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Synthesis, Characterization and Antibacterial Activity of Copper (II) Complex with a Schiff Base Derived From Acetylacetone and Aniline as Ligand

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***Abstract*— The Schiff base (L1) and its copper(II) complex (Cu(L1)2) were synthesized with the percentage yield of 86.96% and 70.65% respectively and characterized by solubility, molar conductivity melting point, UV-Visible spectrometry, FTIR Spectroscopy and Thin Layer Chromatography (TLC). Agar well diffusion method was adopted for the susceptibility testing. The physical characterization of the synthesized ligand L1 and its copper complex Cu(L1)2 indicates that, both Cu(L1)2 and L1 are non-electrolytic compounds with molar conductivity of 0.00 µS. Cu(L1)2 and L1 are stable to heat with melting point of 2280C and 1880C respectively. Both Cu(L1)2 and L1 are crystalline and possess black and yellow colour respectively. The complex (Cu(L1)2) is insoluble in water and in polar organic solvent but soluble in non-polar solvent (chloroform) while the ligand L1 is insoluble in water but soluble in polar organic solvent (methanol, ethanol, petroleum ether etc). UV-visible spectroscopy of the complex (Cu(L1)2) and ligand (L1) shows a λmax(nm) of 203 and 2010 respectively. FTIR spectroscopy indicate the present of νM-O, ν(M-N) and ν(-HC=N , νC=O, νC=C, CH3 str. and νC-H) bonds in the synthesized complex and the absence of νM-O, and ν(M-N) bonds in the ligand. The metals ligands bonds ν(M-O) and ν(M-N) indicate the formation of a complex. The carbon-nitrogen double bond ν(-C=N) indicate the formation of the Schiff base. Gentamicin (10 µg/disc) was used as control. Copper(II) Complex of the Schiff base was found to posses significant antimicrobial activity on the tested microorganisms (Pseudomonas aeruginosa, Klebsiella pneumonia, Bacillus aerius and E.coli. The ligand and the solvent (DMSO) indicate non antimicrobial property when subjected to same organisms.**

***Index Terms*— Antimicrobial resistance, Copper (II).**

I. INTRODUCTION

Antimicrobial resistance is the ability of a microorganism to stop an antimicrobial from working against it. As a result, standard treatments become ineffective, infections persist and may spread to others. This phenomenon causes millions of death worldwide and it is a major health concern nowadays.

According to Cleiton., *et al* (2010) Schiff bases are the compounds containing azomethine group (-HC=N-). They are condensation products of ketones or aldehydes with primary amines and were first reported by Hugo Schiff in 1864. Nowadays, Schiff bases are used as intermediates for the synthesis of amino acids or as ligands for preparation of metal complexes having a series of different structures. Schiff bases are some of the most widely used organic compounds.

According to Savithri, *et al*., (2018), metal complexes have biological activity in many mammalian cell systems. Metal complexes with Schiff bases derived from substituted aldehydes and heterocyclic compounds containing nitrogen, sulfur, and/or oxygen as ligand atoms are of interest because they were found to have effective biological activities in biological systems. Therefore, much attention has been given on the synthesis of Schiff base metal complexes. Copper is an important biocompatible metal ion found in the living system which may be less toxic than the other nonessential metal ions. Cu(II) complexes are preferred candidates for various pharmacostudies due to the presence of its bio relevant ligands which can bind and cleave deoxyribonucleic acid (DNA). The biological activities of Cu(II) complexes has been subjected to intense investigation for DNA binding and cleavage activities for chemotherapeutics and highly sensitive diagnostic agents. The structural and donor atoms of the complex may affect significant factors such as the lipophilic or hydrophilic nature of the complex, the oxidation state of the copper center, and the biological reactivity of the complexes. Cobalt use in the regulation of DNA synthesis indirectly and in cobalt-dependent proteins which make it an essential biological element. Cobalt(III) complexes have been studied in coordination chemistry and biochemistry due to their therapeutic activities. Copper and cobalt are essential metals in all living systems which show antifungal and antibacterial properties against several pathogenic fungi and bacteria depending on the reaction with the DNA system. The binding of fluorescent polycyclic molecules to DNA can be conveniently investigated by many techniques, including absorption and fluorescence spectroscopic methods due to their absorption and emission properties significantly change on complex formation.

