Original Research Article

Copper (II) Complex of Salicylaldehyde Semicarbazone: Synthesis, Characterization and Antibacterial Activity

ABSTRACT

Salicylaldehyde semicarbazone ligand and its Cu (II) complex have been synthesized and characterized by a range of physicochemical methods. Experimental data shows the complex is monomeric and the copper atom is four coordinated in a square planar geometry.

The ligand chelates the copper in a tridentate fashion through the carbonyl O, imine N, and phenolato O with the fourth position being occupied by coordinated Cl. Antibacterial activity of the prepared compounds are tested against the microbes Enterobacter Aerogenes and *Bacillus Cereus.* The metal complex shows antibacterial activity higher than that of the free ligand.

Keywords: *Semicarbazone; Tridentate ligand; Complexation; Antibacterial activity*

1. INTRODUCTION

Semicarbazones are an important class of compounds formed from the condensation of semicarbazide with suitable aldehyde or ketone. Most of these compounds have a wide spectrum of biological activity including activity against tuberculosis[1] bacterial[2] and viral infections[3], psoriasis[4] and malaria[5]. Salicylaldehyde semicarbazone is obtained by the condensation of "–NH_2" group of second position to the low electron dense carbonyl carbon and "-C=O" group of salicylaldehyde (Schiff base formation). It is described below in scheme 1.

Metal complexes with potential biological activity are the focus of extensive investigation. Remarkably, complexation with copper improves the biological activity of a wide range of organic ligands [6, 7]. Copper complex of salicylaldehyde benzoylhydrazone (H_2sb), [Cu(Hsb)Cl].H_2O, is an example, which shows tumour inhibitory activity [8]. [Cu(Hsb)Cl].H_2O was first found to be a potent inhibitor of cell growth and DNA synthesis [9, 10] in a number of human and rodent cell lines [11]. The cytotoxicity of this complex was exposed to exceed many other compounds which were previously known to have such properties, including those used clinically. The Cu(II) complex of the structurally related ligand salicylaldehyde acetylhydrazone (H_2sa) has also exhibited biological activity [12].

A group of vanadium complexes of salicylaldehyde semicarbazone derivatives were reported for their selective potency on human

kidney TK 10 tumour cells[13]. The results obtained with this study showed that modification of the semicarbazone backbone could have a significant effect on the cytotoxicity of the complexes.

The spectral and analytical characterization of the synthesized complex was carried out to propose the most probable stereochemistry of the complex around the Cu(II) ion. In this study, an antibacterial study has also been involved to follow the biological potency of the coordination compound synthesized.

2. EXPERIMENTAL

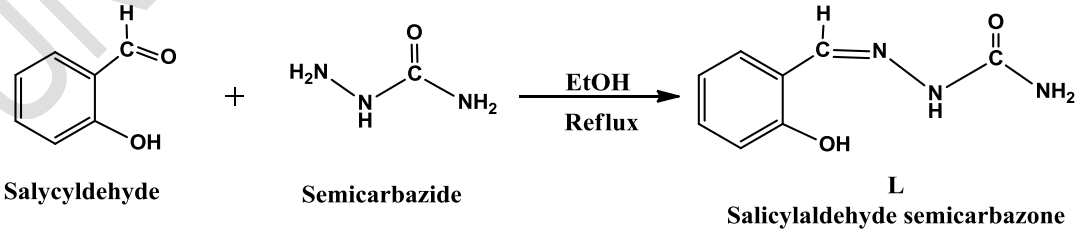
Semicarbazide (analytical grade), salicylaldehyde, and copper chloride were used without further purification.

Methanol (GRP), Ethanol (95%), Dichloromethane (WINLAB GRG 98%) and DMSO (BDH lab, England 99%) were used as solvents. Nutrient agar medium (Include-Peptone, Agar, sugar, marmite) was used to check anti-microbial activity.

Melting points were measured on a digital melting point apparatus. Elemental analyses for CHN were performed using a Vario EL cube [Germany elements (Elemental) analysis system]. FT-IR spectra were recorded on a FT-IR spectrophotometer [JASCO, FT-IR/4100] Japan using KBr pellets as the standard reference. ESI-MS spectra were done with an Agilent Technologies MSD SL Trap mass spectrometer with ESI source coupled with an 1100 Series HPLC system. Magnetic susceptibilities of the metal complexes were measured using a Sherwood Scientific MX Gouy magnetic susceptibility apparatus.

