

Synthesis and Antibacterial Screening of 2-Hydroxybenzylidene-Aminophenols and its Cu(II) Complexes

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ABSTRACT

Background: The design of metal containing antimicrobial agents is currently of interest due to the growing reports of drug resistance reported for known antimicrobial agents. Structure activity relationship is crucial in the investigation of potential candidates in drug therapy.

Objective: In line with structure activity relationship, a new series of copper(II) complexes of Schiff base 2-hydroxybenzylidene-2-aminophenol (L¹), 2-hydroxybenzylidene-3-aminophenol (L²), and 2-hydroxybenzylidene-4-aminophenol (L³) have been synthesised. The goal of this study was to investigate the effect of position of substituent on the antibacterial activities of the ligands and its metal complexes.

Methods: The compounds were prepared using condensation method. Structural and spectroscopic properties were studied by elemental analysis, spectral (FT-IR, ¹H NMR, UV-Vis) and magnetic susceptibility measurements. The Schiff base ligands and its complexes were screened *in-vitro* against *E. coli*, *S. aureus*, *P. aeruginosa*, *B. cereus*, *E. faecalis* and *K. pneumonia* bacteria.

Results: The metal complexes exhibited higher antibacterial activities than the parent ligand. This can be attributed to the greater lipophilic nature of the complexes.

Conclusion: The results show that the investigated metal complexes can be employed as broad spectrum antibacterial agents.

Keywords: Antimicrobial agents, copper (II), metal complex, Schiff base, aminophenol.

INTRODUCTION

Salicylaldehyde Schiff bases and metal complexes show a wide spectrum of antimicrobial properties (1–3). Antimicrobial activity is a property of both inorganic and organic substances, and the exploitation of such activity is a matter of considerable practical importance in the development of antiseptics, sanitizers, germicides, bactericides, sporicides, virucides and disinfectants (4). Schiff bases have played a marvellous role in the development of coordination chemistry as they readily form stable complexes with most of the transition metals (5). Metal complexes of Schiff bases are specifically of interest in bioinorganic chemistry because many of these complexes provide biological models in understanding the structure of biomolecules and biological processes (6). These complexes may statistically mimic the spectroscopic or other physical properties of the enzyme (7). Many researchers have studied the synthesis, characterisation and structure activity relationship (SAR) of Schiff base metal complexes (8-11), but much work has not been reported on the effect of position of substituents on the Schiff base metal complexes of aminophenol derivatives. In line with this, we report the synthesis and characterisation of a new series of copper (II) complexes with

the Schiff bases 2-hydroxybenzylidene-2-aminophenol (L¹), 2-hydroxybenzylidene-3-aminophenol (L²), and 2-hydroxybenzylidene-4-aminophenol (L³). The effect of position of substituents on the antimicrobial activity was studied to determine the derivative that exhibit better activity. This study reports the stereochemistry of the metal complexes and provides baseline data for further study of structure activity relationship of elated complexes.

MATERIALS AND METHODS

Materials

All reagents and solvents were of analytical/spectroscopic grade and used without further purification. Ethanol, chloroform, dimethylsulfoxide, salicylaldehyde, 2-aminophenol, 3-aminophenol, 4-aminophenol and copper (II) chloride were purchased from Aldrich-Sigma Company Limited (Missouri, USA).

Physical Measurements

Microanalytical data were obtained on a Perkin Elmer model 2400 series II CHNS/O elemental analyzer. Infrared (IR) spectra of the compounds were recorded on a Bruker FT-IR (ATR) tensor

27 spectrophotometer directly on small samples of the compounds in the range 400 to 4000 cm^{-1} . $^1\text{H-NMR}$ spectra in DMSO-d_6 solution of the ligands were recorded on a Bruker Avance III 400 MHz. Chemical shifts were reported as δ relative to TMS as internal standard. Electronic absorption spectra of the compounds were recorded from 200 to 800 nm on a freshly prepared CHCl_3 solution using a Cary Model 50 spectrophotometer. Melting points ($^\circ\text{C}$) were determined on a Reichert Thermovar melting-point apparatus and are uncorrected. Magnetic susceptibility measurements were made on powdered samples using a Sherwood Scientific magnetic susceptibility balance. $\text{Hg}[\text{Co}(\text{SCN})_4]$ was used as the calibrant and corrections for diamagnetism were calculated from Pascal's constants.

