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Schistocerca gregaria-derived Chitosan: Antimicrobial Activity and Cytotoxicity Studies of its Schiff Bases and Metal Complexes

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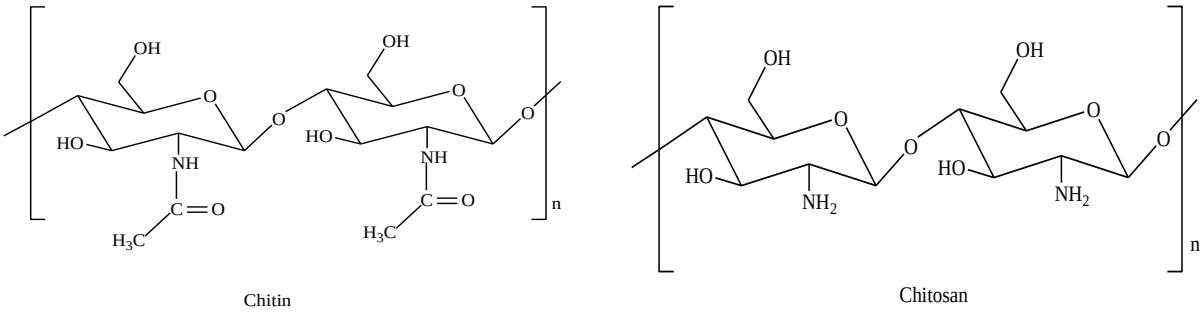
ABSTRACT

Chitosan prepared by deacetylation of chitin extracted from *Schistocerca gregaria* (desert grasshopper) has been coupled with different aldehydes to afford the corresponding Schiff bases and consequently reacted with metal (II) salts to provide the corresponding complexes. The compounds were evaluated for their *in vitro* antimicrobial activity against two Gram positive (*Escheria coli* and *Salmonella typhii*), one Gram negative bacterial strains (*Staphylococcus aureus*), and three fungal strains (*Aspergillus niger, Aspergillus flavus*, and *Tricodma spp*). Chitosan and its derivatives were evaluated for their in vitro antimicrobial activity against two Gram positive bacterial (*Escheria coli and Salmonella typhii*), one gram negative (*Staphylococcus aureus*) and three fungi *(Aspergillus niger, Aspergillus flavus, Tricodma spp*) The MIC, MBC, and MFC values revealed that compared to the parent chitosan, the Schiff bases and their complexes have higher antibacterial and fungal activities which are in the order of chitosan metal complexes >chitosan Schiff bases > non-modified chitosan with BMC values of >500, >250 and 125µg/ml respectively, where MIC and MFC results showed similar trend. The cytotoxicity assay using brine shrimps revealed that parent chitosan was the most active against at all concentrations, 10µg/ml, 100µg/ml, and 1000µg/ml compared to chitosan Schiff bases, metal complexes.

Keywords: Antimicrobial activity, Chitosan, Complexes, Desert grasshopper, Schiff bases, *Schistocerca gregaria*

INTRODUCTION

Chitosan is a non-toxic, biocompatible and biodegradable polymer that exhibits promise in a wide range of biomedical applications including wound dressing, tissue engineering, implant coatings, and therapeutic agent delivery systems (Park and Kim, 2010). Chemically, chitosan is a linear heteropolysaccharide composed of β-1, 4-linked –D-glucose amine (GlcN) and N-acetyl-D-glucosamine (GlcNac) at varying ratios. The proportion of GlcNac in relation to GlcN is defined as the degree of acetylation (DA).



Chitin Chitosan

Figure 1: Structure of chitin and chitosan

Chitosan is found naturally in certain fungi (*Mucoraceae*), shrimps, and recently from few species of insects (Liu *et al*., 2012; Majtan *et al.*, 2007). But it is easily obtained by the thermochemical deacetylation of chitin in the presence of alkali. One of the methods involves hydrolysis of the acetylated analogue (chitin) using sodium or potassium hydroxide solutions as well as a mixture of anhydrous hydrazine and hydrazine sulfate. These conditions were used for determinations of the polymer molecular weight and the degree of deacetylation (Muzzarelli, 1977; Muzzarelli and Rochetti, 1985).

The degree of deacetylation (DD) is the key property that affects the physical and chemical properties of chitosan, such as solubility, chemical reactivity and biodegradability and consequently their applications (Acharyulu *et al* 2013; Wanule *et al*., 2014). Thus, chitosan and its derivatives have attracted considerable interest due to their

antimicrobial activities. But it had majorly been found that chitosan exhibits antibacterial activity only in an acidic medium because of its poor solubility above pH 6.5. Furthermore, the antibacterial activity of chitosan had been found to be influenced by a number of factors that include the type of chitosan, the degree of chitosan polymerization and some of its other physicochemical properties.

