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Synthesis, Characterization and Antimicrobial Study of Chitosan Schiff Base Derivatives

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ABSTRACT

Chitosan is natural, biodegradable, and biocompatible hydrophilic polysaccharide of â(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine prepared from chitin. The remarkable biological properties have paved the way for the introduction of chitosan in the biomedical and pharmaceutical fields. Chitosan, a bio-based polymer which has similar characteristics to those of cellulose, exhibits cationic behaviour in acidic solutions and strong affinity for metals ions. Various chemical modifications have been investigated to improve the properties of chitosan and thus to increase its range of applications. The antimicrobial activity of chitin , chitosan and their derivatives against different groups of microorganisms such as bacteria, yeast and fungi has received considerable attention in the present time. Keeping in view the ample potentials of the antimicrobial activity of chitosan, we have made an attempt to prepare, a Chitosan-base Schiff-based (CSB) compound with an aromatic substituent group. This was synthesized by the reaction of chitosan with an aromatic aldehyde p-dimethylaminobenzaldehyde. The Chitosan-based Schiff base metal complexes were subsequently obtained through the reaction of newly synthesized Schiff bases with metal chlorides. The obtained products were characterized by Fourier transform infrared (FTIR) spectroscopy. The antifungal activity of Chitosan Schiff base and its metal complexes were measured against Rhizopus fungus and antibacterial activity against Gram positive and Gram negative bacteria. These results show that it has both antifungal and antibacterial activity. Chitosan Schiff metal complex have higher antibacterial activity against gram positive and gram negative bacteria.

Key words: *Chitosan, Anti microbial property, Schiff base, Rhizopus fungus*

Introduction

Organic waste from seafood processing units is produced in large quantities in terms of wastewater and solid waste. Waste reduction to zero-waste is crucial for developing a sustainable environment. One of the major waste from the seafood industry is exoskeleton of shrimp. The major components (dry weight basis) of shrimp waste contain protein (35–50%), chitin (15–25%), minerals (10–15%) and carotenoids (Abdou *et al*., 2008). Chitin the most abundant aminopolysaccharide polymer occurring in nature, is the building material that gives strength to the exoskeletons of crustaceans, insects, and the cell walls of fungi. Chitin can be converted to chitosan by enzymatic or chemical deacetylation. The conversion chitin shells of shrimp and other crustaceans is done by treating it with sodium hydroxide (Islam *et al*., 2016). The wide array of its properties like biocompatibility, biodegradability, and nontoxicity make it suitable for various applications by causing no harm to life forms or nature. Antimicrobial activity of chitosan is one of its important use which increases the commercial value of the product. Based on the difference in the characteistics of the microorganism the antimicrobial activity of chitosan also differs (Cai-Ling *et al*., 2021, Bautista-Banos *et al*., 2017). The antibacterial property focus its ability to alter the membrane permeability of the cells. Chitosan which is positively charged interact with anionic components like lipopolysaccharides, phospholipids and bacterial cell surface proteins (Chung *et al*., 2004, Kong *et al.,* 2010). The action of chitosan to disrupt the cell membrane resulting in the death of the bacterial cell reveals its action to mitigate microbe like Burkholderia pseudomallei (Watcharaporn Kamjumphol *et al*., 2014).

Due to its physical and chemical properties, chitosan is being used in a vast array of widely different products and application, ranging from pharmaceutical and cosmetic products to water treatment and plant protection. Chitosan has also received considerable attention as a possible pharmaceutical excipient in recent decades, due to its good biocompatibility and low toxicity properties in both conventional excipient applications as well as in novel application. These properties changes with degree of acetylation and molecular weight as well (Kumar *et al*., 2004).