II. EXPERIMENTAL

A. *Materials and instrumentation*

All chemicals and reagent used were of analytical grade obtained from chemistry and biochemistry laboratory of the Gombe State University and used without further purification.

IR spectra were recorded on Perking Elmer FTIR Spectrophotometer (4000– 400 cm^{-1}) in KBr pellets. UV–vis, spectra were determined in the DMSO and chloroform solvent for the lidand and the complex respectively with concentration (1.0×10-3 M) using CE7400 AQUARIUM

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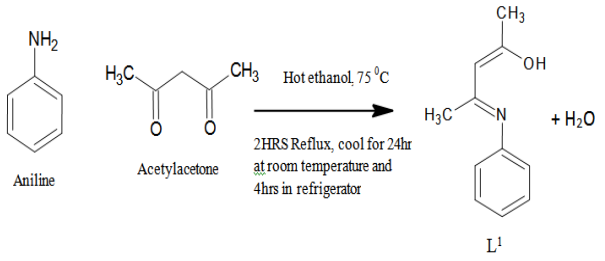
Spectrophotometer with 1cm quartz cell, in the range 100–800 nm The purity of the ligand L^1 was determine using Thin Layer Chromatography.(TLC).

B. Synthesis of L^1

Procedure

Ethanol (10 ml) was added to 20 ml of acetyl acetone, 20 ml of aniline was added to 10 ml of ethanol, and the two mixtures was reflux for 2hrs at 75 ^{0}C, mixed together and allowed to cool for 24hrs at room temperature. 60 ml of distilled water was added to the resulting mixture and cooled in a refrigerator for 4hrs (Scheme 1). As reported by Adnan, (2013) with some modifications.



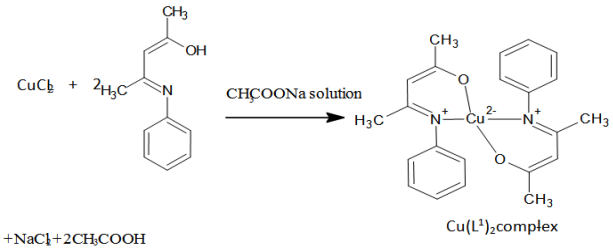
Scheme 1: Synthesis of L^1

The purity of the Schiff base was ascertained using Thin Layer Chromatography (TLC). The TLC of the starting material (aniline and acetyl acetone) together with the Schiff base was conducted with ethanol as a mobile phase. Aniline, acetyl acetone and the synthesized Schiff base separately gave single spot with different Rf values on the same TLC plate. Thus, indicate the absence of the starting material in the product.

C. Synthesis of $Cu(L^1)_2$

Procedure

Copper(II) chloride dihydrate ($Cucl_2.2H_2O$) of 2.23 mmole was dissolved in 10 ml of distilled water, over a period of 4 minutes with stirring, a solution of 10 ml L^1 in 10 ml of Methanol was added. 4.28 mmole of sodium acetate (CH_3COONa) in 10ml of distilled water was added to the resulting mixture over a period of 4minutes. The mixture was heated for 15minutes at 80 ^{0}C in a water bath, cooled in an ice and filtered using ultra membrane filtration. The product was washed with cold distilled water and dried in an oven at 100^{0}C for 1hr (Scheme II).



Scheme 2: Synthesis of $Cu(L^1)_2$ complex

III. ANTIMICROBIAL SUSCEPTIBILITY TESTING

A. Preparation of Mc Farland Standard

Procedure

A $BaSO_4$ 0.5 McFarland standards were prepared as follows;

A 0.5-ml aliquot of 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2.2H_2O$) was added to 99.5 ml of 0.18 mol/L H_2SO_4 (1% v/v) with constant stirring to maintain a suspension. The correct density of the turbidity standard was verified by using a spectrophotometer with a 1-cm light path and matched cuvette to determine the absorbance. The absorbance at 625 nm was within the standard range (0.008 to 0.10) (Lalitha, 2004).

