



# Synthesis, Characterization, Antimicrobial and Toxicological Study of Mn(II), Ni(II) Complexes of Tridentate Ligand Derived from Ethylenediamine, Chlorobenzaldehyde and Aminobenzophenone

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## Abstract

Two novel metal complexes of tridentate ligand derived from ethylenediamine, chlorobenzaldehyde and aminobenzophenone were synthesized and characterized by FT-IR, NMR, UV-Vis, Mass spectroscopy, Magnetic susceptibility measurement and Elemental analysis. The data obtained from the study shows that both N atoms of the Schiff base ligand and NH<sub>2</sub> group coordinated with metal(II) ions, indicating the tridentate nature of the ligand; tetrahedral geometry proposed for the complexes. The biological activity of the metal complexes and the ligand compared with the standard drugs (Amoxicillin and Nystatin) were screened against isolates of two g(+), *Staphylococcus aureus*, *Bacillus subtilis*, three g(-) *Escherichia coli*, *Klebsiella pneumonia*, *Shigella flexneri* bacteria and two fungi strains: *Candida albicans* and *Aspergillus fumigatus*. The obtained results indicated that both the metal complexes and the ligand showed good to moderate activity against the tested microorganism. Toxicology tests against the liver of the Wistar rats showed that the prepared compounds are not toxic compared with the standard drug (Amoxicillin). The levels of AST and ALT in the tested livers of the rats are within the permissible range.

**Keywords:** Metal complexes, ligand, antimicrobial, ethylenediamine, chlorobenzaldehyde

## Introduction

Schiff base - metal complexes have gained significant attention in recent time due to their stability, ease of modification and numerous biological properties [1:2] They are widely studied due to their selectivity and sensitivity towards the central metal atom coupled with their structural similarities with natural biological compounds and also due to the presence of azomethine group (HC=N) [3]. Research has established significant progress in the application of transition metal complexes as drugs. The potency of some therapeutical agents has been reported to have increased upon coordination to transition metals [4:6]. It has been found that metals such as copper, nickel, cobalt, manganese and iron bound to a ligand containing oxygen, nitrogen and sulphur showed enhanced antimalarial, antimicrobial, antifungal and anticancer activity [7].



## Material and Methods

All reagents are purchased from Sigma-Aldrich, Germany and used without further purification. IR spectra (4000-400  $\text{cm}^{-1}$ ) using KBr discs were recorded with a Varian 660 MidIR Dual /MCT /DTGS bundle with ATR. Electronic spectra were recorded using Spectrum lab 7525 spectrophotometer in the 0 –400, 400-900 range for ligand and complexes. The magnetic measurements were carried out at room temperature on Gouy balance at room temperature using Swissmake-H-1640 with maximum capacity 80g and precision  $\pm 0.01\text{mg}$ . The Mass spectra of the ligand and its metal complexes were recorded using GC – MSQP - 2010 (Shimazu). The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of the Schiff base ligand were recorded in deuterated DMSO (Internal standard TMS) on Bruker spectrometer. Melting points were recorded on a gallenkamp apparatus and are uncorrected. Elemental analysis was determined using Perkin Elmer, USA 2400 Series II. Conductivity measurements were recorded at (R.T) $^\circ\text{C}$  for solution of samples in DMSO/ water. The organisms used were obtained from the Department of Microbiology, Federal Medical Centre, Owo. The Wister rats used were collected from the Central Laboratory, Federal University of Technology, Akure.