Chitosan being the only pseudo natural cationic polymer finds many applications that follow from its unique character. Since, last decade so many modifications have been carried out due to presence of free –NH2 and –OH groups on chitosan. Such as preparation of complexes, drug delivery, coating hydrogel, cross linking polymer, nanoparticles and nanocomposites, and quaternary salt. The presence of both reactive amino and hydroxyl groups make the chemical alteration of its properties under wild reaction conditions. Schiff's bases obtained by the reaction of free amino groups of chitosan with an active carbonyl compound such as aldehyde and ketone is one of its substituted biopolymer (Thatte *et al.*, 2014). Biologically important Schiff bases possess antimicrobial, antibacterial, antifungal, anti-inflammatory, anticonvulsant, antitumour and anti HIV activities (Aliasghar Jarrahpour *et al.*, 2007).

The synthesis and characterization of new metal complexes with antibacterial and antifungal agents are of great importance for understanding the drug-metal ion interaction and for their potential pharmacological use. New kinds of chemotherapeutic agents containing Schiff bases have gained significant attention among biochemists due to its anticancer and anti tumour prorperties (Hu *et al*., 2012 and Creaven *et al*., 2010).

Schiff bases with antibacterial activity were derived from different aldehydes and amines (Wenling *et al*., 2013). The silane complexes are better inhibitors than the corresponding free ligands. Antibacterial activity of Schiff bases and their metal complexes, varied from inactive to highly active form with regards to ligands and metal content. The detailed cell viability studies of Schiff base complexes of different metal salts with chitosan and salicyl aldehydes were studied by Hellen et al (2017). The molecular weight (MW) and the degree of acetylation (DA) are important factors in determining the biological activity. In general the lower the MW and the DA, the higher will be the effectiveness on reducing microorganism growth and multiplication Rejane *et al*., 2009).

Keeping in view the ample potentials of the anti microbial properties of chitosan and its derivatives, we have made an attempt to prepare Chitosan-Schiff base derivative and its three metal complexes. These compounds were characterized by Fourier transform infrared (FTIR) spectroscopy. The antifungal activity against Rhizopus fungus and antibacterial activity against gram positive and gram negative bacteria by Agar well diffusion method was done.

Materials and Methods

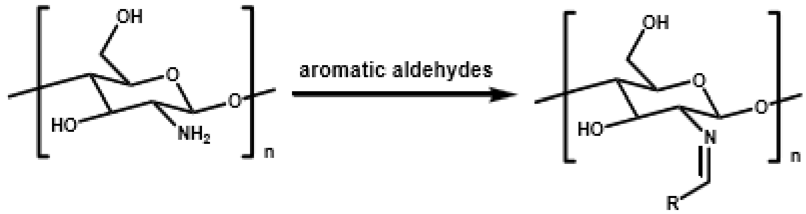
Reagents and Instrumentation

Ethanol, p-dimethyl amino benzaldehyde, Glacial acetic acid, Cupric chloride, colbaltous chloride, nickel chloride used were products of Nice Chemicals. Chitosan was obtained from the Chitin division, Indian Seafoods, Kochi, Kerala.

The FTIR data were obtained from Sophisticated Test and Instrumentation Centre, Cochin University of Science and Technology. The Antibacterial and Antifungal studies have been carried out at the Microbiology wing of the Departement of Zoology, St. Albert's College. Microorganisms used for antibacterial study are Gram-positive bacteria , Gram negative bacteria. Fungus used: Rhizopus.

Synthesis of Chitosan Schiff Base (CSB)

2 g of Chitosan was dissolved in 250 ml of 1% acetic acid in 1:1 ethanol water mixture and stirred at room temperature for 3hrs. Filtered the solution using a white cloth. Then, 2.5g of aromatic aldehyde (p-



dimethylaminobenzaldehyde) dissolved in 10 mL ethanol and was added to the chitosan solution. The mixture was stirred and heated at 60 °C for 12 h using water bath. Deep yellow gels of Schiff base were formed and it was dried and washed several times with ethanol to remove any unreacted materials. The product was dried at 60 °C in vacuum for 24 h giving a yellow powder.