In this work, an attempt has been made to explore alternative chitosan derivatives with the view of addressing some of the limitations reported by the previous workers.

MATERIALS AND METHODS

Materials

Desert grasshoppers (*Schistocerca gregaria*) were obtained from Maigatari local market from Jigawa State, Nigeria. 2-hydroxybenzaldehyde (salicylaldehyde), 2-methoxybenzaldehyde (*O*-anisaldehyde) from Sigma- Aldrich. Metal (II) chloride salts (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 conductivity meter and MK1 Sherwood for magnetic susceptibility respectively. Scanning electron microscope (SEM) was accessed at Umaru Musa Yar' Adua University, Katsina, Katsina State. Bacterial and fungal isolates were obtained and identified at the Department of Microbiology, Faculty of Life Sciences, Bayero University, Kano, Nigeria. Brine shrimp eggs (Artemia Species) were obtained from Sigma-Aldrich. All solvents were of analytical grade and used as received. Stuart SMP10 melting point machine, Jenway Conductivity meter, MK1 Sherwood Machine were all accessed from Department of Pure and Industrial Chemistry, Bayero University, Kano-Nigeria. Nutrient Broth (NB) and potato dextrose agar (PDA) were used as bacterial and fungal media respectively.

Methods

Grasshoppers wings were removed separately and the exoskeletons were sun-dried for three days, and then grounded into small paste/powder (Liu *et al*., 2012). 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 24 h. The demineralized powder was deproteinized with 3.5% NaOH solution in the ratio of 1:15(w/v) for 30-40 mins 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 24 h. The removal of the acetyl group from the chitin was achieved by autoclaving for 2 h at 121° C using 50% NaOH with a solid to liquid ratio of 1:15(w/v).The resulting chitosan was neutralized with tap water followed by rinsing with distilled water and oven dried at 60° C for 24 h (Shilratan *et al*., 2014).

Preparation of chitosan Schiff bases

Previously purified chitosan (1g) was dissolved in 50 ml of 2% acetic acid and stirred at room temperature for 30 mins. Then 10 ml of ethanol containing 0.87g of aromatic aldehyde was added to the mixture (in case of 2-hydroxy-benzaldehyde, 2 ml was used). The reaction mixtures were left to react at temperature of 60° C with stirring for 6 h. 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-methoxy benzaldehyde (*o*-anisaldehyde), and 2-hydroxybenzaldehyde (salicylaldehyde) and coded as CASB and CSSB respectively.

Preparation of chitosan metal complexes

To a suspension of Schiff base ligands (0.2g) in 10 ml of ethanol, 0.15g of the metal (II) salt solution in ethanol was added. The mixture was heated on water bath (60° C) for 6 hrs with stirring. 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 differently and were coded as follows: $[Fe(CASB)Cl_2].3H_2O$, $[Cu(CSSB)Cl_2].2H_2O$.

Solubility Test

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; Pillai *et al.*, 2009).

Conductivity Measurement

Conductivity measurements of the metal complexes were determined using Jenway conductivity meter model 4010. For each metal complex 0.02g 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).

Magnetic Susceptibility Measurement

The magnetic susceptibility was determined using MK1 Sherwood 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. The magnetic moment was calculated using the following equation; $\sqrt{n(n+2)}$ BM, based on the number of unpaired electron.

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 SMP10 melting point machine by introducing 0.02g of each metal complex into a capillary tube and then inserted into the Stuart SMP10 melting machine, the temperature at which the ligands melts and the complexes decomposes were recorded (Ahmed *et al*., 2017).

Scanning Electron Microscopy (SEM)

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

Minimum Inhibitory Concentration (MIC)

The antimicrobial minimum inhibitory concentrations were determined using micro dilution method. (Chikezie, 2017). In which the nutrient broth (NA) and potato dextrose agar were used for bacterial and fungal assays respectively. Thus, the samples (20 mg) were dissolved in 2 ml DMSO. Each stock solution was diluted with standard method broth to prepare serial two fold dilutions of the broth containing 106 cfu/ml of the test bacteria/fungi. The stock solution concentration of the samples was 10 mg/ml. The liquor of 1ml of broth media was placed into each test-tube, sealed with cotton wool and sterilized for 45 minutes before antimicrobial agent were added. The concentration of the antimicrobial agents was adjusted to 1000, 500, 250 and 125 µg/ml. Thereafter, each test-tubes was inoculated with 5 µl of suspensions containing 106 cfum/l (equivalent to McFarland 0.5) of the culture and incubated at 37°C for 18-24 h for bacterial and 48 h 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 ketoconozole for fungi with a dosage of 500 mg 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).