### Preparation of Ligand (L) and Metal complexes

#### Synthesis of Schiff Base Ligand (L)

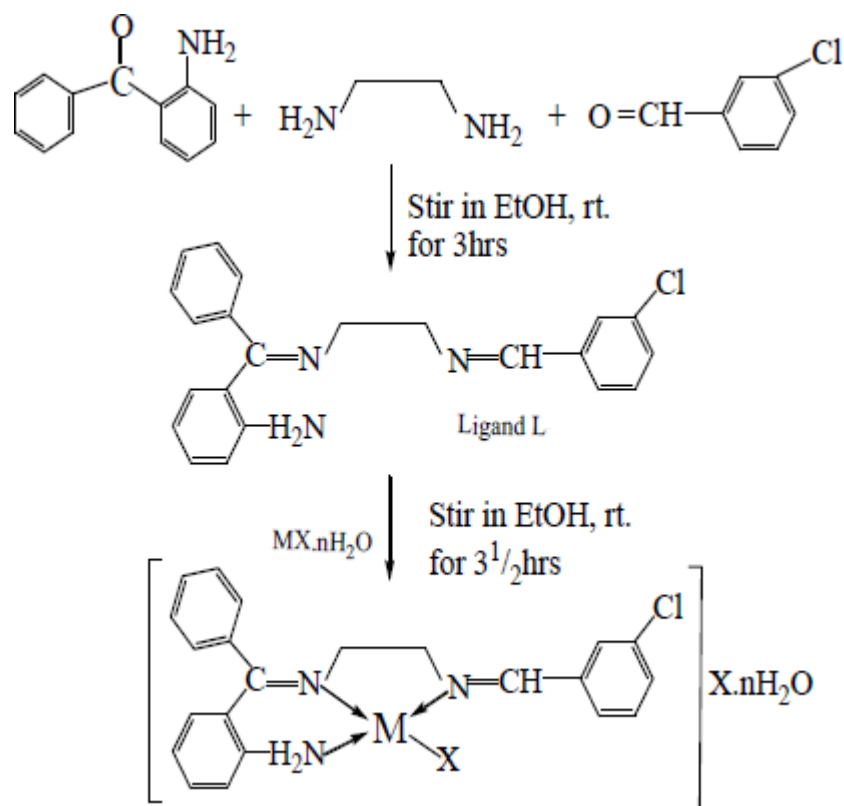
The ligand (L) (Figure 1) was prepared according to the literature [8] with slight modification. Ethylenediamine (0.008mol, 0.5g) in 20ml ethanol was gently added to ethanol solution (20ml) containing 3-chlorobenzaldehyde (0.007 mol, 1.17g), followed by slow addition of 2- aminobenzophenone (0.014mol,1.64g) dissolved in 20ml ethanol. The mixture was stirred for 3hrs. The yellow coloured precipitate formed was separated by filtration and purified by recrystallization and dried overnight in air.

#### Synthesis of Ni(II) and Mn(II) Complexes

The complexes were prepared by addition of (0.0013mol, 0.34g)  $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ ; (0.00 13mol, 0.22g)  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  dissolved in 20ml absolute ethanol in a round bottom flask and added to ethanolic solution (0.00 262mol, 0.4g) of N, N<sub>1</sub>-bis(3-chlorobenzylidene)ethylenediamine ligand (L) in molar ratio of (1:1). The colour change was observed immediately. The resulting mixture was reflux for 3½ hours. The precipitate formed was filtered, washed with ethanol and dried overnight in a desiccator.

#### Antimicrobial Study

The bacterial cultures of three Gram-negative (*Escherichia coli*, *Klebsiella pneumonia* and *Shigella flexneri*), two Gram positive (*Staphylococcus aureus* and *Bacillus substilis*) while the fungal cultures of *Candida albicans* and *Aspergillus fumigatus* were maintained on nutrient broth while the fungal cultures were maintained on Sabouraud liquid medium. Inoculum size containing 10cfu/ml for bacterial and 10sfu/ml for fungal were used to seed already solidified petri plates of Mueller-hinton agar. The antimicrobial activities of the ligand and its complexes were determined using agar well diffusion method. A sterile 6mm cork borer was used to make well on already solidified agar. The wells were filled with the prepared compounds ensuring that there was no spill on the agar surface surrounding the well. The plates were allowed to stand for about 2 hours to allow absorption of the synthesized compounds into the medium after which bacterial were incubated for 24 hours at 37 $^\circ\text{C}$  and fungi 25 $^\circ\text{C}$  7 days.



Where M = Ni (II), Mn (II); X = SO<sub>4</sub>, H<sub>2</sub>O, n = 1, 5

Figure 1: Synthesis of Schiff base N-(3-chlorobenzylidene)-N'-(2-aminobenzophenylidene) Ethylenediamine Ligand (L) and its metal (II) complexes

### Toxicology Study

The toxicology activity of the ligand and its complexes are carried out following [9] method. The level of safety of the ligand and its complexes was compared with standard antibiotic drug, Amoxicillin. Fifteen Wistar rats of average weight 150g were used. They were weighed and group into five, fed for one month; three weeks to stabilize them with normal food and one week with food dosed with the prepared compounds. The level of safety of the ligand and complexes were compared with Amoxicillin and negative control, DMSO. The tested rats were grouped as follows:

Group 1: Negative control (DMSO)

Group 2: Ligand (L)

Group 3: MnL

Group 4: NiL

Group 5: Positive control (Amoxicillin)

They were administered orally two times daily for 7 days at a dose level of 5mg/kg body. Enzyme activity (ALT and AST) in the liver were determined as described by [10:11].

