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Extraction and Characterization of Chitosan Obtained from Scales of *Clarias gariepinus* (Catfish)

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Fish waste is considered to be one of the major bio-pollutants which are generally discarded in coastal regions through local markets and fish processing industries. Nowadays, it is being eyed as a newer bioresource. Fish scales are good source of chitin and chitosan. Very little information is available related to extraction of chitosan from fish scales. In this study fish scale waste from markets around Chennai was used for extraction of chitosan. Chitosan is extracted from the fish scales and the physicochemical properties of the extracted chitosan were characterized by FTIR, UV-Visible spectroscopy, and Scanning Electron Microscopy. The antibacterial activity of chitosan against different sea food pathogens pathogens like *Vibrio parahaemolyticus, Vibrio cholerae, Staphylococcus aureus, Salmonella typhii*, *Escherichia coli* and *Shigella dysenteriae* was evaluated by calculation of minimum inhibitory concentration (MIC). Antioxidant activity for chitosan was

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performed with various concentration of 20 µm-100 µm and the scavenging activity of the sample is calculated as 15%. The physiochemical properties and FTIR and XRD studies confirm the production of chitosan, because of its high antibacterial activity against pathogens, hence chitosan can be widely used in food preservation, manufacture of wound dressing and in antimicrobial finished textiles.

Keywords: Fish scales; chitosan; antimicrobial activity; scanning electron microscope; fourier transform infrared spectra; antioxidant activity.

1. INTRODUCTION

The sea food industries waste poses environmental hazard due to easy deterioration, even though the wastes generated are biodegradable, it degrades slowly. About 130 million tons of fish waste is generated each year in the world [1]. Fish waste is nothing but pile of the leftover parts of dead fish - heads, tails, internal organs etc. So quick and effective method to utilize these wastes could be to produce biological sustainable materials is the current challenge in research. Chitin is a major structural component of all the crustaceans. Chitosan can be produced by partial deacetylation of chitin with alkali solutions at elevated temperature [2-4]. It is a Bio-polymer composed of β-(1-4)-linked D-Glucosamine. Chitosan has many industrial application based on the modification of reactive functional group. These modifications can be carried out using chemical or biological method [5,6]. Chitosan and its derivatives are inhibiting the growth of many bacteria, fungi and pathogens [7,8]. This important property of acting as antimicrobial agent is useful for food and pharmaceutical industry. Some of the important applications of chitosan are as biomaterial in tissue engineering, food preservatives, drug delivery. Numerous research studies have been undertaken to extract chitosan from prawn shell and lobster. The commercial production of chitosan from shrimp and crab shells requires high production cost and multiple chemical processes such as demineralization, deproteinization, and decolourization [9]. Therefore fish scale waste is another low cost raw material for chitin production. “Fish scales” are good source of chitin and chitosan. Generally, fish scales consist of protein (type I collagen and ichthylepidin) and apatite (calcium phosphate, magnesium carbonate and calcium carbonate) [10]. Research on extraction of chitosan from fish scale is scares and the present investigation focuses on its extract from *Clarias gariepinus* sps and determination of it physico-chemical properties along with antimicrobial and antioxidant properties [11].

2. MATERIALS AND METHODS

2.1 Collection of Fish Scales

Fish scale from *Clarias gariepinus*, was collected from the fish market in Chennai, India. The scales and meat portions were separated and cleaned. The scales were dried at 50°C in an oven for 24 hours. The dried material was homogenized in a laboratory mixer. The moisture content was determined by weighing before and after being dried. The scales were then crushed and preserved in air tight containers.

2.2 Extraction of Chitin and Chitosan

The extraction of chitin and chitosan involved sequence washing of crushed fish scales. These were placed in 1000 ml beakers and soaked in boiling sodium hydroxide (2 and 4% w/v) for one hour in order to dissolve the proteins and sugars thus isolating the crude chitin. 4% NaOH is used for chitin preparation [12]. After the samples were boiled in the sodium hydroxide, the beakers containing the fish scales samples were allowed to cool for 30 minutes at room temperature.