Synthesis of Schiff Base

Equimolar quantities (10 mmol) of salicylaldehyde and corresponding amine were dissolved in ethanol (50 ml) and stirred under reflux at 70°C for 6 h. The precipitate formed was separated by filtration, re-crystallised from ethanol, dried and stored in a desiccator.

Synthesis of Metal Complexes

An ethanolic solution (40 ml) of Schiff base (4 mmol) was mixed with an ethanolic solution (20 ml) of Cu(II) chloride (2 mmol). The solution was made slightly alkaline with triethylamine. The mixture was refluxed for 4 h. The solid product obtained was filtered hot, washed in ethanol and dried in vacuum.

Biological Studies

The Schiff bases and metal complexes were individually tested against a panel of standard microorganisms namely *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 19582), *Bacillus cereus* (10702), *Enterococcus faecalis* (ATCC 29212) and *Kliformella pneumoniae* (ATCC 10031).

Disc Diffusion Assay

Antibacterial activity of Schiff bases and metal complexes were carried out in triplicate using the disc diffusion method

(12). Molten Mueller-Hinton agar was inoculated with the bacteria suspension which had been adjusted to the 0.5 McFarland standard and poured into sterile 90 mm Petri dishes. Schiff bases and metal complexes were dissolved in DMSO to obtain a final concentration of 10 mg/ml. Sterile Whatman No. 1 (6 mm) discs were separately impregnated with each sample to be tested and placed on the inoculated agar. The plates were incubated at 37°C for 24 h and the zones of inhibition measured at the end of the incubation period. Ampicillin was used as reference compound.

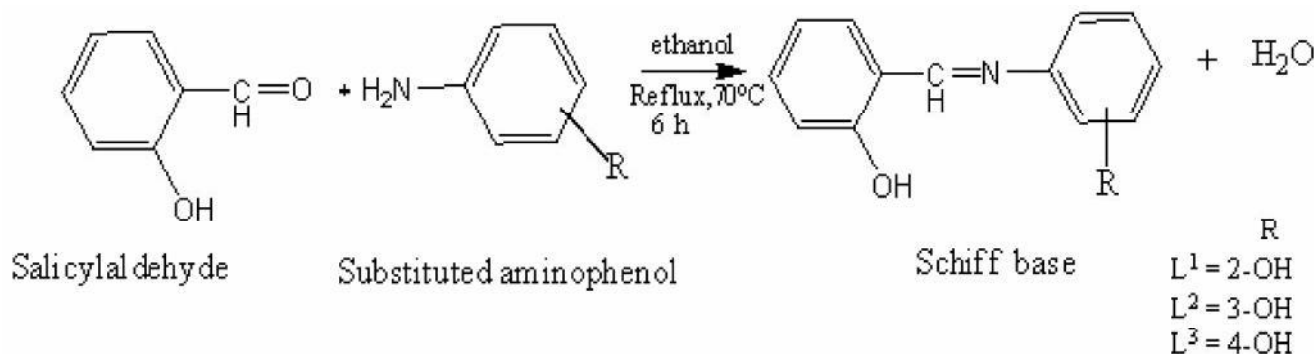
Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of the Schiff bases and metal complexes were determined using the 96-well micro-plate dilution method (13). Serial plate concentrations of 5.0, 2.5, 1.25, 0.625, 0.312, 0.157, 0.078 and 0.039 mgmL^{-1} were prepared for each compound. Each was inoculated with 1.5×10^8 CFU/mL of 0.5 McFarland standard bacteria suspension and incubated for 24 h at 37°C . As an indicator of bacterial growth, 20 μL of 0.2 mgmL^{-1} *p*-iodonitrotetrazolium solution (a colourless tetrazolium) was added to each well and incubated at 37°C for 30 min. Growing bacteria metabolise this salt to give a red product (formazan). Inhibition prevents this conversion resulting in a clear well. MIC values were recorded as the lowest concentration of compound preventing bacterial growth.

