



Synthesis, Characterization, Antimicrobial and Cytotoxicity Evaluation of Mn (II) and Ni (II) Complexes with Schiff base Prepared from Chitosan and 2-hydroxy-1-naphthaldehyde and *O*-vanillin aldehydes

Umar Ali^{1*}, Maimuna Usman B.², and Abdussalam Abubakar S.²

¹Department of Chemistry, Sule Lamido University Kafin Hausa

²Department of Pure and Industrial Chemistry, Bayero University Kano

*Corresponding Author's Email: umar.ali@slu.edu.ng, dumarali4@gmail.com

Abstract

Chitosan was prepared from *Schistocerca gregaria* (desert grasshopper) and chemically modified with different aldehydes and their metal (II) complexes were prepared. The chitosan Schiff bases and their metal complexes were characterized on the basis of physicochemical properties, FT-IR and SEM. Chitosan and its derivatives were evaluated for their *in vitro* antimicrobial activity against two Gram positive bacteria (*Escheria coli* and *Salmonella typhi*), one gram negative (*Staphylococcus aureus*) and three fungi (*Aspergillus niger*, *Aspergillus flavus*, *Tricodma spp*) by MIC and MBC/MFC method. The chitosan Schiff bases have a melting point range of 235-252⁰C, decomposing temperature of the chitosan metal complexes 278-291⁰C. Magnetic moment of the complexes suggested the paramagnetic nature of the complexes, also molar conductivity obtained revealed the non-electrolytic nature of the complexes. The infrared bands at 3257 cm⁻¹, 1622 cm⁻¹ and 1626 cm⁻¹, 1603 cm⁻¹, and 1611 cm⁻¹ confirmed the chitosan, chitosan Schiff bases and its co-ordination with the metal ions via the azomethane nitrogen respectively. The results revealed that chitosan Schiff bases and their complexes have higher antifungal/antibacterial activities than the non-modified chitosan, which are in the order of chitosan metal complexes >chitosan Schiff bases>non modified chitosan. Cytotoxicity test *in vivo* was also carried out for the samples and results revealed that chitosan non modified sample was the most active against the brine shrimps at all concentrations 10µg/ml, 100 µg/ml, 1000 µg/ml compared to chitosan Schiff bases and chitosan metal complexes.

Keywords: Antimicrobial, Cytotoxicity, Chitosan, Complexes, Schiff base

1. Introduction

Chitosan is a natural-based biopolymer derived from chitin, the second most abundant polymer in nature next to cellulose. Chitin can be found from many sources in nature, including exoskeletons of crustaceans, insects, as well as mollusks and fungi. Chitin and chitosan share very similar backbone structures, as shown in (Figure 1). The similarity among these two molecules is the functional group at C-2 position. In molecular chain of chitin, it consists of linear structures of 2-acetamido-2-deoxy-β-D-glucose through β (1 → 4) linkage, by replacing hydroxyl group at C-2 position in cellulose molecular chain with acetamido group. Chitosan is the chitin derivative produced by the *N*-deacetylation process, resulting in the amino

group at C-2 position on its backbone. Both chitin and chitosan are considered as naturally-occurring polysaccharides, and they are of particular commercial interest because of great nitrogen content (Acharyulu *et al.*, 2013).

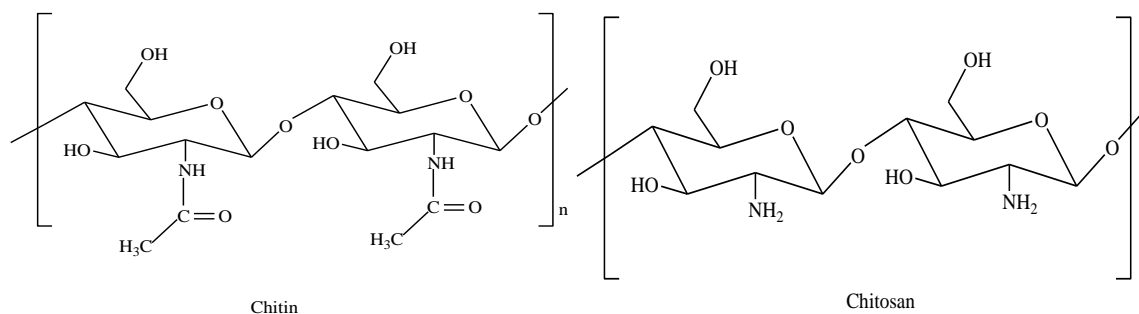


Figure1: Structure of chitin and chitosan

Due to the hydroxyl and amino groups on its backbones, chitosan is an amenable molecule that can be easily modified by various methods. Modifications of chitosan are aimed to improve physicochemical properties of chitosan and thus expand its applications in different situations.

This work, informed other studies on the most recent advancement of applications of chitosan and its derivatives in antimicrobial activities and cytotoxicity, as well as food preservation that improved shelf life.

