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Synthesis, Spectral and Thermal Characterization of Cu(II) Complexes with Two New Schiff Base Ligand towards Potential Biological Application

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ABSTRACT

The Cu(II) complexes $[C_{15}H_{11}N_3O_3Cu]$ *and* $[C_{15}H_{11}N_3O_2SCu]$ *were prepared with two new Schiff base ligands* $HL^1(C_{15}H_{11}N_3O_3)$ *and* HL^2 $(C_{15}H_{11}N_3O_2S)$*. Schiff base ligands* HL^1 *and* HL^2 *were derived from the condensation reaction of salicylaldehyde with semicarbazide and thiosemicarbazide respectively. Where,* HL^1*=bis(2-hydroxybenzylidene)hydrazinecarboxamide and* HL^2*=bis(2-hydroxybenzylidene) hydrazinecarbothioamide. The ligands and complexes were isolated from the reaction in the solid form and characterized by IR,UV-visible,1H NMR, thermal analysis and some physical measurements. Spectroscopic evidence indicated that the Schiff bases were behaved as N, O coordinating chelating agents. Magnetic susceptibility data coupled with electronic spectra suggested a distorted square planar structure of the complexes. The overall reaction is monitored by TLC and UV-Visible spectral analysis. The Schiff bases and their metal complexes have been shown moderate to strong antimicrobial activity.*

Keywords: Schiff base, Semicarbazone, Thiosemicarbazones, Transition metal complexes, UV-visible, IR, ^1H NMR TGA, DTG, Antibacterial activity

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INTRODUCTION

Schiff bases are condensation products of primary amines with carbonyl compounds and they were first reported by German chemist noble prize winner Hugo Schiff. The common structural feature of these compounds is the azomethine group with a general formula RHC=N-R/ where R and R/ are alkyl, aryl, cyclo alkyl or heterocyclic groups which may be variously substituted. These compounds are also known as anils, imines or azomethines [1]. Metals have played a significant role in biological systems over the years. Many are important to our diets in varying quantities, although people have only recently realized their significance. Introducing metal ions into a biological system may be carried out for therapeutic or diagnostic purposes, although these purposes overlap in many cases. Metals not only provide path for synthesis, but they also introduce functionalities that enhance drugs action. The chemistry of the transition metal complexes of thiosemicarbazones became largely appealing because of their broad profile of pharmacological activity that provides a diverse variety of compounds with different activities [2-5]. Bis-Schiff base ligands and their coordination compounds having multifunctional groups play an important role in the areas of stereochemistry, structure of science, spectroscopy, and magnetic fields [6]. In recent years, sulfur containing ligands such as thiosemicarbazones and their transition metal complexes have received more attention in the area of medicinal chemistry, due to their pharmacological properties, such as antiviral, antibacterial, antifungal, antiphrastic, antitumor, anticancer and anti HIV activities [7-19]. Mei-Hsiu Shih et al [20] were synthesized Nickel (II) complexes from novel semicarbazone ligands with chloroformyl arylhydrazine, benzimidazole and salicylaldehyde moieties. Sulekh Chandra, et al. [21] synthesized copper (II) complexes with semicarbazones and thiosemicarbazones also determined their biological activity. Raman, et al. [22] synthesized Cu(II), Co(II), Ni(II) and Zn(II) complexes with Schiff base ligand derived from semicarbazide hydrochloride and shown their microbial activity. Recently, we have synthesized Mn(II), Fe(II), Co(II) and Sn(II) complexes of Schiff base ligand [23] and Cr(III), Sn(II) complexes of N,O coordinating novel Schiff base ligand [24] also investigated their thermal and antibacterial activity. Keeping these facts in view the significance of transition metal complexes in biological system, we here in report the synthesis and characterization with antibacterial activity of two novel Schiff base ligands and their Cu(II) complexes.

MATERIALS AND METHODS

Reagents and chemicals

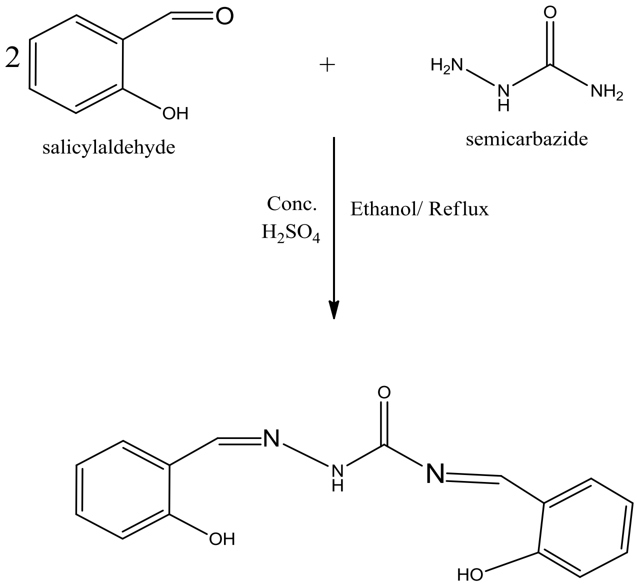
All the reagents used were of AnalaR grade or chemically pure grade. All metal (II) salts were used as chloride and sulphate. The solvents such as ethanol, methanol, chloroform, diethyl ether, petroleum ether, DMSO (dimethyl sulfoxide), dichloromethane and acetonitrile were purified by distillation procedure.

Physical measurement

The melting point or the decomposition temperature of all the prepared ligand and metal complexes were observed in an electro thermal melting point apparatus model No.AZ6512. Vibrational spectra (IR) were recorded with SHIMADZU-8400, FTIR spectrophotometer, (Japan), in the range 4000-400 cm^{-1} with a KBr disc as reference. UV-Visible spectra of the complexes in DMF (0.0005 molar) were recorded in the region 200-800 nm on a THERMOELECTRON NICOLET evolution 300 UV-Visible spectrophotometer. The SHERWOOD SCIENTIFIC Magnetic Susceptibility Balance that following the Gouy Method were used to measure the magnetic moment of the solid complexes. The electrical conductance measurements were made at room temperature in freshly prepared solution (10^{-3} M) in DMF using a WPACM35 conductivity meter and a dip-cell with a platinum electrode. The thermogravimetric analysis (TGA) was performed on Perkin Elmer Simultaneous Thermal Analyzer, STA-8000. The purity of the ligand and metal complexes was tested by Thin Layer Chromatography (TLC).

