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Full Length Research Paper

Chitosan derived from the tunic of ascidian *Phallusia nigra* (Savigny, 1816) showing antibacterial activities and its characterization

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The present study was to evaluate the *in-vitro* antibacterial activity of chitosan against clinical pathogens. Chitosan was extracted from the tunic of *Phallusia nigra* the marine black tunicate by utilizing standard protocol. Chitosan is a versatile natural polysaccharide, the second most abundant, biocompatible, biodegradable and non-toxic natural polymer which made wide applicability in conventional pharmaceutics as a potential formulation excipient. The extracted chitosan was affirmed through Fourier Transform Infra-red (FT-IR) spectroscopy. The FT-IR spectrum of chitosan was resolved and the characterization was done and it was compared with standards. Chitosan, those prepared in this study may display potential antibacterial activity against various pathogens. Through testing, the crude chitosan demonstrated good inhibition activities against clinical, *Vibrio cholarae* (9.5 mm), and *Vibrio parahaemolyticus* (8.9 mm) at the concentration of 1 mg/ml by determining the zone of inhibition. These outcomes showed that the high potential of chitosan biopolymer as an antibacterial agent against different clinical pathogens.

Key words: Ascidians, chitosan, antibacterial activities, Fourier transform infra-red (FT-IR).

INTRODUCTION

Ascidians are the wide diverse group of sessile filter feeding marine invertebrates which belonged to phylum Chordata, subphylum Urochordata, and class Ascidiacea called “Sea Squirts” or tunicate usually firmly attached with natural and artificial substrates in the intertidal and subtidal zones of marine ecosystem (van Name, 1945). Presently, nearly about 3000 ascidian species are known. Ascidians are entirely strong spatial competitors and once they are brought into the new environment, they might persist and get to be the prevailing group to that location (Nandakumar, 1996; Lambert and Lambert, 2003). They demonstrate a large dissimilarity in not only the external appearance and colour, but also exhibit huge morphological variation in its structure of dominant internal organ and in the branchial basket (Kott, 1989; Monniot et al., 1991). The body is secured with a smooth thick and tough mantle called tunic which is quite rigid in nature, made up of cellulose like substance called tunicin,

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a polysaccharide. The components of tunic are very strange, composed of water, proteins, mucoploy-saccharides and carbohydrates at various proportions (Welsch, 1984). The intricacy of tunic fluctuated among species, they may be rigid or flexible, transparent or opaque, smooth or in a molded surface. Consistency of tunic extends from delicate to cartilaginous, because of varieties in the fibrous components (Burighel and Cloney, 1997). Fibrous materials consist of a microfibrillar protein polysaccharide complex (Van Daele et al., 1992). Tunic is a dynamic living tissue working as a defensive covering and a supporting exoskeleton (Goodbody, 1974).

The extensive number of living organisms synthesized chitosan in the form of polysaccharides and it was pulled in by establishing researchers due to its functional properties (Abdou et al., 2008). Chitosan is the deacetylated derivative of chitin and it has a few significant properties, for example, antimicrobial activity, low toxicity, admirable biocompatibility, biodegradability, wound healing that initiate its applications in various fields such as pharmaceutical commercial ventures and food industries (Muzzarelli and Muzzarelli, 2005; Quattara et al., 2000; Tokura and Tamura 2007). Sagheer et al. (2009) reported about the extraction and characterization of chitin and chitosan from numerous marine sources of Arabian Gulf. However, chitosan having a few confinements in its utilization, in view of its insolubility nature in water and chitosan is just dissolvable in organic acids, for example: hydrochloric acid, acetic acid, and in formic acid which is highly viscous and affinity to coagulate proteins at high pH (Ravi kumar, 2000; Kurita, 1998; Rabea et al., 2003).

The aim of this study was the extraction of chitosan from the tunic of ascidian *Phallusia nigra.* FT-IR spectrum of chitosan was taken and degree of acetylation was figured by the stretching of IR groups and the antibacterial activity of chitosan extracted from marine ascidians was assessed against clinical pathogens.

MATERIALS AND METHODS

Sample collection

The ascidian *Phallusia nigra* samples were collected from Mandapam coast line (Lat. 9° 17' N; Long. 79° 10' E), Palk Bay region, Southeast coast of India during the low tide of intertidal area. Samples were washed altogether with distilled water and gathered into polythene sacks and transferred aseptically to the research facility. After the samples came to the laboratory, the tunic of ascidians were evacuated aseptically and again washed well with distilled water and air dried.