2. Materials and Methodologies

2.1 Materials

Desert grasshopper (*Schistocerca gregaria*) was obtained from Maigatari market, Jigawa State, Nigeria. 2- hydroxy -1-nathaldehyde, *O*-vanillin from Sigma- Aldrich. Metal (II) Chloride (Analytical grade) was used to synthesize the complexes. Stuart SMP10 apparatus was used for melting/decomposition temperature determination for Schiff bases and metal (II) complexes, Jenway 4010 apparatus for conductivity and MKI Sharewood for magnetic susceptibility respectively. Surface Electronic Microscope analysis (SEM) was carried out in Katsina. Bacterial and fungal isolates were obtained from Department of Microbiology, Faculty of Life Sciences, Bayero University Kano, Kano State. Brine shrimp eggs (*Artemia* Species) from Sigma-Aldrich. All solvents were of analytical grade and used as received. Stuart Mplo Melting Point Machine, Jenway Conductivity Meter, MKI Sharewood Machine (Chemistry laboratories) of Pure and Industrial Chemistry Department, Bayero University, Kano-Nigeria. Nutrient Broth (NB) and potato dextrose agar (PDA) were used as bacterial and fungal media respectively.

2.2 Methods

Desert grasshoppers' wings were removed separately and the exoskeletons were sun-dried for three days, and then grounded into small paste/powder (Rawda and Hadeer, 2015). Initially, the grasshopper exoskeleton powder was demineralized with 5% HCl, with solid to solvent ratio of 1:15(w/v) with constant stirring for 30mins at ambient temperature followed by vacuum filtration. The residue was washed in a running tap water, rinsed with distilled water and oven dried at 60°C for 24hours. Further, demineralized powder was deproteinized with 3.5% NaOH solution in the ratio 1:15(w/v) for 30-40mins with constant stirring. The mixture was vacuum filtered and the residue was washed in a running tap water, rinsed with distilled water and oven dried at 60°C for 24hours. The removal of the acetyl group from the chitin was achieved by autoclaving for 2hrs at 121°C using 50% NaOH with a solid to liquid



ratio of 1:15(w/v). The resulting chitosan obtained was washed and neutralized with tap water followed by rinsing with distilled water and oven dried at 60°C for 24hours (Shilratan *et al.*, 2014).

A. Preparation of chitosan Schiff bases

Previously purified chitosan (1g) was dissolved in 50ml of 2% acetic acid and stirred at room temperature for 30mins. Then 10ml of ethanol containing 0.87g of aromatic aldehyde was added to the mixture (in case of 2-hydroxy-benzaldehyde, measurement was in ml due to its liquid form). The reaction mixtures were left to react at temperature of 60°C with stirring for 6hrs. The formation of a deep yellow gel refers to formation of chitosan Schiff base. The resulting product was filtered, washed several times with ethanol to remove unreacted aldehydes and dried in vacuum oven at 60°C. The obtained yellow products were kept in a desiccator over silica gel for further analysis (Mohy Eldrin *et al.*, 2015).

Two different chitosan-Schiff bases were prepared with two different aldehydes (2-hydroxy-1-naphthaldehyde and *O*-vanillin) and coded as CNSB and CVSB.

B. Preparation of Chitosan-metal complexes

To a suspension of Schiff base ligands (0.2g) in 10ml of ethanol, and was added to the solution of metal complexes (0.15g in ethanol). The mixture was stirred and heated for 6hrs under water bath at 60°C. The product was filtered off, washed with excess of ethanol and dried in vacuum oven at 60°C (Vadivel *et al.*, 2015). Two different chitosan-metal complexes were synthesized and was coded as follows: $[Mn(CVSB)Cl_2] \cdot 2H_2O$, $[Ni(CNSB)Cl_2] \cdot 2H_2O$

The metal complexes and their Schiff bases were tested for their solubility in various solvents i.e. dimethylformamide, dimethylsulfoxide, carbon tetrachloride, distilled water, ethanol, methanol, acetone, acetonitrile, chloroform. About 0.02g of each sample was transferred into a test tube to which about 5ml of the corresponding solvent was added. The content was shaken vigorously and the solubility was determined (Nagesh *et al.*, 2015).

C. Conductivity Measurement

Conductivity measurements of the metal complexes were determined using Jenway conductivity meter model 4010. 0.02g of each metal complex was dissolved in 10ml of DMSO and the corresponding conductance values were recorded, molar conductance of each metal complex was calculated. (Hellen *et al.*, 2017).

D. Magnetic Susceptivity Measurement

The magnetic susceptibility was determined using MK1 Sharewood machine. The prepared metal complexes were introduced into a given capillary tube up to a given mark and the reading recorded using the magnetic susceptibility balance (Nagesh *et al.*, 2015).

E. Melting Point /Decomposition Temperatures Determination

Melting point of the chitosan Schiff bases as well as decomposition temperatures of the chitosan metal complexes were determined using Stuart Mplo melting point machine by introducing 0.02g of each metal complex into a capillary tube and then inserted into the Stuart Mplo melting machine, the temperature at which the ligands melts and the complexes decomposes were recorded (Ahmed *et al.*, 2017).