## Results and Discussion

The chemical equation showing the preparation of the ligand and the metal (II) complexes are presented in Figure 1. The Schiff base ligand is yellow in colour and soluble in butanol. The metal complexes are coloured solid which are stable in air, soluble in ethanol and water respectively. Disparity in the melting point of the products (metal complexes) from the ligand as shown in Table 1 indicate the formation of a coordination compound. The purity of the prepared ligand and complexes can be confirmed from the sharp melting point. Molar conductivity data Table 4 for the complexes in aqueous solution at room temperature were observed to be  $46 \text{ ohm}^{-1}\text{cm}^2\text{mol}^{-2}$  and  $124 \text{ ohm}^{-1}\text{cm}^2\text{mol}^{-1}$  for Ni (II) and Mn (II) complexes. These values suggested 1:1 electrolyte for Mn (II) and non electrolytic nature for Ni (II) complexes.

### FTIR Spectra

The IR band assignments are given in Table 2. The IR spectra of metal (II) complexes displays the ligand characteristic bands with appropriate shifts due to the complex formation. The IR band at  $1639\text{cm}^{-1}$  of the free Schiff base ligand is due to the presence of azomethine group. Upon complexation, this band shifts to lower frequencies  $1600$  and  $1601\text{cm}^{-1}$  indicating a stronger double bond character of the imine band and a coordination of azomethine nitrogen atom to the metal (II) ions [12]. The coordination of M - L is further supported by the appearance of medium intensity bands at  $518$  and  $539\text{cm}^{-1}$  assigned to  $\nu(\text{M} - \text{N})$  vibration. The band in the region  $3420\text{cm}^{-1}$  in the spectrum of the ligand which can be attributed to stretching frequency of primary amine ( $\text{NH}_2$ ) shifted to higher frequencies in the spectra of the complexes, indicating the involvement of  $\text{NH}_2$  in coordination to metal ions [13:14]. This observation was further confirmed by the absorption band at  $688\text{cm}^{-1}$  in the spectrum of the ligand, due to  $\text{NH}_2$  deformation shifted to lower frequencies  $619$  and  $618\text{cm}^{-1}$  after coordination to metal ions through nitrogen atom [15]. The new bands  $3624$  and  $3624\text{cm}^{-1}$  in the spectra of metal complexes are assignable to  $\nu(\text{H}_2\text{O})$  of lattice water molecules.

### NMR Spectral Analysis

The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra and spectroscopic assignments of the ligand are shown in Figure 2 and Table 3 respectively. The characteristic resonance signals at  $\delta$  8.5ppm and 6.5 - 7.9ppm attributed to  $\text{CH}=\text{N}$  proton showing formation of Schiff base ligand and aromatic benzylidene ring [16]. The signals at  $\delta$  4.0 and  $\delta$  2.8ppm were due to methylene and N-H proton respectively. In  $^{13}\text{C}$  NMR spectrum, signals at  $\delta$  114.59 - 140.01ppm region are assigned to aromatic carbons of benzylidene ring. The recorded signal at  $\delta$  164.00ppm is assigned to azomethine carbon atom [17] which confirmed the formation of Schiff base ligand.