2.2.1 Demineralization

The grounded scales were demineralized using 1% HCl with four times its quantity. The samples were allowed to soak for 24 h to remove the minerals (mainly calcium carbonate) [13]. The remaining chitin was washed with deionized water, which is then drained off. The chitin was further converted into chitosan by the process of deacetylation.

2.2.2 Deacetylation

The deacetylation process was carried out by adding 50% NaOH and then boiled at 100°C for 2 h on a hot plate. The samples were then

placed under the hood and cooled for 30 min at room temperature. Then the samples are washed continuously with the 50% NaOH and filtered in order to retain the solid matter, which is the chitosan. The samples were then left uncovered and oven dried at 110°C for 6 h. The chitosan obtained was in a creamy-white form [9].

2.2.3 Purification of chitosan

The processed chitosan has to be purified to make it suitable for further Industrial applications. The purification process was designed in three steps (a) Removal of insoluble with filtration. (b) Reprecipitation of chitosan with 1N NaOH (c) Demineralization with hydrochloric acid and NaOH.

2.3 Characterization of Prepared Chitosan

2.3.1 pH

The pH measurements of the chitosan solutions was carried out using a microprocessor pH meter.

2.3.2 Ash value

To determine the ash value of chitosan, 2.0 g of chitosan sample were placed into previously ignited, cooled, and tarred crucible. The samples were heated in a muffle furnace preheated to 65°C for 4 hr. The crucibles were allowed to cool in the furnace to less than 200°C and then placed into desiccators with a vented top. Percentage of ash value is calculated using the following [14].

$$\%Ash = weight(g)/sampleweight(g)\times 100$$

2.3.3 Loss on drying

Loss on drying of the prepared chitosan was determined by the gravimetric method. The water loss has been determined by drying the sample to constant weight and measuring the sample after and before drying. The water mass (or weight) will be the difference between the weights of the wet and oven dry samples [15].

$$\%\ Loss\ on\ drying = Wet\ weight\ (g) - Dry\ weight(g)/\ Dry\ weight(g)\times 100$$

2.3.4 Fourier transform infrared spectra (FTIR) studies of chitosan

The samples of Chitin and Chitosan produced were characterized in KBr pellets by infrared spectrophotometer in the range of 400 to 4000 cm^{-1} (Brucker Equinox 55).

2.3.5 Scanning electronic microscopy (SEM)

Scanning electron microscope (model 2360, Leo Oxford, England) was used to evaluate the surface and shape characteristics of the particles.

2.3.6 UV-Vis absorption

Each sample was analyzed by UV-visible spectrophotometer (Optima 3200, Tokyo, Japan) in the range 200-750 nm and the wavelength corresponding to maximum absorption (μ max) was recorded. 0.1% (w/v) and 0.5% (w/v) chitosan in 1% (v/v) acetic acid solutions irradiated at the same dose (20±2 kG) were used as blank samples.

2.4 Screening for Antimicrobial Activity

The Chitosan was tested for their antimicrobial activity by the agar well diffusion method and the Minimum inhibitory Concentration was calculated by the lowest concentration of chitosan that inhibits the growth of bacteria was considered as the minimum inhibitory concentration or MIC. The microbial strains *Vibrio parahaemolyticus, Vibrio cholerae, Staphylococcus aureus, Salmonella typhi, Escherichia coli, Shigella dysenteriae* were used for this analysis. This culture suspension was seeded in agar plates by the pour plate technique. Three cavities were made using a cork borer (10 mm diameter) at equal distance and were filled with the chitosan solution and then incubated at room temperature for 24 hrs. The formation of a clear zone (restricted microbial growth) around the cavity is an indication of antimicrobial activity.

2.5 Scavenging Ability on 1, 1-diphenyl 1-2-picryl Hydroxyl Radicals (DPPH)

The scavenging effect of chitosan on DPPH radical was examined using the modified method described earlier [16,17]. Each chitosan sample (0.1-10 mg/ml) in 0.2% acetic acid solution was mixed with 1 ml of methanolic solution containing DPPH radicals, results/resulted in a final concentration of 10 mM DPPH. The mixture was shaken vigorously and left to stand for 30 min in the dark and the absorbance was then measured at 517 nm against a blank [18]. Ascorbic acid was used as standard. The scavenging ability was calculated as follows:

Scavenging ability (%) = [ΔA517 of control-ΔA517 of sample) /ΔA517 of control] X100.

