Research Article

Synthesis, Characterization and Biological Activities of Chitosan Based Schiff Base Complex

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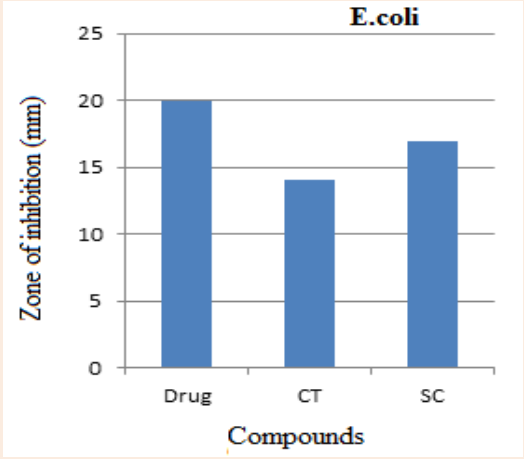
Abstract

Chitosan is a natural, eco-friendly potential bioactive polymer. The development of new applications for chitosan and its derivative is due to the fact that these are renewable source of natural biodegradable, biocompatible polymers. Currently, chitosan has been attracted more attention for its unique physico-chemical characters, versatility, non-toxic, economical, easy availability and bioactivities. Based upon the above reports, in this investigation we have carried out the isolation process of chitosan from prawn shells of chitin source collected from Tuticorin, India using the conventional methods of pretreatment, demineralization, deprotienization and deacetylation. The resultant chitosan was used to synthesize schiff base complex, salicylalchitosan by the reaction of chitosan with salicylaldehyde. The chitosan and its schiff base ligand were characterized by UV- visible, IR spectroscopy, elemental analytical techniques. Further the compounds were screened for the antibacterial and antifungal activities. The results obtained revealed that deacetylation value of chitosan was 74.4% calculated from the elemental analysis. The antimicrobial activities of synthesized chitosan and its schiff base were significantly higher.



Keywords: Azomethine, Chitosan, Chitin, Deacetylation, Demineralization, Deprotienization.

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Introduction

Schiff base ligands represent one of the most widely utilized classes of ligands in coordination chemistry which are derived from an amino and carbonyl compounds [1-2]. Schiff base ligands, as a variety of compounds with imine group, have gained importance because of the physiological and pharmacologist activities associated with them [3-4]. Schiff base complexes have been extensively used in wide applications including food industry, analytical industry, catalysis, clinical and biological activities [5-7]. It has been suggested that azomethine linkage (CH=N) might be responsible for the biological activities of schiff bases [8] such as antibacterial [9], antifungal [10], antiviral [11], antitumor [12], anti-HIV-1 [13] etc. The importance of schiff base complexes has been expanded in variety of fields such as bioinorganic chemistry, biomedical applications, supramolecular chemisty, material sciences, marine applications, polymer industries [14-17] etc. A considerable number of schiff base complexes have potential biological interest, being used as more or less successful models of biological compounds.

Chitosan is a versatile natural abundant polymer. Chitosan is produced commercially by deacetylation of chitin, naturally occurring polysaccharides which is the structural element in the exoskeleton of crustaceans (crabs, shrimp, mussel shells etc.) [18-20]. The development of new applications for chitosan and its derivative is mainly due to fact that these are renewable source of natural biodegradable polymers, nontoxic, as well as linear nitrogenous polysaccharides [19]. Chitosan is readily soluble in dilute solution of most of the organic acids such as acetic acid,

citric, tartaric acid and sparingly soluble in inorganic acids [20]. Chitosan displays unique polycationic, chelating, and film-forming properties due to the presence of active amino and hydroxyl functional groups [21]. For these reasons, chitosan is widely used in many different fields, including medicine, foods and chemical engineering, pharmaceuticals, agriculture and a number of biological activities [22-24]. But it also has several limitations to be utilized in biological system, including its poor solubility under physiological conditions. To overcome these limitations, researchers focused on the derivatization of chitosan by chemical modifications and results in increased solubility in water as well as in organic solvents [25].