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 24 hrs. The MBC/MFC is defined as the lowest concentrations required to kill 99.9% of the cell.

Cytotoxicity Test

Preparation of the samples

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

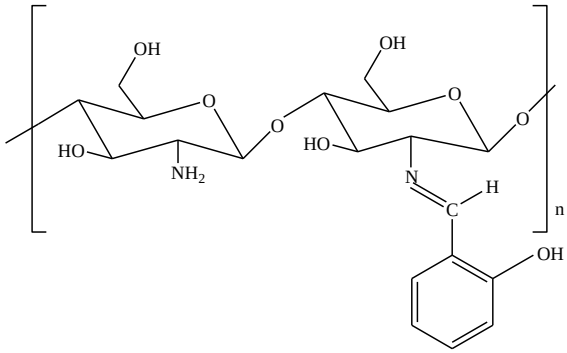
Brine Shrimp Lethality Assay (BSLA)

Brine shrimp lethality bio-assay was carried out according to the procedure described by Meyers *et al*., (1982). In this test, 4 g of brine shrimp eggs were weighed and dissolved in 250 ml 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. 2 ml 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 5 ml per test tube. The test-tubes were left uncovered under light. The number of surviving shrimps were counted and recovered after 24 hrs. 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.

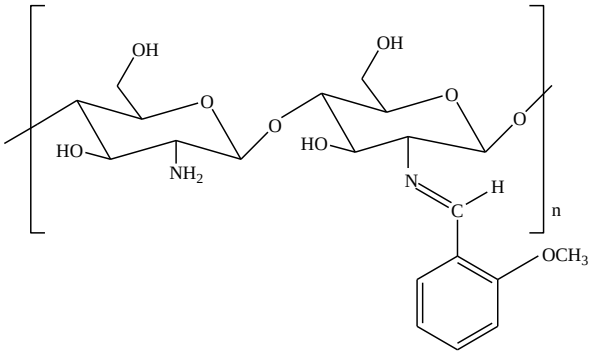
RESULTS AND DISCUSSIONS

Coupling of chitosan with aldehydes

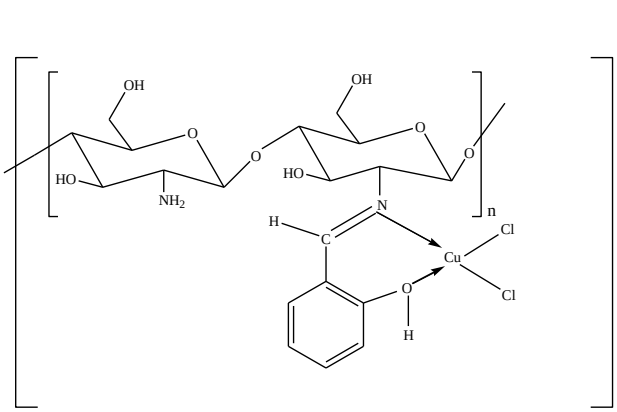
The goal of the synthesis part is coupling of aldehyde molecules with chitosan. Chitosan primary amine reacts with the active carbonyl groups of aldehyde to produce corresponding Schiff bases via condensation reaction and later complexed with metal (II) complexes (Figure 2).



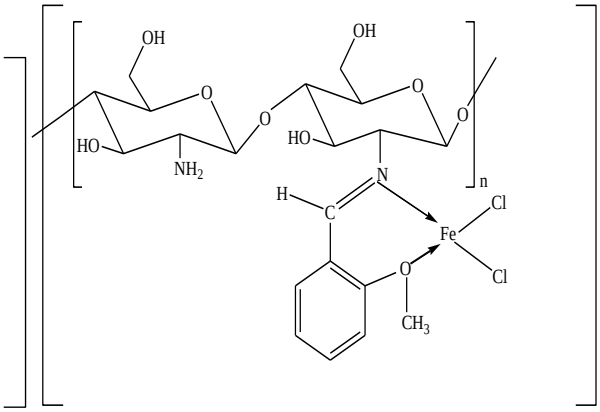
Chitosan-salicyladehyde Schiff base



Chitosan –*o*-anisaldehyde Schiff base



Chitosan salicyladehyde Schiff base copper (II) complex



Chitosan-*o*-anisaldehyde Schiff base iron (II) complex

Figure 2: Proposed structures of chitosan Schiff bases and their metal (II) complexes

