



## Synthesis, characterization and antibacterial activity of Cu(II) and Fe(III) complexes of a new tridentate Schiff base ligand

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### ABSTRACT

Copper (II) and Iron (III) complexes of Schiff base ligand namely (Z)-3-hydroxy-4-((5-methylthiophen-2-yl) methyleneamino) benzoic acid derived from the condensation of 5-methyl-2-thiophene carboxaldehyde with 4-amino-3-hydroxy benzoic acid were synthesized. The Schiff base and its metal complexes were structurally characterized based on elemental analysis, molar conductance, IR, UV-Visible and EPR spectral and thermal (TG-DTA) analyses. The FTIR spectra showed that the ligand is coordinated to the metal ions in a tridentate manner through azomethine nitrogen, thiophene sulphur and phenolic OH groups. The molar conductance measurements of the complexes in DMF showed non-electrolytic nature. From the EPR spectra an octahedral geometry has been proposed for Cu(II) complex. The synthesized Schiff base ligand and its metal complexes were screened for their biological activity against bacterial species, two Gram -ve bacteria (*Escherichia coli*, *Enterobacter aerogenes*) and one Gram +ve bacteria (*Staphylococcus aureus*). The activity data showed that the metal complexes are more potent /antibacterial than the parent Schiff base ligand. It has also been observed that concentration of compounds played an important role by increasing the degree of inhibition as the concentration increases.

**Keywords:** Schiff base, EPR spectra, Thermal analysis, Bioassay.

### INTRODUCTION

Schiff bases, an important class of ligands plays an important role in the development of coordination chemistry as they can easily form stable complexes with most of the transition metals [1]. Metal complexes of the Schiff bases possess numerous applications including antibacterial, antifungal [2-5] and other biological applications, as well as clinical, analytical and industrial in addition to their important roles in catalysis [6-8]. It is known that N and S atoms play a key role in the coordination of metals at the active sites of numerous metallo-biomolecules. Metallo-organic chemistry is becoming an emerging area of research due to the demand for new metal based antibacterial and antifungal compounds [9, 10]. It is known that thiophene derivatives exhibit intensive antibacterial and antitumor activities [11, 12]. Also metal complexes of Schiff bases with heterocyclic compounds find applications as potential drugs [13]. Keeping in view of the above facts, we have synthesized a new Schiff base, thiophene carboxaldehyde derivative viz (Z)-3-hydroxy-4-((5-methylthiophen-2-yl) methyleneamino) benzoic acid and its Cu(II) and Fe(III) metal complexes. The Schiff base and its metal complexes were characterized and screened for the biological activity. The complexes exhibited higher lethal effect on bacteria than their parent ligand.

## EXPERIMENTAL SECTION

**Materials and physical measurements**

All the chemicals and solvents used were of analar grade. The IR spectra of the compounds were recorded using a Thermo-Nicolet FT-IR, Nicolet-200 USA spectrophotometer as KBr discs. Electronic spectra of the compounds in DMF solution were recorded with Shimadzu UV 1800 spectrophotometer. Molar conductance ( $10^{-3}\text{M}$ ) of the complexes in DMF solution was measured using Elico Conductivity bridge at room temperature. EPR spectra of the Cu(II) complex was recorded as polycrystalline sample at room temperature using Geol XA 200 spectrometer. TGA analysis was carried out under nitrogen atmosphere with a heating rate  $10^{\circ}\text{C}/\text{min}$  using a SDT Q 600 V 20.9 Build 20 analyzer instrument.

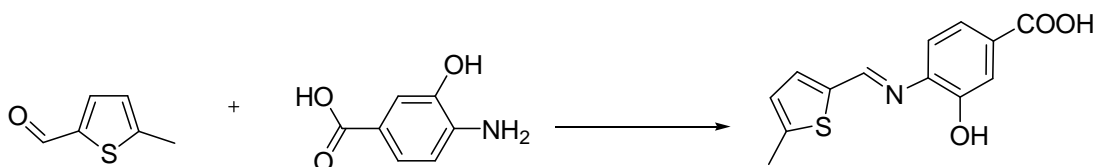
**Synthesis of ligand**

The scheme of preparation of ligand, (Z)-3-hydroxy-4-((5-methylthiophen-2-yl) methyleneamino) benzoic acid is shown in (Fig.1). A mixture of 4-amino-3-hydroxy benzoic acid (0.05mol) and 5-methyl-2-thiophene carboxaldehyde (0.05mol) was taken in methanol and refluxed for 4 hrs at a temperature of  $50\text{-}60^{\circ}\text{C}$ . The yellow coloured precipitate obtained was removed by filtration, washed several times with ethanol and dried under vacuum over  $\text{P}_4\text{O}_{10}$ .