**Table 1:** Physical and analytical data of Schiff base ligand and its complexes

Compound	M. wt. gmol <sup>-1</sup>	M.P (°C)	Colour	Yield (%)	Solubility	Elemental Analysis Calc. (found) %			
						C	H	N	M
L : C <sub>22</sub> H <sub>20</sub> N <sub>3</sub> Cl	362	220	Yellow	49	Buthanol	73.03 (73.12)	5.53 (5.51)	11.61 (11.68)	—
[NiL(H <sub>2</sub> O)]SO <sub>4</sub> .5H <sub>2</sub> O	625	320	Grey	69	Elthanol	42.24 (41.82)	5.12 (5.31)	6.72 (6.56)	9.44 (9.51)
[MnL(SO <sub>4</sub> )].H <sub>2</sub> O	531	290	Pink	72	Water	49.71 (49.86)	4.14 (4.12)	7.91 (7.87)	10.36 (10.43)

**Table 2:** FTIR Spectra bands of Schiff base and its metal complexes

Compound	ν(C=N)	ν(NH <sub>2</sub> )	ν(M – N)	ν(H <sub>2</sub> O)	δ(H <sub>2</sub> O)	δ(NH <sub>2</sub> )
				(Hydrated)	(coordinated)	
L : C <sub>22</sub> H <sub>20</sub> N <sub>3</sub> Cl	1639	3420	—	—	—	688
[NiL(H <sub>2</sub> O)]SO <sub>4</sub> .5H <sub>2</sub> O	1600	3574	518	3624	926	619
[MnL(SO <sub>4</sub> )].H <sub>2</sub> O	1601	3574	539	3624	—	618

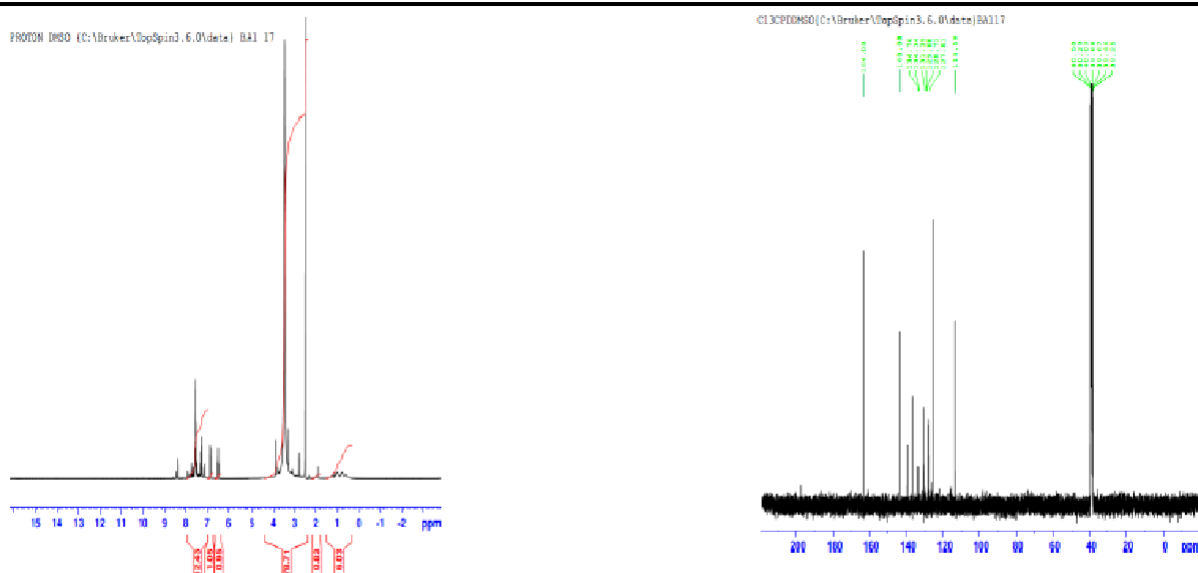


Figure 2: (i) <sup>1</sup>H NMR Spectrum of ligand (L)

(ii) <sup>13</sup>C NMR Spectrum of ligand (L)

Table 3: Spectroscopic data of ligand (L)

Compound	<sup>1</sup> H NMR, $\delta$ (ppm)	<sup>13</sup> C NMR $\delta$ (ppm)
L: C <sub>22</sub> H <sub>20</sub> N <sub>3</sub> Cl	8.5 (HC = N)	164.00 (HC = N)
	4.0 and 2.8 (N – H)	114.59 – 140.01 (Ar – C)
	6.7 – 7.9 (Ar – H)	

### Electronic Spectra and Magnetic Studies

The results of electronic spectra of the ligand and metal complexes in water at room temperature are presented in Table 4. The ligand showed bands in the region 318, 340 and 365nm. The band appearing at lower energy is

attributed to  $n \rightarrow \pi^*$  transition of conjugation between the lone pair of electron of p orbital of N atom in azomethine

group and conjugation  $\pi$  bond of the benzene ring [18]. The bands appearing at higher energy are attributed to  $\pi \rightarrow$