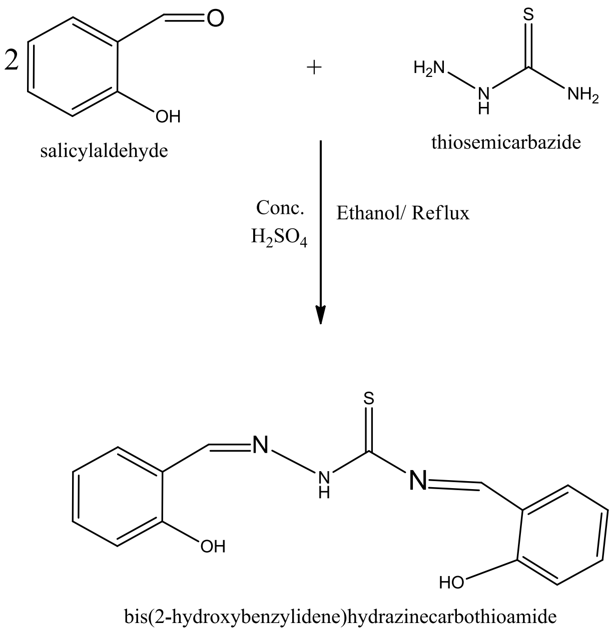
Preparation of Schiff base ligand HL^1 and HL^2

Salicyaldehyde (20 mmol, 2.4 mL) dissolved in absolute ethanol/(20 mL) was added slowly to a constant stirring solution of semicarbazide (0.75 g,10 mmol)/thiosemicarbazide (0.91 g, 10 mmol) in 20 mL hot ethanol in a two necked round bottom flask with 5 ml of conc. H_2SO_4 and the mixture was refluxed for 6 h. On cooling, a solid orange product was formed which was filtered, washed with ethanol and diethyl ether and dried in vacuum over anhydrous $CaCl_2$. The synthesized reaction of ligand and complexes were monitored by TLC using petroleum ether, ethyl acetate, toluene and methanol solvents. The product was found to be soluble in methanol, DMF and DMSO and insoluble in ethanol, acetone, diethyl ether, petroleum ether and isopropanol. The synthesis route of Schiff base is shown in **Schemes 1 and 2** respectively.



bis(2-hydroxybenzylidene)hydrazinecarboxamide

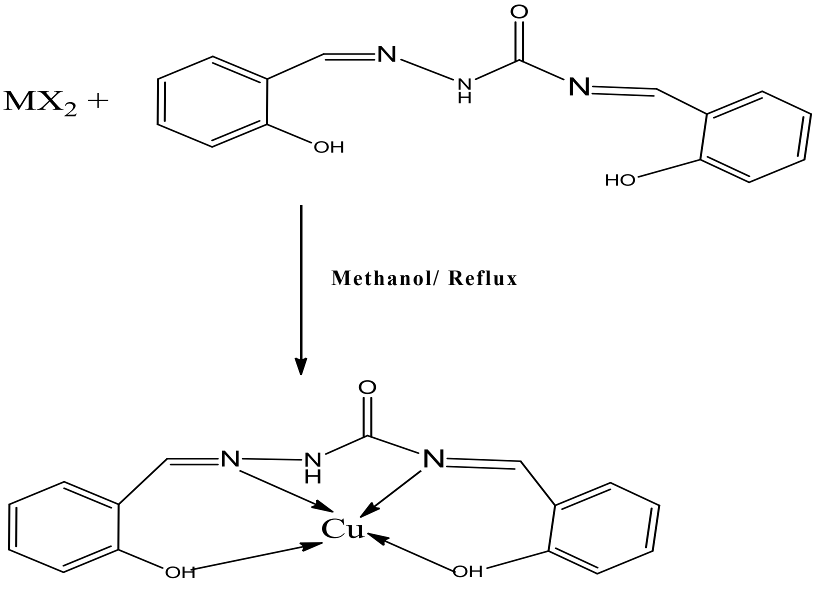
Scheme 1: Synthesis pathway of Schiff base HL^1 ($C_{15}H_{11}N_3O_3$).



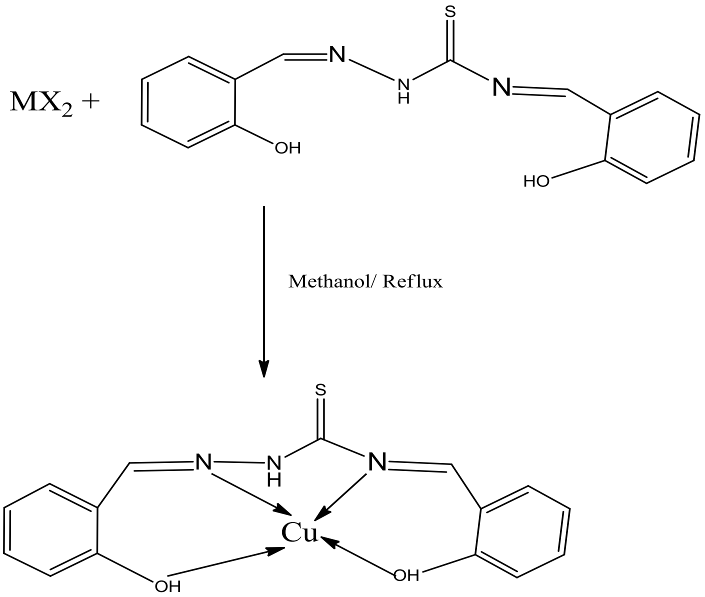
Scheme 2: Synthesis pathway of Schiff base HL^2 ($C_{15}H_{11}N_3O_2S$).