Colour: Yellow, Yield: 80-85 %, Elemental analysis, found (calcd.) for  $\text{C}_{13}\text{H}_{11}\text{NO}_3\text{S}$ : C, 59.59 (59.76); H, 4.20 (4.24); N, 5.20 (5.36); O, 18.37 (18.12); S, 12.19 (12.27) %. IR data (KB,  $\text{cm}^{-1}$ ); 3325  $\nu(\text{O-H})$ , 1585  $\nu(\text{C=N})$ , 765  $\nu(\text{C-S-C})$ .  $^1\text{H-NMR}$  ( $\text{DMSO-d}_6$ );  $\delta$ 12.53 (broad 1H, -COOH),  $\delta$ 9.459 (broad 1H, Ar-OH),  $\delta$ 8.698 (1H, s, azomethine),  $\delta$ 7.521-7.395 (3H, Aromatic individual peaks),  $\delta$ 7.109-6.931 (2H, thiophene ring d, d d),  $\delta$ 2.527-2.502 (3H, s,  $\text{CH}_3$  group of thiophene).

UV-Vis (DMF, nm); 246, 309, 410.

Figure 1. Synthesis of ligand.

**Synthesis of metal complexes**

The metal complexes were synthesized by the following general procedure. Hot methanolic solution of the ligand (0.1mol) and hot methanolic solution of corresponding metal salts (0.05mol) ( $\text{MX}_2/\text{X}_3$  Where M= Cu(II), Fe(III); X=chlorides) were mixed together with constant stirring. The mixture was refluxed for 2-3 hrs at  $50$  to  $60^{\circ}\text{C}$ . On cooling, coloured solid metal complexes were precipitated out. The products were filtered, washed with cold methanol and dried. .

**1.  $\text{CuL}_2$** 

Colour: Bluish green, Yield: 76 %, m.p.  $>300^{\circ}\text{C}$ . Anal. Calc. for  $\text{C}_{26}\text{H}_{20}\text{CuN}_2\text{O}_6\text{S}_2$  found (calcd.); C, 53.41(53.46); H, 3.40 (3.45); N, 4.79 (4.80); O, 16.39 (16.43); S, 10.88 (10.98); Cu, 10.86 (10.88) %. FT-IR (KBr,  $\text{cm}^{-1}$ ); 3157  $\nu(\text{O-H})$ , 1550  $\nu(\text{C=N})$ , 746  $\nu(\text{C-S-C})$ .

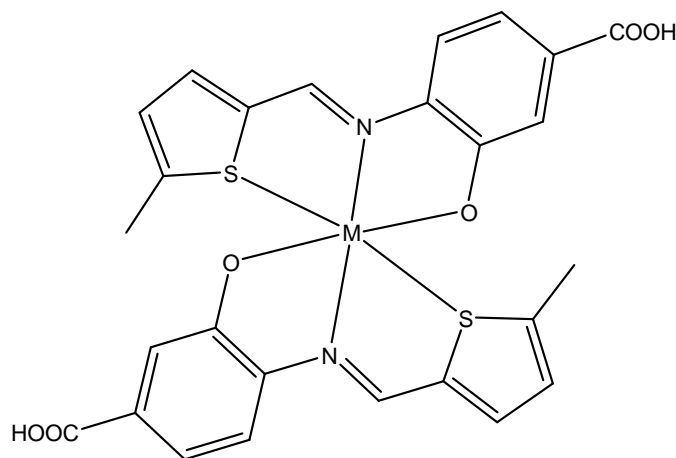
UV-Vis (DMF, nm); 206, 252, 379, 417.

**2.  $\text{FeL}_2$** 

Colour: Brown, Yield: 78 %, m.p.  $250^{\circ}\text{C}$ . Anal. Calc. for  $\text{C}_{26}\text{H}_{20}\text{FeN}_2\text{O}_6\text{S}_2$  found (calcd.); C, 54.12 (54.18); H, 3.49 (3.50); N, 4.78 (4.86); O, 16.61 (16.65); S, 11.11 (11.13); Fe, 9.63 (9.69) %. FT-IR (KBr,  $\text{cm}^{-1}$ ); 3310  $\nu(\text{O-H})$ , 1535  $\nu(\text{C=N})$ , 769  $\nu(\text{C-S-C})$ .