F. Scanning Electron Microscopy (SEM)

The surface morphology of chitosan, chitosan Schiff bases, chitosan metal complexes were observed with Scanning electron microscope to verify the compatibility of the mixtures of chitosan Schiff bases and chitosan metal complexes. To analyze, the samples were cut into pieces of various sizes and coated with a thin gold-palladium layer by a sputter coater unit (UG-microtech, UCK field, UK), (Shashikala and Shafi, 2015).

G. Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was performed based on a micro dilution method in test-tubes according to Chikezie, (2017) in which nutrient broth (NA) was used for bacterial and potato dextrose agar (PDA) for fungi. The samples (20mg) were dissolved in 2ml of DMSO. Each stock solution was diluted with standard method to prepare serial two fold dilutions of the broth containing 106cfu/ml of test bacterial/fungal. The stock solution concentration of the samples was 10mg/ml. The liquor of 1ml of broth media was placed into each test-tube, sealed with cotton wool and sterilized for 45minutes before antimicrobial agent were added. The concentration of the antimicrobial agents was adjusted to 1000 μ g/ml, 500 μ g/ml, 250 μ g/ml and 125 μ g/ml. Thereafter, each test-tubes was inoculated with 5 μ l of suspensions containing 106 cfum/l (equivalent to McFarland 0.5) of the culture and incubated at 37°C for 18-24hrs for bacterial and 48hrs at room temperature for fungi. To account for inhibitory effect, a negative control was included. This was achieved by preparing the control sample with broth in place of test sample material. The commercial drugs amoxicillin as a positive control for bacterial and ketoconazole for fungi with a dosage of 500mg were used. At the end of the incubation period, the minimal inhibitory concentration (MIC) values were recorded as the lowest concentration of the substance that had no visible turbidity (Saroat *et al.*, 2012).

H. Determination of MBC and MFC

The MBC is defined as the lowest concentration where no bacterial growth is seen (indicated by the absence of turbidity). This was determined using the broth dilutions resulting from the MIC tubes by sub-culturing to antibacterial free agar as described by Chikezie, 2017. In this method, the contents of the test tubes resulting from MIC were streaked with a sterile wire loop onto agar plate free antibacterial agents and incubated at 37°C for 24hrs. The lower concentration which produced no bacterial growth was recorded as the MBC. The MBC/MFC was defined as the consequent concentrations required killing 99.9% of the cell.

I. Cytotoxicity Test

Preparation of reagent

The stock solution was prepared by dissolving 20mg of the samples in 2mls of DMSO. Concentrations of 10 μ g/ml, 100 μ g/ml and 1000 μ g/ml were prepared by serial dilution from the stock solution. Artificial sea water was prepared in which 16g of NaCl was dissolved in 1L of distilled water (Quazi *et al.*, 2017).

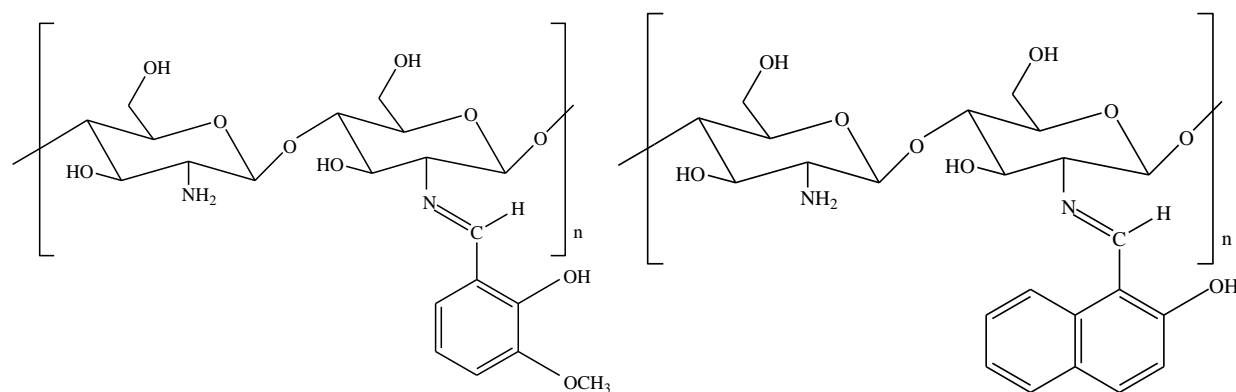
Brine Shrimp lethality Assay (BSLA)

Brine shrimp lethality bio-assay was carried out according to the procedure described by Meyers *et al.*, (1982). In this test, 4g of brine shrimp eggs were weighed and dissolved in 250ml of artificial sea water for hatching the brine shrimp eggs in a source of light. Constant oxygen supply was maintained through the hatching period. Two days were allowed for the shrimp eggs to hatch and mature as nauplii (larva).

