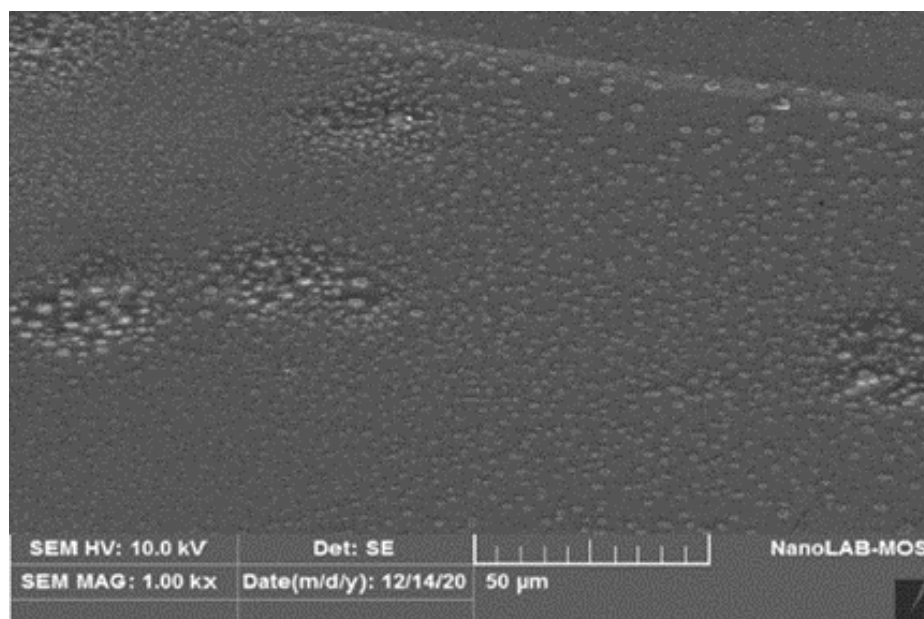


Fig. 3. SEM spectra of Chitosan.

were measured accurately. At (30 mg/mL) the bacteria appear different inhibition zone with high result *Klebsiella Pneumoniae*. The chitosan showed significant activity against *Staphylococcus*, *candida albicans* and *Pseudomononeus*). (Table 1) shows the highest effect of chitosan on the growth of *Klebsilla Pneumoniae* by inhibition zone (30.5 mm) with the (30mg/mL), but the *Salmonella typhi* and *Staphylococcus* show inhibition zone (26.2 mm, 23 mm) respectively at the same concentration, while the growth of *Staphylococcus aureus* and *Acinetobacter baumannii* by inhibition zone. (20.4 mm, 20.6 mm) respectively with (30mg/mL). The highest value of inhibition zone with (*Klebsilla Pneumoniae*, *Salmonella typhi* and *Staphylococcus*) respectively by. (30.5 mm ,26.2 mm, 23 mm) respectively on the growth by inhibition zone. The chitosan showed significant activity against *Staphylococcus*, *candida albicans* and *Pseudomononeus*, Antimicrobial activity local extracted chitosan better antibiotic sensitivity compared Antibiotic sensitivity, antimicrobial isolates was given behavior against different antibiotic, no result of ceftazidime but highly sensitive to vancomycin other given different result.

Antioxidant Activity

The antioxidant activity of Chitosan was evaluated by means of 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) free radical

scavenging assay. In brief, varied concentrations of Chitosan (1.0, 1.2, 1.4, 1.6 and 2.0 mg/mL) were added to 10 mL of 0.1 M DPPH solution and placed under dark at room temperature for 30 min to facilitate the reaction. Thereafter, using ethanol as blank, the absorbance was recorded at 517 nm wavelength. The experiment was conducted in a similar manner using ascorbic acid as standard. Scavenging activity was determined based on the absorbance i.e., the lower the absorbance, the higher is the scavenging activity. The percentage inhibition of free radicals was calculated based on the formula given below: spectrophotometer was then used to measure the optical density (O.D) of the solutions. The zero measurement of the spectrophotometer was set using distilled water (O. D = 0.732 nm). The measurements obtained were compared with the O.D of the blank set and radical scavenging activity was examined.

$$\% \text{ Inhibition} = (\text{Control OD} - \text{Sample OD}) / (\text{Control OD}) \times 100$$

It is evident from the results that the Chitosan exhibited potent antioxidant activity at different concentrations. Fig. 4 was observed difference antioxidant with difference concentration.

