


RESEARCH ARTICLE

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Extraction of Chitosan from Crab Shell and Fungi and Its Antibacterial Activity against Urinary Tract Infection Causing Pathogens

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Abstract

In this study, shells of sea crab and fungus *Aspergillus niger* were subjected for chitosan extraction which has been done following demineralization, deproteination and deacetylation. Chitosan yield from crab shell and fungi was 37.5% and 39.3% respectively and water binding capacity was 58.44% and 60.21% respectively. The extracted chitosan was characterized using Fourier transform infrared spectroscopy (FTIR) and subjected for antibacterial activity against Urinary tract infection (UTI) pathogens - *Klebsiella pneumoniae*, *Proteus mirabilis* and *E. coli*. Chitosan of crab shell showed better antibacterial activity than fungal derived chitosan. Chitosan gel was prepared using the extracted chitosan where it was also showing good antibacterial activity.

Keywords: Crab shell, fungal cell, chitosan, antibacterial activity, UTI pathogens

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INTRODUCTION

Chitin, an abundant natural amino polysaccharide which is a constituent of exoskeleton of shrimp, crab etc and also seen on fungi cell walls^{1,2}. Deacetylated form of chitin is chitosan which consists of polymer of N-acetyl D-glucosamine bonded through β -1,4-glycosidic bond^{3,4}. This chitosan has multiple functional properties and biological activities thus used in pharmaceutical, nanotechnology and agricultural industries⁵⁻⁸. Its antimicrobial activity made them to be used in food preservation as coating agents on wrapper of various foods⁸. Its non-toxicity and non-allergenicity made them to be used in biomedical applications and also used in treating wounds^{9-12,4}. Chitosan coated nanoparticles are used for heavy metal removal, nanocarrier synthesis, drug delivery etc¹³⁻¹⁸. Urinary tract infections (UTI), commonly caused by bacteria and their pathogenicity depends on host biological behavioral factors and properties of the infecting uropathogens¹⁹. The major bacteria causing UTI are *Proteus mirabilis*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*²⁰. Emergence of drug resistance making the treatment harder, and development of new drugs become inevitable²¹⁻²³. If derived from biological sources it might be having less side effect²², chitosan is one such which can be derived from crab shell and fungal cell wall. Thus, in this study, chitosan of crab shells and fungal cell walls was extracted and used for antibacterial activity against UTI pathogens.

MATERIALS AND METHODS

Collection of Crab shells

The crab shells required for the experiments were brought from local fish processing industries in Tuticorin, Tamil Nadu, India. The crab shells were washed, cleaned, dried at 70°C in hot air oven, powdered using blender and stored in air tight container in room temperature.

Isolation and cultivation of fungi

Soil fungi *Aspergillus niger* was isolated from soil and inoculated into 100 mL of yeast peptone glucose (YPG) medium (2.0% glucose, yeast peptone glucose - 0.2% yeast extract, and 1.0% peptone) and incubated at 28°C for 5 days.

The fungal mycelium was transferred into 500 mL of YPG medium. The flasks containing inoculum were kept for incubation without shaking at 28°C for 5 days. Mycelium was obtained through filtration process using Whatman filter paper and harvested mycelia was washed with distilled water and dried in hot air oven and ground to a powder and stored in a sterile airtight container at room temperature for extraction for chitosan. The extraction of chitosan consisted of three steps such as, deproteinization, demineralization and deacetylation.

Extraction, fat binding capacity (FBC) and water binding capacity (WBC) of chitosan

Deproteinization of both the fungi and crab shell was done as prescribed²⁴. Demineralization and deacetylation of chitin were done following the earlier reports²⁵⁻²⁶. Determination of yield of crab shell and fungal biomass chitosan was done²⁷. The fat binding capacity and water binding capacity of chitosan were measured using the protocol of No et al.²⁸.

Characterization of chitosan

FTIR characterization of sample was performed with a Perkin Elmer spectrum RX1 instrument within a frequency range of 400–4000 cm^{-1} . Degree of deacetylation of chitosan was determined²⁹.