Synthesis of Chitosan Schiff Base of Cobalt (II) Complex

0.5g of newly synthesized Chitosan Schiff base was taken in a round bottom flask, 1g of cobaltous chloride was dissolved in 10 ml ethanol and was mixed with the Schiff base and was stirred and heated at 60°C for 7 hrs. The golden brown complex thus obtained was washed several times with alcohol to remove the unreacted material. It is then filtered and dried in vacuum.

Synthesis of Chitosan Schiff Base of Nickel (II) Complex

0.5g of newly synthesized Chitosan Schiff base was taken in a round bottom flask, 1 g of Nickel chloride was dissolved in 10 ml ethanol and was mixed with the Schiff base and was stirred and heated at 60°C for 7 hrs. The green coloured complex thus obtained was washed several times with alcohol to remove the unreacted material. It is then filtered and dried in vacuum.

Synthesis of Chitosan Schiff Base of Copper (II) Complex

0.5g of newly synthesized Chitosan Schiff base was taken in a round bottom flask, 1g of cupric chloride was dissolved in 10 ml ethanol and was mixed with the Schiff base and was stirred and heated at 60 °C for 7 hrs. The deep green coloured complex thus obtained was washed several times with alcohol to remove the unreacted material. It is then filtered and dried in vacuum.

Characterization

Fourier Transform Infrared Studies

Fourier transform infrared spectra of Chitosan Schiff base derivatives using KBR pellet method were recorded in the frequency range of 400 – 4000 cm^{-1} using Thermo Nicolet, Avatar 370 spectrometer.

Anti Fungal and Antibacterial Study

Agar- Well Diffusion Method

Agar well diffusion method was used to evaluate the antimicrobial activity of Chitosan p-dimethylaminobenzaldehyde Schiff base and its four metal complexes. Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. In this assay technique the culture medium were inoculated with the microbial pathogen. Wells were filled with 1ml of Chitosan Schiff base and its complexes. The plates are incubated. This classic method yields a zone of inhibition in mm result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms. It is carried out in Petri plates.

Procedure

The agar plate or petriplate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 µl) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated at 37 °C. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.

Results and Discussion

Chitosan Based Schiff Base (CSB) has been prepared by the condensation reaction between chitosan and an aromatic aldehyde, p-dimethylamino benzaldehyde. The Chitosan-based Schiff base metal complexes of Co, Ni and Cu were subsequently synthesized through the reaction of newly prepared Schiff bases with corresponding metal chlorides. These products were characterized by Fourier transform infrared (FTIR) spectroscopy. The antibacterial activity of Chitosan Schiff base and its metal complexes were measured against Gram positive and Gram negative bacteria and antifungal activity of Chitosan p-dimethylaminobenzaldehyde Schiff base and its four metal complexes were also studied.

Infrared Spectral Analysis

The characteristic band of Schiff base, that is, strong stretching vibration of imine (C=N), has appeared in the spectra of Schiff base at wave number 1639.21 cm^{-1} (Fig. 1). Its metal complexes, i.e. Cu, Co, Ni and Mn complexes show a red shift at 1633.78, 1636.83, 1634.20 and 1636.93 cm^{-1} respectively (Fig. 1-4). The shifting of this band to lower frequency in these complexes, indicate the coordination through azomethine nitrogen[8]. In the spectra of CS there is a strong broad vibration at around 3442 cm^{-1} which can be assigned to the stretching vibration of O-H and N-H groups (Sathisha *et al*., 2008). In addition, some obvious shifts are shown in the spectra of Schiff base and its complexes. There is no evidence for the presence of the free aldehydes that can be characterized by appearing peaks around 1665 cm^{-1} due to free aromatic aldehyde groups. These results confirm the formation of the Schiff bases through reaction of Chitosan with p-dimethylamino benzaldehyde with no traceable residues of free aldehydes.

The characteristic peaks due to stretching of C-O pairing in β (1→4) glycoside bonds of polysaccharide appeared at 1079.94, 1062.68, 1081.19, and 1078.11 cm^{-1} in IR spectra of Chitosan Schiff bases with p-dimethylaminobenzaldehyde and its metal complexes with Cu, Co and Ni respectively.