$\pi^*$  of the benzene ring and LMCT [19:20]. The UV-visible absorption spectra of the metal (II)

Schiff base complexes showed new bands due to d - d transition. The bands 370 and 510nm were observed in Ni(II) complex assigned to LMCT,  $^3T_1(F) \rightarrow ^3T_2(g)$ . This transition is characteristics of tetrahedral Ni(II) complex. Ni(II) exhibits magnetic moment of 4.47BM which was higher than the spin only magnetic moment of 2.83BM. The reason for higher magnetic moment than expected was due to orbital contribution of  $^3F$  ground term of Ni(II) free ion [21]. Mn(II) complex showed absorption bands at 250, 280 and 400nm. The bands were assigned to  $n \rightarrow \pi^*$ ,  $\pi \rightarrow \pi^*$ ,  $^6A_1 \rightarrow ^4T_1$  transitions. The complex exhibits magnetic moment of 5.87BM, which is in agreement with tetrahedral range of 5.9 - 6.2BM, hence the proposed tetrahedral geometry [22].

**Table 4:** Electronics Spectral of Ligand (L) and its Complexes

Compound	Electronic Transition $\lambda_{\max}(\text{nm, DMF})$	Band Assignment	Configuration	$U_{\text{eff}}$ (B.M)	Molar Cond. ( $\Omega^{-1}\text{Mol}^{-1}\text{cm}^2$ )	Geometry
L: $\text{C}_{22}\text{H}_{20}\text{ClN}_3$	318, 340, 365	$n \rightarrow \pi^*, \pi \rightarrow \pi^*$ , LMCT	—	—	—	—
$[\text{NiL}(\text{H}_2\text{O})]\text{SO}_4 \cdot 5\text{H}_2\text{O}$	370, 510	LMCT, ${}^3\text{T}_1(\text{F}) \rightarrow {}^3\text{T}_2$	$d^8$ (h.s) ( $e^4 t_2^4$ )	4.47	46	T.h
$[\text{MnL}(\text{SO}_4)] \cdot \text{H}_2\text{O}$	250, 280, 400	$n \rightarrow \pi^*$ $\pi \rightarrow \pi^*$ ${}^6\text{A}_1 \rightarrow {}^4\text{T}_1$	$d^5$ (h.s) ( $e^2 t_2^3$ )	5.87	124	T.h

### Mass Spectra

The mass spectra of the ligand and its metal(II) complexes are shown in Figure 3. The recorded mass spectra with molecular ion peaks have been used to confirm the proposed structures shown in Figure 4. The ligand and its complexes produced molecular ions at ( $m/z$  361.83) for ligand, while ( $m/z$  626) and ( $m/z$  531.42) for Ni and Mn respectively. The ligand fragmented to give other peaks at 322, 226.03 and 132.68 corresponding to  $\text{M} - [2\text{CH} + \text{CH}_2]^+$ ,  $\text{M} - [\text{C}_6\text{H}_5\text{NH}_2 + \text{HCN} + 2\text{H}]^+$  and  $\text{M} - [\text{C}_6\text{H}_4\text{Cl} + \text{C}_6\text{H}_5 + \text{HCN} + \text{CH}_2]^+$ . The fragmentation of the complexes produced other relevant peaks at 622.18, 534.94 and 515.36 for nickel complex corresponding to  $\text{M} - [3\text{H}]^+$ ,  $\text{M} - [\text{C}_6\text{H}_5 + \text{CH}]^+$  and  $\text{M} - [\text{C}_6\text{H}_5 + \text{H}_2\text{O} + \text{CH}_2 + \text{H}]^+$ . Manganese complex fragmented to produce peaks at 527.39, 490.38 and 469.74 indicating  $\text{M} - [4\text{H}]^+$ ,  $\text{M} - [\text{HCN} + \text{CH}_2]^+$  and  $\text{M} - [\text{Cl} + 2\text{CH}]^+$  respectively.