From the literature many of the aldehydes tested were found to have highly potent antimicrobial activity. Among the various aldehyde compounds salicylaldehyde is a key precursor in the formation of various bidentate ligand coordinated metal complexes, some of which are commercially important [28]. In 1971, Hodnett [29] synthesized a series of schiff base metal complexes and carried out antitumor experiments, which indicated that aldehyde substituent was superior to amine substituent in anticancer effect and salicylaldehyde schiff bases were superior to other aldehyde schiff bases.

Recently, a number of reports have appeared describing the use of natural materials in the field of coordination chemistry to enhance the biological and medicinal activities of the material. Based upon this we were focused on the chitosan due to its significant medicinal purposes to prepare bioactive schiff base complexes. On the other hand, many research papers are describing the use of salicylaldehyde in the preparation of various significant schiff base complexes. In combining these two areas of interest, in the present investigation we showed more attention to deal the synthesis process of bioactive schiff base complexes by using chitosan and salicylaldehyde. Firstly, chitosan was isolated from chitin prepared from prawn shells and then it was reacted with salicylaldehyde under certain conditions to obtain salicylalchitosan (SC) schiff base ligand. We found that the synthesized chitosan and its schiff base ligand were shown better yield and significant biological activities.

Experimental

Materials and Methods

The chitin source, prawn shells were obtained from local sea food markets at Tuticorin, India. All other reagents and solvents were purchased from Merck (I) Ltd and used as received. The experiment is carried out by normal refluxed method.

Synthesis of chitosan from chitin

According to the preparation method of Brine & Austin, 1981 [30]; Muzzarelli & Jeuniaux, 1986 [31], chitosan was isolated from prawn shells of chitin source with slight modifications. Double distilled water has been used throughout the synthesis process. Firstly, prawn shells were washed several times with water and dried in oven at 60-70^{0}C overnight. The dried shells were grinded into a powdered form and this powder was de-mineralized by using aqueous hydrochloric acid solution (1M) with constant stirring. Then the solution was washed by double distilled water and dried in oven at 60^{0}C for 3-4 hrs. Again this dried powder was deprotienized with aqueous sodium hydroxide (1M) with constant stirring and kept in hot magnetic stirrer at 60^{0}C for an hour. The colour of the solution slowly changed to light pink colour then it was washed several times with double distilled water and dried in oven at 60^{0}C overnight. The dried chitin powder was deacetylated with aqueous solution of 40% sodium hydroxide for 2 hrs at 60^{0}C and the obtained dirty whitish precipitate of chitosan was washed several times and dried in oven at 60^{0}C for 3-4 hrs.

Synthesis of chitosan schiff base complex

Double distilled water has been used throughout the synthesis process. 100 mg of synthesized dried chitosan powder was dissolved in 25 ml of acetic acid solution (0.5 M) and kept in magnetic stirrer for 2hrs at room temperature to get

complete dissolved solution. Then another mixture of 10 ml of salicylaldehyde (0.1 M) in ethanol was prepared and stirred well for an hour at room temperature. The ethanol mixture was added to the chitosan mixture and kept in hot magnetic stirrer at 50-60^{0}C for 12 hrs and refluxed well. The obtained yellowish green coloured precipitate of schiff base complex of salicylalchitosan was cooled and filtered then washed several times with ethanol and dried under vacuum at 60^{0}C for 12 hrs. The synthesized yellowish green powder of schiff base ligand was kept in a desiccator over silica gel for further analyses. The synthesis process was followed according to the scheme represented in figure 1.