Antibacterial Activity

The antibacterial activities of Chitosan, its Schiff bases and its metal complexes against the gram-positive bacteria and gram negative bacteria are shown in Table 1 and 2. It has been found that the Schiff base metal complexes have got higher antibacterial activity than that of Chitosan Schiff base which in turn shows more antibacterial activity than Chitosan alone. The degree of acetylation and pH determines the antimicrobial activity of chitosan (Kong *et al*., 2010, Sathisha *et al*., 2008, Hosseinnejad and Jafari 2016). Distorted morphology and degra-

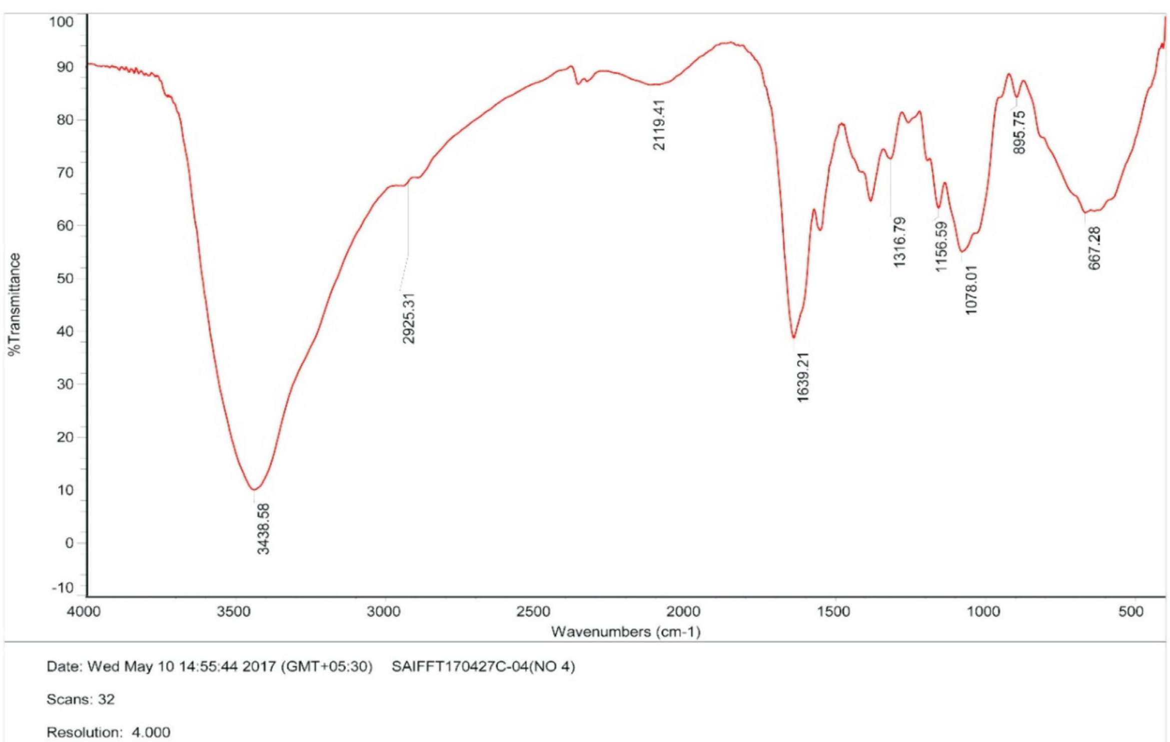


Fig. 1. FTIR Spectra of Chitosan Schiff base with p-dimethylaminobenzaldehyde

Table 1. Antibacterial study with Gram negative bacteria

Antibacterial agent	Zone of inhibition (mm)
Chitosan	18
Chitosan Schiff base	19
Chitosan Schiff base Co complex	14
Chitosan Schiff base Ni complex	18
Chitosan Schiff base Cu complex	20

Table 2. Antibacterial study with Gram positive bacteria

Antibacterial agent	Zone of inhibition (mm)
Chitosan	18
Chitosan Schiff base	22
Chitosan Schiff base Co complex	23
Chitosan Schiff base Ni complex	18
Chitosan Schiff base Cu complex	21

dation of cell membrane was noticed in Chitosan-treated *Candida albicans* cells (Pei *et al.*, 2019).