RESULTS AND DISCUSSION

Synthesis

The Schiff base ligands were prepared by the reaction of salicylaldehyde and aminophenol in a 1:1 stoichiometric ratio as illustrated in Scheme 1 below. Analytical and physical data is represented in Table 1. The compounds were isolated in good yield. $^1\text{H NMR}$ data revealed that the desired Schiff bases were isolated while microanalysis confirmed the purity of the ligand. Treatment of the ligands L^1 , L^2 and L^3 with Cu(II) chloride afforded complexes (Scheme 2) corresponding to the general formula $[\text{CuL}_2]\text{X}_n$ where $\text{X} = \text{H}_2\text{O}$ or Cl and $n = 2$. The analytical data show that the metal to ligand ratio is 1:2. The complexes were soluble in common organic solvents; chloroform, DMF, DMSO.



Scheme 1: Synthetic Route to Schiff Bases $\text{L}^1 - \text{L}^3$

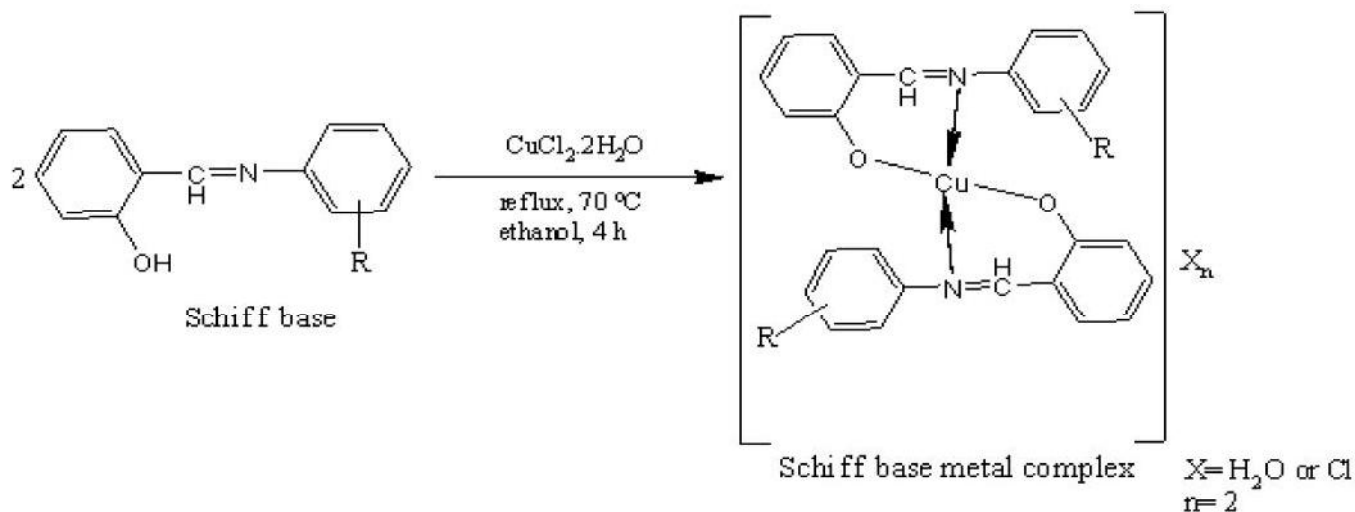
Scheme 2: Synthetic Route to Schiff Base Metal Complexes $\text{Cu L}^1 - \text{Cu L}^3$