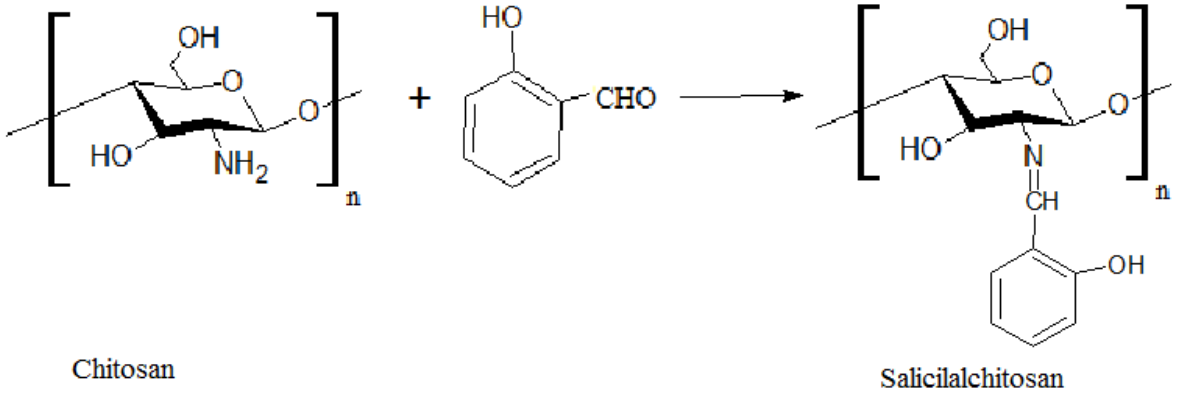

Figure 1 Synthesis route of chitosan base schiff base

Characterization

All samples were stored in a desiccator containing silica gel for at least 48 hr at room temperature to ensure minimal moisture content before spectroscopic analysis. The UV-Visible absorption spectra of the chitosan and its schiff base complex were measured on a Shimadzu UV-Vis V-530A spectrophotometer in the range of 300 to 600 nm.
Elemental analyses were carried out with Elementar Vario EL III series used to collect the micro analytical data (C, H and N) and compared with the calculated theoretical values. The unmodified and chemically modified chitosan and its schiff base complex were examined for FT-IR spectra analysis and recorded on a jasco FT-IR/4100 spectrophotometer with 4cm^{-1} resolution in the range of 4000 to 400 cm^{-1}.

Biological assay

The antibacterial and antifungal activity of the synthesized Chitosan and Salicylalchitosan were tested against two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), two gram negative bacteria (*Escherichia coli* and *Staphylococcus bacillus*) and four fungies (*Candida albicans*, *Curvularia lunata*, *Aspergillus niger* and *Trichophyton simii*). Generally chitosan possesses antimicrobial activity against many bacteria and fungi at pH < 6, therefore the value of pH for chitosan and its schiff base ligand was maintained at 5-6 throughout the biological assay tests. A comparative study of the growth inhibition zone values for chitosan and its schiff base were evolved.

Antibacterial activity test

The disc diffusion method (Bauer *et al.,* 1966) was used to screen the antimicrobial activity [32]. Stock cultures were maintained at 4^{o}C on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loopful of cells from the stock cultures to test tube of Muller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 hrs at 37^{o}C and 25^{o}C respectively. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to 2.0×10^6 colony forming units (CFU/ml) for bacteria. The Muller Hinton Agar (MHA) plates were prepared by pouring 15 ml of molten media into sterile petri plates. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly and allowed to dry for 5 minutes.

The concentration of sample at 40 mg/disc was loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

Antifungal activity test

The fungal strains were inoculated separately in sabouraud's dextrose broth for 6 hrs and the suspensions were checked to provide approximately 10^5 CFU/ml. The agar well diffusion method (Perez, 1993) was modified. Sabouraud's dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with the sample and solvent blanks (hydro alcohol, and hexane). Standard antibiotic (Fluconazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37°C for 72 h. The diameters of zone of inhibition observed were measured.

Results and Discussion

UV-Vis spectra studies

The UV-Vis spectra of chitosan and salicylalchitosan are given in figure 2 and 3 respectively. Chitosan exhibits the absorption peaks at 220, 270, 350 nm with medium intensities can be due to n - π* transition (figure 2). Salicylalchitosan exhibits the absorption bands in the UV spectrum at 330-355 nm with low intensities. This absorption band could be assigned to π - π* and n - π* transitions in the aromatic ring or azomethine (figure 3).