Preparation procedure of Schiff base metal complexes

The complexes have the general formula $[Cu(HL^1)/HL^2]$; where HL^1 =Synthesized Schiff base ligand $C_{15}H_{11}N_3O_3$ and HL^2 = Synthesized Schiff base ligand $C_{15}H_{11}N_3O_2S$. Methanolic solution 20 mL of (HL^1, 0.562 g, 2 mmol/ HL^2,0.594g, 2 mmol)was taken in a two necked round bottom flask and kept on magnetic stirring. A warm methanolic solution (10 mL) of $Cu(NO_3)_2.6H_2O$ (0.482 g, 2 mmole) was added drop wise and stirred with heating for 4 h. On cooling, precipitates were formed which were filtered, washed with ethanol, acetone, and diethyl ether and dried in vacuum desiccators over anhydrous $CaCl_2$. The purity of each complexes were tested by TLC using petroleum ether, ethyl acetate, toluene and methanol solvents. The complexes were soluble in DMSO and DMF. The synthesis pathway of complexes is shown in **Schemes 3 and 4**.



Scheme 3: Synthesis route of $[C_{15}H_{11}CuN_3O_3]$ complex.



Scheme 4: Synthesis route of [$C_{15}H_{11}CuN_3O_2S$] complex.

RESULTS AND DISCUSSION

Physical properties

Some physical properties of the Schiff base ligand and its metal complexes such as melting point, color, magnetic moment etc. are shown in **Table 1**. The complexes are intensely colored, powdered solids, which decomposes above 300°C. Molar conductance values in DMSO (10^{-3} M) showed low values (25-41 µS/cm) indicating [25] them to be non-electrolyte.

Table 1: Physical and analytical data of the Schiff bases and metal complexes.

Compound/Mol. formula	Color	Yield (%)	Melting Point/^{0}C	Conductivity /(µS/cm)
HL^1 $C_{15}H_{11}N_3O_3$	Orange	59	195	25
HL^2 $C_{15}H_{11}N_3O_2S$	Gold	62	205	35
Cu- HL^1 [$C_{15}H_{11}$ CuN_3O_3]	Saddle brown	65	>300	38
Cu- HL^2 [$C_{15}H_{11}CuN_3O_2S$]	Coffee	60	>300	41

Selected ^{1}H NMR spectral component of ($C_{15}H_{11}N_3O_2S$)

^{1}H-NMR spectra of Schiff base ligand ($C_{15}H_{11}N_3O_2S$) was recorded in DMSO-d_6 on a Bruker 300 MHz spectrophotometer using TMS as internal standard δ 6.786(s OH), δ 6.803(s CH), δ 8.303(s NH), δ 7.173-7.221(t, ph-H), 7.777-7.846 (d, ph-H),

Infrared spectral analysis

IR-Spectral analysis of Schiff base $C_{15}H_{11}N_3O_3$ and [$C_{15}H_{11}CuN_3O_3$] complex:

The strong band 1627 cm^{-1} that was assigned to ν(CH=N) in the Schiff base ligand (**Figure 1**) indicated that the free-NH_2 group of semicarbazide was converted to azomethine group. The appeared of band 3445 cm^{-1} that was assigned to ν(O-H) in free Schiff base ligand confirmed that hydroxyl group of salicylaldehyde was present in Schiff base. The strong band 1697 cm^{-1} for ν(C=O) indicated that C=O bond for amide group was present in the Schiff base ligand. In $C_{15}H_9CuN_3O_3$ complex the band 3445 cm^{-1} for ν(O-H) in free Schiff base ligand was shifted to lower absorption frequency revealed that it was coordinated to metal atom (**Figure 2**). The shifting of bands from 1627 cm^{-1} to lower absorption frequency suggested that CH=N group was also coordinated to metal atom.

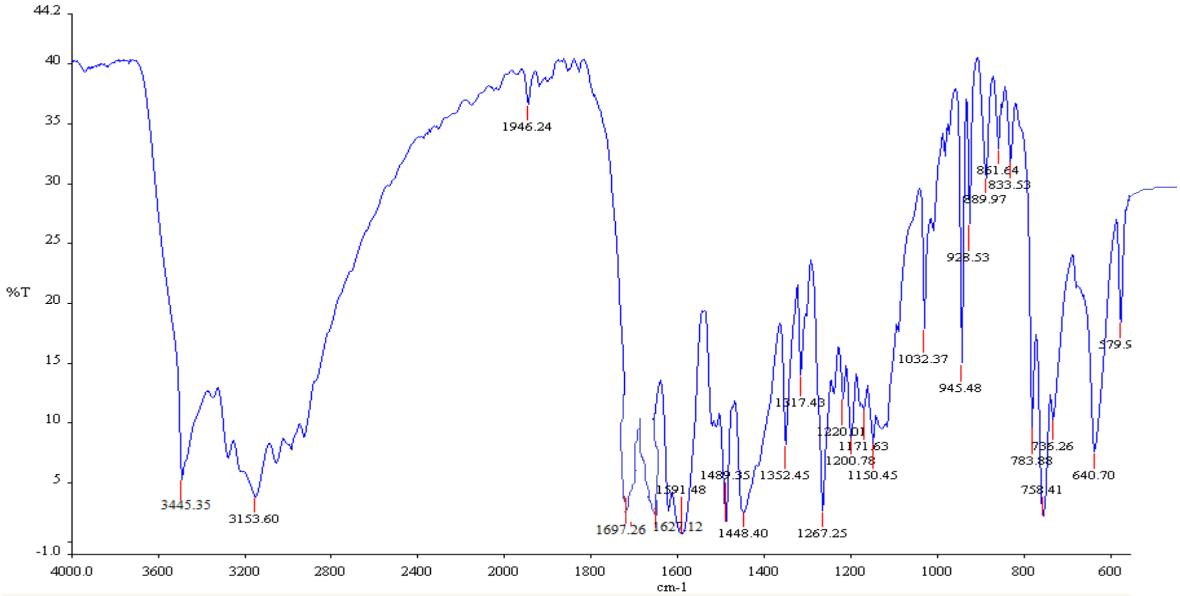


Figure 1: IR Spectra of Schiff base ligand HL1 ($C_{15}H_{11}N_3O_3$).