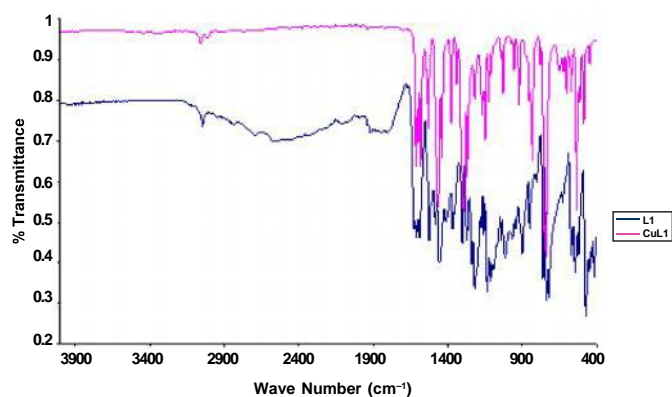
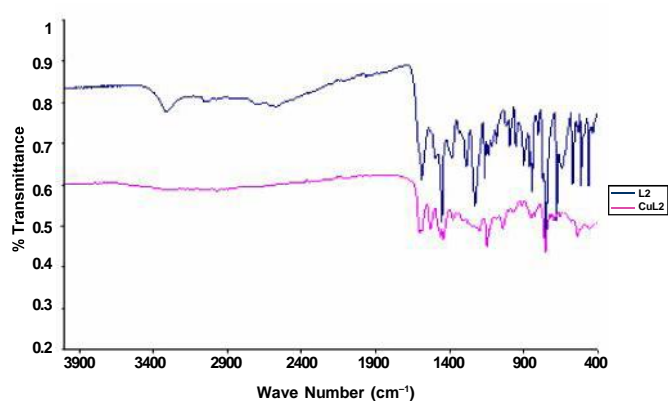
Table 1: Physical Properties and Analytical Data of Prepared Compounds

Compound	Formula Weight	Yield %	Colour	M.pt $^{\circ}\text{C}$	Micro-analysis Data Found (calcd)		
					%C	%H	%N
L^1	$\text{C}_{13}\text{H}_{11}\text{NO}_2$	74	Red	146–147	73.43(73.23)	6.44(6.57)	5.10(5.20)
CuL^1	$\text{C}_{26}\text{H}_{20}\text{Cl}_2\text{CuN}_2\text{O}_4$	57	Green	Decomp>293	56.43(55.87)	2.96(3.61)	4.89(5.01)
L^2	$\text{C}_{13}\text{H}_{11}\text{NO}_2$	71	Orange	82–83	73.01(73.23)	5.16(5.20)	6.57(6.57)
CuL^2	$\text{C}_{26}\text{H}_{22}\text{Cl}_2\text{CuN}_2\text{O}_4$	61	Brown	Decomp>250	55.32(55.67)	3.97(3.95)	5.54(4.99)
L^3	$\text{C}_{13}\text{H}_{11}\text{NO}_2$	65	Orange	98–99	73.23(73.23)	5.11(5.20)	6.61(6.57)
CuL^3	$\text{C}_{26}\text{H}_{24}\text{CuN}_2\text{O}_6$	79	Brown	Decomp>273	60.32(59.59)	4.95(4.62)	5.71(5.35)

Infra-red Spectroscopy

In order to study the binding mode of Schiff base to metal in the complexes, IR spectrum of the free ligand was compared

with the spectra of the metal complexes. IR spectra bands of the ligands and their metal complexes are presented in Figures 1–3 and diagnostic bands reported in Table 2.