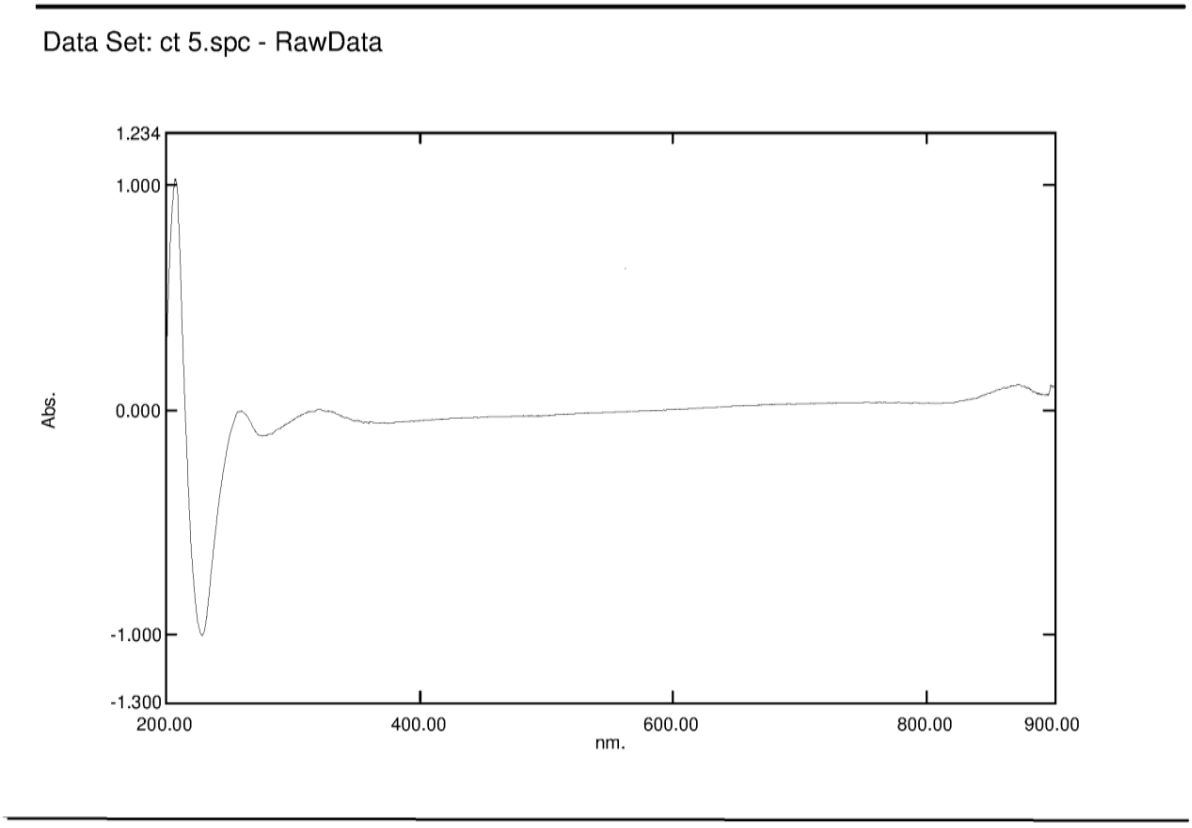

Figure 2 UV-Vis spectrum of chitosan

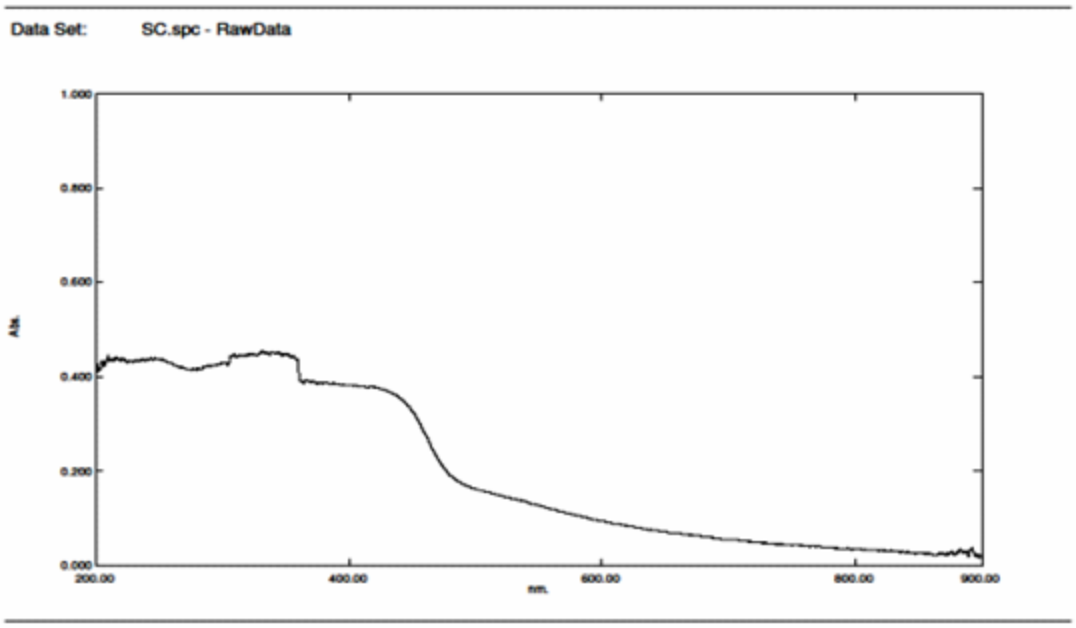

Figure 3 UV-Vis spectrum of salicylalchitosan

Elemental Analysis

The analytical data of chitosan and its schiff base complex are summarized in table 1. The complexes prepared are stable at room temperature and non-hygroscopic. From the result it can be concluded that the synthesized chitosan having medium molecular weight.

Table 1 Elemental analysis data of Chitosan (CT) and Salicylalchitosan (SC)

Sample code	Experimental value			Theoretical value		
	C	H	N	C	H	N
CT	40.13	6.75	7.19	46.16	6.88	8.97
SC	52.42	5.85	5.37	57.10	5.70	6.11

Degree of Deacetylation (DD)

Deacetylation is the most important one to form the soluble chitosan product. Generally the degree of deacetylation for chitosan must be 70% and above for better reaction. In fact, the biological activity of chitosan depends on many factors including the deacetylation degree. The elemental analysis data is used to find out the degree of deacetylation more accurate than the other methods. The calculation of DD value by elemental analysis was carried out by the following relationship given as,

$$DD = \frac{6.857 - C/N}{1.7143} \times 100 \qquad \text{---------- (1)}$$

The D.D value can also be calculated from N/C ratio according to Kasaai equation (Abdou et al, 2008),

$$\frac{N_{obs}}{C_{obs}} = \frac{14}{96 - 24\ (DD)} \qquad \text{---------- (2)}$$

The values of C, H and N calculated for the formula based on 1 H2O per glucosamine residue were in agreement with the observed values. From the both equations the deacetylation value was obtained same as 74.4%.