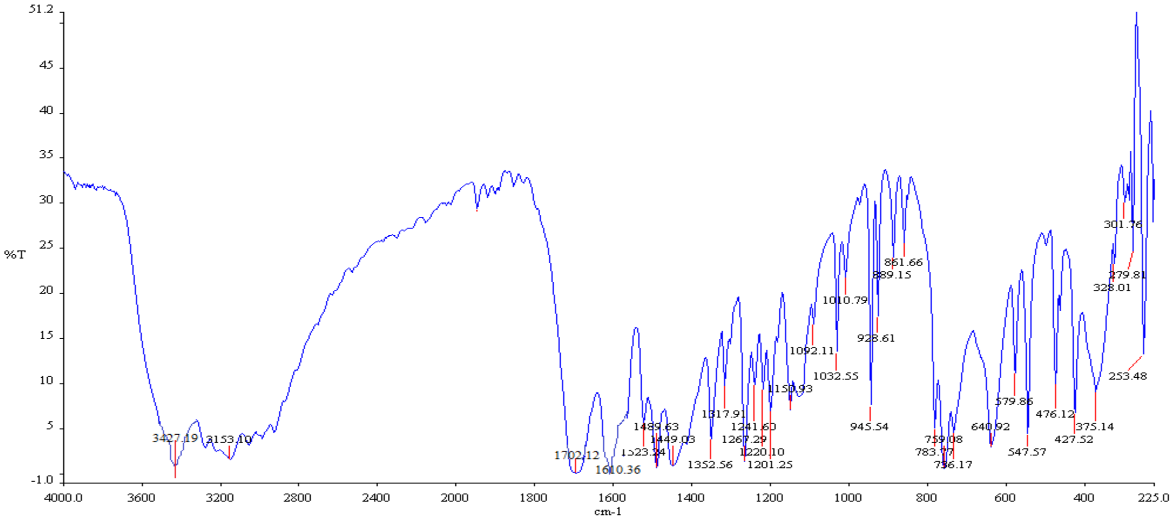


Figure 2: IR spectra of Cu(II) complex of Schiff ligand HL[1] ($C_{15}H_{11}CuN_3O_3$).

IR-Spectral analysis of Schiff base $HL^2(C_{15}H_{11}N_3O_2S)$ and $[C_{15}H_{11}CuN_3O_2S]$ complex

The IR-absorption bands 3371 and 3280 cm^{-1} was due to free -NH_2 group of thiosemicarbazide (**Figure 3**) was absent in the free Schiff base ligand (**Figure 4**). The strong band 1615 cm^{-1} that was assigned to ν(CH=N) in the Schiff base ligand represented that free -NH_2 group of thiosemicarbazide was converted to azomethine group. The appeared of band 3468 cm^{-1} that was assigned to ν(O-H) in Schiff base confirmed that hydroxyl group of salicylaldehyde was present in ligand. The strong band 1266 cm^{-1} for ν(C=S) indicated that C=S bond was present in the Schiff base ligand [26]. During complexation the band 3468 cm^{-1} for ν(O-H) shifted to lower absorption frequency evident that it was coordinated to metal atoms. The shifting of bands from 1615 cm^{-1} to lower absorption frequency suggested that CH=N group was coordinated to central metal atom. Also the appearance of new low absorption bands at (468-494), (317-389) that was assigned to ν(M-N), ν(M-O) respectively [27] confirmed that O and N atoms are coordinated to central metal atoms. IR spectra of $C_{15}H_{11}CuN_3O_2S$ complex was shown in **Figure 5**. IR spectral data of Schiff base and metal complexes were shown in **Table 2**.

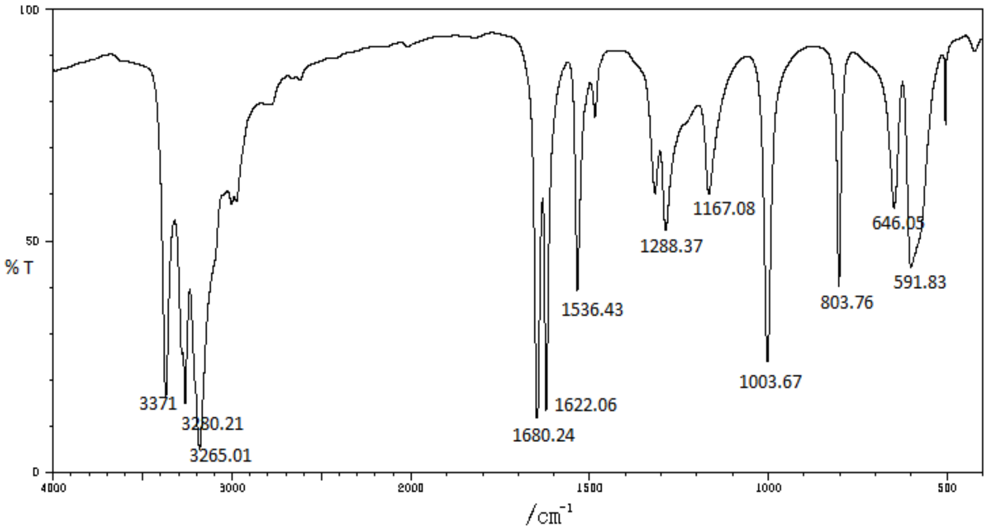


Figure 3: IR spectra of thiosemicarbazide.