B. Normal Saline

The normal saline solution is simply the 0.85% Sodium chloride (NaCl) solution and was prepared by dissolving 0.85g Sodium chloride crystals in 100ml of distilled water (Lalitha, 2004).

C. Preparation of Stock Solution

0.1g/ml (W/V) of the synthesized compounds in DMSO was prepared as a stocks solution for antimicrobial susceptibility testing.

The following microorganisms *Pseudomonas aeruginosa (Gram-negative), Escherichia coli (E. coli) (Gram-negative,) Klebsella pneumoni(Gram-negative) and Bacillus aureus (B. Aureus) (Gram-positive)* were identified using gram-test and used for antimicrobial testing

D. Inoculation of Test Plates

Optimally, within 15 minutes after adjusting the turbidity of the inoculums suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculums from the swab. (Lalitha 2004)

The dried surface of a Müeller-Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculums (Lalitha. (2004).

E. Control

Gentamicin (10 µg/disc) was used as control for antibacterial susceptibility testing . The antimicrobial activity of the solvents dimethyl sulphure oxide (DMSO) was measured to know whether the antimicrobial activity was only associated with the complex and not the solvent. The percentage activity index;

$$\%\text{Activity index} = \frac{\text{zone of inhibition by test compound diameter}}{\text{zone of inhibition of Antibiotic}} \times 100;$$ was obtained

IV. RESULTS AND DISCUSSION

In all the tables and discussion the ligand was denoted by L^1 the complex $Cu(L^1)_2$

L^1 =Schiff base of acetylacetone and aniline and

$Cu(L^1)_2$ = corresponding copper(II) complex of the Schiff base

The result of the physical characterization of the synthesized ligand (L^1) and it copper(II) complex (Cu (L^1)$_2$) are shown in Table 1. The ligand and the complex are stable to heat and air non- electrolytic and insoluble in water with a percentage yield of 86.96 and 70.65 respectively. Condensation of acetylacetone and aniline produced bidentate ligand L^1 while the reaction of the L^1 and Cu^{2+} from $CuCl_2.2H_2O$ salt resulted in the formation of the bidentate complex $Cu(L^1)_2$ this was identified by FTIR Spectroscopy.

Table 1: Molar conductivity, Melting points, Colour, Solubility and percentage yield;

Comp	Molar conductivity (µS)		Melting points	Colour	% Yields	solubility
	Metal Comp	Solvents				
L^1	L^1 = 0.00	DMSO = 0.00	188^0C	Yellow	86.96	Insoluble in water but soluble in polar organic solvent
$Cu(L^1)_2$	$Cu(L^1)_2$ = 0.00	DMSO = 0.00	228^0C	Black	70.65	Insoluble in water and in polar organic solvent but soluble in chloroform

A. Molar conductivity of the $Cu(L^1)_2$ complex

From Table 1 the molar conductivity of the complex with chloroform as a solvent (1.0×10^{-3} mol) were 0.00 µs which implies that the complex was non-electrolytic. Conductivity measurements reveal whether the complex will possess counter ions or not (ion outside the coordination sphere to counter balance the charge on the complex) they helped in choosing the method of testing degree of ionization of the complex.

B. UV-Visible Spectroscopy of The Complexes

UV-visible spectroscopy of the complex ($Cu(L^1)_2$) and ligand(L^1) shows a maximum absorption within the ultraviolet region as shown in figure 1 and figure 2. This implies that the colour of the complex was due to n→π* transition and not d-d. This indicate that the complex obeyed LaPorte rule or the Orbital rule which states that, in a molecule or ion possessing a centre of symmetry, transitions are not allowed between orbitals of the same parity, for example d to d. In other words, there must be change in parity (Δl=±1), i.e. the orbital quantum number should differ by 1. The forbidden transitions are s → s, d → d, p → f. etc. The geometries affected by this rule include octahedral and square-planar complexes. The rule is not applicable to tetrahedral complexes as it they do not contain a center of symmetry.