After two days, the shrimps were ready for assay. 2ml of the artificial sea water was added to each test tube and 10 living brine shrimps were introduced into each test tube with the help of a Pasteur pipette. Thus there was total of 30 per dilutions. Then, the volume was adjusted with the artificial sea water up to 5ml per test tube. The test-tubes were left uncovered under light. The number of surviving shrimps were counted and recovered after 24hrs. Negative control was run parallel to the samples using 5ml of sea water and 2 drops of DMSO and a positive control using potassium dichromate.

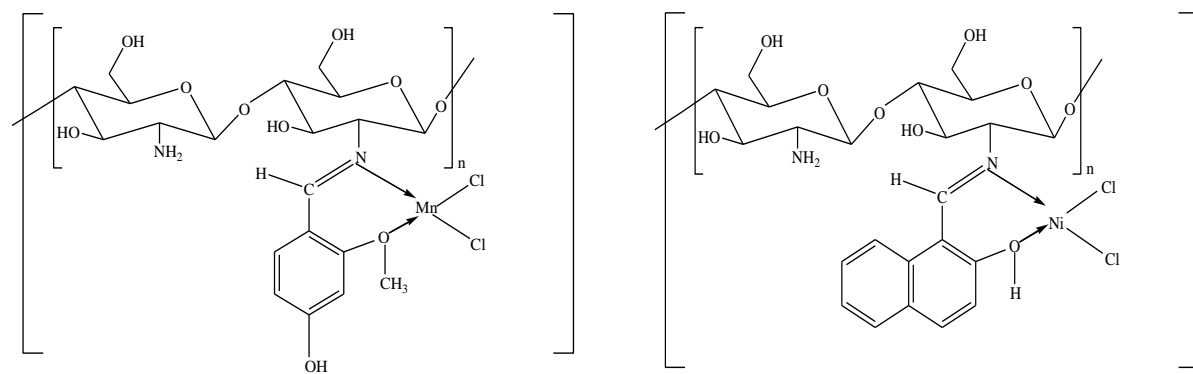
3.0 Results

An effort has been made to synthesize chitosan Schiff bases and its metal (II) complexes using chitosan from grasshopper. It served as the main source of imine and *O*-Vanillin aldehyde, 2-hydroxy-1-naphthaldehyde as aldehydes and Mn(II) and Ni(II) as metal (II) Complexes. The proposed structures were given below.



Chitosan-*o*-vanillin Schiff base

Chitosan-2-hydroxy-1-naphthaldehydeschiff base



Chitosan-*o*-vanillin Schiff base manganese (II) complex and Chitosan-2-hydroxy-1-naphthaldehyde Schiff base nickel (II) complex.

Figure 2: Proposed structure of chitosan Schiff bases and its metal (II) complexes

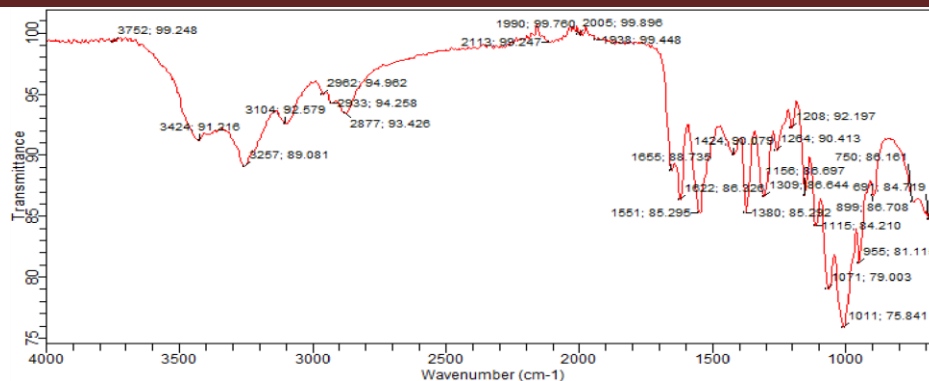


Figure 3: FTIR of chitosan from *Schistocerca gregaria* (desert grasshopper)

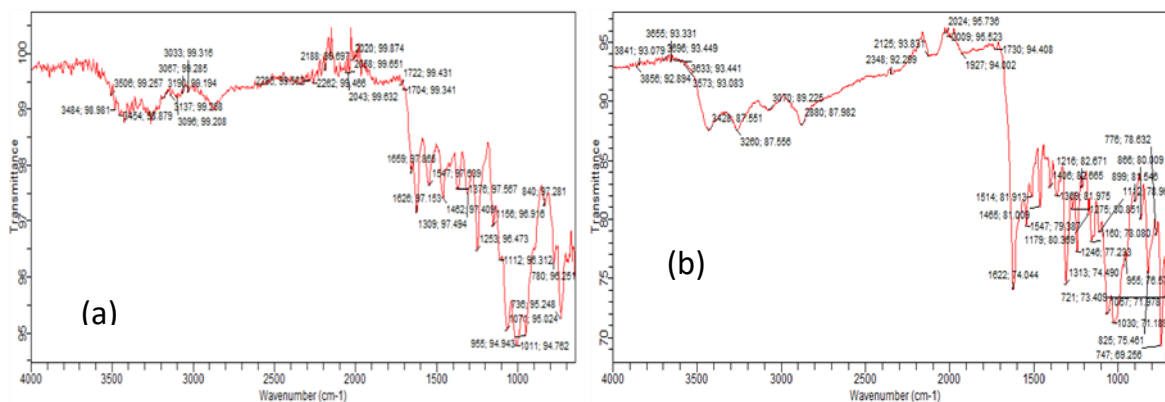


Figure 4:(a)FTIR of Chitosan-2-hydroxy-1-naphthaldehyde Schiff base Figure 4:(b)FTIR of Chitosan- o- vanillin Schiff base