However, free radical scavenging activities of nanoparticles were lower to the standard (ascorbic acid). The highest scavenging activity was observed in ascorbic acid (20% with 1gm/ml concentration

Table 1. Antimicrobial effect of local extracted Chitosan from fish shell compered withe stander chitosan measured by mm.

0	Chitosan (local extracted)			Chitosan (Standard)		
	10mg/mL	20mg/mL	30mg/mL	10mg/mL	20mg/mL	30mg/mL
<i>Klebsilla Pneumoniae</i>	no inhibition zone	18.6	30.5	no inhibition zone	18	30
<i>Pseudomonas aeruginosa</i>	no inhibition zone	10.3	15.6	no inhibition zone	10	15
<i>Staphylococcus aureus</i>	6	12.6	20.4	5	12	20
<i>Acinetobacter baumannii</i>	10.5	14	20.6	10	13	20
<i>Escherichia coli</i>	no inhibition zone	no inhibition zone	16	no inhibition zone	no inhibition zone	10
<i>Staphylococcus epidermidis</i>	17.5	21	23	17	20	22
<i>Salmonella typhi</i>	no inhibition zone	19.2	26.2	no inhibition zone	18	25
<i>Candida albicans</i>	no inhibition zone	12	18	no inhibition zone	12	17

Table 2. Antibiotic Sensitivity Test measured by mm.

Name of Bacteria	CEFTAZIDIME	CEFOTAXIME	AMPICILLIN	TICARA+CLAUV	CEFOXITIN	VANCOMYCIN
<i>Klebsilla Pneumoniae</i>	0	8	13	11	0	16
<i>Pseudomonas aeruginosa</i>	0	12	10	13	8	10
<i>Staphylococcus aureus</i>	0	10	12	14	16	18
<i>Acinetobacter baumannii</i>	0	7	10	11	13	20
<i>Escherichia coli</i>	0	0	5	11	8	10
<i>Candida albicans</i>	0	14	7	10	5	12
<i>Staphylococcus epidermidis</i>	0	13	10	14	7	12
<i>Salmonella typhi</i>	0	5	10	14	8	17