Antibacterial Activity Against Gram Negative Bacteria

Antibacterial activity of Chitosan and its Schiff base derivatives against gram negative bacteria are shown in Fig.5. The Table 1 shows that all the five compounds synthesized exhibit antibacterial activity against gram negative bacteria. It indicates that out of the five compounds, Chitosan Schiff base Cu complex has got higher antibacterial activity against gram negative bacteria. Also it is found that the Chitosan Schiff base has more inhibition than Chitosan itself. The inhibition of Chitosan Schiff Base Ni complex is same as that of Chitosan whereas the activity of Co complex is somewhat less. The increasing order of antibacterial property against gram negative bacteria is as follows.

Chitosan Schiff Base Co complex < Chitosan = Chitosan Schiff Base Ni complex < Chitosan Schiff Base < Chitosan Schiff Base Cu complex.

Antibacterial Activity Against Gram Positive Bacteria

Antibacterial activity of Chitosan and its Schiff base derivatives against gram positive bacteria are shown in Fig.6. The Table 2 shows that the synthesized Schiff base and its metal complexes possesses antibacterial activity against gram positive bacteria. In this case, out of the five compounds, Chitosan Schiff Base Co complex have got more antibacterial property against gram positive bacteria, which is against that found in Gram negative bacteria. Also inhibition of Chitosan Schiff base is higher than that of Chitosan. Cu complex has also got higher in-