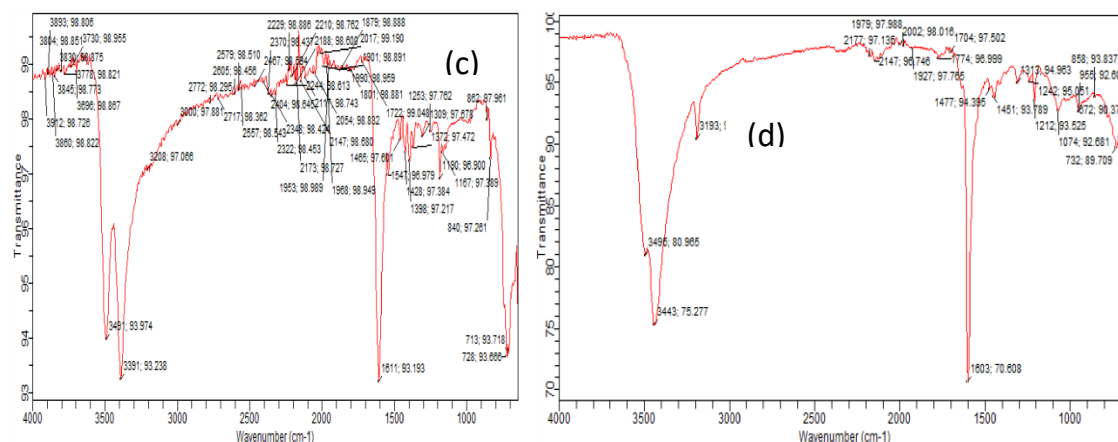


Figure 5:(c): FT-IR of Chitosan -2-hydroxy-1-naphthaldehyde (d) FTIR of Chitosan-o-vanillin Schiff base manganese (II) chloride and Schiff base nickel (II) complex.

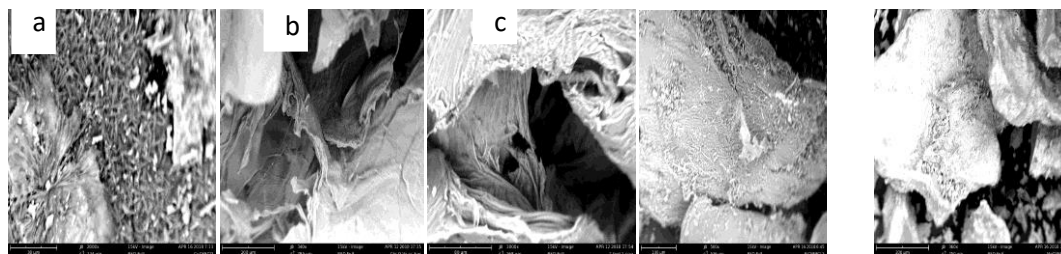


Figure 6: SEM for (a) chitosan (b) *O*-vanillin Schiff base (c) 2-Hydroxyl-1-naphthaldehyde Schiff base (d) Chitosan-2-hydroxy-1-naphthaldehyde Schiff base Ni (II) complex (e) Chitosan-*o*-vanillin Schiff base Mn (II) Complex

Table 1: IR spectral data of chitosan Schiff bases Ligand (L) and its metal (II) complexes

Compounds	ν_{OH}/ν_{H_2O}	Amide $\nu(NH)$ cm^{-1}	$\nu(C=O)$ cm^{-1}	$\nu(C=N)$ cm^{-1}	$\nu(M-N)cm^{-1}$	$\nu(M-O)cm^{-1}$	$\nu(M-Cl)cm^{-1}$
CVSB	3428	3260	1730	1622	-	-	-
CNSB	3484	3137	1722	1626	-	-	-
[Mn(CVSB)Cl ₂].2H ₂ O	3443	3193	1722	1603	732	541	369
[Ni(CNSB)Cl ₂].2H ₂ O	3491	3208	1722	1611	784	514	302

Table 2: Physical properties of the synthesized ligands and metal (II) complexes

Compounds	Color obtained	B.M U _{eff}	Melting Temp (°C)	Decomposition Temp (°C)	Molar conductance Ohm ⁻¹ cm ² mol ⁻¹	Percentage yields (%)
CNSB	Deep yellow		235	-	-	74.42
CVSB	Deep yellow		252	-	-	62.28
[Mn(CVSB)Cl ₂].2H ₂ O	Light brown	5.21	-	278	14.69	50.23
[Ni(CNSB)Cl ₂]. 2H ₂ O	Grey	3.46	-	291	17.42	64.85