UV-Vis (DMF, nm); 213, 367, 441.

Figure 2. Proposed structure for metal complexes. Where, M = Cu, Fe.



### Antibacterial assay

The ligand and its complexes were tested against the bacterial species *Escherichia coli* (Gram –ve), *Staphylococcus aureus* (Gram +ve) and *Enterobacter aerogenes* (Gram –ve). These studies were carried out using Tetracycline as standard antibacterial agent by diffusion method [14, 15]. All strains were isolated from patients in SVS Medical college and hospital, Mahabubnagar, India. The identity of all the strains was confirmed. The test solutions were prepared in DMSO. A bacterial suspension was prepared from overnight cultures (12 hr) in Nutrient Broth (Himedia) and turbidity was adjusted equivalent to 0.5 McFarland Units (approximately  $10^8$  cfu/ml). Aliquots (100  $\mu$ L) of inoculums were spread over the surface of Nutrient Agar (Himedia) plates with a sterile disposable plastic spreader (Himedia). Sterilized paper discs were wetted in 10  $\mu$ L of each compound to be tested in the concentration of 2.0, 1.0, 0.5 mg/10  $\mu$ L in DMSO. The plates were then incubated for 24 h at 37  $^{\circ}$ C in Bacteriological incubator (Thermo Lab). Antibacterial activity was indicated by the presence of clear inhibition zones around the samples and the zones of inhibition formed were measured in mm.

### RESULTS AND DISCUSSION

The analytical and physical data of the Schiff base ligand and its complexes are presented in Table 1. The complexes are soluble in DMF and DMSO and are insoluble in some common organic solvents. The analytical data clearly show that the metal to ligand ratio is 1:2 in all the complexes. The composition of the complexes is  $ML_2$ , where (M = Cu(II) and Fe(III) and L = ligand). The low molar conductance values (Table 1) of  $10^{-3}$  M solutions in DMF showed that the complexes are non-electrolytes.

Table 1 : Analytical data and other details of the ligand and metal complexes

S. No.	Molecular formula	Formula weight	Colour % yield	m.p $^{\circ}$ C	Elemental Analysis % Found (calculated)						Molar conductance ( $\text{ohm}^{-1}\text{mol}^{-1}\text{cm}^2$ )
					C	H	N	O	S	M	
1.	HL	261.30	Yellow 73%	126	59.69 (59.76)	4.11 (4.25)	5.29 (5.36)	17.97 (18.37)	12.21 (12.27)		
2.	Cu(L) <sub>2</sub>	584.12	Bluish green 76%	> 300	53.41 (53.46)	3.40 (3.45)	4.79 (4.80)	16.39 (16.43)	10.88 (10.98)	10.86 (10.88)	16
3.	Fe(L) <sub>2</sub>	576.42	Brown 68%	250	54.12 (54.18)	3.49 (3.50)	4.78 (4.86)	16.61 (16.65)	11.11 (11.13)	9.63 (9.69)	21