Extraction of chitosan

The air dried ascidians tunic was thoroughly dried by placing it in a hot air oven at 60°C for 24 h and the dried tunic was powdered by using blender. A common extraction of chitosan comprises of 3 steps: demineralization, deproteinization and deacetylation. Powdered tunic was treated with 5% sodium hydroxide solution and kept at 60°C for 2 h to uproot the proteins and other organic materials present in it. After this treatment, the sample was washed over and over with distilled water until the subsequent solution became neutral and then after that allowed to dry to evacuate the excess water content present in the sample. The deproteinated samples were treated with 1M HCL solution for 24 h. Samples were again washed thoroughly with distilled water until the solution came to neutral pH and dried to expel water content from the demineralised sample. Demineralised ascidian samples were deacetylated by using treated 50% sodium hydroxide at 60°C for 8 h. At that point, the tunic samples were repeatedly washed with distilled water until the solution becomes neutral. 200 ml of 10% acetic acid was added with the sample and kept for 12 h at room temperature. The dissolving chitosan was precipitated by adding 40% NaOH at pH 10.

Fourier transform-infra red spectroscopy (FT-IR)

The FT-IR spectroscopy of chitosan was determined using FT-IR spectrophotometer (SHIMADZU). 10 µg of chitosan sample was mixed with 100µ g of potassium bromide (KBr) and compressed to prepare a disc.

Determination of antibacterial activity

Suspension cultures (50 µl) of clinical pathogenic bacteria were used to inoculate the petridish containing Muller Hinton agar medium. Wells of 6 mm diameter was punched over the agar plates by using a sterile well puncture. Chitosan solutions was added to different wells in the plate and kept for incubation for 24 h at room temperature. After the incubation period, the antibacterial activity of chitosan against clinical pathogens was affirmed by the measurement of zone of inhibition (mm).

RESULTS AND DISCUSSION

Chitosan is a biocompatible, non-toxic, cationic polysaccharide produced by deacetylation of chitin. For the development of novel biomaterials, chitin and chitosan were synthetically modified because such procedure would not modify the fundamental skeleton of chitin and chitosan; it keeps the originality in the biochemical and physiochemical properties (Muzzarelli, 1973). The structure of ascidian derived chitosan was affirmed using FT-IR spectroscopy by comparing it with the standard one (Figure 1). FT-IR spectrum of standard chitosan demonstrated 11 major peaks in between 3429 and 613 cm^{-1} (Subhapradha et al., 2013); since the chitosan obtained from this study also showed 11 major peaks lying in between 3842.20 and 675.09 cm^{-1}, the specific peaks revealed at 3381.21, 2933.73, 1678.07, and 1535.34 cm^{-1} corresponds to H-bonded NH_2 and OH stretching, aliphatic CH stretching, amide C=O stretching and NH bending, respectively which elucidates the structure of chitosan (Subhapradha et al., 2013; Brugnerotto et al., 2001). The assimilation bands at 1292.31 and 918.12 cm^{-1} were illustrated as amide III band and β-anomer, respectively (Wang et al., 2008).