### Antimicrobial Activity

The antimicrobial activity of the prepared compounds were tested against five bacterial strains (two Gram-positive and three Gram-negative) and two fungi as show in Table 5-7 respectively. Synthesized metal (II) complexes and the ligand were screened against (*Escherichia coli*, *Klebsiella pneumonia* and *Shigella flexneri*); two Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*), the inhibitory results are tabulated in Table 5. The effect of the tested compounds on Gram-negative bacterial showed that the metal complex of Ni(II) was found to be more effective than Mn(II) and the free ligand.

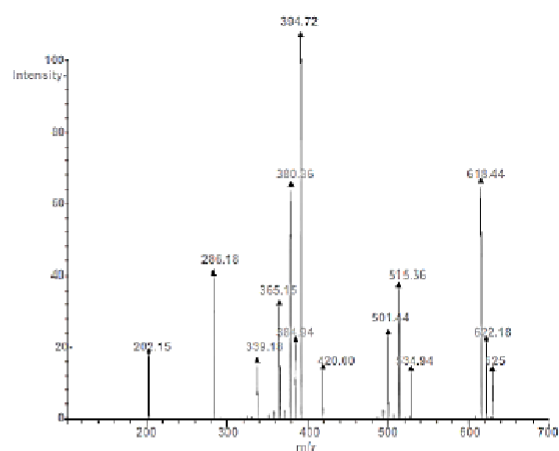
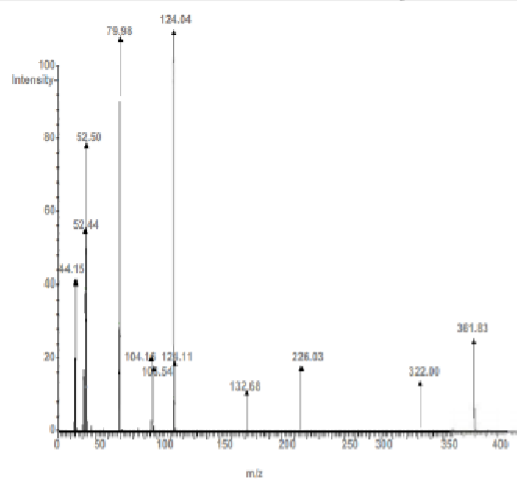
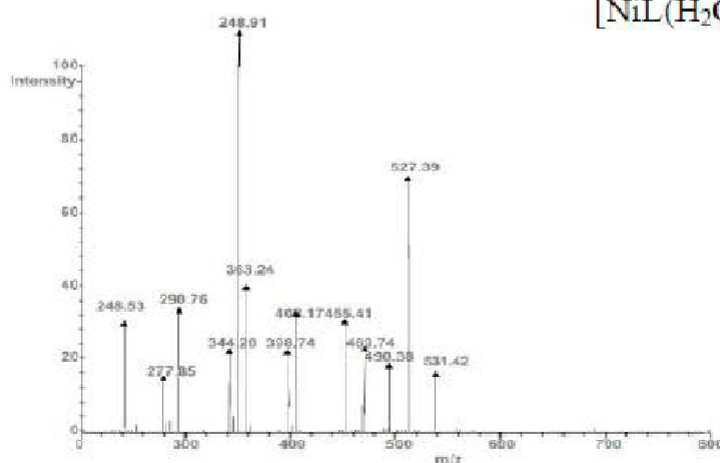


Figure 3: (i) Mass spectrum of Ligand (L):

(ii) Mass spectrum of



(iii) Mass spectrum of  $[MnL(SO_4)] \cdot H_2O$

This enhanced bacterial activity is due to chelation which makes the Schiff base complexes to act as more powerful and potent bacteriostatic agents [23:25] thus inhibiting the growth of bacteria more than the free Schiff base. The activity of the free ligand is higher than Mn(II) complex. For Gram-positive bacteria, the ligand possessed good antibacterial activity than the complexes, this is contrary to what is usually reported in the literatures. This activity of Mn(II) is greater than Ni(II) complex on *staphylococcus aureus* while both complexes have the same activity on *Bacillus subtilis*. Both the ligand and the complexes were potent against the tested fungi as shown in Table 6. Mn(II) produces least activity against *Aspergillus fumigatus*.