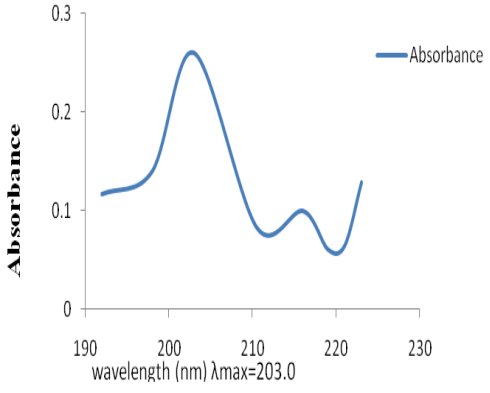


Figure 1 A Plot of Absorbance against Wavelength for $Cu(L^1)_2$ Complex

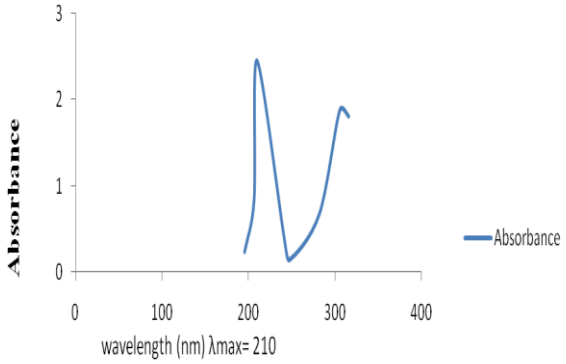


Figure 2 Plot of Absorbance against Wavelength for $L^{1`}$ Schiff base

Table 2: UV-Visible spectroscopy for L^1 and $Cu(L^1)_2$

compounds	λmax(nm)	transition
L^1	210	n→π*
$Cu(L^1)_2$	203	n→π*

From Table 2: the difference in λmax (nm) between the ligands and the metals complexes can be explained as, when ligands bond to a transition metal ion to form a complex, electrons in the ligands and electrons in the five d-orbitals of the metal ion repel each other. As a result the energies of the d-orbitals are raised; and split into two groups of differing energy. For instance when white light is passed through a solution of the Cu^{2+} ion, some of the energy is used to promote (or excite) an electron from an orbital in the lower group to an available orbital in the upper group. The energy that is absorbed is equal to the energy gap between the two groups, the size of the energy gap between the two groups of the d orbital will vary with the transition metal ion, its oxidation state and the nature of the ligands. The result of the UV- visible spectroscopy suggested that acetylacetone was a weak lidand hence formed a high spin complex with low λmax (nm) value (203) as compared with the λmax (nm) value of the ligand (2010)

C. Fourier Transform Infrared spectra

The major FTIR data of the L^1 and $Cu(L^1)_2$ are shown in Table 3 and FTIR spectra are shown in Figure 3and figure 4 respectively. The stretching vibration ν(-HC=N) in (L^1)which appears at 1595.44 is shifted in the spectra its corresponding complex ($Cu(L^1)_2$) which indicate the sharing of HC=N in the formation of the complex