Table 3: Solubility of chitosan Schiff bases and their metal (II) complexes

Compounds	CCl ₄	DMF	DMSO	EtOH	MeOH	CHCl ₃	Acetone	Acetonitrile	H ₂ O
CNSB	IS	S	S	SS	S	SS	SS	SS	IS
CVSB	IS	S	S	SS	S	IS	SS	SS	IS
[Mn(CVSB)Cl ₂].2H ₂ O	IS	S	S	S	S	S	SS	SS	IS
[Ni(CNSB)Cl ₂].2H ₂ O	IS	S	S	SS	S	SS	SS	SS	IS

Keys:

S =soluble, SS=slightly soluble, IS=insoluble, DMF=Dimethylformamide, DMSO= Dimethylsulfoxide, CVSB = chitosan-*O*- vanillin Schiff base CNSB = chitosan-2-hydroxy-1-naphthaldehyde Schiff base, [Mn (CVSB)Cl₂].2H₂O = Chitosan salicylaldehyde Schiff base copper (II) complex, [Ni (CNSB)Cl₂].2H₂O = Chitosan- *O*-anisaldehyde Schiff base iron (II) complex



Table 4: MIC and MBC Result of Antibacterial Activity of Chitosan, Chitosan Schiff bases and Complexes

Samples	Test organisms	MIC (Concentrations($\mu\text{g/ml}$))				MBC($\mu\text{g/ml}$)
		500 ($\mu\text{g/ml}$)	250 ($\mu\text{g/ml}$)	125 ($\mu\text{g/ml}$)	62.5 ($\mu\text{g/ml}$)	
Chitosan	<i>E.coli</i>	-	-	-	+	125
	<i>S. typhii</i>	-	-	-	+	125
	<i>S.aureus</i>	-	-	-	+	125
CVSB	<i>E.coli</i>	-	-	-	+	125
	<i>S. typhii</i>	-	-	+	+	>250
	<i>S.aureus</i>	-	-	+	+	>250
CNSB	<i>E.coli</i>	-	-	-	-	MIC=MBC
	<i>S. typhii</i>	+	+	+	+	>500
	<i>S.aureus</i>	+	+	+	+	>500
[Mn(CVSB)Cl ₂].2H ₂ O	<i>E.coli</i>	-	-	-	-	MIC=MBC
	<i>S. typhii</i>	+	+	+	+	>500
	<i>S.aureus</i>	-	-	-	-	MIC=MBC
[Ni(CNSB)Cl ₂].2H ₂ O	<i>E.coli</i>	-	-	+	+	>250
	<i>S. typhii</i>	+	+	+	+	>500
	<i>S.aureus</i>	-	-	+	+	250

Table 5: MIC and MFC Result of Antifungal Activity of Chitosan, Chitosan Schiff bases and its Metal Complexes

Samples	Test organisms	MIC (Concentrations($\mu\text{g/ml}$))				MFC($\mu\text{g/ml}$)
		500 $\mu\text{g/ml}$	250 $\mu\text{g/ml}$	125 $\mu\text{g/ml}$	62.5 $\mu\text{g/ml}$	
Chitosan	<i>A.flavus</i>	-	-	+	+	>250
	<i>A. niger</i>	-	-	-	+	125
	<i>Tricodema spp</i>	-	-	+	+	>250
CVSB	<i>A.flavus</i>	-	-	+	+	>250
	<i>A.niger</i>	-	-	+	+	>250
	<i>Tricodema spp</i>	+	+	+	+	>500
CNSB	<i>A.flavus</i>	-	-	+	+	>250
	<i>A.niger</i>	-	-	+	+	>250
	<i>Tricodema spp</i>	-	-	+	+	>250
[Mn(CVSB)Cl ₂].2H ₂ O	<i>A. flavus</i>	-	-	+	+	>250
	<i>A.niger</i>	-	-	+	+	>250
	<i>Tricodema spp</i>	-	-	+	+	>250
[Ni(CNSB)Cl ₂].2H ₂ O	<i>A.flavus</i>	-	+	+	+	>500
	<i>A.niger</i>	-	-	+	+	>250
	<i>Tricodema spp</i>	-	-	+	+	>250

Keys *A.niger* = *Aspergillus niger*, *A.flavus* = *Aspergillus flavus*, *S.T* = *Salmonella Typhii*, *S.A* = *Staphylococcus aureus*, - =no growth of organism, + =growth of organism

Table 6: The number of shrimp nauplii that survived after treating with chitosan and its derivatives and the percentage mortality