2.1 Synthesis of ligand salicylaldehyde semicarbazone (L)

To a stirring solution of *o*-Phenylenediamine (0.32g, 3 mmol) dissolved in about 20 mL ethanol, a solution of salicylaldehyde (0.64 mL, 6 mmol) in 10 mL of ethanol was added drop wise. This has resulted an orange color solution, which was refluxed for three hours (Scheme 1). The reaction mixture was cooled and kept for evaporation at room temperature leading to isolation of solid orange product. The product thus formed was filtered and washed several times with ethanol and dried in oven under 60^{o}C[14, 15]. The product was found to be soluble in DCM, DMF and DMSO.



Salycyldehyde + Semicarbazide → L (Salicylaldehyde semicarbazone)

Scheme 1. Synthesis of ligand salicylaldehyde semicarbazone.

2.2 Synthesis of Copper (II) complex with salicylaldehyde semicarbazone

To the warm ethanolic solution (10 mL) of ligand L (2 mmol), 10 mL warm ethanolic solution (2 mmol) of Cu(II) chloride was added and the resulting mixture was refluxed for about 3-4 hours. The obtained precipitates were filtered, washed with ethanol and dried under vacuum on anhydrous $CaCl_2$.

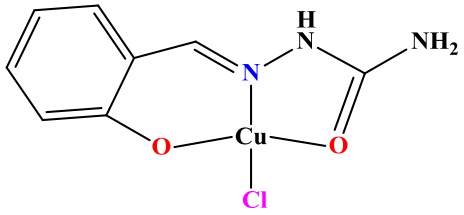


Fig. 1: Proposed structure of the synthesized complex.

2.3 Metal Weight Estimation

A known weight of the metal complex was taken into a conical flask and concentrated H_2SO_4 (500 μL) was added to it. It was fumed down to dryness and the process was repeated. Concentrated HNO_3 (500 μL) and $HClO_4$ (500 μL) were then added and the mixture was fumed to dryness. The process of adding acids and fuming down to dryness was continued until there was no black materials. 100 mL distilled water was added to dissolve the residue. Finally, the weight of the metal was estimated complexometrically [16, 17] using EDTA (Ethylenediamine tetra acetic acid. Excellent agreement of results were found.

2.4 Antibacterial Activity Study

Antibacterial activity was checked by the Agar-ditch method [18]. The *in vitro* antibacterial screening effects of the examined compounds were tested against *Bacillus cereus* and Enterobacter Aerogenes. The compounds were dissolved in dimethyl sulfoxide (DMSO) to get final concentration of 5 $mgmL^{-1}$. In order to activate the bacterial strain, it was inoculated in 25 mL of Mac Conkey agar and incubated for 24 h at 37^0 C. Activated bacterial strain solution was prepared in normal saline (0.9% NaCl solution). The bacterial density was adjusted to 0.5 McFarland standard units. Mueller-Hinton agar was transferred over sterile 90 mm Petri dishes. Then 1 mL of activated bacterial strain solution was inoculated into the media at 40-45^0 C. The medium was permitted to solidify. Fine well was made with the help of cork borer in the plates and

then the plates was filled with test solution (synthesized compounds dissolved in DMSO solution). Controls were run for the solvent and each bacteria. The plates were then incubated at 37^0 C for 24 h. The inhibition zones produced by the tested compounds were measured at the end of the incubation period.

3. RESULTS AND DISCUSSIONS

3.1 Synthesis

The Schiff base ligand, L was prepared in good yield from the condensation reaction of salicylaldehyde and semicarbazide in a 1: 1 stoichiometric ratio. Treatment of the Cu(II) chloride salt with the ligand L, formed the complex corresponding to 1:1 metal-ligand ratio. Physical and analytical data of studied compounds are presented in Table 1 and 2.

Table 1. Physical data of the ligand, L and its metal complex.

Compound	Empirical Formula	FW (g/mol)	Colour (%yield)	m.p. (^{0} C)
L	$C_8H_9N_3O_2$	179.18	White (83%)	218
ClCuL	$C_8H_8ClCuN_3O_2$	277.17	Brown (78%)	265

Table 2. Analytical data of the compounds.