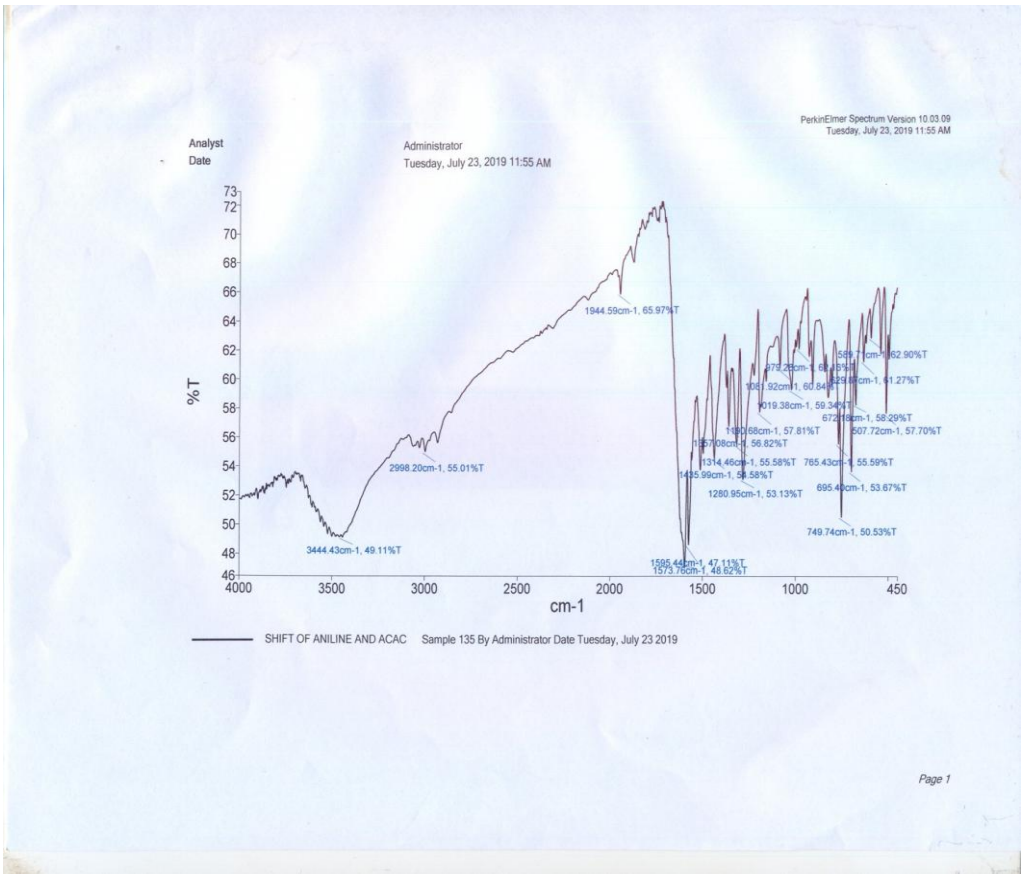


Figure 3.FTIR Spectra of L^1 (Schiff base)