Samples	Concentration ($\mu\text{g/mL}$)	Number of surviving Nauplii after 24hrs			TTL of survivors	% Mortality	LC_{50} ($\mu\text{g/ml}$)
		T1	T2	T3			
Chitosan	10	8	8	10	25	17%	4547.478
	100	8	6	8	24	20%	
	1000	6	8	7	21	70%	
CVSB	10	8	5	4	10	66%	21.397
	100	2	2	2	6	80%	
	1000	0	0	0	0	100%	
CNSB	10	5	8	7	20	33%	5.112
	100	4	4	4	12	60%	
	1000	1	0	0	1	96%	
[Mn(CVSB)Cl ₂].2H ₂ O	10	5	3	3	11	63%	12.110
	100	2	2	1	5	83%	
	1000	0	0	0	0	100%	
[Ni(CNSB)Cl ₂].2H ₂ O	10	6	4	7	17	43%	26.460
	100	2	5	2	9	70%	
	1000	0	0	0	0	100%	

4. Discussions

The formation of the chitosan was confirmed by the FTIR spectra (Table 1 and Figure 3) which exhibits strong peak at 3257cm^{-1} which can be assigned due to coxial stretching vibration of O-H superimposed to the N-H stretching band and inter hydrogen band of the polysaccharide. The C-H axial stretching band arises at 2873cm^{-1} . Primary amines have two weak adsorption peaks at 1655cm^{-1} and 1551cm^{-1} , corresponded to amide (I) and amide (II) respectively, which indicates that the prepared chitosan have high degree of deacetylation. Besides, the bands at 1115cm^{-1} and 1071cm^{-1} are due to C-O stretching (1-4)-linked- β -D-glucosamine unit (Vadivel *et al.*, 2015)

The FTIR spectrum of the chitosan Schiff bases (ligands) (Table 1 and Figure 4) showed strong peaks at, 1622 and 1626cm^{-1} which are characteristics stretching frequency of azomethine group (C=N). These bands are shifted to higher frequency such 1603 and 1611cm^{-1} due to azomethine group complexes with metal ion. This indicated the co-ordination of metal Centre with azomethine nitrogen group (Thatte *et al.*, 2015).

In the IR spectra of the metal complexes (Table 1 and Figure 5) medium intensity weak bands appeared at 3443 , 3491cm^{-1} and 3193 , 3208cm^{-1} were due to OH and NH which appeared almost at about the same position as in the case of ligands confirming their non-involvement in the co-ordination. The shift of the phenolic $\nu(\text{C}=\text{O})$ from 1730 to 1722cm^{-1} of the chitosan Schiff bases indicates the formation of co-ordinate bond between the metal ion and the phenolic oxygen atom via deprotonation. The new bands at 732 , 784cm^{-1} and 541 , 514cm^{-1} appeared in the far infrared red region in the spectra of the complexes, which suggest the formation of M-N and M-O (Sani and Dailami, 2015). Also the bands 369cm^{-1} and 302cm^{-1} were due to M-Cl bond respectively (Patrick *et al.*, 1969).

4.1 Physical properties of Schiff bases and metal complexes

The chitosan Schiff bases and its metal (II) complexes were prepared in good yields, ranging from 50.23 - 74.42%. The chitosan Schiff bases obtained (Table 2), have varying colours in which they were predominantly yellow in color while the metal (II) complexes have varying colours; light brown and grey. The effective magnetic measurement result showed that all the complexes are paramagnetic (Table 2). Sharp melting /decomposition temperatures indicated that the compounds are pure and stable. Conductivity measurement of the complexes revealed the non-electrolytic nature compounds (Table 2). Solubility test (Table 3) revealed that the compounds are soluble in protonic solvents such as methanol, and ethanol, insoluble in distilled water, carbon tetrachloride, while slightly soluble in acetone, acetonitrile (Sani and Dailami, 2015).

4.2 Antimicrobial Analysis

The antimicrobial effects of chitosan and its derivatives were assessed separately in vitro against two Gram positive bacterial (*Escheria coli*, *Salmonella typhii*), one gram negative (*Staphylococcus aureus*) and three fungi (*Aspergillus niger*, *Aspergillus flavus*, *Tricodma spp*) using ciprofloxacin as the standard antibiotic for bacterial and Ketoconazole for fungi, using MIC and MBC method. The obtained result indicates that chitosan and its derivatives had significant antibacterial and antifungal effects.

4.3 Antibacterial Activity

The antibacterial effects of chitosan and its derivatives have been reported by many authors (Vadivel *et al.*, 2016; Verlee *et al.*, 2017)). Although several mechanisms for their antibacterial activity have been suggested, the exact mode of action is still not known in detail. However, there is clear evidence of these compound having molecular-level interactions with the cell membrane. Ionic and or hydrophobic interactions are often considered to be common interactions that lead to the damage or breakage of the cell membrane (Kong *et al.*, 2010).