3. RESULTS AND DISCUSSION

Chitosan was extracted from the fish scales which are discarded daily as waste materials from fish markets of Kanathur. Extraction of chitosan from fish scales was done using chemical treatments. The results of physiochemical properties of chitosan are given in (Table 1). The prepared chitosan was confirmed as reported by Sunithakumari et al. (2014).

Table 1. Physiochemical parameters of chitosan

Physiological parameters	Chitosan
Yield	45.56 %
Moisture content	5%
Ash value	1.26%
Loss on drying	3%
pH	7
Solubility	Acetic acid

The absorption bands of FTIR of chitosan were observed in the range of 3643 -3938 cm^{-1} related to associated in N-H bond primary amines, 2920 cm^{-1} – 3440 cm^{-1} was associated with C═O of carboxylic acid, 1787-2522 cm^{-1} was associated with C ═ N, C ≡ N of aliphatic amine and 433 cm^{-1} - 860 cm^{-1} C ─ N Aromatic (Bending) (Fig. 1). The band at 1567 cm^{-1} has a larger intensity than at 1655 cm^{-1}, which suggests effective deacetylation. When chitin deacetylation occurs, the band observed at 1656 cm^{-1} decreases, while a growth at 1597 cm^{-1} occurs, indicating the prevalence of NH2 groups [19,20].

Fig. 2 show the SEM photographs of prepared chitosan from fish. The sample exhibited rough and thick surface morphology under electron microscopic examination at 50X magnification. This was in accordance to the previous data [21].

The antibacterial activity of the extracted chitosan samples against gram negative and gram positive bacteria are shown in Fig. 3. The plates were checked for anti-bacterial activity after 24 hours of incubation. Data indicated that, chitosan markedly inhibited the growth of most of gram negative bacteria tested; however, the inhibitory effects differed depending on the types of chitosan and the tested bacteria. It could be reported that, good antibacterial activity was observed in the plates containing test strains *Vibrio parahaemolyticus* (20 mm), *Vibrio cholera* (17 mm), *Staphylococcus aureus* (18 mm), *Salmonella typhii* (17 mm), *Escherichia coli* (15 mm), *Shigella dysenteriae* (16 mm). The bacterial pathogen *V. parahaemolyticus* for chitosan showed 20 mm inhibition zone whereas the Chitosan showed little inhibition for test strain *E. coli* compared to others (Table 2). Chitosan from fish scales show efficient antibacterial property due to their extremely large surface area, which provides better contact with microorganisms [22,23].