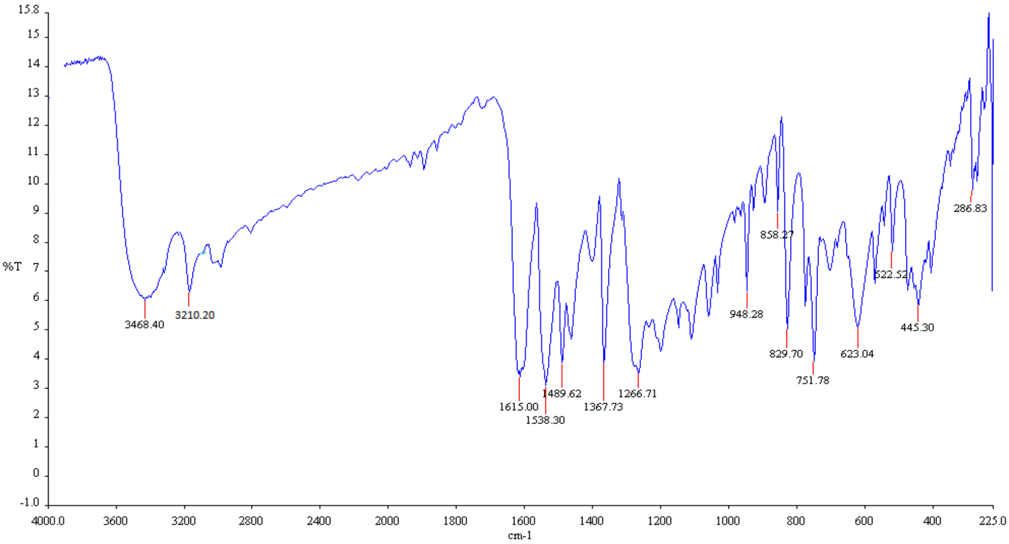


Figure 4: IR spectra of Schiff base ligand HL^2 ($C_{15}H_{11}N_3O_2S$).

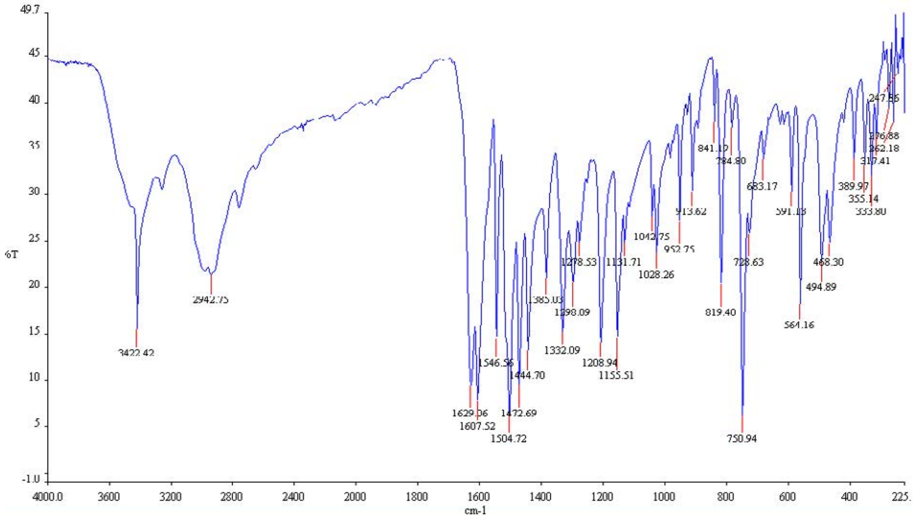


Figure 5: IR spectra of Cu(II) complex of Schiff ligand HL^2 ($C_{15}H_{11}CuN_3O_2S$).

Table 2: Selected IR spectral data of the Schiff base and metal complexes.

Ligand/complex	IR(cm^{-1})			
	ν(O-H)	ν(C=N)	ν(M-N)	ν(M-O)
($C_{15}H_{11}N_3O_3$)	3445	1627	-	-
($C_{15}H_{11}N_3O_2S$)	3468	1615	-	-
($C_{15}H_{11}CuN_3O_3$)	3427	1610	476	375
[$C_{15}H_{11}CuN_3O_2S$]	3422	1607	494	389

Magnetic moment and UV-visible spectral analysis

The spectra of both Schiff base ligand and their metal complexes [$C_{15}H_{11}CuN_3O_3$] and [$C_{15}H_{11}CuN_3O_2S$] were taken in DMSO. At room temperature magnetic moment value of Cu(II) complex HL^1 and HL^2 was found to 1.04 B.M and 1.10 B.M respectively, representing one unpaired electron per Cu(II) ion. The [$C_{15}H_{11}CuN_3O_3$] complex showed d-d transition at 350 and 500 nm (**Figure 6**). The [$C_{15}H_{11}CuN_3O_2S$] showed d-d transitions at 410 and 300 nm (**Figure 7**) which indicated the square planner structure of the complex.

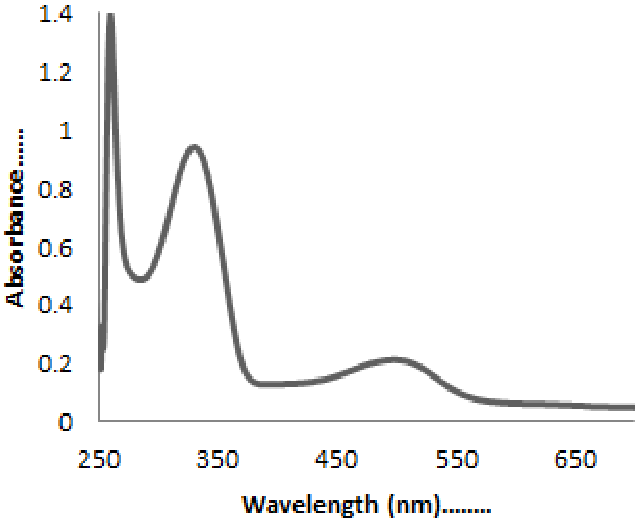


Figure 6: UV-Visible Spectra of [$C_{15}H_{11}CuN_3O_3$]complex.