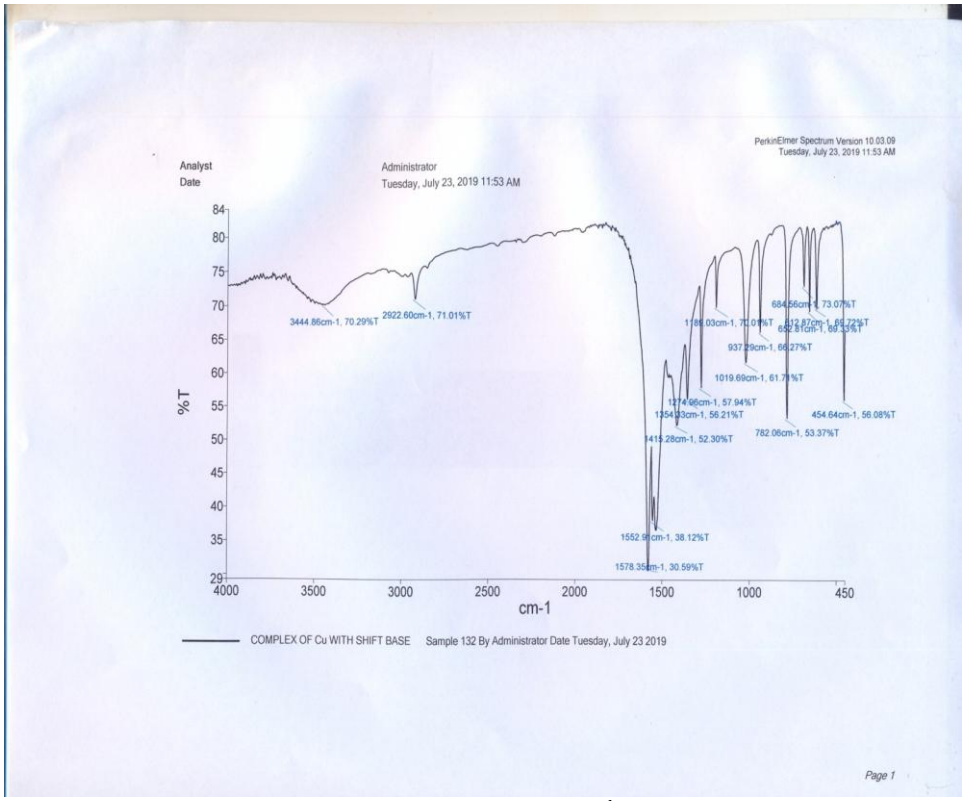


Figure 4 FTIR Spectra of $Cu(L^1)_2$ Complex

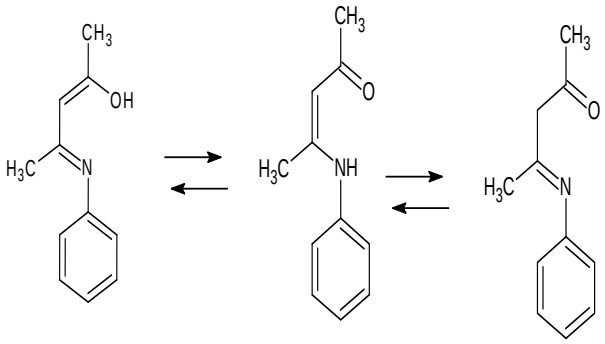
Table 3: FTIR Data for ^{1} and Complexes $Cu(L^{3})_2$

Compounds	functional groups						
	νC=O	ν C=C	CH_2 str.	νC-H	ν M-O	ν(M-N)	ν(-HC=N)
L^1	-	1435.99	2998.2	1314.46	-	-	1595.44
$Cu(L^3)_2$	-	1415.42	2922.64	1354.05	454.64	782.06	1578.48

4.4 FTIR Result for Schiff Base (L1)

The FTIR result for L1 compound indicate the present of azomethene bond (ν(-HC=N)) at 1595.44 cm-1 the νC=C, CH3str., and νC-H bonds were observed at 1435.99, 2998.2 and 1314.46 respectively . Most azomethene bond FTIR absorption was observed at 1600-1650. This implies that, the azomethene at 1595 is a weak (ν(-HC=N)) bond resembling aromatic azomethene due to the resonance in the proposed structure of the Schiff base as shown in the figure 5 below

Figure 5 proposed resonance structures of L^1 Schiff base



Therefore the weakness of the azomethene bond was attributed to the present of these proposed resonance structures.

The Metal-Oxygen (M-O) and Metal-Nitrogen (M-N) stretching vibration at 454.64 and 782.06 respectively which are absent in the spectra of the Ligand (L1) were observed in the spectra of the complex (Cu(L1)2) which indicate the bond formation between the ligand and the copper(II) ion

V. ANTIMICROBIAL SUBSEPTIVITY TESTING

The antimicrobial activity was determined using Agar well diffusion method dimethyl sulphure oxide (DMSO) was use as solvent to prepare the concentrations of the tested compounds. Clinical bacterial isolate was obtained from the microbiology laboratory of the federal teaching hospital Gombe. The bacterial isolates were subculture to before the inoculation. Gentamacin (10 µg/disc) was use as control. The result antimicrobial activity of the solvent (DMSO) ligand L1 and the complex (CuL1)2 were presented in Table 4, 5 and 6, respectively

A. *Antimicrobial Activity of method dimethyl sulphure oxide (DMSO)*

Table 4: Antimicrobial Activity of DMSO Mean ZI (mm)

Concentration (%)/(% activity index)

	100	75	50	CONTR
PA	00(00)	00(00)	00(00)	20
KP	00(00)	00(00)	00(00)	20
BA	00(00)	00(00)	00(00)	20
EC	10(50)	00(00)	00(00)	20

PA= *speudomona auregenosa*, KP= *Klebsiella Pneumoniae*, BA= *Bacillus aureus*, EC= *Escherichie Coli*, , *and* ZI = zone of inhibition

Antimicrobial activity of dimethyl sulphure oxide (DMSO) was investigated to know whether there was solvent contribution to the observed antimicrobial properties. *Pseudomonas auregenosa, Klebsiella pneumonia Bacillus aerius* was found to be 100% resistance to DMSO. A zone of inhibition of 10mm was observed with *E. coli* at 100% DMSO concentration. It therefore means that as for the *E.coli* DMSO has contributed a little for the antimicrobial activity that were observed. The result was compared with the work of Hendric., *et al* (2010) in which the author was able to identify several Bacterial isolates capable of growing on DMSO as a sole source of carbon and energy.