FT-IR Spectra Studies

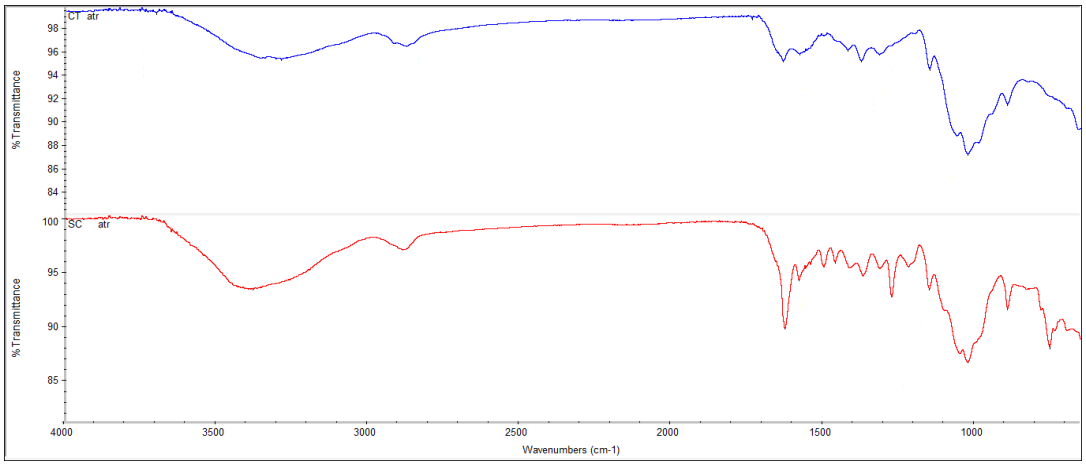

Figure 4 FT-IR spectra of Chitosan (Top); Salicylalchitosan(Bottom)

FTIR spectra of chitosan and salicylalchitosan are shown in figure 4. The IR spectrum of chitosan exhibits characteristic band of O-H stretching at 3284.82 cm^{-1}. It showed a peak at 2873.14 cm^{-1} which corresponds to C-H axial stretching band. The peak observed at 1632.35cm^{-1} which can be assigned to the amide I stretching of C=O which associated with N-H deformation of amide II at 1576.55 cm^{-1} and C-N stretching coupled with N-H plane deformation at 1418.76 cm^{-1}. The band at 1374.19 cm^{-1} corresponds to symmetrical deformation of CH_3 group and a band at 1313.51 cm^{-1} for C-N amino group axial stretching. The other important observed two bands at 1149 cm^{-1} and 891 cm^{-1} are the characteristic peaks of β (1-4) glycosidic bridge. The C-O-C stretching vibration was observed at 1023.87 cm^{-1}. In the FTIR spectrum of salicylalchitosan, the following significant bands were observed. The axial vibration of O-H was observed at 3379.11 cm^{-1}, the C-H stretching band observed at 2884.67 cm^{-1} and the important sharp peak arrived at 1626.15 cm^{-1} which can be assigned to azomethine group. The new bands arised at 1274.18 cm^{-1} and 1219.45 cm^{-1} due to the presence of phenolic C=O stretching and phenolic O-H stretching respectively. The obtained important and common peaks for chitosan and salicylalchitosan are summarized in **Table 2**.

Table 2 IR spectra bands of chitosan and salicylalchitosan

Compounds	Functional groups (cm^{-1})						
	υ(O-H)	υ(C-H)	υ(N-H)	υ(CH_3)	υ(C-O-C)	υ(β(1-4)-glucoside)	υ(CH=N)
Chitosan (CT)	3284.82	2873.14	1576.55	1374.19	1023.87	1149.07 891.94	-
Salicylalchitosan (SC)	3379.11	2884.67	1579.58	1368.78	1023.19	1150.94 892.38	1626.15

Antibacterial activity

The antibacterial activities of chitosan and its schiff base against two of the gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two of the gram-negative bacteria (*Escherichia coli* and *Staphylococcus bacillus*) were evaluated and their activity was compared to a well-known commercial antibiotic Chloramphenicol. The results are reported in **Table 3**.

Table 3 Effect of chitosan and salicylalchitosan on antibacterial activity

Bacterial Species	Zone of inhibition diameter (mm sample^{-1})		
	Standard drug (C)	Chitosan(CT)	Salicylalchitoan(SC)
S. aureus	21	19	22
B.subtilis	19	17	19
E. coli	20	14	17
S.bacillus	19	16	18

From these results, it can be indicated that the inhibition activity of both chitosan and salicylalchitosan were higher against all gram positive bacteria tested comparing with that on gram negative bacteria. Moreover, chitosan schiff base have higher inhibition efficiency than the non-modified chitosan. It showed a greater effect against *S.aureus*, *B.subtilis* and *S.Bacillus* than the chitosan. The zone of inhibition observed for those synthesized products (CT & SC) against *E.coli* showed the moderate antibacterial action when compared to the results obtained against other bacterial species. Therefore the activity exhibited by the chitosan and its schiff base ligand were significantly appreciable. The results compared with standard drug (chloramphenicol) have been indicated that the synthesized schiff base was more active and showed almost similar activity to the standard drug, especially, it exhibited highest inhibition activity against *S.aureus* and also more than the standard drug. The result of antibacterial evaluation is summarized in **Figure 5**.