Interpretation of the IR spectra

Table 1: IR Spectral Data of Chitosan Schiff bases Ligand (L) and its Metal (II) Complexes

Compounds	υOH/ υH_2O	Amine υ(NH)	υ(C=O)	υ(C=N)	υ(M-N)
CASB	3420	3257	1711	1629	-
CSSB	3447	3104	-	1599	-
[Fe(CASB)Cl_2].3H_2O	3335	3182	1722	1592	776
[Cu(CSSB)Cl_2].2H_2O	3361	3167	1774	1629	765

CASB = chitosan-o-anisaldehyde Schiff base CSSB = chitosan-salicylaldehyde Schiff base

The FTIR of the chitosan (Figure 3) 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 2873 cm^{-1} ·Primary amines have two weak adsorption peaks at 1655 cm^{-1} and 1551cm^{-1}, corresponded to amine (1) and amine (11) respectively, which indicates that the prepared chitosan have high degree of deacetylation. Besides, the bands at 1115 cm^{-1} and 1071cm^{-1} are due to C-O stretching (1-4)-linked-β-D-glucosamine unit (Vadivel *et al*., 2015)

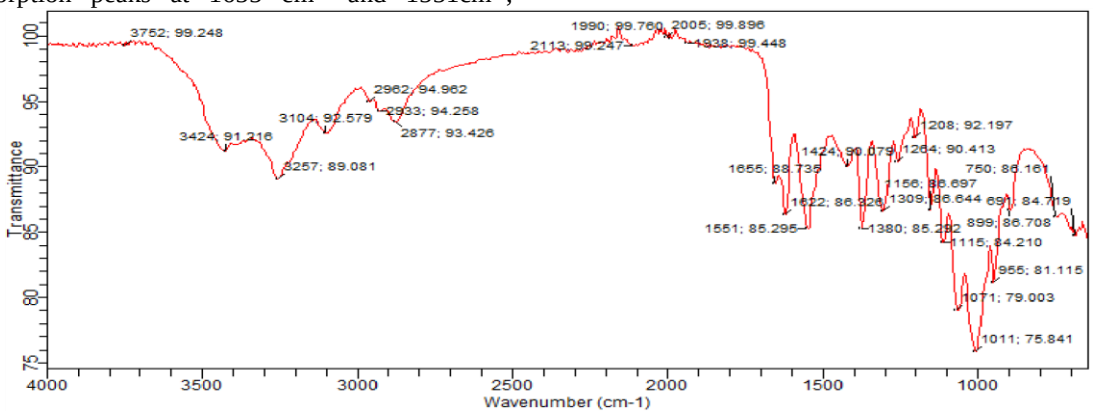


Figure 3: FTIR spectrum of chitosan

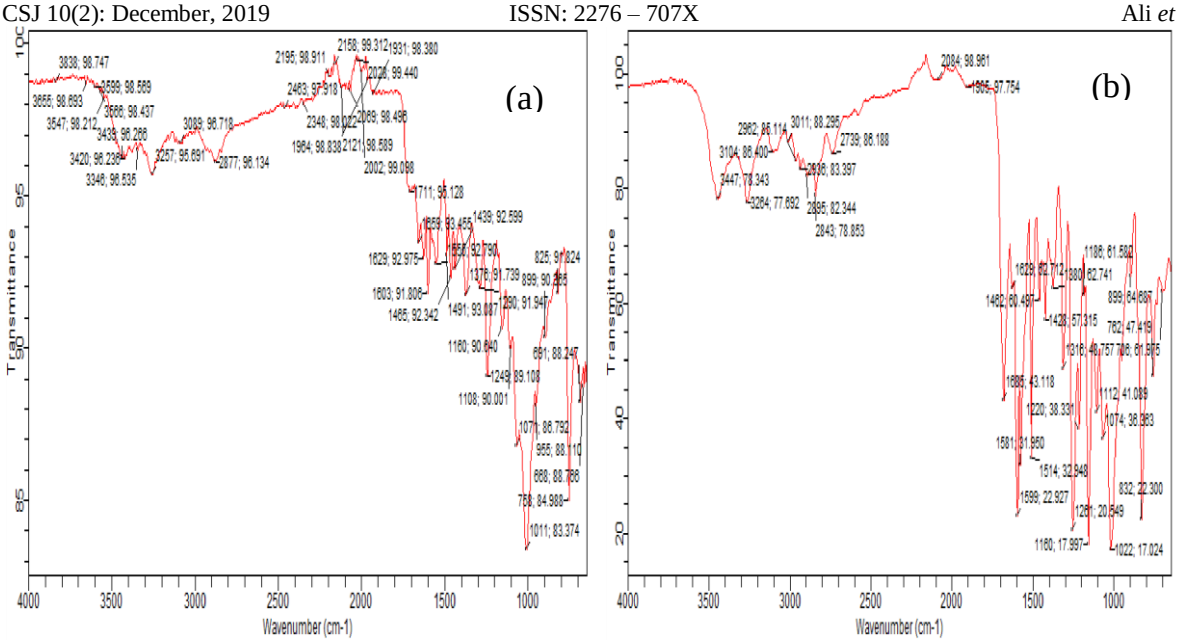


Figure 4: FTIR spectrum of Salicyladehyde Schiff base (a) and FTIR spectrum of Chitosan-*o*-anisaldehyde Schiff base (b)

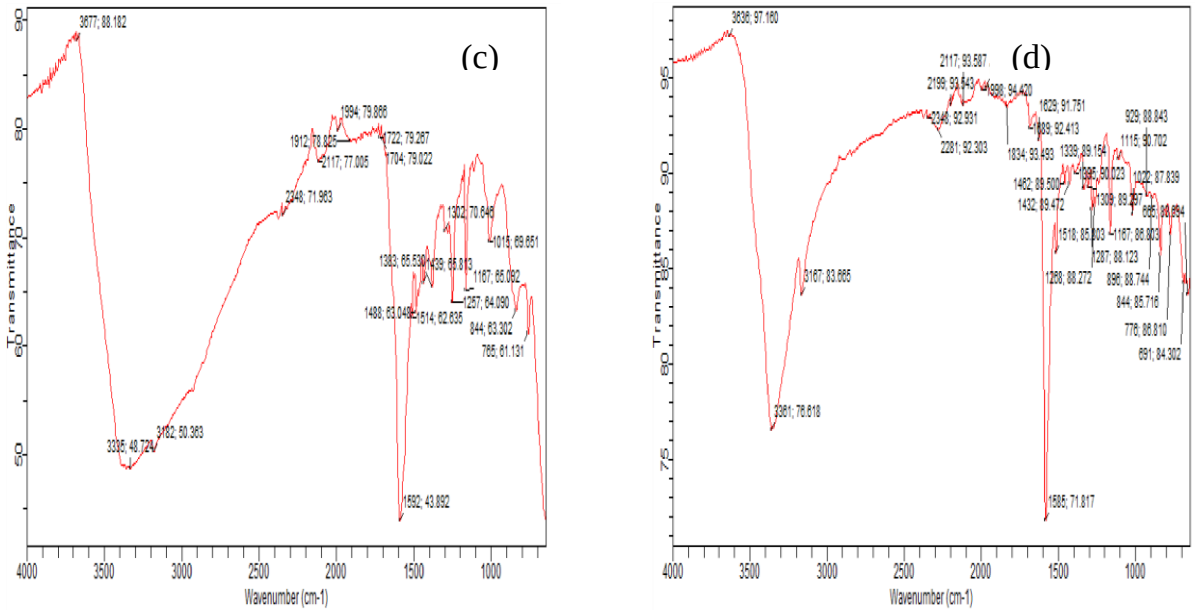