Figure 3(a). IR spectrum of the ligand

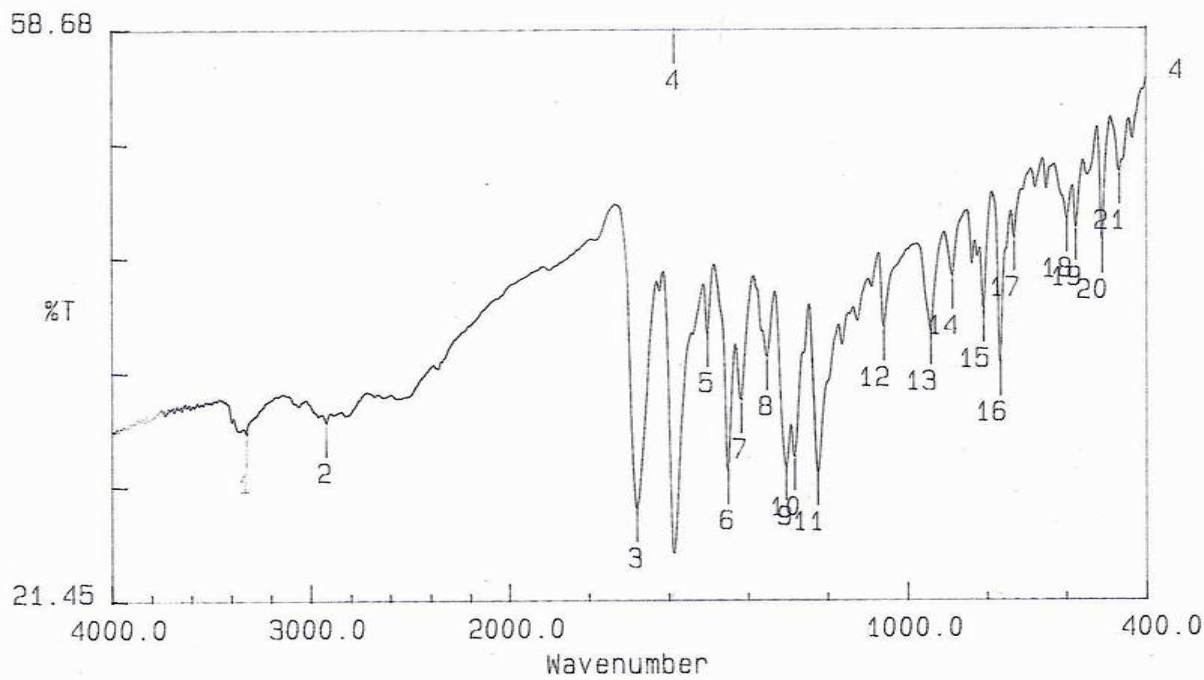
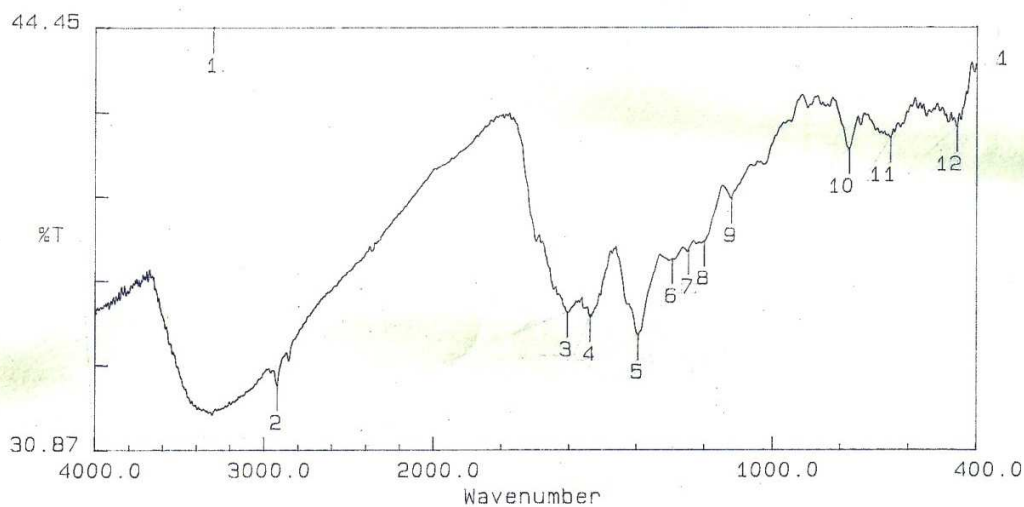


Figure 3(b). IR spectrum of the Fe(III) complex.



### Infrared spectra

The IR spectral data of the ligand and its complexes are presented in Table 2. The IR spectrum of the Schiff base ligand and a representative system of Fe(III) complex are shown in Fig. (3(a) and (b)). In the Schiff base ligand, the strong band observed at  $1584\text{ cm}^{-1}$  can be assigned to  $\nu(\text{C}=\text{N})$  azomethine stretching vibration [16]. On complexation, this band was shifted to lower frequency in the range  $1537\text{-}1550\text{ cm}^{-1}$ , indicating the coordination of azomethine nitrogen to the central metal ions. A broad band observed at  $3325\text{ cm}^{-1}$  due to phenolic  $-\text{OH}$  group of the free ligand was found to be absent in case of the complexes which gives a strong evidence for the ligand coordination around the metal ion in its deprotonated form. Thiophene ring (C-S-C) showed absorption band at  $780$