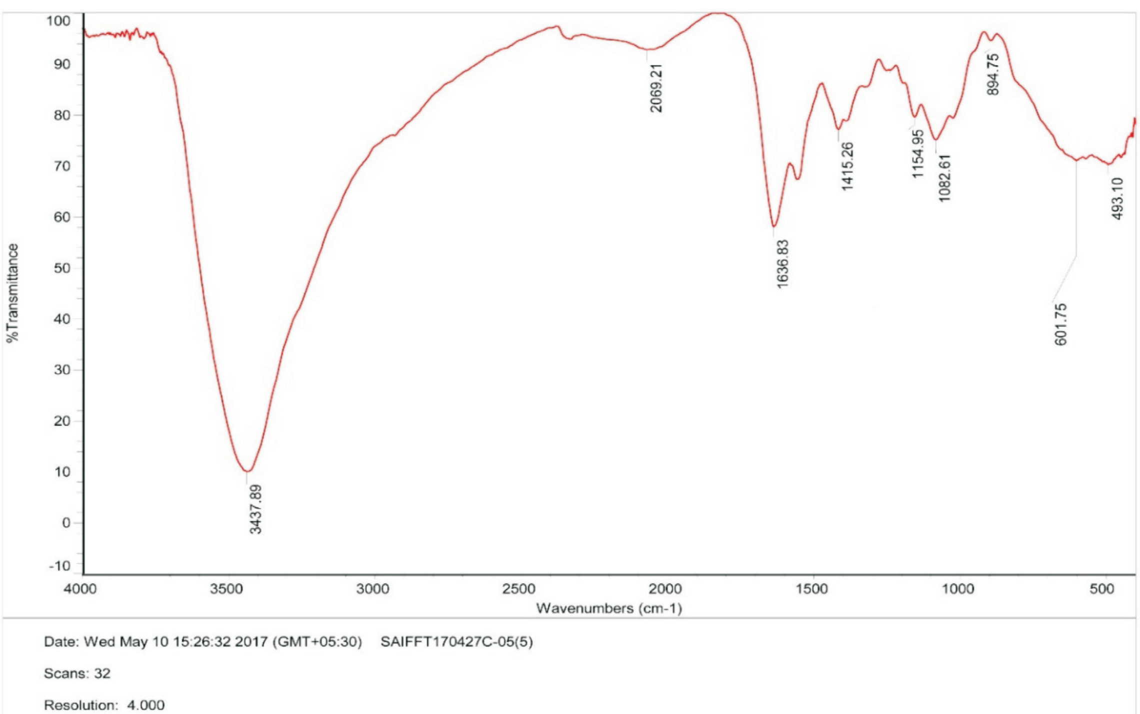


Fig. 2. FTIR Spectra Chitosan Schiff base complex

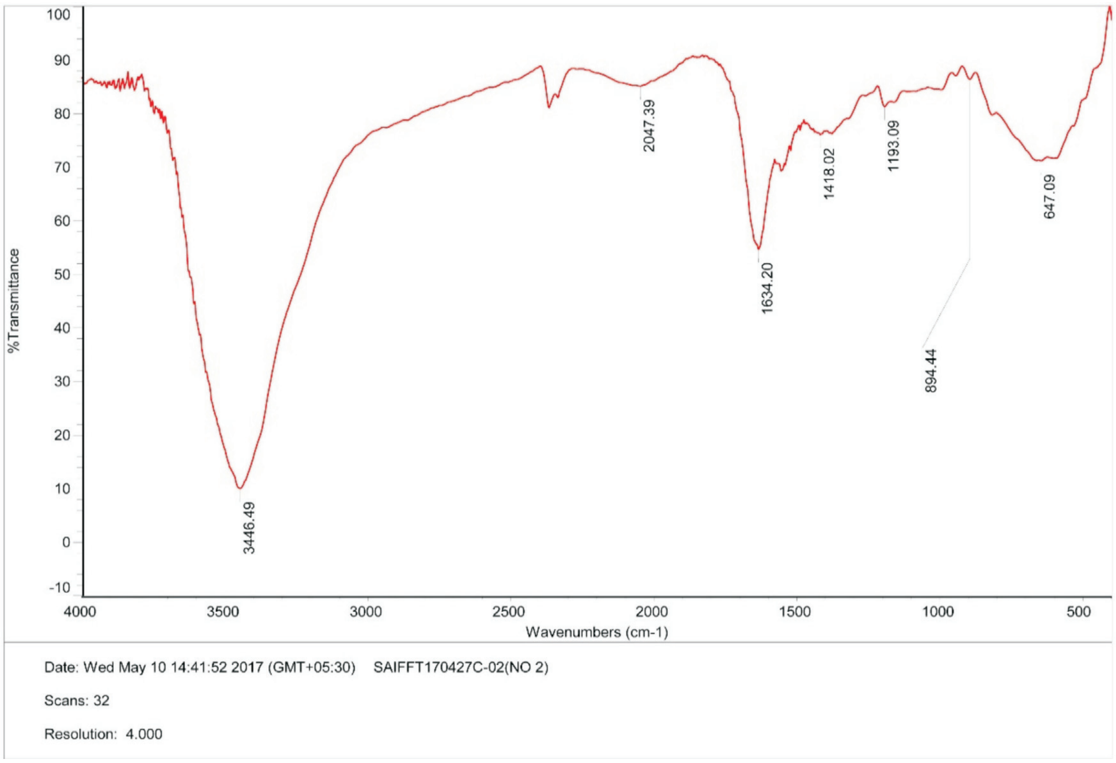


Fig. 3. FTIR Spectra Chitosan Schiff base Ni complex

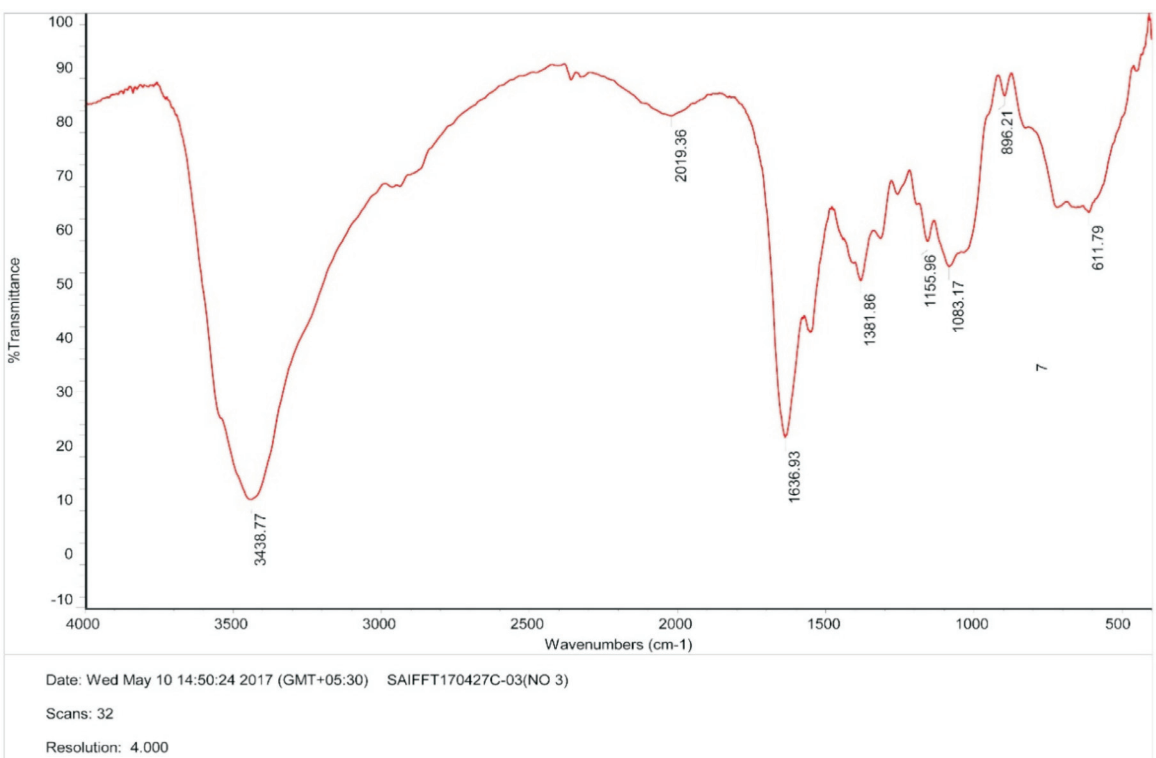


Fig. 4. FTIR Spectra Chitosan Schiff base Cu complex

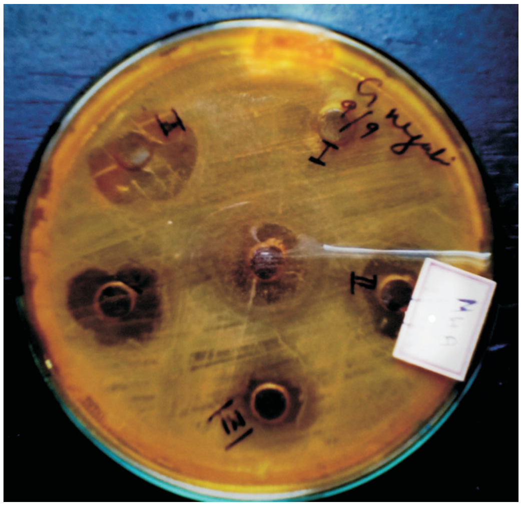


Fig. 5. Photograph of antibacterial inhibition against gram negative bacteria

hibition than Chitosan whereas Ni complex has got the same inhibition activity as that of Chitosan. The increasing order of antibacterial property against gram negative bacteria is as below.

Chitosan = Chitosan Schiff Base Ni complex < Chitosan Schiff Base Cu complex < Chitosan Schiff Base < Chitosan Schiff Base Co complex.