In the antibacterial analysis (Table 4) results showed that the chemical modification of chitosan decreased its antibacterial activity where chitosan metal complexes had MIC/MBC ranging from 250 to 500 $\mu\text{g/ml}$ with $[\text{Mn}(\text{CVSB})\text{Cl}_2]\cdot 2\text{H}_2\text{O}$ complex having MIC=MBC which completely resist the attack of the bacterial, chitosan Schiff bases also had MIC/MBC ranging from 125 to 250 $\mu\text{g/ml}$ with chitosan-*o*-anisaldehyde Schiff base (CVSB) which completely also resist the attack of bacterial organism *E.coli* compared to non-modified chitosan having MIC/MBC ranging from 125 to 62.5 $\mu\text{g/ml}$. All chitosan Schiff bases and their metal complexes were more active than the non-modified chitosan, in inhibiting the growth of bacterial and fungi. The most active compound among the chitosan metal-complexes was $[\text{Ni}(\text{CNSB})\text{Cl}_2]\cdot 2\text{H}_2\text{O}$ complex while among chitosan Schiff bases was chitosan-*o*-anisaldehyde Schiff base (CNSB) and chitosan was less active when comparing chitosan –metal complexes to the Schiff bases and non-modified chitosan to the antibacterial effects. Results of the antibacterial analysis showed that the hydrophobic chitosan metal complexes had stronger antibacterial effects than the chitosan Schiff bases and the non-modified chitosan; moreover, large effects were observed for more hydrophobic derivatives (Pavinatto *et al.*, 2014).

4.4 Antifungal Activity

In contrasts to the results of the antibacterial assay, here the hydrophobic interactions appeared to be less crucial for the action of chitosan and its derivatives. Thus, charge density seems to be an important factor for the antifungal effect, where chitosan Schiff bases with fewer available amine groups were less active



than the non-modified chitosan and the major mode of action is the electrostatic interaction between the positive charge of chitosan and cell surface of the fungi.

In antifungal assay (Table 5), where chitosan-metal complexes had MIC ranging from 250 to 500µg/ml and Schiff bases had MIC ranging from 250 to 125 µg/ml and MIC ranging from 125 to 62.5ug/ml for the non-modified chitosan.

4.5 Brine Shrimp Lethality Assay

Brine Shrimp (*Artemia Spp*) Lethality Assay (BSLA) is used as an indicator for general toxicity and also as a guide for the detection of antitumor and pesticidal compounds (Meyer *et al.*, 1982).Chitosan and its derivatives chitosan were tested *in vivo* for their cytotoxicity effect against the brine shrimp nauphii and relative toxicity result (Table 6). The percentage mortality (%M) was calculated by dividing the number of dead nauplii by the total number and then, multiplied by 100 (Lilybeth and Olga, 2013).

$$\%M = \frac{\text{Number of dead nauplii}}{\text{Number of total nauplii}} \times 100 \dots\dots\dots (I)$$

The lethality concentration (LC₅₀) of the samples was 10µg/ml, 100µg/ml, 1000 µg/ml respectively. The degree of lethality was directly proportional to the concentration of the samples (Itrat *et al.*, 2013). Maximum mortalities (100%) were observed at a concentration of 1000 µg/ml in both samples except chitosan sample. Based on the results obtained, the brine shrimp lethality of the samples was found to be concentration-dependent. Chitosan has an LC₅₀ of 4745.47 which is above 1000 and considered as non-toxic and both the Schiff bases (CVSB)-21.397, (CNSB)-5.112) and metal complexes of chitosan ([Mn(CVSB)Cl₂].2H₂O)-12.110, ([Mn(CVSB)Cl₂].2H₂O)-26.460 has an LC₅₀ value less than 1000 and considered as toxic (active). According to Meyers *et al.*, (1982), an LC₅₀ value of less than 1000 is toxic while LC₅₀ value greater than 1000 is non-toxic. This may also be due to the fact that tertiary amines are less toxic than primary amines as chitosan derivatives containing primary amines versus tertiary aliphatic amines. Moreover, chitosan metal complexes were also active against the brine shrimps analysis; this could be attributed to the presence of metal (II) chlorides which were complexes with the chitosan Schiff bases.

4.6 Scanning Electron Analysis

The surface morphology analysis of chitosan, chitosan Schiff bases and chitosan metal complexes were studied using scanning electron microscopy. The SEM plates (b-c), of the chitosan Schiff bases showed rough surfaces clearly indicating gelation appearance and so also covered with benzene derivatives as there is change in sizes and shapes when compared to the chitosan particles in plate (a). Also we can noticed that the surface morphology of the chitosan metal complexes plates (d-e) differs from that of chitosan Schiff bases which appears smoother and brighter, confirming the presence of Mn (II), Ni(II), ions as indicated by the FT-IR results (Sashikala and Shafi, 2017).

5. Conclusion

A biopolymer chitosan was extracted from *Schistocerca gregaria* (Desert Grasshopper) and two Schiff bases were prepared using 2-hydroxy-1-naphthaldehyde and *O*-vanillin aldehydes as well as its Mn (II) and Ni (II) complexes have been synthesized successfully. Antimicrobial activity results indicated that the prepared chitosan and its derivatives are promising antibacterial and antifungal agents. There was enhanced antimicrobial activity in the chitosan metal (II) complexes than the chitosan Schiff bases and non-modified chitosan, this have been explained on the basis of chelation theory. The cytotoxicity test *in vivo* confirmed that the prepared chitosan is non-toxic compared to its derivatives thus, suggesting its potential application in improvement of food quality, preservation as well as packaging.



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