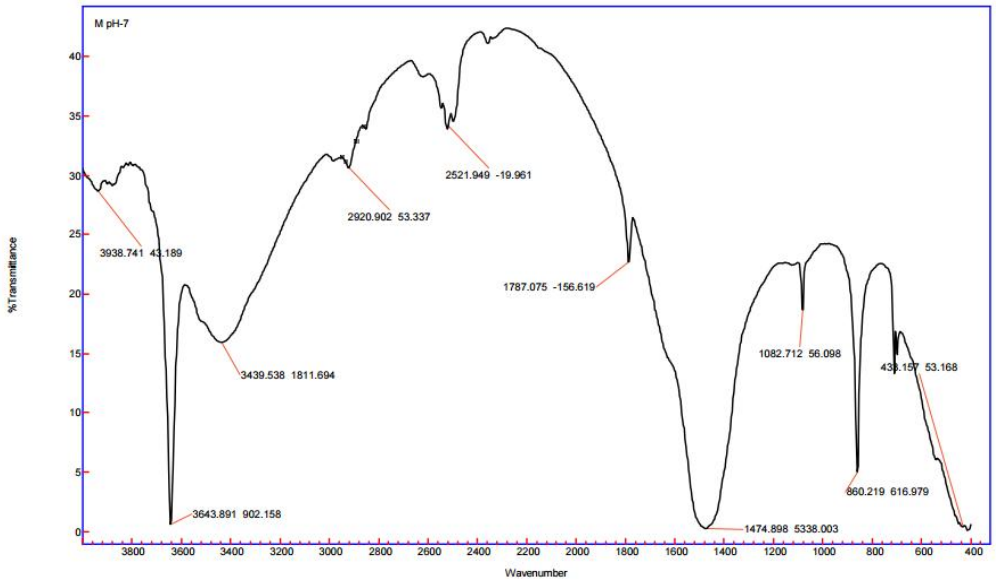

Fig. 1. FTIR of chitosan

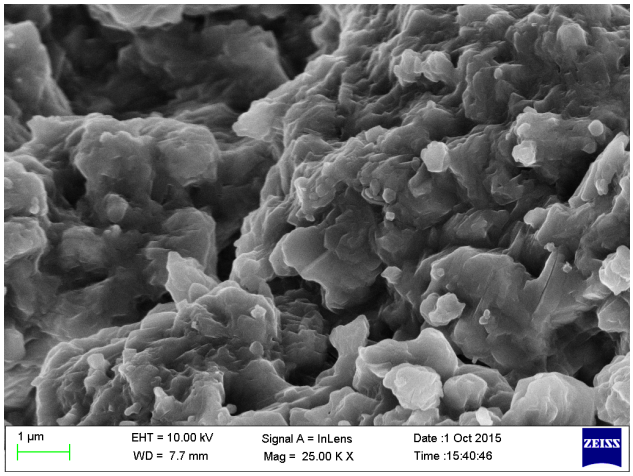

Fig. 2. SEM image of chitosan

Table 2. Antimicrobial activity of chitosan derived from fish scales

Test organism	Concentration of chitosan (µg/mL)	Concentration of acetic acid (µL/mL)
	0.001	20
	Zone of inhibition(mm)	
V. parahaemolyticus	20	
Vibrio cholera	17	
S. aureus	18	
S. typhii	17	
E. coli	15	
Shigella dysenteriae	16	

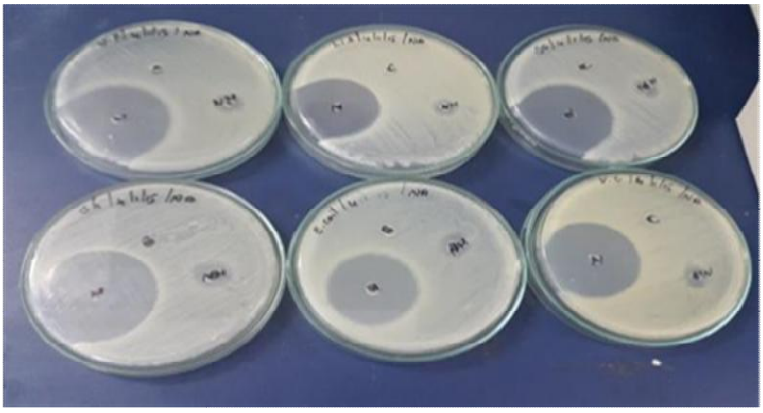

Fig. 3. Zone of inhibition of chitosan derived from fish scales against various pathogens

Antioxidant activity of chitosan at different concentration was shown in Fig. 4. Yen et al. [18] reported that the scavenging activity of the extracted chitosan sample on (DPPH) radicals was increased with their concentration increased. The DPPH radical scavenging activity of the chitosan was significantly lower than that of Ascorbic acid at the same concentration. The activity varied from 2.5 to 15%, which corresponds to 0.2 to 1.0 mg of the chitosan per mL. Chitosan scavenges various free radicals through the action of nitrogen on the C-2 position of the chitosan [24,25].