Identification of UTI organisms and determination of antibiotic susceptibility

Urine sample isolates were collected from AVM hospital, Tuticorin, Tamil Nadu, India. Sterile nutrient agar plates were prepared and inoculated with urine sample. After 24 h of incubation, observed colonies were identified through the morphological and biochemical characteristics. All the organisms were checked for their antibiotic resistance pattern against commercial antibiotic discs. Antibiotic susceptibility was determined using commercially available disc diffusion assay.

Antibacterial activity of chitosan

Chitosan solution was done by dissolving chitosan in 1% of acetic acid. This solution was used for testing antibacterial activity³⁰. For antibacterial activity, Mueller Hinton agar (MHA) medium was prepared and sterilized. The culture of pathogenic microorganisms was swabbed onto the surface of sterile MHA plates. The antibacterial activity was performed by agar well diffusion method³¹. Chitosan solution of different concentration (10 μg ,

20 µg, 30 µg) was added using a micropipette into the wells (6 mm) punched over the MHA plates using a sterile cork borer. These agar plates were incubated for 24 h at 37°C. Zone of inhibition was observed and measured around the wells after the period of incubation.

Preparation of the chitosan gels and its antibacterial activity

In order to form the gel, method described by Samano-Valencia et al.³² was followed and antibacterial activity was performed by agar well diffusion method.

RESULTS

The yield of extracted chitosan of crab shell and fungi was found to be 38% and 39.3% respectively (Table 1). The moisture content of the chitosan extracted from crab and fungi was found to be 64.34% and 66% respectively. The water binding capacity and fat binding capacity of crab and fungal chitosan was found to be 58.44%, 60.21% and 40%, 41% respectively (Table 2).

Table 1. Percentage of extracted crab and fungal chitosan

S.No	Source	Initial weight (g)	Final weight (g)	% of extracted Chitosan
1.	Crab shell	20	7.5	37.5
2.	Fungal biomass	20	7.86	39.3

Table 2. Physicochemical characterization of extracted chitosan

S.No	Physicochemical Characteristics	Values	
		Crab Shell chitosan	Fungal Biomass Chitosan
1	% Water binding capacity of Extracted Chitosan	58.44	60.21
2	% Fat binding capacity of extracted Chitosan	40	41
3	Moisture content	64.34	66
4	Degree of deacetylation (%)	90	60

The appearance of extracted chitosan from crab shell was yellowish white in color (Fig.1a) and from fungi it was black in color due to its conidial nature (Fig.1b). FTIR spectrum of crab chitosan recorded major peaks lying between

500.05 cm⁻¹ and 3880.60 cm⁻¹ (Fig. 2). FT-IR spectrum of the fungal chitosan recorded major peaks lying between 564.67cm⁻¹ and 3451.92 cm⁻¹. In this way, our study of FT-IR analysis showed spectral peaks of extracted chitosan from crab