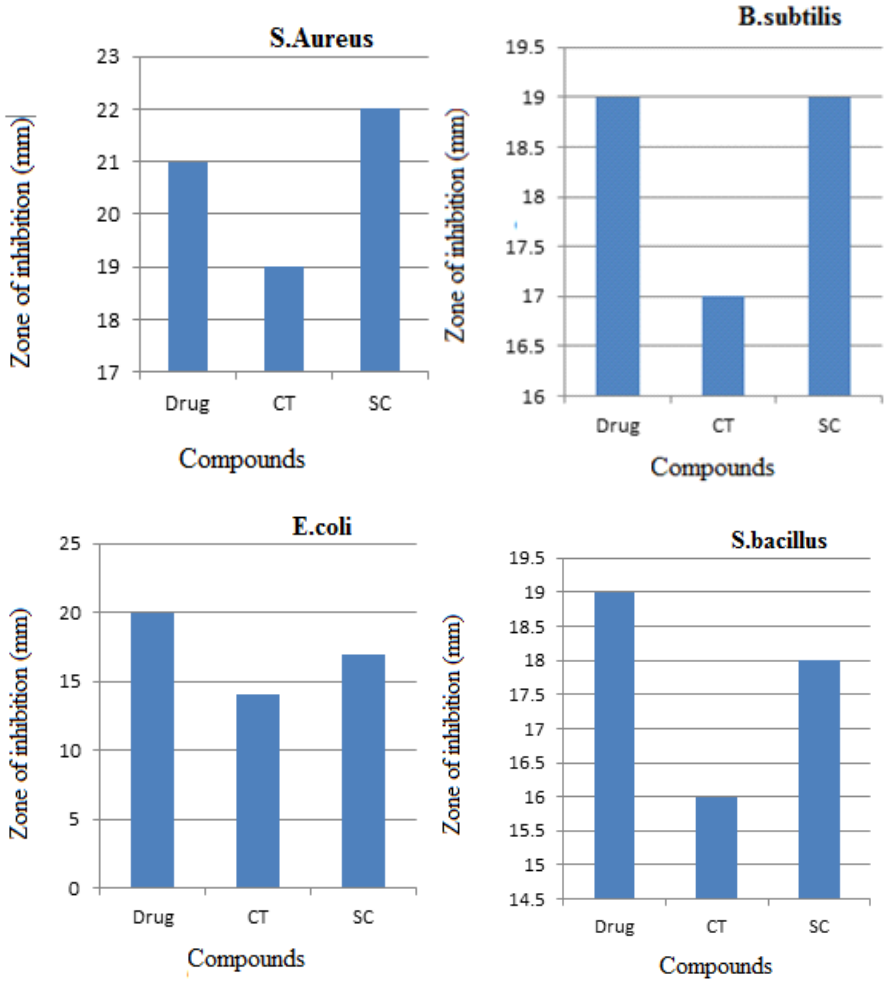

Figure 5 Antibacterial activity data of complexes

Antifungal activity

Chitosan and its schiff base ligand were determined for their antifungal activity against four fungal strains *Candida albicans*, *Curvularia lunata*, *Aspergillus niger* and *Trichophyton simii* and their activity was compared with standard antifungal drug fluconazole. The results were shown in **Table 4**.

Table 4 Effect of Chitosan and its schiff base on antifungal activity

Fungal Species	Zone of inhibition diameter (mm sample^{-1})		
	Standard drug (C)	Chitosan(CT)	Salicylalchitosan(SC)
C.albicans	19	15	18
C.lunata	19	22	16
A.niger	20	11	17
T.simii	21	18	20

From the results, it is indicated that the activity of the schiff base ligand salicylalchitosan was found to be moderate active against all the four fungal strains compared with the synthesized raw material chitosan. The zone of inhibition observed for the schiff base ligand against *C.albicans* and *T.simii* was higher and also nearly similar activity to standard drug (fluconazole). Interestingly, the inhibition efficiency against the fungal species *C.lunata* and *T.simii* for chitosan was significantly active than the standard drug. However schiff base showed better inhibition activity than chitosan over all. The result of antifungal evaluation is summarized in **Figure 6**.