Fig. 1: IR Spectra of Schiff Base L^1 with Copper Metal Ion.Fig. 2: IR Spectra of Schiff Base L^2 with Copper Metal Ion

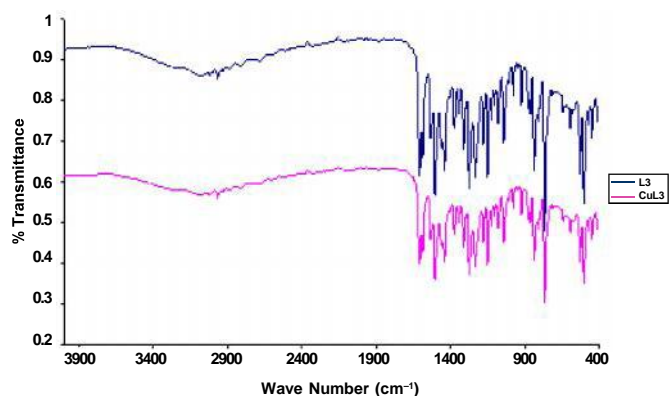


Figure 3: IR Spectra of Schiff Base L³ With Copper Metal Ion

Magnetic Susceptibility Measurements and Electronic Absorption Spectra

The electronic spectra and magnetic moments of Schiff bases and their metal complexes are presented in Table 3. The electronic spectra of the free ligands and its metal complexes were studied in chloroform (CHCl₃) solution. In all the spectra of the ligand, three absorption bands appear in the region 240–268 nm, 270–303 nm and 343–355 nm assigned to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$. Electronic spectra of Cu(II) complexes showed absorption bands in the region 306–328 nm assigned to charge transfer (CT) and 391–446 nm due to $d \rightarrow d$ transitions attributed to ${}^2B_{1g} \rightarrow {}^2A_{1g}$ transition of four coordinate, square-planar geometry. This $d \rightarrow d$ transition is in the region of that observed for structurally well characterized complexes of copper (II) N-alkylsalicyladiminates with square planar geometry (17). This was further corroborated with the magnetic moments values of

Table 2: IR and ¹H-NMR spectra data of prepared compounds

Compound	(C=N)	(C-O)	(O-H)	(Cu-N)	(Cu-O)	Chemical shift δ (ppm)	
						HC=N	OH
L ¹	1616	1287	3040	–	–	8.69	9.11
CuL ¹	1612	1268	3050	487	536	–	–
L ²	1584	1225	3294	–	–	8.38	8.77
CuL ²	1581	1190	–	442	532	–	–
L ³	1607	1272	3104	–	–	8.36	8.78
CuL ³	1601	1266	3204	513	540	–	–

Infrared spectra of the Schiff base ligands, L¹, L² and L³ shows characteristics absorption bands at 1616, 1584 and 1607 cm⁻¹, assignable to C=N. These bands due to the azomethine nitrogens of the Schiff base underwent a shift to lower frequencies 1612, 1581 and 1601 cm⁻¹ upon complexation with Cu(II), indicating the involvement of nitrogen of the azomethine group in coordination (14). The band for C-O stretching which occurs at 1287, 1225 cm⁻¹ for the ligand L¹, L² was moved to lower frequencies 1268, 1190 cm⁻¹ (CuL¹ and CuL²) after complexation which indicates that the shifts are due to the coordination of the phenolic oxygen of the ligand to the metal ion (15). It can be concluded that the Schiff base acts as a bidentate ligands coordinating via the azomethine N and the phenolic O. The broad band at 3204 cm⁻¹ for CuL³ complex indicates the presence of coordinated water molecule. The new bands observed in the complexes in the region 442–513 and 532–536 cm⁻¹ were assigned to Cu–N and Cu–O bonds respectively (16).

¹H NMR Spectra

The ¹H NMR spectrum of the ligands (L¹, L² and L³) in DMSO show signal at 8.36, 8.38, 8.69 ppm (s, 1H, N=C-H) assigned to the azomethine protons. The peaks at 8.77, 8.78, 9.11 ppm are attributed to phenolic protons (s, 1H, phenolic-OH) respectively.

1.76–1.88 B.M which falls within the range normally observed for one unpaired electron of Cu²⁺ complexes with square-planar geometry (18).