Compound	Found (Calculated) (%)				μ_{eff} (B.M.)	Conductivity (μScm^{-1})
	Cu	C	H	N		
L	-	53.56 (53.63)	5.10 (5.06)	23.74 (23.45)	-	-
ClCuL	22.64 (22.93)	34.71 (34.67)	2.89 (2.91)	15.06 (15.16)	1.76	8

3.2 Elemental Analysis

The micro analysis data of the synthesized compounds are given in Table 2. The analytical data suggest that the complex was mononuclear. The data also reveal that metal to ligand ratio for the complex is 1:1. Moreover, these data also supports the proposed structure of the ligand and complex.

3.3 Magnetic Measurements

The magnetic moment, 1.76 BM is an additional evidence for the proposed square planar geometry of the complex, ClCuL where the ligand act as tridentates [19, 20] [21, 22].

3.4 Molar Conductivity Measurements

The molar conductance values of 10^{-3} M solution of the ligand and metal complex in DMSO are presented in Table 2. The low molar conductance value revealed that the metal complex was non-electrolyte in nature [23].

3.5 FT-IR Studies

FT-IR spectrum of the studied compounds are shown in Fig. 2-3. IR spectrum of the free ligand, L was compared with the spectra of the complex to determine the binding mode of the ligand to metal in the complexes. Characteristic IR peaks of the ligand and its metal complex are given in Table 3. The spectrum of the ligand shows the IR bands at 3458, 3161 and 3104 cm^{-1} due to v as(NH_2), v s(NH_2) stretching and v as(NH) vibration of free NH_2 groups respectively. The spectrum also shows bands at 3284, 1692 and 1594 cm-1 due to v(Phenolic-OH), v (>C=O) and v (>C=N) groups respectively. A medium intensity band in the IR spectrum of the ligand at 3284 cm^{-1} is assigned to an intramolecular hydrogen bond v(O-H). This band is absent in the spectrum of the complex, indicating that the phenolic-OH group is deprotonated. In complex, a new peak corresponding to phenolic v(C-O) is observed at 1317 cm^{-1}. The position of ligand band due to (>C=N), 1594 cm-1 and (>C=O), 1692 cm^{-1} is shifted towards lower side to 1581 cm^{-1}, 1687 cm^{-1} respectively, indicating the coordination through the nitrogen atom of the imine group and oxygen atoms of the ketonic (>C=O) and phenolic -OH groups.[24][25, 26]. The coordination through the azomethine nitrogen and phenolic oxygen to metal atom were further supported by the appearance of additional M-N & M-O vibrations in the region 740 cm^{-1} and 548 cm^{-1}, respectively in the IR spectra of the metal complex.

Table 3. IR (cm^{-1}) and ESI-MS data of the compounds.

Compound	*v* (O-H)	*v* (C=O)	*v* (C=N)	v (Cu-N)	v (Cu-O)	ESI-MS
L	3284	1692	1594	-	-	179.0759
ClCuL	-	1687	1581	740	548	277.0253