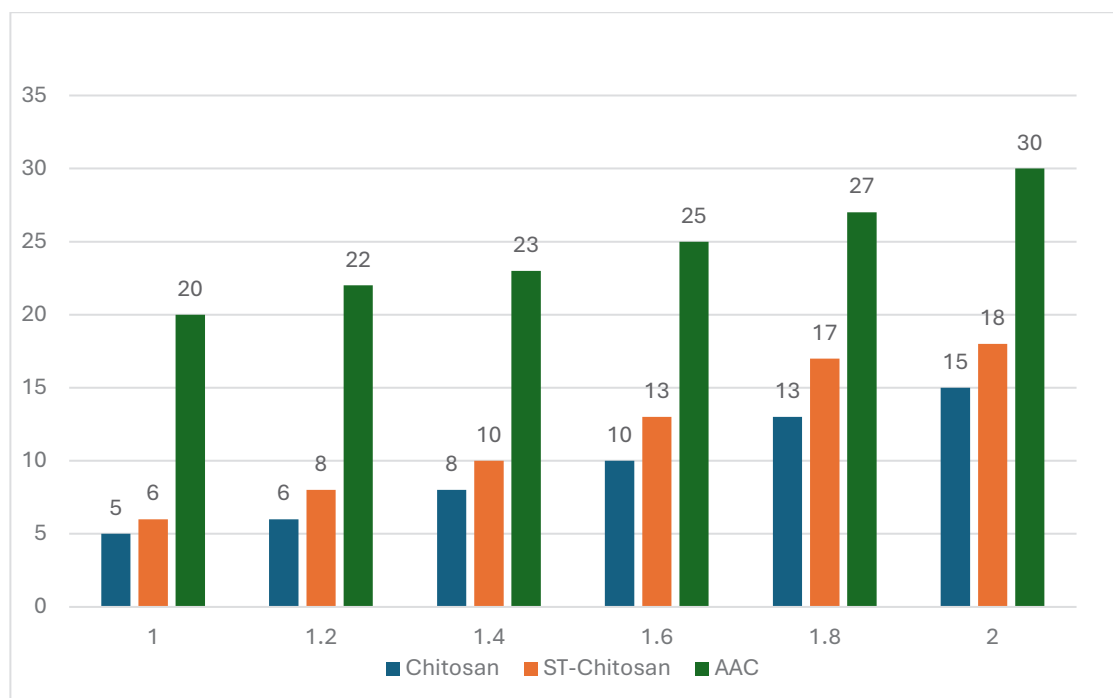


Fig. 4. 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) free radical scavenging activities of ascorbic acid, Chitosan.

(30%) with 2mg/ml. the highest scavenging activity was observed in Chitosan extracted, Chitosan (4%,5%) with 1mg/ml concentration (16%,18%) with 2mg/ml. This antioxidant activity could be linked to the transfer of free electrons from the oxygen atom of nanoparticles to free radicals present at the nitrogen atom of DPPH molecules. It has been reported that many of the metal nanoparticles can scavenge free radicals and act as antioxidants [33].

One of these products' chitin. Chitosan

derivative from chitin, which is found in the exoskeletons of insects, the shells of crustaceans, and the cell walls of fungi. While Chitosan has low toxicity in terms of its antibacterial action, with antimicrobial properties, high bioactivity, biodegradability. Chitosan has been local extracted from Fish Shells by simple and a green approach. Chitosan reduces the growth of bacteria by obstructing the formation of bacterial cell walls (Disruption of the membrane.). By interacting with the polysaccharides in bacterial cell walls, chitosan

prevents the manufacture of new cell walls and stops the development of bacteria. Chitosan has the ability to block the actions of enzymes that help bacteria produce their cell walls and essential proteins, which prevents the bacteria from growing and reproducing. The chemical and structural characteristics of chitosan should be taken into account when evaluating its inhibitory efficacy against bacteria. Since the bacterial outer membrane serves as an effective exterior permeability barrier against macromolecules, chitosan, being a polymeric macromolecule, cannot flow through it [34].

Permeabilizers are chemicals that increase outer membrane permeability through a variety of methods without being intrinsically harmful [35]. For instance, it has been demonstrated that when EDTA is used to disrupt the outer membrane, bacteria's resistance to the biocidal activity of lysozyme decreases [36,37].

CONCLUSION

Adopt simple friendly with environment and effective method for local extraction of high purity chitosan from fish shell waste by. It observes the XRD studies elucidate the highly amorphous nature of the Chitosan. From FT-IR results it was evident that, in the prepared the peaks were observed due to the various functional groups (OH, COO⁻, NH) present in added polymeric components, and the rough surface morphology was identified from the SEM studies. In addition, study the antioxidant activity compared to ascorbic acid (AAc). Fish shells can be utilized for ecologically friendly biosynthesis. These nanoparticles are effective antioxidants and antibacterials. Large-scale synthesis is another option. It may be used to target resistant infectious bacteria and is highly recommended as a less expensive alternative to traditional anti-bacterial medicines. It can also be utilized as a preservative in fisheries goods. Nevertheless, the inhibitory effects varied according to the chitosan kinds and the tested bacteria, with gram positive bacteria showing more antimicrobial activity than gram negative bacteria.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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