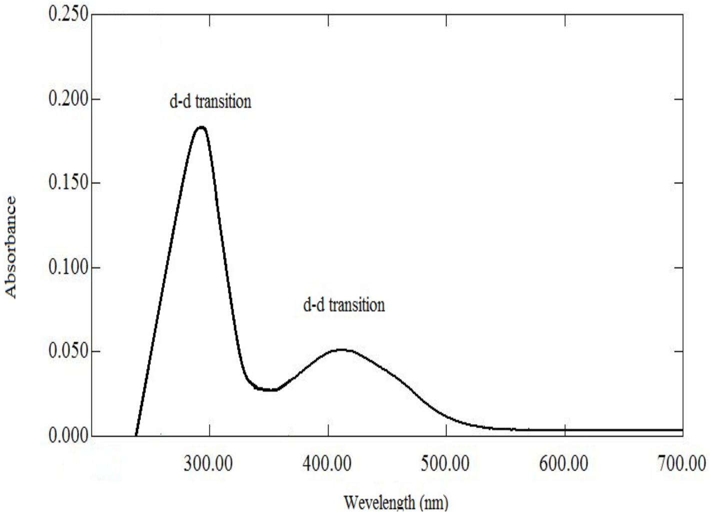


Figure 7: UV-Visible Spectra of [$C_{15}H_{11}CuN_3O_2S$] complex.

Thermogravimetric analysis

TGA was carried out for solid [$C_{15}H_{11}CuN_3O_3$] and [$C_{15}H_{11}CuN_3O_2S$] metal complexes under N_2 flow. Thermal data of the complexes were shown in **Table 3**. The heating rate was suitably controlled at 30°C min^{-1} and the weight loss was measured from the ambient temperature up to 800°C. The TGA and DTG curve of [$C_{15}H_{11}CuN_3O_2S$] complex was shown in **Figure 8**, the curve indicated that the complex was decomposed into 3 main steps. Where, the 1st step involves the removal of $C_{12}H_8O_2$ (calculated 53.25%, experimental 52.12% weight) at temperature range 180-320^{0}C [23,24,28]. The part of complex -NH_3S- was decomposed between temperature range 400-580^{0}C (calculated 21.37%, experimental 20.25% weight) at 2nd Step of decomposition. In 3rd step the complex is completely decomposed and removed as CuO at above 670^{0}C. The TGA and DTG curve of [$C_{15}H_{11}CuN_3O_3$] complex was shown in **Figure 9**. The curve indicated that the $C_{15}H_9CuN_3O_3$ complex was decomposed into 3 or 4 main steps. The major fragmentation occur at 280-330^{0}C temperature range involves the decomposition of the part of complex $C_{12}H_8O_2$ (calculated 55.75%, experimental 53.12% weight). The other part of complex -NH_3O- was decomposed between temperature range 400-540^{0}C (calculated 24.09%, experimental 21.20% weight) at 2nd Step of decomposition. The complex is completely decomposed and removed as CuO at above 520^{0}C.

Table 3: Thermal data of [$C_{15}H_{11}CuN_3O_3$] and [$C_{15}H_{11}CuN_3O_2S$] complexes.

Complexes	Steps	Temperature Range/ °C	TG mass loss% calc./found	Assignments
[$C_{15}H_{11}CuN_3O_2S$]	1st 2nd 3rd	180-320 400-580 >670	53.25/52.12 21.37/20.25 23.01/22.12	$C_{12}H_8O_2$ NH_3S- CuO
[$C_{15}H_{11}CuN_3O_3$]	1st 2nd 3rd	280-330 405-650 >580	55.75/53.12 24.09/21.20 26.33/24.45	$C_{12}H_8O_2$ -NH_3O- CuO

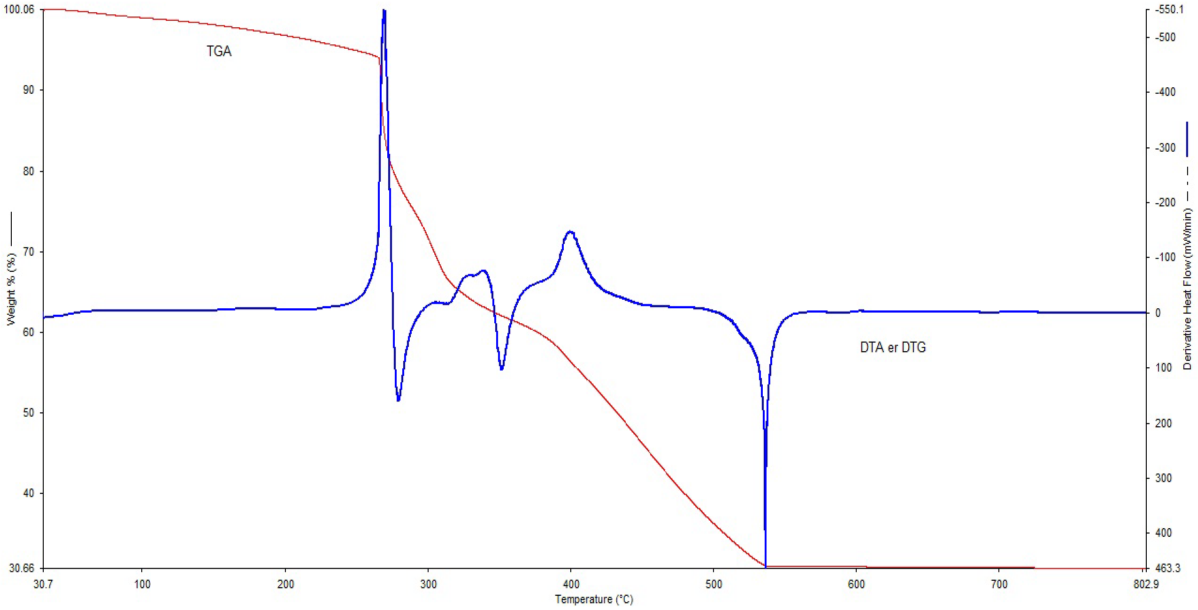


Figure 8: TGA and DTG curve of [$C_{15}H_{11}CuN_3O_2S$]..