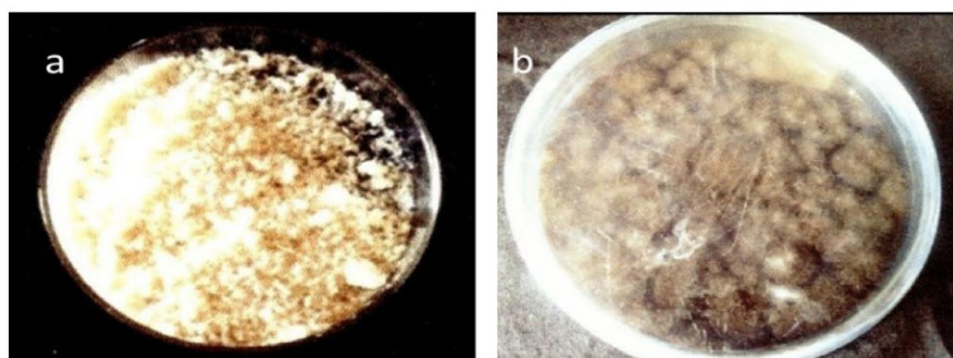


Fig. 1. Extracted chitosan a) Crab shell derived b) fungal derived

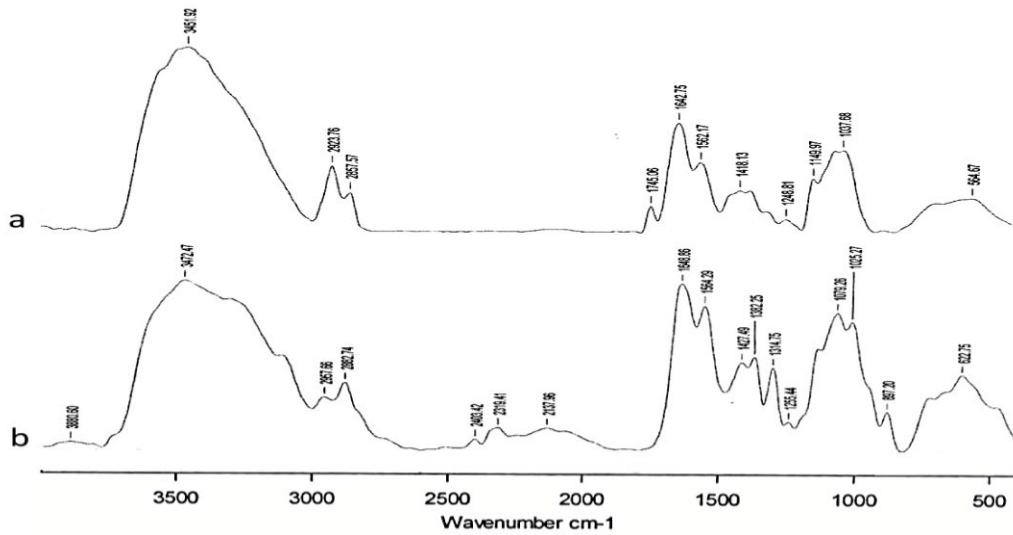


Fig. 2. Fourier transform infrared spectroscopy (FTIR) of chitosan a) Crab shell derived b) fungal derived

and fungi were confirmed with their functional groups and crab and fungal chitosan with degree of deacetylation (DD) of 90%, 60% respectively.

The identified organisms of urine samples were *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumoniae* through its biochemical characteristics. All the organisms were showing resistance to one or two antibiotics used in

this study (Table 3). 20 µg of crab shell derived chitosan showed 6 mm zone of inhibition against *Escherichia coli* whereas 30 µg showed 8 mm against *K.pneumoniae* (Table 4 and Fig. 3). The highest zone of inhibition was observed at 30 µg of both crab shell chitosan and fungal chitosan against all bacteria (Table 4, Fig. 3 and 4).

Table 3. Antibiotic susceptibility of UTI pathogens

S.No	Pathogens	Tetracycline (30mcg)	Streptomycin (10mcg)	Norfloxacin (10mcg)	Methicillin (10mcg)
1	<i>E. coli</i>	R	I	R	R
2	<i>P. mirabilis</i>	I	I	R	R
3	<i>K. pneumoniae</i>	R	R	I	R

R- resistant, I - intermediate

Table 4. Antibacterial activity of crab chitosan against the urinary pathogens

Organism used	Zone of inhibition (mm)					
	Crab chitosan (10 µg)	Fungal chitosan (10 µg)	Crab chitosan (20 µg)	Fungal chitosan (20 µg)	Crab chitosan (30 µg)	Fungal chitosan (30 µg)
<i>E. coli</i>	-	2	6	5	8	6
<i>P. mirabilis</i>	-	-	-	2	5	2
<i>K. pneumoniae</i>	-	-	1	-	2	3