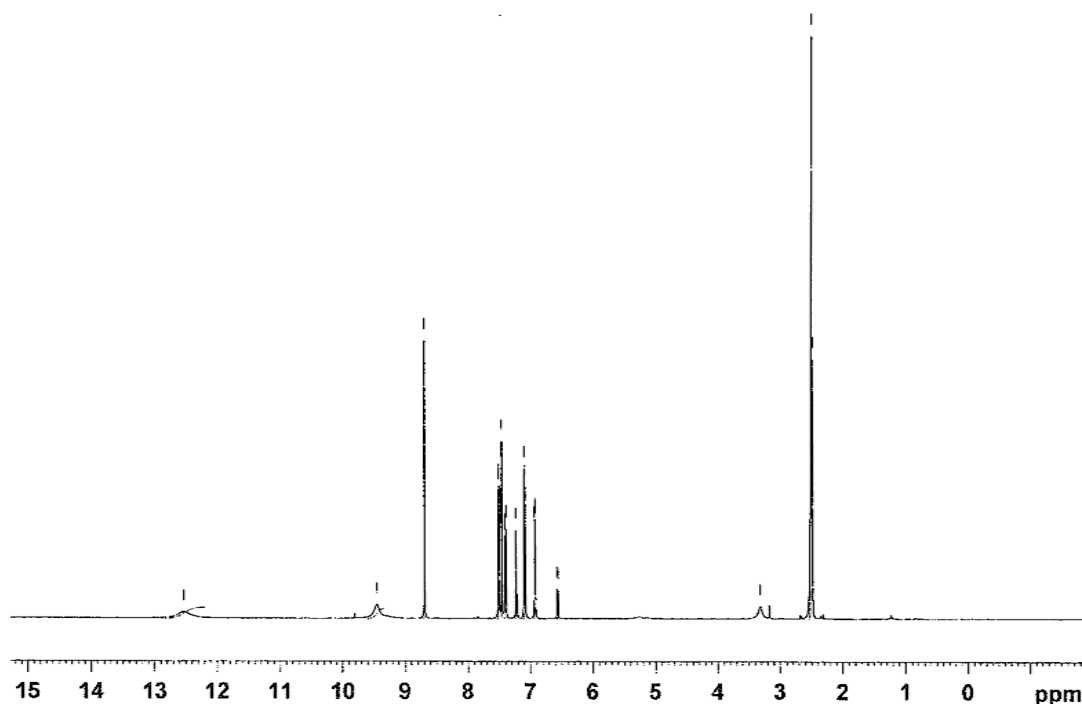
$\text{cm}^{-1}$  in the ligand spectra. This band shifted to lower frequency  $746 \text{ cm}^{-1}$  in case of Cu(II) complex and to higher frequency in Fe(III) complex, suggesting the participation of thiophene sulphur in complexation [17]. Coordination of azomethine nitrogen and phenolic OH groups are further confirmed by the presence of new bands in the region  $450\text{-}457 \text{ cm}^{-1}$  and  $547\text{-}652 \text{ cm}^{-1}$  which are assignable to  $\nu(\text{M-N})$  and  $\nu(\text{M-O})$  stretching vibrations. From the IR spectra, it may be concluded that the Schiff base ligand is tridentate and coordinates through the phenolic oxygen, azomethine nitrogen and thiophene sulphur.

**Table 2. IR-spectral data of the ligand and its metal complexes**

Compound	$\nu(\text{O-H})$	$\nu(\text{C=N})$	$\nu(\text{C-S-C})$	$\nu(\text{M-N})$	$\nu(\text{M-O})$
Ligand	3325	1585	765		
Cu(L) <sub>2</sub>	3157	1550	746	450	547
Fe(L) <sub>2</sub>	3310	1535	769	457	652

### <sup>1</sup>H NMR Spectra

The structure of ligand was confirmed by <sup>1</sup>H NMR spectra. The <sup>1</sup>H NMR spectra of ligand recorded in DMSO-d<sub>6</sub> solvent (Fig.3) showed well resolved signals for the protons present in the ligand. The spectrum exhibited broad signals at  $\delta$  9.459 and at 12.53 ppm which may be assigned to phenolic OH and carboxylic COOH protons respectively. A sharp singlet observed at  $\delta$  8.698 ppm may be assigned to the azomethine group. A singlet corresponding to the three protons of methyl groups of thiophene is appeared at  $\delta$  2.527-2.502 ppm. A doublet and doublet of doublet are appeared in the range at  $\delta$  7.109-6.931 ppm due to two protons of thiophene ring. Signals observed at  $\delta$  7.521-7.395 ppm are due to three protons of aromatic ring. In case of all the complexes, the protons due to azomethine and thiophene ring undergo shift towards downfield, indicating the coordination of these groups with the metal ions. And also signals due to hydroxyl group (OH) were found absent in the spectra of complexes indicating the deprotonation of the hydroxyl group and the involvement of oxygen atom in complexation.