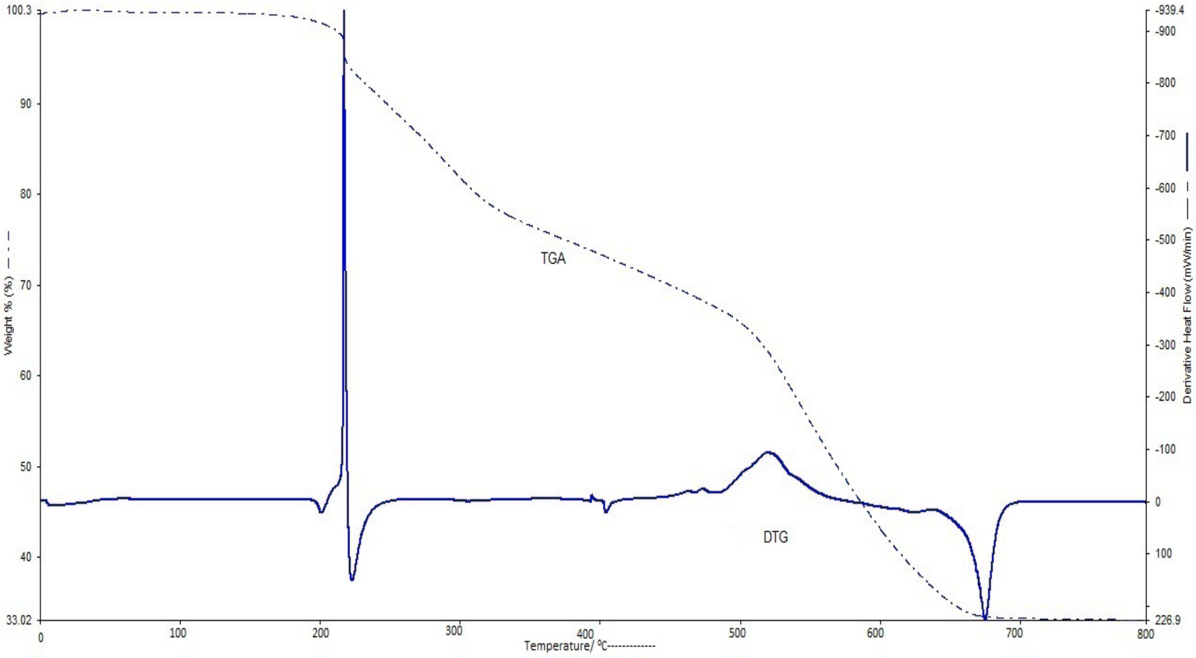


Figure 9: TGA and DTG curve of [$C_{15}H_{11}CuN_3O_3$]

Antibacterial activity

The prime objective of performing the antibacterial screening is to determine the susceptibility of the pathogenic microorganism to test the compound which, in turn is used to selection of the compound as a therapeutic agent. The free Schiff base ligand and their metal complexes were screened for their antibacterial activity against strains the *Bacillus cereus* ATCC25923*, Escherichia coli* ATCC 25922, *Shigella sonnei*, *Shigella boydii, Enerobacter* and *Salmonella typhinium* of 4500. The compounds were tested at a concentration of 50 µg/0.01 mL in DMSO solution using the paper disc diffusion method [29-31]. The susceptibility zones (**Figures 10 and 11**) were measured in diameter (mm) and the result are listed in **Table 4**. The susceptibility zones were the clear zones around the discs killing the bacteria. Copper(II) complex of the Schiff base ligand HL^2 exhibited more inhibitory effects than that of Cu-HL^1 complex on the growth of tested bacterial species. The graphical representation of antibacterial activity of Cu-HL^1 and Cu-HL^2 complexes against seven pathogenic bacteria with kanamycin standard was shown in **Figure 12**.

Table 4: Antibacterial screening activity of Schiff base and metal complexes.

Tested Bacteria	Diameter of zone inhibition(mm) of tested compounds				Kanamycin (30 µg/disc)
	HL^1	HL^2	Cu- HL^1	Cu- HL^2	
Bacillus cereus	11	8	12	17	20
Bacillus subtilis	10	9	10	18	25
E.coli	-	8	11	17	30
Shigella sonnei	8	7	10	18	26
Shigella boydii	9	-	12	20	25
Enterobacter	-	10	8	20	25
Salmonella typhinium	10	7	11	18	30
DMSO control	-	-	-	-	30
Where, HL^1=$C_{15}H_{11}N_3O_3$ and HL^2= $C_{15}H_{11}N_3O_2S$					

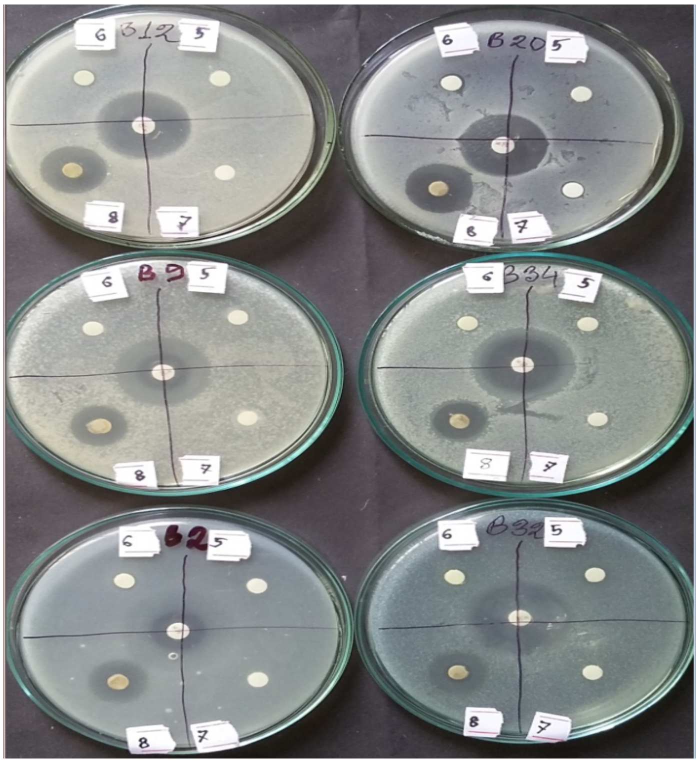


Figure 10: Photographic representation of zone of inhibition of $C_{15}H_{11}CuN_3O_3$ complex against *Bacillus cereus* ATCC25923, *Escherichia coli* ATCC 25922, *Shigella sonnei*, *Shigella boydii, Enterobacter* and *Salmonella typhinium* of 4500 bacterial species.