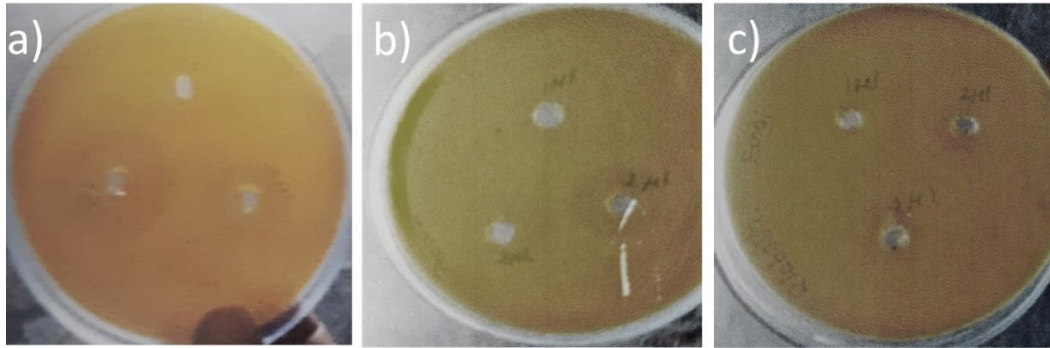


Fig. 3. Antibacterial activity of crab shell chitosan a) *E.coli* b) *Proteus mirabilis* c) *K.pneumoniae*

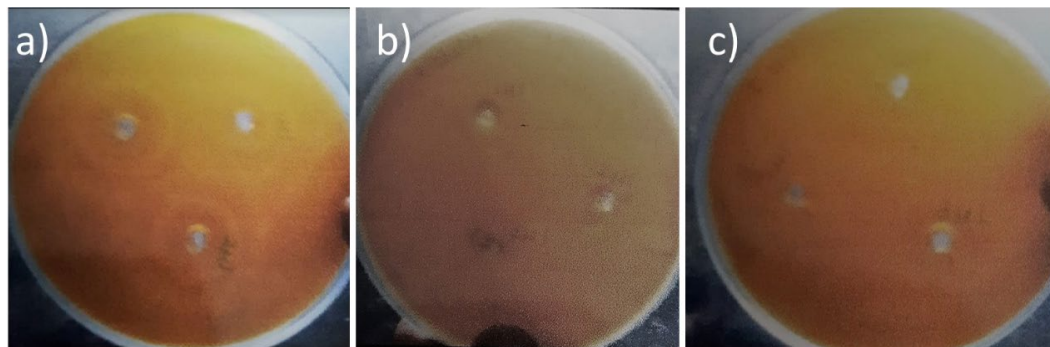


Fig. 4. Antibacterial activity of fungal chitosan a) *E. coli* b) *Proteus mirabilis* c) *K. pneumoniae*

Antibacterial activity of crab shell chitosan gel showed maximum zone of inhibition i.e. 6 mm against *Escherichia coli* (Fig. 5) where fungal

chitosan showed maximum zone of inhibition at 100 µg against *Proteus mirabilis* (4 mm).