Table 3: Magnetic Moments and Electronic Spectra Data (nm) of Schiff Bases and their Metal Complexes

Compound	CHCl ₃ λ_{max} (nm)	Assignment	Magnetic Moments	Geometry
			μ_{eff}	
L ¹	240, 270 355	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	–	–
CuL ¹	306 420, 446	CT d – d	1.79	Square-Planar
L ²	242, 270 343	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	–	–
CuL ²	329 422	CT d – d	1.88	Square-Planar
L ³	268, 303 346	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	–	–
CuL ³	308 391	CT d – d	1.76	Square-Planar

Antibacterial Activity

Antibacterial activity of the ligands and complexes were tested *in-vitro* against six human pathogenic bacteria. The activities were compared with that of ampicillin. The compounds were tested at a concentration of 10 mg/ml in DMSO using the paper disc diffusion method. The diameters

of growth inhibitory zones were measured and the results are presented in Figure 4. The susceptibility zones measured were clear zones around the disc killing the bacteria; this is represented in Table 4. The complexes showed comparable activity with the reference compound ampicillin.

Table 4: Diameter of Zones of Inhibition of Bacteria in different Compounds (mm)

Bacterial Strain	L ¹	CuL ¹	L ²	CuL ²	L ³	CuL ³	Ampicillin
<i>S. aureus</i> (ATCC 6538)	10±0.70	13±0.00	10±0.00	10±0.70	10±0.35	10±0.00	13±0.00
<i>E. faecalis</i> (ATCC 29212)	7±0.35	11±1.41	0±0.00	10±0.00	0±0.00	9±1.41	11±0.20
<i>B. cereus</i> (ATCC 10702)	10±1.41	10±0.70	10±0.70	10±0.70	0±0.00	10±0.00	14±0.20
<i>E. coli</i> (ATCC 8739)	11±0.70	13±0.70	0±0.00	11±0.10	0±0.00	11±0.70	10±0.00
<i>P. aureginosa</i> (ATCC 19582)	0±0.00	12±0.00	0±0.00	10±0.28	0±0.00	8±0.28	9±0.10
<i>K. pneumonia</i> (ATCC 10031)	0±0.00	11±0.00	0±0.00	10±0.35	0±0.00	10±0.70	11±0.10

Diameter of zones of inhibition ≥ 7 = active, diameter of zones of inhibition ≤ 6 = not recorded (low activity = 0).