Figure 5: FTIR spectrum of salicyladehyde Schiff base copper (II) complex (C) and FTIR spectrum of chitosan -*o*-anisaldehyde iron (II) complex (d).

The FTIR spectrum of the chitosan Schiff bases (ligands) (Table 1) showed strong peaks at, 1626 and 1599 cm^{-1} which are characteristics stretching frequency of azomethine group (C=N).These bands are shifted to higher frequency such as 1592 cm^{-1}, 1626 cm^{-1} due to axomethine group complexed with metal ion. This indicated the co-ordination of metal centre with azomethine nitrogen group was successful (Thatte *et al*., 2014).

In the IR spectra of the metal complexes (Table 1) medium intensity weak bands appeared at 3335-3361 cm^{-1} and 3182-3167 cm^{-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 υ(C=O) at 1711-1722 cm^{-1} of the chitosan Schiff bases indicates the formation of co-ordinate bond between the metal ion and the phenolic oxygen atom via deprotonation. This is further confirmed by the increase in absorption frequency in the range region 1249-1268 cm^{-1} (Guibal *et al.*, 2014: Wakil *et al*., 2017).

The new bands of 776 cm^{-1} (Figure 5 (d)) and 765 cm^{-1} (Figure 5 (c)) appeared in the far infrared red region in the spectra of the complexes; suggest the formation of M-N (Sani and Dailami, 2015).