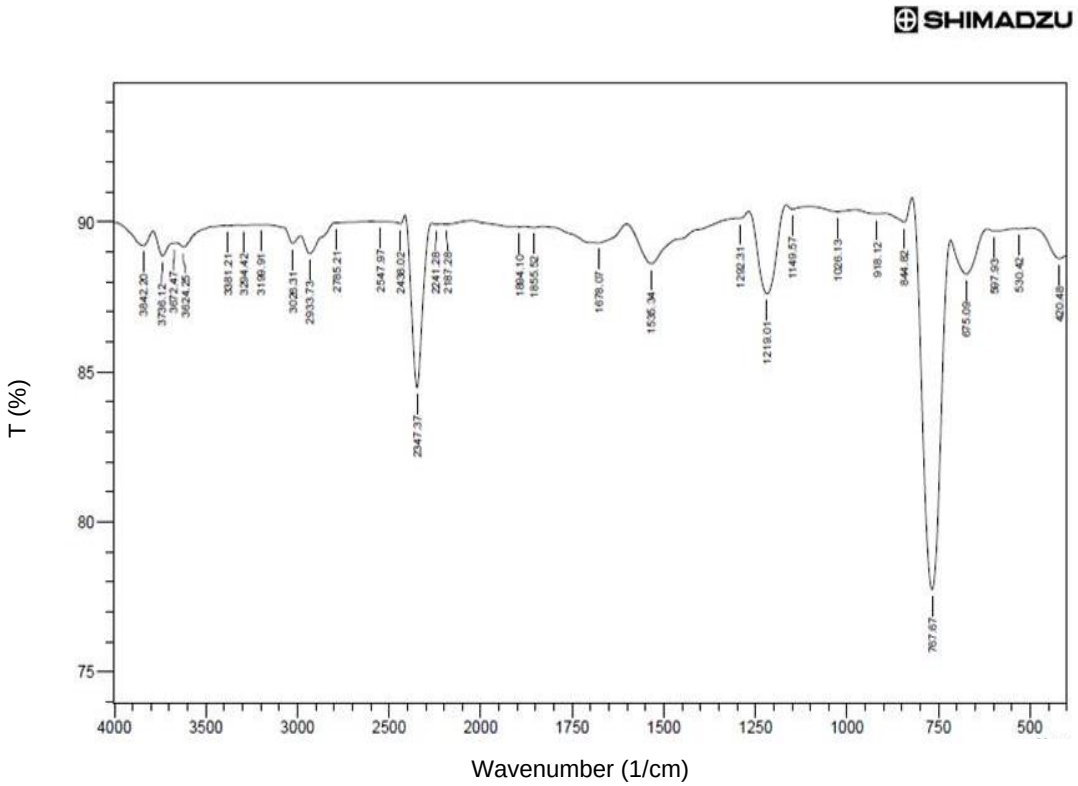

Figure 1. FT-IR spectrum of chitosan extracted from marine ascidian.

Table 1. Antibacterial activity of chitosan against various clinical pathogens.

S/N	Zone of Inhibition (mm)		
	Clinical pathogens	Concentration (mg/ml)	
		0.5 mg/ml	1 mg/ml
1	*V. parahaemolyticus*	6	8.9
2	*V. cholarae*	5.9	8.1
3	*K. oxytoca*	6.4	9.5
4	*K. pneumoniae*	-	1
5	*E. coli*	1.3	1.5
6	*P. mirabilis*	-	-
7	*V. vulniticus*	2.1	4.8

The above assimilation bands and its stretching and bending initially ascertained the item to be chitosan.

Antibacterial activity of chitosan prepared from tunic of marine ascidian *P. nigra* was tested against some clinical pathogens shown in Table 1. Jeon and Kim (2006) reported that chitosan is a well effective antibacterial agent against gram positive and gram negative bacterial pathogens. Maximum zone of inhibition came out against *Klebsiella oxytoca* (9.5 mm), *Vibrio parahaemolyticus* (8.9 mm), *Vibrio cholarae* (8.1 mm), *Vibrio vulniticus* (4.8 mm), *Escherichia coli* (1.5 mm), *Klebsiella pneumoniae* (1 mm) at the concentration of chitosan in 1 mg/ml. There is no activity observed against *P. mirabilis* at none of the concentrations. Crude chitosan exhibited higher zone of inhibition (9.5 mm) against *K. oxytoca* among all the clinical pathogens (Figure 2). When compared with other pathogens tested, this observation showed the excellent antibacterial inhibition mechanism of chitosan.

Recently, chitosan has received a very significant attention as an antimicrobial agent against various microorganisms (Khanafari et al., 2008; Yang et al., 2010). Two major mechanisms involved in the

Figure 2. Antibacterial activity of chitosan against clinical pathogens.

antimicrobial activity of chitosan are those causing growth inhibition of microbial cells. Due to its polycationic nature, chitosan molecules interact with anions present around the cell surface making an impermeable layer around the cell and it confines the transportation of vital solutes from all through the cell membranes.

This mechanism was proved by using electron microscopy; the site of this mechanism occurred in the outer membrane of gram negative bacteria (Helander et al., 2001). The other one is adsorption and blocking the transcription of DNA by penetrating the chitosan with DNA molecules. And in this case, the molecular weight of the chitosan molecules must be low, to be able to prevade into cells (Jeon and Kim, 2006). Antimicrobial ability of chitosan essentially relies on its molecular weight and degree of deacetylation. Chitosan with proficient degree of deacetylation indicated the highest antimicrobial activity (Liu et al., 2001). The present study verified that, chitosan having a phenomenal potential to interact and damage bacterial cell membranes via membrane disruption or pore formation leads to lysis of bacterial cell. Therefore, chitosan shows a promising result, as a natural and alternative antibacterial agent against various clinical pathogens involved in human infections.

CONCLUSION

This study proved that ascidian derived chitosan was a potent antibacterial agent against many gram positive and negative human clinical pathogenic microorganisms. This chitosan could be a very effective source of drug, which can be used as an antibacterial agent against pathogenic bacterial infection.

Conflict of interests

The author has not declared any conflict of interests.

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