Table 7 shows the MIC of the ligand and metal complexes. It can be seen from the results that all the ligand and Mn(II) produced similar bactericidal effect at 50 and 25(mg/mL) on all the tested microorganism except *Escherichia coli* and *Shigella flexneri*. Ni(II) displays bacteriocidal effects at 50 and 25(mg/mL) for all the tested organisms.

### Liver Function Indices

The effect of oral administration of the ligand and its complexes on the enzymes activity (ALT and AST) in the rat are shown in Table 7. It can be observed from the Table that the concentration of ALT in the liver of the rats administered with ligand is lower when compared with those administered with metal complexes and the standard drug (Amoxicillin). Similarly, the concentration of AST in the liver of rats administered with ligand is



lower when compared with manganese complex of the ligand and the standard drug (Amoxicillin). It can be seen from the results that the standard drug, Amoxicillin possesses higher level of ALT and AST (18.00 – 17.00 IU/L). This observation shows that the drug possesses higher level of toxicity when compared with all the tested compounds. In general, all the tested compounds are within the control range which means they can be tolerated in the body.

**Table 5:** Antimicrobial activity of ligand (L) and its metal complexes at 150mg/mL

Compound	Zone of inhibition (mm)				
	Gram - negative			Gram - positive	
	<i>Escherichia coli</i>	<i>Klebsiella pneumonia</i>	<i>Shigella flexneri</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
L: C <sub>22</sub> H <sub>20</sub> ClN <sub>3</sub>	17.67 ± 0.58	21.00 ± 2.00	19.33 ± 3.51	24.00 ± 1.00	21.00 ± 1.00
[NiL(H <sub>2</sub> O)]SO <sub>4</sub> .5H <sub>2</sub> O	24.00 ± 2.00	21.33 ± 0.57	19.67 ± 0.58	21.00 ± 2.00	20.00 ± 1.00
[MnL(SO <sub>4</sub> ).H <sub>2</sub> O	15.33 ± 2.51	13.33 ± 1.53	13.33 ± 1.53	25.00 ± 1.00	20.33 ± 0.58
Amoxicillin	33.33 ± 0.58	29.00 ± 1.00	29.00 ± 1.00	31.67 ± 3.511	33.33 ± 3.06

Values are mean ± standard deviation of three replicates.

**Table 6:** Antifungal activity of ligand (L) and its metal complexes at 150mg/mL

Compound	Zone of inhibition (mm)	
	<i>Candida albicans</i>	<i>Aspergillus fumigatus</i>
L: C <sub>22</sub> H <sub>20</sub> ClN <sub>3</sub>	26.00 ± 1.00	26.33 ± 0.578
[NiL(H <sub>2</sub> O)]SO <sub>4</sub> .5H <sub>2</sub> O	24.00 ± 1.00	26.00 ± 1.00
[MnL(SO <sub>4</sub> ).H <sub>2</sub> O	26.00 ± 3.00	20.00 ± 3.00
Nystatin	32.33 ± 7.23	34.00 ± 2.00

Values are mean ± standard deviation of three replicates.