Physical Properties of the Synthesized Ligands and Metal (II) Complexes

The chitosan Schiff bases and their metal (II) complexes were prepared in good yields,

ranging from 59.55 -80.15%. The chitosan Schiff bases obtained (Table 1), have colours in while the metal (II) complexes exhibited deep green and reddish brown colours. 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 of the compounds (Table 2).

Table 2: Physical Properties of the Synthesized Ligands and Metal (II) Complexes

Compounds	Color obtained	B.M U_{eff}	Melting Temp (^{o}C)	Decomposition Temp (^{o}C)	Molar conductance Ohm $^{-1}$ cm^2 mol^{-1}	Percentage yields (%)
CASB	Light yellow		227	_	_	80.15
CSSB	Light yellow		243	_	_	71.32
[Fe(CASB)Cl_2].3H_2O	Deep green	1.83	_	293	15.12	60.02
[Cu(CSSB)Cl_2].2H_2O	Reddish brown	5.42	_	296	17.02	59.55

CASB = chitosan-o-anisaldehyde Schiff base CSSB = chitosan-salicylaldehyde Schiff base

Solubility Test

Solubility test revealed that the compounds were soluble in protic solvents such as methanol and ethanol, insoluble in distilled water, carbon tetrachloride, while slightly soluble in acetone and acetonitrile (Table 3)

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

Compounds	CCl_4	DMF	DMSO	EtOH	MeOH	$CHCl_3$	Acetone	Acetonitrile	H_2O
CASB	IS	S	S	SS	S	SS	SS	SS	IS
CSSB	IS	S	S	SS	S	IS	SS	SS	IS
[Fe(CASB)Cl_2].3H_2O	IS	S	S	S	S	S	SS	SS	IS
[Cu(CSSB)Cl_2].2H_2O	IS	S	S	SS	S	SS	SS	SS	IS

Keys: S = soluble SS = slightly soluble IS = insoluble
DMF = Dimethyl formamide DMSO = Dimethyl sulfoxide
CASB = chitosan -*o*- anisaldehyde Schiff base CSSB = chitosan-salicylaldehyde Schiff base
[Cu (CSSB)Cl_2].2H_2O = Chitosan salicyladehyde Schiff base copper (II) complex
[Fe (CASB)Cl_2].3H_2O = Chitosan -*o*-anisaldehyde Schiff base iron (II) complex

Table 4: Antibacterial activity of chitosan, chitosan Schiff bases and complexes

Samples	Test organisms	MIC (Concentrations(µg/ml))				MBC(µg/ml)
		500 (µg/ml)	250 (µg/ml)	125 (µg/ml)	62.5 (µg/ml)	
Chitosan	*E.coli*	-	-	-	+	125
	S. typhii	-	-	-	+	125
	S.aureus	-	-	-	+	125
CASB	*E.coli*	-	-	-	+	125
	S. typhii	-	-	+	+	>250
	S.aureus	-	-	+	+	>250
CSSB	*E.coli*	-	-	-	+	>125
	S. typhii	-	-	+	+	>250
	S.aureus	-	-	-	+	>125
[Fe(CASB)Cl_2].3H_2O	*E.coli*	-	-	-	-	MIC=MBC
	S. typhii	+	+	+	+	>500
	S.aureus	-	-	+	+	>250
[Cu(CSSB)Cl_2].2H_2O	*E.coli*	-	-	-	+	>125
	S. typhii	+	+	+	+	>500
	S.aureus	-	-	+	+	>250

CASB = chitosan -*o*- anisaldehyde Schiff base CSSB = chitosan-salicylaldehyde Schiff base
E. coli = Escheria coli *S. typhii = Salmonella typhii* *S. aureus = Staphylococcus aureus*

The antibacterial activities (Table 4) showed that the chemical modification of chitosan decreased its antibacterial activity where chitosan metal complexes had MIC/MBC ranging from 250 to 500µg/ml particularly $[Cu(CSSB)]Cl_2].2H_2O$ complex which exhibited similar MIC and MBC values. The chitosan-o-anisaldehyde Schiff base (CASB) demonstrated MIC and MBC values from 125 – 250 µg/ml against *E.coli* compared to the parent chitosan (125 to 62.5 µg/ml). All chitosan Schiff bases and their metal complexes were more active than the parent chitosan. The most active compound among the chitosan metal-complexes was $[Cu(CSSB)Cl_2].2H_2O$ complex while among chitosan Schiff bases was chitosan-o-anisaldehyde Schiff base (CASB).