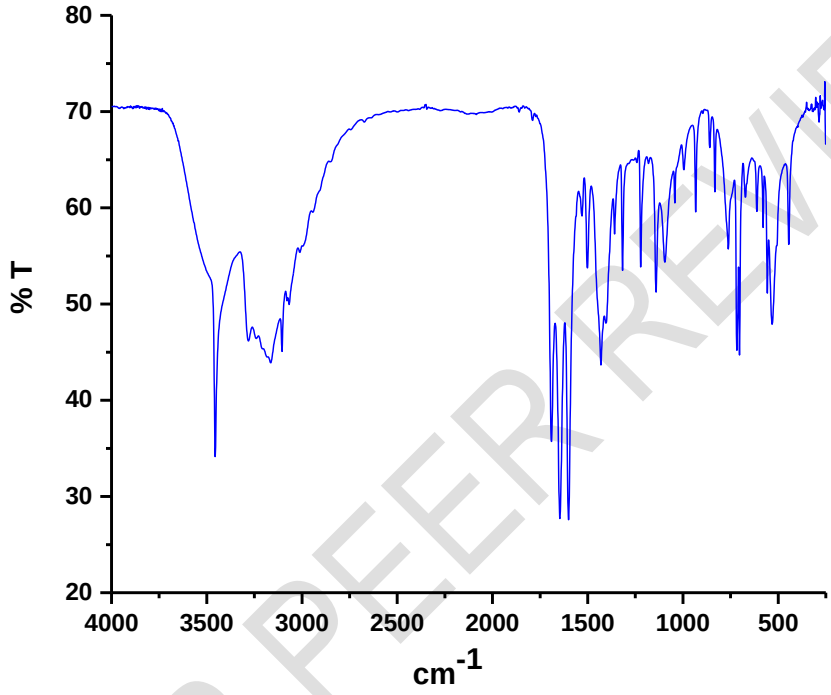


Fig. 2: IR spectrum of the ligand, L.

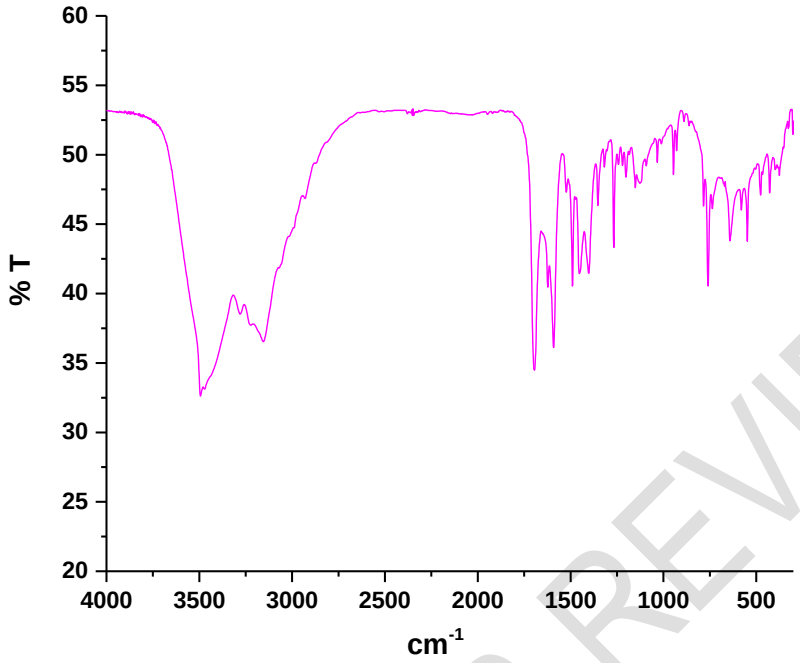


Fig. 3. IR spectrum of the complex, ClCuL.

3.6 ESI-Mass Spectra

The ESI-Mass spectra of the ligand and complex are presented in Fig. 4. The obtained m/z values are similar to the formula weight (Table 1 and 3) which further supports the proposed structure of the synthesized compound.