Table 5: Antimicrobial Activity of L^1 Mean ZI (mm)

Concentration (mg/ml)/(% activity index)

	10	15	20	CONTR
PA	00(00)	00(00)	00(00)	25
KP	00(00)	00(00)	00(00)	25
BA	00(00)	00(00)	00(00)	25
EC	00(00)	00(00)	00(00)	20

PA= pseudomonas auregenosa, KP= Klebsiella Pneumoniae, BA= Bacillus aureus, E. coli = Escherichie Coli, , and ZI = zone of inhibition

From table 5, at a maximum concentration of 20 mg/ml, L^1 indicates non antimicrobial property on all the tested organisms (*Pseudomonas auregenosa, Klebsiella Pneumoniae Bacillus aerius and E.coli.*)

Table 6: Antimicrobial Activity of $Cu(L^1)_2$ Mean ZI (mm)

Concentration (mg/ml)/(% activity index)

	1.0	1.5	2.0	CONTR
PA	11(64.4)	16(88.9)	19(105)	18
KP	15(125)	20(216)	26(266)	12
BA	10(71.5)	15(107)	22(157)	14
EC	12(120)	15(150)	20(200)	10

PA= speudomona auregenosa, KP= Klebsiella Pneumoniae, BA= Bacillus aureus, E. coli = Escherichie Coli, , and ZI = zone of inhibition

From Table 6 at a maximum concentration of 2.0 mg/ml for $Cu(L^1)_2$, *Pseudomonas auregenosa, Klebsiella pneumoniae Bacillus aureus* and *E.coli* were found to be highly susceptible by $Cu(L^1)_2$ with very clear zone of inhibition at a very low concentration (2.0 mg/ml) as compared to the control (Gentamicin). The result was compared with the work of (Rajasekar., *et al* 2010). Comparing the antimicrobial activity of $Cu(L^1)_2$ with the standard drug (Gentamicin) indicated that $Cu(L^1)_2$ complex was more effective as shown in figure 6

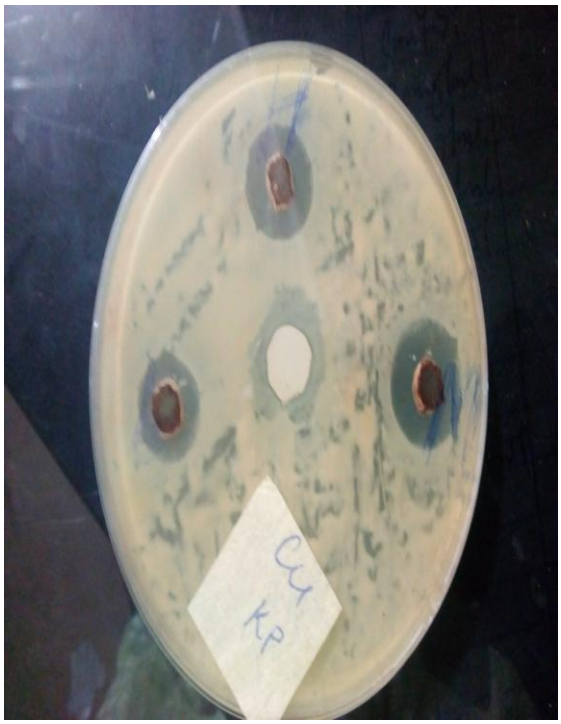


Figure 6: Photograph of the result for the antimicrobial susceptibility test after incubation for 18-24hr of Klebsiella Pneumoniae in Cu(L1) concentration.

The effective antimicrobial activity observed $Cu(L^1)_2$ complexes while the ligand (L^1) had been resisted by the all the tested bacteria was explained on the basis of chelation theory. Chelation reduces the polarity of the metal ion, because positive charges of the metal are partially shared with the donor atoms present in the ligands and there may be π-electron delocalization over the whole chelate ring. This phenomenon increases the lipophilic character of the metal chelate and favors its permeation more efficiently through the lipoid layer of the microorganism, thus destroying them more forcefully.

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