Antifungal Activity

In contrasts to the results of the antibacterial assay, antifungal assay (Table 5), showed that the 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 62.5 µg/ml for the parent chitosan.

Table 5: Antifungal activity of chitosan, chitosan Schiff bases and their Metal Complexes

Samples	Test organisms	MIC (Concentrations(µg/ml)				MFC(µg/ml)
		500µg/ml	250µg/ml	125µg/ml	62.5µg/ml	
Chitosan	*A.flavus*	-	-	+	+	>250
	A. niger	-	-	-	+	125
	Tricodema spp	-	-	+	+	>250
CASB	*A.flavus*	-	-	-	-	MIC=MFC
	A.niger	-	-	+	+	>250
	Tricodema spp	-	-	-	-	MIC=MFC
CSSB	*A.flavus*	+	+	+	+	>500
	A.niger	-	-	-	+	125
	Tricodema spp	-	-	+	+	>250
$[Fe(CASB)Cl_2].3H_2O$	*A.flavus*	-	-	-	-	MIC=MFC
	A.niger	-	-	-	-	MIC=MFC
	Tricodema spp	+	+	+	+	>500
$[Cu(CSSB)Cl_2].2H_2O$	*A.flavus*	-	-	+	+	>250
	A. niger	-	-	+	+	>250
	Tricodema spp	+	+	+	+	>500

Keys:
A.niger = *Aspergillus niger* A.flavus = *Aspergillus flavus* -: no growth of organism
+: growth of organism CASB = chitosan-*o*-anisaldehyde Schiff base CSSB = chitosan-salicylaldehyde

The difference in effects observed in the antifungal and antibacterial analysis suggests that the tested chitosan metal complexes acted by different mechanism depending on the tested microorganisms, such that hydrophobic interactions seem to be more crucial activity against *E.coli*, but electrostatic interactions seem to be more important for *Salmonella typhii.* So also, the same in *Aspergillus niger* and *Tricodema spp* (Chikezie, 2017).

The enhancement in the antimicrobial activity may be rationalized on the basis that ligands mainly possess azomethine (C=N) bond. Moreover, in chitosan-metal complex, the positive charge of the metal ion is partially shared with the hetero donor atoms (N and O) present in the ligands and there may be pie electron delocalization over the whole chelating system.

Hence, the increase in the lipophilic character of the metal chelates favor its permeation through the lipoid layer of the bacterial membranes and blocking of the metal binding sites in the enzymes of micro-organisms (Nagesh *et al*., 2015).

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 were tested for their cytotoxicity effect against the brine shrimp nauplii and relate 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, 2017).

$$\%M = \frac{\text{Number of dead nauplii}}{\text{Number of total nauplii}} \times 100 \ldots (1)$$

Table 6: Brine shrimp lethality assay

Samples	Concentration (µg/mL)	Number of surviving Nauplii after 24 h			TTL of survivors	% Mortality	LC_{50} (µg/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%	
CASB	10	8	5	4	10	66%	36.297
	100	2	2	2	6	80%	
	1000	0	0	0	0	100%	
CSSB	10	5	8	7	20	33%	9.396
	100	4	4	4	12	60%	
	1000	1	0	0	1	*96%*	
$[Fe(CAB)Cl_2].3H_2O$	10	5	3	3	11	63%	82.533
	100	2	2	1	5	83%	
	1000	0	0	0	0	100%	
$[Cu(CSSB)Cl_2].2H_2O$	10	6	4	7	17	43%	26.460
	100	2	5	2	9	70%	
	1000	0	0	0	0	100%	

CASB = chitosan-o-anisaldehyde Schiff base CSSB = chitosan-salicylaldehyde Schiff base

The concentration of the samples investigated were 10µg/ml, 100µg/ml, 1000µg/ml and it was found that the 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 were found to be concentration-dependent. Chitosan has an LC_{50} of 4745.47 which is above 1000 and considered as non-toxic. Both the Schiff bases (CASB)-36.297, (CSSB)-9.396) and metal complexes of chitosan ($[Cu(CSSB)Cl_2].2H_2O$)-82.533, ($[Fe(CASB)Cl_2]3H_2O$)-26.460) has an LC_{50} value less than 1000 and considered as toxic (active). According to Meyers *et al*., (1982), an LC_{50} value of less than 1000 is toxic while LC_{50} value greater than 1000 is non-toxic. 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 complexed with the chitosan Schiff bases.

Scanning Electron Microscopic 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 Cu(II), Fe(II), ions as indicated by the FT-IR results (Sashikala and Shafi, 2017).