**Table 7:** Minimum inhibitory concentration (MIC) of the ligand and its complexes

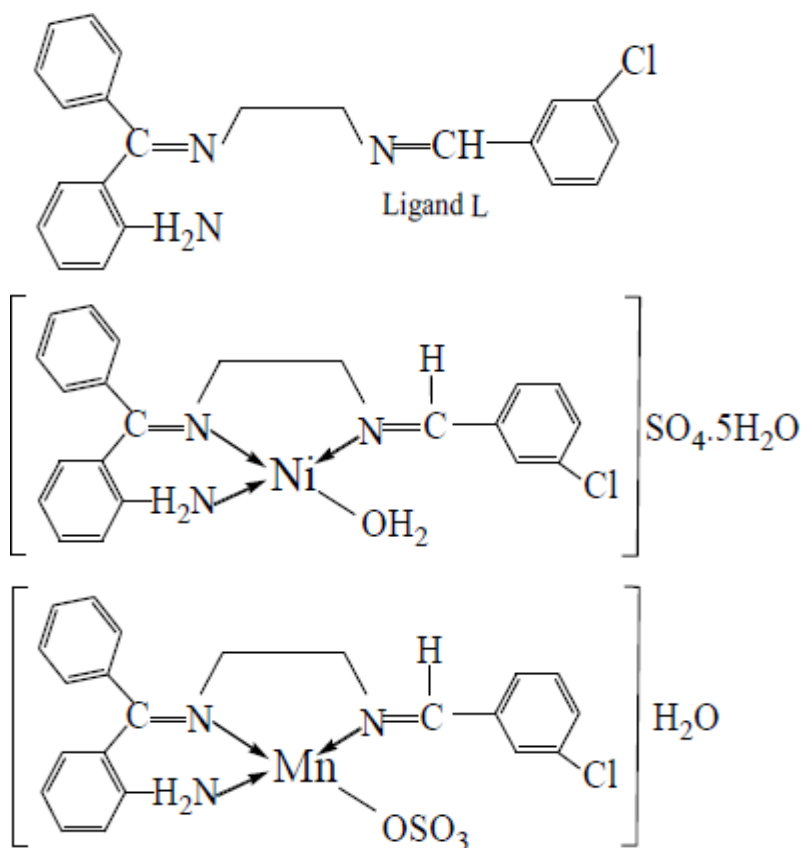
Compounds/ Concentration (mg/mL)	Organisms						
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumonia</i>	<i>Shigella flexneri</i>	<i>Candida albicans</i>	<i>Aspergillus fumigatus</i>
<b>C<sub>22</sub>H<sub>20</sub>ClN<sub>3</sub></b>							
50	+	+	+	+	+	+	+
25	+	+	-	+	-	+	+
12.5	-	-	-	-	-	-	-
6.5	-	-	-	-	-	-	-
3.2	-	-	-	-	-	-	-
<b>[NiL(H<sub>2</sub>O)]SO<sub>4</sub>.5H<sub>2</sub>O</b>							
50	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+
12.5	-	-	-	-	-	-	-
6.5	-	-	-	-	-	-	-
3.2	-	-	-	-	-	-	-
<b>[MnL(SO<sub>4</sub>)].H<sub>2</sub>O</b>							
50	+	+	+	+	+	+	+
25	+	+	-	+	-	+	+
12.5	-	-	-	-	-	-	-
6.5	-	-	-	-	-	-	-
3.2	-	-	-	-	-	-	-
<b>Amoxicillin</b>							
50	+	+	+	+	+		
25	+	+	+	+	+		
12.5	+	+	+	+	+		
6.5	+	+	+	-	-		
3.2	-	-	-	-	-		
<b>Nystatin</b>							
50						+	+
25						+	+
12.5						+	+
6.5						+	+
3.2						+	-
<b>30% DMSO</b>							
50	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-
12.5	-	-	-	-	-	-	-
6.5	-	-	-	-	-	-	-
3.2	-	-	-	-	-	-	-

Key: + : Clear (no growth); - : Turbid (there is growth)

**Table 8:** Effects of ligands and their metal complexes on selected liver function indices in Wistar rat.

Compounds	Enzyme (ALT) Conc. (IU/L)	Enzyme (AST) Conc. (IU/L)
L:C <sub>22</sub> H <sub>20</sub> ClN <sub>3</sub>	8.00 ± 0.00	9.00 ± 0.00
[NiL(H <sub>2</sub> O)]SO <sub>4</sub> .5H <sub>2</sub> O	11.00 ± 0.00	8.66 ± 0.57
[MnL(SO <sub>4</sub> )].H <sub>2</sub> O	16.33 ± 0.58	14.33 ± 0.58
Amoxicillin	18.00 ± 0.00	17.00 ± 0.00
DMSO	11.00 ± 0.00	13.00 ± 0.00

Normal range of ALT and AST: 0 - 40 IU



**Figure 4:** Proposed Structures for Ligand (L) and its Metal complexes

## Conclusion

This study shows justification for synthesis of novel Schiff base and its complexes using ethylenediamine, chlorobenzaldehyde and aminobenzophenone. The ligand acts as tridentate coordinating via N atoms of azomethine groups and NH<sub>2</sub> to form stable complexes with proposed tetrahedral geometry for Mn(II) and Ni(II) respectively. The ligand and the complexes produced good activity against the tested bacteria. All the synthesized compounds were potent against the tested fungi, hence promising candidates for development of new active antifungal agents. The ligand and its compounds produced bacteriocidal activity at higher inhibitory concentration. Toxicological results indicated that the prepared compounds are safe to be developed as antimicrobial agents.

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