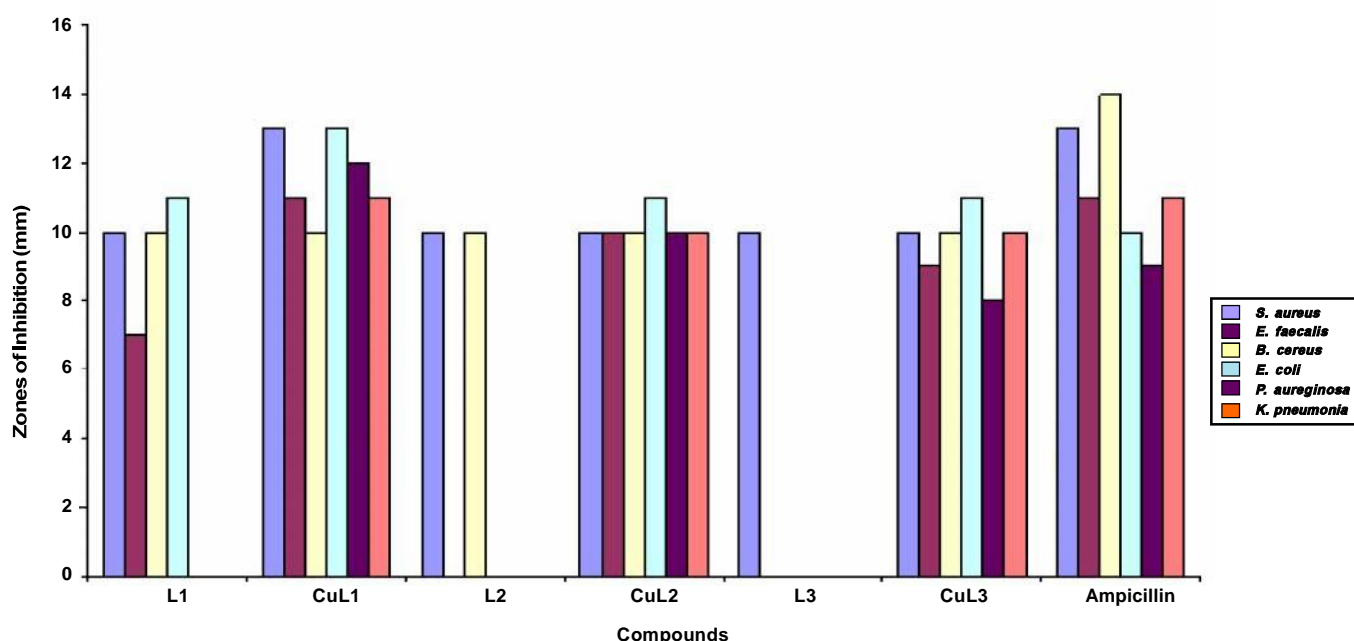


Fig. 4: Histogram showing the Comparative Activities of the Compounds

Table 5: Minimum Inhibitory Concentration (mg/ml)

Bacterial Strain	L ¹	CuL ¹	L ²	CuL ²	L ³	CuL ³	Ampicillin
<i>S. aureus</i> (ATCC 6538)	2.50	0.15	2.50	2.50	5.00	2.50	2.50
<i>E. faecalis</i> (ATCC 29212)	5.00	1.25	2.50	2.50	5.00	2.50	5.00
<i>B. cereus</i> (ATCC 10702)	5.00	0.62	5.00	5.00	5.00	2.50	5.00
<i>E. coli</i> (ATCC 8739)	2.50	2.50	2.50	2.50	2.50	2.50	1.25
<i>P. aureginosa</i> (ATCC 19582)	5.00	0.62	2.50	2.50	2.50	2.50	5.00
<i>K. pneumonia</i> (ATCC 10031)	2.50	0.62	2.50	2.50	2.50	2.50	2.50

The Schiff base ligands and their metal complexes all have the capacity of inhibiting the metabolic growth of the investigated bacteria to different extent. MIC values of the all the compounds against six bacteria strains used in the study are presented in Table 5. A minimum inhibitory concentration value of 0.28–1.27 mg/ml has been attributed with extremely strong activity while MIC values of 1.81–8.85 mg/ml are attributed with weak activities (19). The MIC result showed that the CuL¹ (copper(II) complex of Schiff base 2-hydroxybenzylidene-2-aminophenol) exhibited an extremely strong activity against the tested bacteria except against *E. coli* in respect of which it exhibited weak activity (19). It is known that chelation tends to make ligands act as more powerful and potent bactericidal agent and this was confirmed by the fact that the metal complexes showed enhanced antimicrobial activity against one or more strains with CuL¹ remarkably showing good activity. This can be attributed to the position of substituents, CuL¹ having the OH group at the *ortho* position (20–21). Increase in activity of this complex can be attributed to the ease of chelate formation.

CONCLUSION

Copper(II) complexes involving Schiff bases derived from 2-hydroxybenzaldehyde and aminophenols have been synthesised and characterised. Comparison of the IR spectra of the Schiff bases and their metal complexes indicate that the Schiff bases act as bidentate ligands coordinating via the azomethine *N* and the phenolic *O*. The electronic spectra of the complexes indicate square-planar geometry. The antibacterial (MIC) results show that the Cu(II) complexes were more active than the Schiff bases, with CuL¹ complex exhibiting a very low MIC compared to the other complexes. This can be attributed to the position of the substituent (OH) at the *ortho* position. The reported Cu(II) complexes particularly the 2-aminophenol complex, CuL¹ complex can be used in development of broad spectrum antimicrobial agents.

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