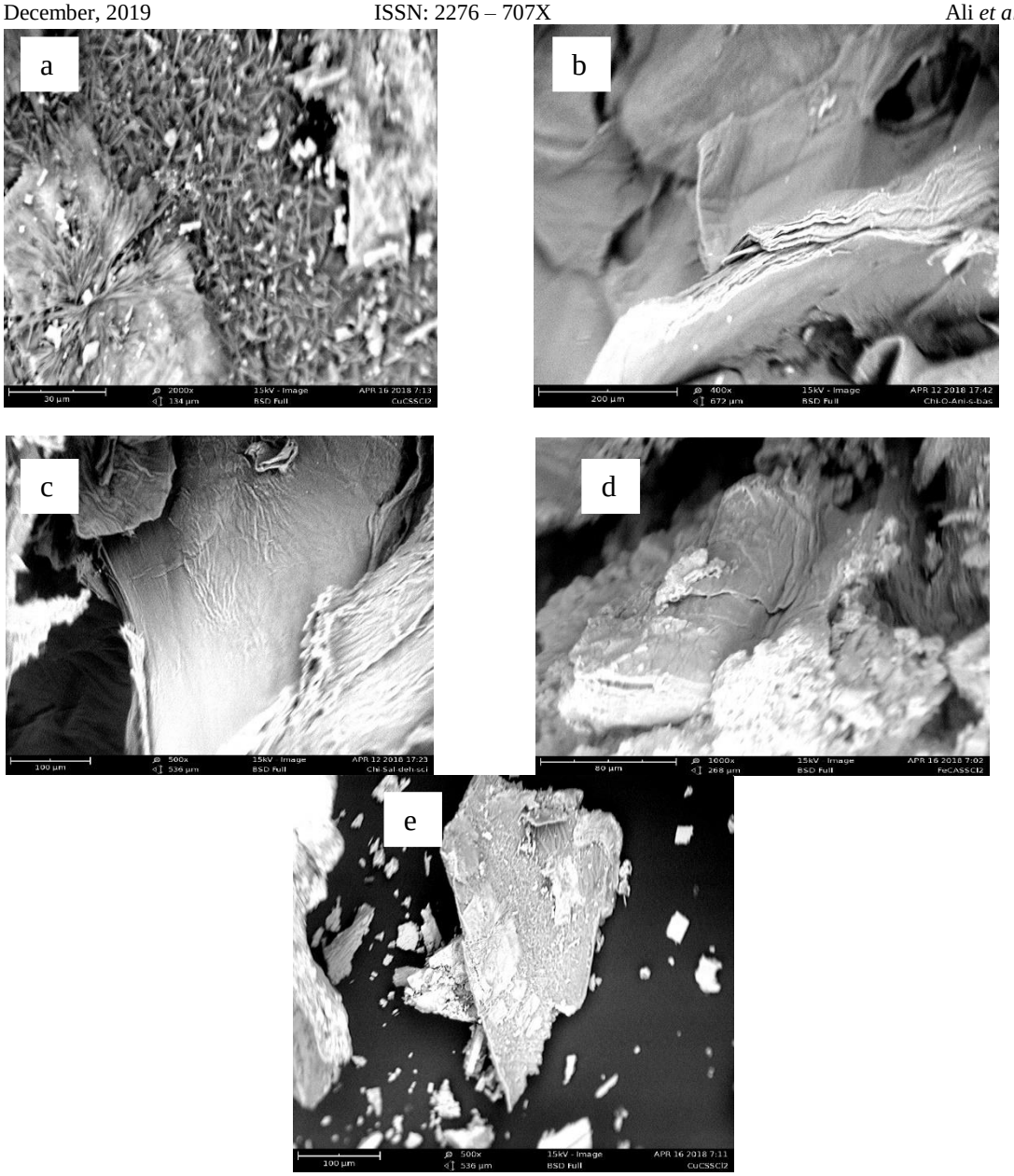


Figure 6: SEMs of chitosan (a), *o*-anisaldehyde Schiff base (b), salicyladehyde Schiff base (c) *o*-anidaldehyde Schiff base Fe(II) complex (d), salicyladehyde Schiff baseCu(II) complex (e)

CONCLUSION

The results of this investigation showed that Schiff bases and metal (II) complexes of chitosan from *Schistocerca gregaria* (Desert Grasshopper) were successfully synthesized. The use of the Schiff bases and the metal complexes as antimicrobial agents indicated great improvement in the antimicrobial activities of the chitosan and decreases its cytotoxicity. However, the outcome of the research reveals that the biopolymer chitosan could be used effectively as antimicrobial agent in basic medium

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