Chitosan a natural biopolymer which is derived from chitin by deacetylation has widespectrum antimicrobial activity against many antibioticresistant microorganisms both gramnegative and gram-positive by disintegrating the bacterial wall (Muzzarelli *et al*., 1990, Liu *et al*., 2004, Raafat *et al*., 2009, Li *et al*., 2010 and Tao *et al*., 2011) without altering resistance by increased efficiency (Ma *et al*., 2016). The title compound confirmed increased antimicrobial activity in a stable manner. Biodegradability and limited toxicity to mammalian cells made chitosan effective to control microbial plant pathogens as well (Jovanovic *et al*., 2016) and to degrade infectious disease causing agents like Staphylococcus aureus (Han *et al*., 2016). Moreover increased shelf life is noticed in fruits and vegetables which are coated with chitosan (Romanazzi *et al*., 2017).

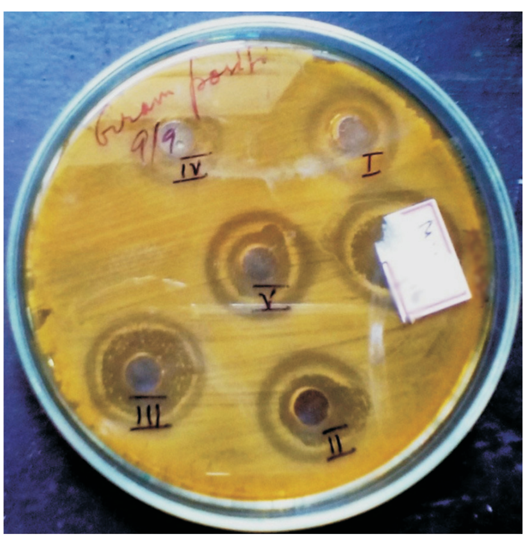


Fig. 6. Photograph of antibacterial inhibition against gram positive bacteria

Antifungal Activity

Agar- Well Diffusion Method

Agar well diffusion method was used to evaluate the antifungal activity of Chitosan p-dimethylaminobenzaldehyde Schiff base and its four metal complexes. Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts and in this assay technique the culture medium were inoculated with the fungal pathogen rhizopus. Wells were filled with 1mL of chitosan Schiff base and its complexes. The plates are incubated. This classic method yields a zone of inhibition in mm result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms. It is carried out in Petri plates. The chitosan Schiff base and its metal complexes have high antifungal activity. From this picture of petriplate (Fig. 7) shows that all have equal antifungal property against Rhizopus fungus. The

Fig. 7. Photograph of antifungal property against Rhizopus fungus

antifungal activity of chitosan biopolymer is reported on various fruits and vegetables (Guo *et al*., 2017, Gutierrez *et al*., 2017, Irkin and Guldas 2013, Jiang *et al*., 2018) and the chitosan Schiff base and its complexes in the study is stable in its antifungal activity.

Conclusion

The chitin, chitosan and their derivatives have received considerable attention in the present time due to their antimicrobial activity against different groups of microorganisms such as bacteria, yeast and fungi. Chitosan based Schiff base compound was successfully synthesized by the condensation of High viscous Chitosan with p-dimethylaminobenzaldehyde under mild acidic conditions. Chitosan based Schiff base Co, Ni and Cu metal complexes were also prepared by treating the newly synthesized Schiff base with Cobaltous chloride, Nickel Chloride and Cupric chloride respectively. Chitosan based Schiff base and its three metal complexes were characterized by FTIR. Bioactivities of Chitosan based Schiff base and its metal complexes were evaluated against Rhizopus fungus. It indicates that all the synthesized compounds have an antifungal property against Rhizopus. Bioactivities of Chitosan based Schiff base and its metal complexes were evaluated against gram positive bacteria and gram negative bacteria. It indicates that all the synthesized compounds have an antibacterial property against both bacteria. Chitosan Schiff Base Metal complex have got higher antibacterial activity in both cases. The inhibition of metal complexes may be due to the effect of ionic strength in the medium. Chitosan based Schiff base exhibits higher antibacterial activity than Chitosan itself, against both gram positive and gram negative bacteria. So it can be an eco-friendly alternative as an antimicrobial agent replacing the synthetic agents.

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