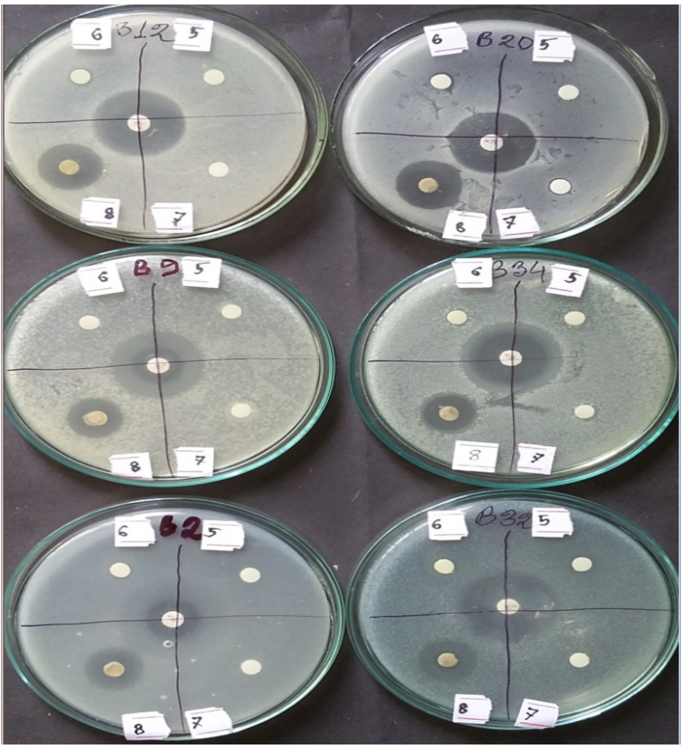


Figure 11: Photographic representation of zone of inhibition of $[C_{15}H_{11}CuN_3O_2S]$ complex against *Bacillus cereus* ATCC25923, *Escherichia coli* ATCC 25922*, Shigella sonnei, Shigella boydii, Enerobacter* and *Salmonella typhinium* of 4500 bacterial species.

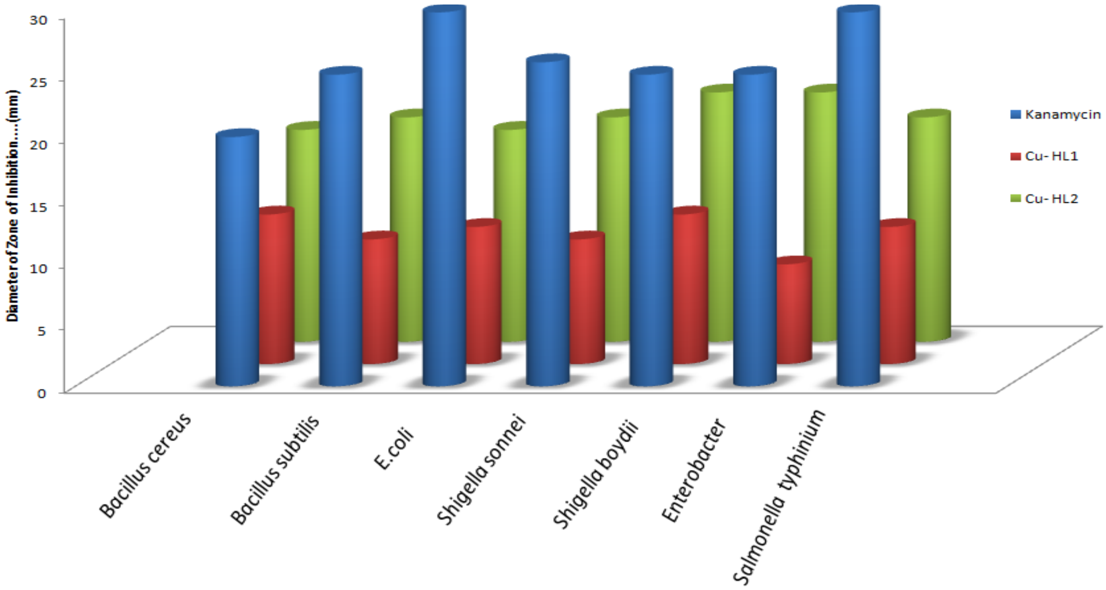


Figure 12: Graphical Representation of Antibacterial activity of Cu-HL[1] and Cu-HL[2] complexes against seven pathogenic bacteria with kanamycin standard.

CONCLUSION

In this paper we have explored the synthesis and coordination chemistry of Cu(II) complex with two new Schiff base ligand bis(2-hydroxybenzylidene)hydrazinecarboxamide and bis(2-hydroxybenzylidene)hydrazinecarbothioamide derived from the condensation reaction of salicylaldehyde with semicarbazide and thiosemicarbazide respectively. The physicochemical analysis indicated the formation of four coordinated metal complexes. IR spectral analysis indicated that N and O atoms were coordinated to central metal atom. Magnetic moment, UV-Visible and Thermogravimetic analysis confirmed the proposed structure of metal complexes. TGA analysis indicated that all the complexes are thermally stable up to 200^0C. Biological activity revealed that the Cu- HL^2 complex had more antibacterial activity as compared to Cu- HL^1 complex. The metal complexes were more antibacterial activity than its free Schiff base ligand.

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CONFLICT OF INTEREST

The authors have no conflict of interest to publish the article.

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