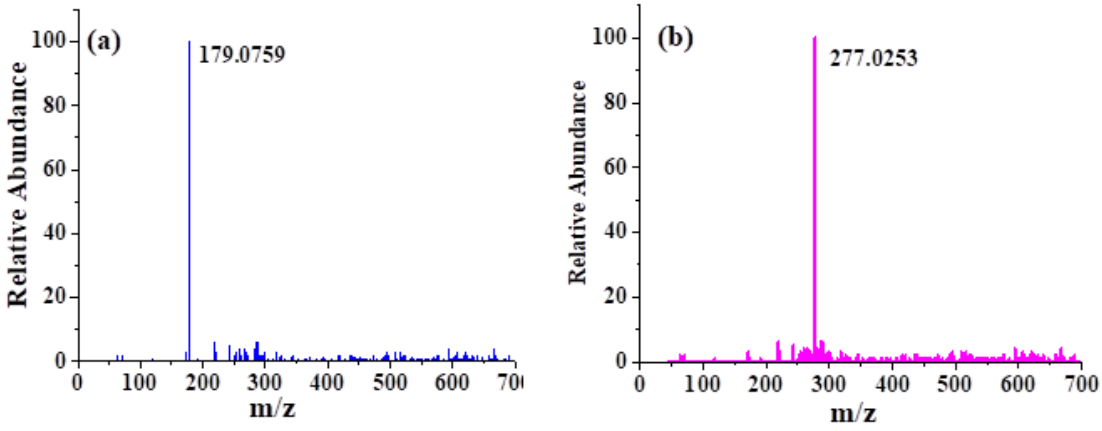


Fig. 4. ESI-Mass spectra of the (a) L and (b) ClCuL

3.7 Antibacterial Activity

The antibacterial activity of the compounds were investigated against the microorganism Bacillus Cereus and Enterobacter Aerogenes with the concentration of 5 mgmL^{-1} employing agar ditch method. The zone of inhibition were measured in diameter (mm). The antibacterial activity results are presented in Table 4. The metal complex showed anti-bacterial activity over the free ligand. The ligand, L exhibited very little activity against both the organisms. The complex, ClCuL showed high activity against the microbes Enterobacter Aerogenes. The variation in the activity of metal complex against tested organisms depends on either the impermeability of cells of organisms or the difference in ribosomes of bacterial cell [27]. The reasons of showing higher anti-bacterial activity of the complex than that of free ligand can be explained on the basis of Overtone's concept and Tweedy's chelation model [28]. Polarity of metal ion is reduced to a greater extent due to the overlapping of the ligand orbital and partial sharing of positive charge of metal ion with donor atoms of the ligand on chelation [29]. The lipophilic character of the central metal atom is also increased upon chelation, which consequently favors the permeation through the lipid layer of cell membrane [30].

Table 4. Antibacterial activity of the ligand L and its Cu(II) complex (5 mg mL^{-1}).

Compound	Diameter of inhibition zone of bacteria (mm)	
	Gram positive	Gram negative
	Bacillus cereus	Enterobacter aerogenes
L	+	+
ClCuL	+ + +	+ + +

Control (DMSO): No activity (There was no inhibition zone)

Note: High activity = + + + (Inhibition zone > 12mm and Sight = + (Inhibition zone = 4-8 mm).

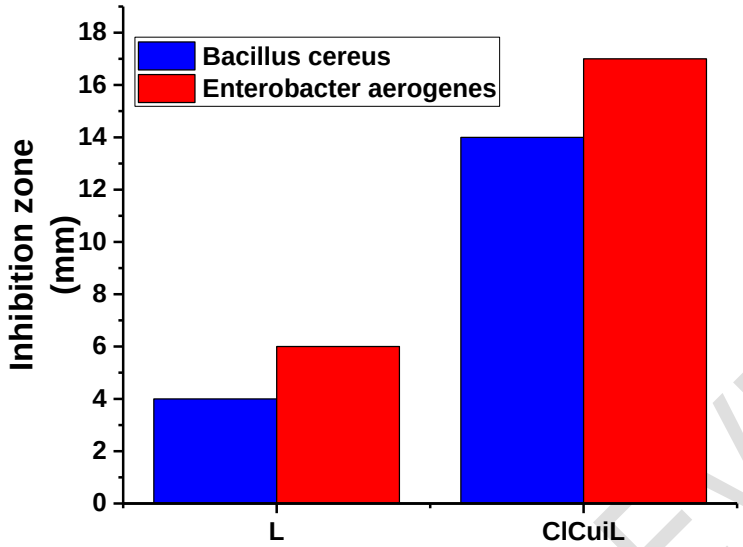


Fig. 5. Statistical representation for antibacterial activity for the ligand (L) and its Cu (II) complex.

4. CONCLUSION

The spectral, elemental analysis, conductivity and magnetic measurements data of the synthesized metal complex of Cu(II) with the tridentate ligand, salicylaldehyde semicarbazone have shown square planar geometry. The metal complex is biological active and exhibit enhanced antibacterial activity compared to free ligand.

The antibacterial activity and chemical properties is dependent on molecular structure of the compound. Hence, substitution at the aromatic ring of the ligand can modify the electronic and steric properties of the resulting complexes, which can enable fine-tuning of chemical and biological properties of the ligands and metal complexes.

It is important to note that numerous salicylaldehyde semicarbazone ligands can be readily synthesized using commercially available derivatives of semicarbazide and salicylaldehyde. A more systematic investigation of such type of metal complexes could be valuable for different biological applications.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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