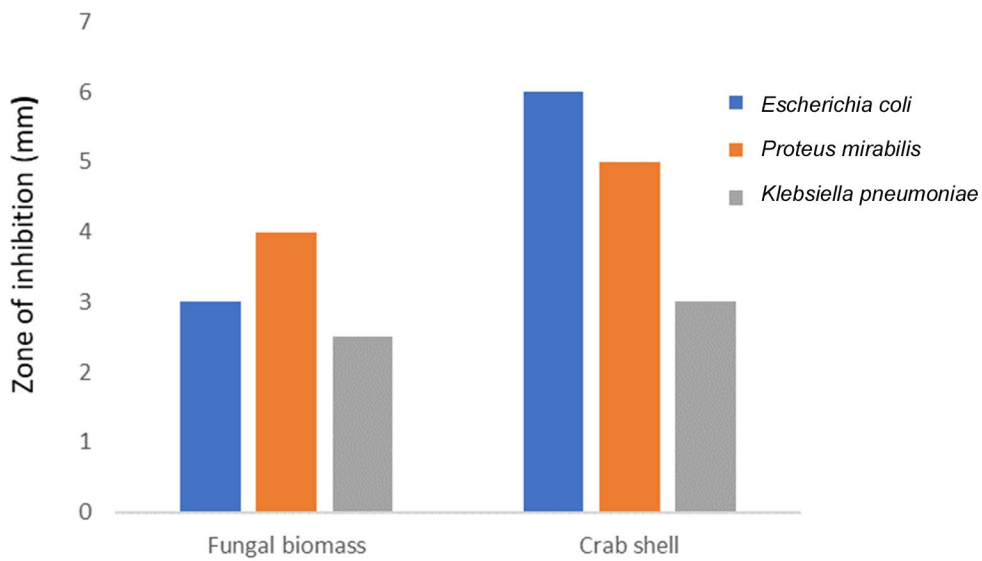


Fig. 5. Antibacterial activity of chitosan gel against UTI pathogens

DISCUSSION

The wider applications of chitosan are because of its biodegradability, biocompatibility and low toxicity³³. The maximum yield of chitin and chitosan in fungi was obtained in late logarithmic phase³⁴. Yield% of chitosan from *Scylla serrata* was 38.23%^{35,36} where in this study, it has also shown crab chitosan yield% of 37.5%. Chitosan extraction using NaOH helps in binding with acetyl group of chitins, forms sodium acetate aids in the extraction of chitosan³⁷. It was found that the fat binding capacity was more with fungal derived chitosan than the crab derived chitosan. No et al.²⁸ reported this as a physical property of chitosan to hold water or fat held and getting trapped in the structure and swells, thus can be used for drug delivery. Chemical methods for deproteination and demineralization are effective³⁸, the colour of chitosan obtained was different which might the influence of conidia of fungal strain. Kobayashi et al.³⁹ pointed that increased advantage of chitosan of fungi made them to receive great attention compared to chitosan extracted from crustacean shell. The major advantage of using chitosan extracted from fungi mycelium is, it is obtained easily by fermentation process and available at all seasons and geographical location but collection of crustacean waste supplies is limited by fishing industry locations and seasons⁴⁰. chitin from fungal species can be derived from Phycomycetes, Basidiomycetes, and Ascomycetes⁴¹.

Peaks at 564.67cm^{-1} and 3451.92cm^{-1} of fungal chitosan and 500.05cm^{-1} and 3880.60cm^{-1} of crab shell derived chitosan where there are on par with the earlier reports of chitosan extracted from various sources⁴²⁻⁴⁴. The degree of deacetylation (DD) is calculated by identifying the functional group present in the extracted chitosan⁴⁵. The DD of crab shell derived chitosan and fungal chitosan were 90% and 60% respectively, Thus, DD was more with crab shell chitosan than fungal, so crab shell chitosan was purer than fungal chitosan. Chitosan behaves like a polyelectrolyte by getting dissolved in the acid aqueous solution when the degree of deacetylation reaches above 50%⁴⁶. In our study, both extracted chitosan was insoluble in water and highly soluble in acidic aqueous solutions. Chitosan molecules do have a pH below 6 with a strong positive charge when the DD is high (> 85%)⁴⁷. Chitosan extracted from crab shell

showed excellent antibacterial activity compared to fungal chitosan.

Escherichia coli, *Proteus mirabilis* and *Klebsiella pneumoniae* were identified by their biochemical characteristics⁴⁸. The highest zone of inhibition was observed for crab shell chitosan as well as the chitosan gel than fungal chitosan / chitosan gel against all bacteria. Kong et al.⁴⁹ reported that crab shells are rich source of chitosan and this chemical constituent greatly effects antibacterial activities. And also, Klaykruey et al.⁵⁰ reported that smaller oligomeric chitosan can penetrate into the cell membrane and inhibits RNA transcription and prevents the cell growth therefore it is observed that molecular weight plays a major role in determining the antibacterial activities of chitosan. Moreover, chitosan absorbs the electronegative substrate of proteins and may disrupt the physiological activities of microbes and leads to cell death⁵¹. Lim and Hudson⁵² reported that the interaction between the anionic components and intracellular components are due to the leakage caused by changing the cellular permeability by the polycationic nature of chitosan and leading to cell death.

CONCLUSIONS

In this study, crab shell and fungi were used for deriving chitosan. The derived chitosan were characterized using FTIR and used for making chitosan gel. Both the chitosan and chitosan gel were found to have antibacterial activity against UTI pathogens, where crab shell derived chitosan and chitosan gel were showing more activity than the fungal chitosan.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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