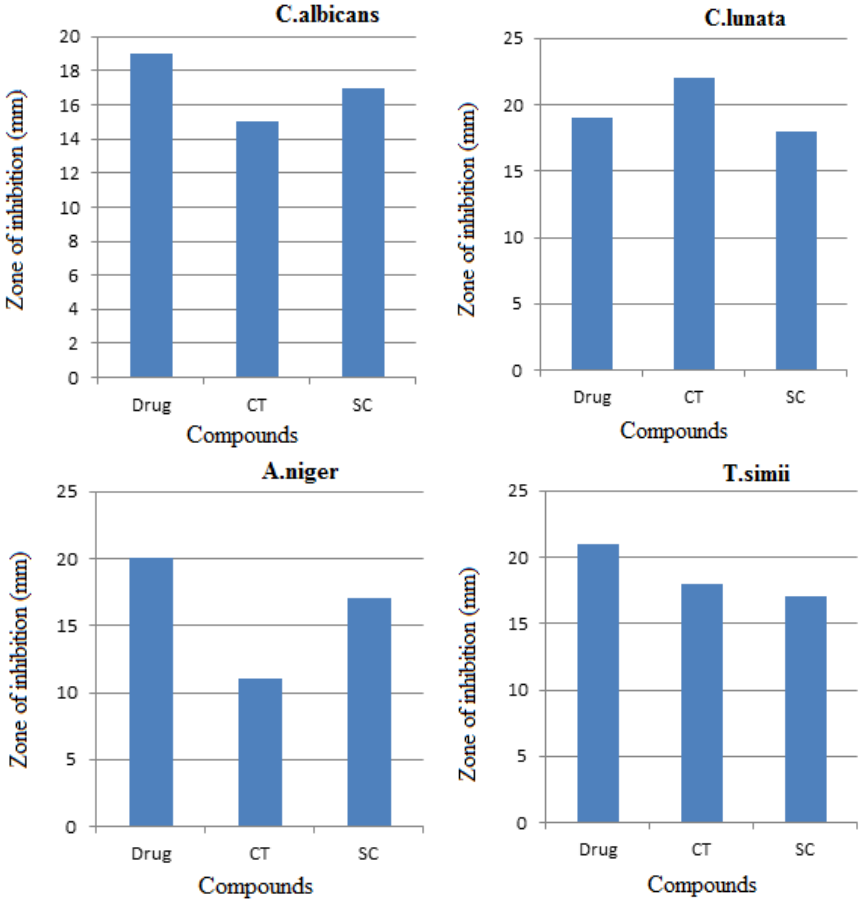

Figure 6 Antifungal activity data of complexes

Conclusions

In summary, we have isolated bioactive chitosan from natural prawn shells by the proper methods and the resultant chitosan was used to prepare the schiff base ligand salicylalchitosan. The synthesized chitosan and schiff base ligand have been characterized by UV, FTIR, elemental analytical techniques and deacetylated value was found as 74.4%. Moreover, bioactivities of chitosan and its schiff base were evaluated against two gram positive bacteria (*S.aureus* and *B.subtilis*), two gram negative bacteria (*E.coli* and *S.bacillus*) and four fungal species (*Candida albicans*, *Curvularia lunata*, *Aspergillus niger* and *Trichophyton simii*). Both chitosan and schiff base ligand showed greater antibacterial activity against two gram positive bacteria. There was an appreciable higher inhibition activity observed for synthesized schiff base against all four bacterial strains which was found to be similar to chloramphenicol activity. The significant antifungal activity was also exhibited for chitosan against (*C.lunata* and *T.simii*) and for salicylalchitosan against (*C.albicans* and *T.simii*). Therefore, the synthesized schiff base complex is more bioactive ligand for utilizing in the preparation of complexes and other biological applications also.

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