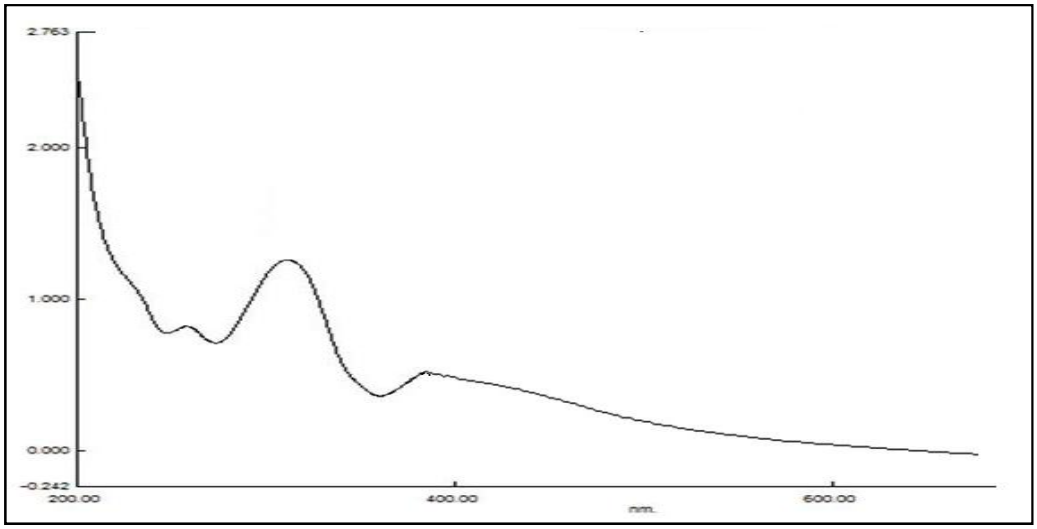

Fig. 4. UV visible spectroscopy of chitosan scales (x-axis wavelength in nm, y-axis absorbance in nm)

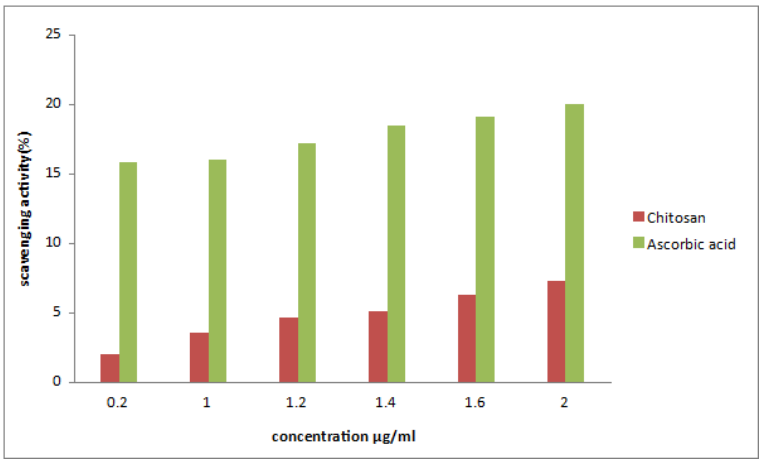

Fig. 5. Antioxidant activity of chitosan scales from *Clarias gariepinus* (X axis = concentration of DPPH µg/mL, Y axis = % of scavenging activity)

4. CONCLUSION

In this study, waste fish scale collected from markets were used for extraction of chitosan. Chitosan was synthesized from the fish scales and the physicochemical properties of the extracted chitosan were characterized by FTIR, UV-Visible spectroscopy, and Scanning Electron Microscopy. The antibacterial activity of chitosan against different sea food pathogens pathogens was evaluated by calculation of minimum inhibitory concentration (MIC). Antioxidant activity for chitosan was performed with various concentrations and the scavenging activity of the sample is calculated. This study shows the production of chitosan from fish scales, would successfully reduce the environmental pollution. The physiochemical properties and the FTIR and XRD studies confirm the production of chitosan. Because of its high antibacterial activity against sea food pathogens chitosan can be widely used in food preservation, manufacture of wound dressing and in antimicrobial finished textiles. Further plan of work would be on exploiting the use of chitosan in the field of environmental studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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