**Figure 4. <sup>1</sup>H-NMR Spectrum of the ligand**

### EPR spectra

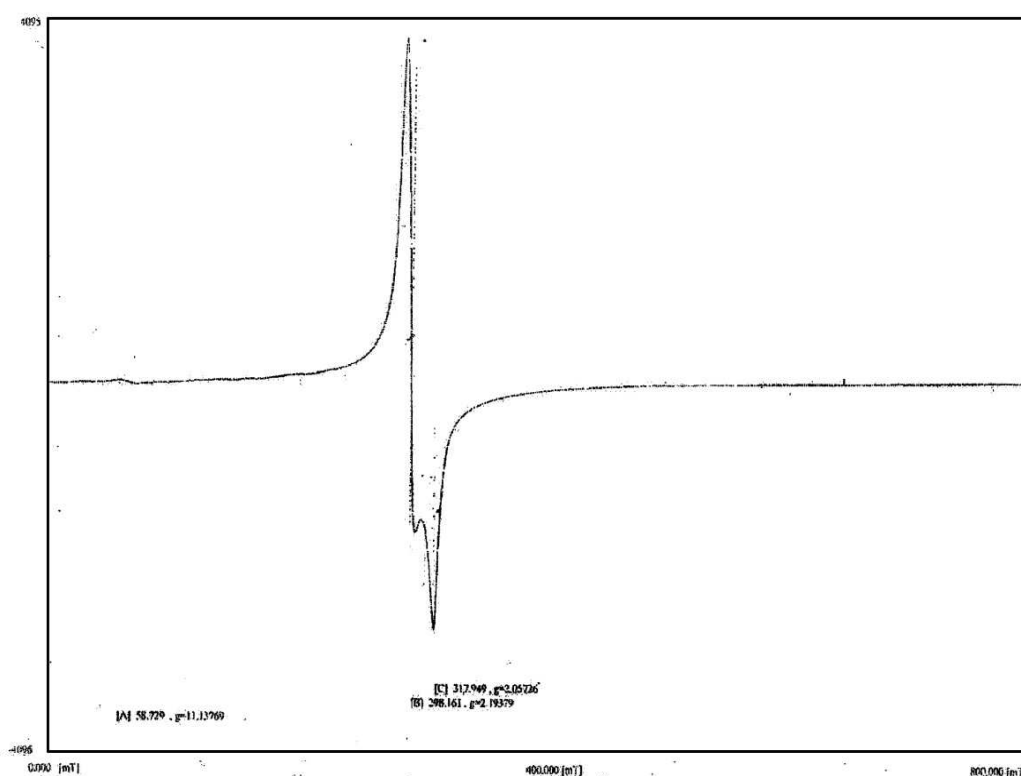
The EPR spectra of the complex provide information about the extent of delocalization of unpaired electron. The g-values calculated for Cu(II) complex from EPR spectra (Fig.4) are presented in Table 3. The geometric parameter G, which measures the exchange interaction between the copper centres in the polycrystalline sample for Cu(II)

complex has been calculated by using the expression  $G = (g_{11}-2)/(g_{\perp}-2)$ . According to Hathaway [18-20], if  $G > 4$ , the exchange interaction is negligible, if  $G < 4$ , it indicates considerable exchange interaction in the solid complexes. In case of Cu(II) complex, the calculated  $G$  value is less than four, indicating the existence of considerable exchange interaction in the solid complex. The trend  $g_{11} > g_{\perp} > g_e$  (2.0023) observed in case of Cu(II) complex showed that the unpaired electron is localized in  $d_{x^2-y^2}$  orbital of the Cu(II) ion [21]. The spectral studies revealed that the Cu(II) ion coordinated to the ligand is in an octahedral geometry as shown in fig 1.

**Table 3. EPR-spectral data of the metal complexes**

Compound	$g_{11}$	$g_{\perp}$	$G$	$g_1$	$g_2$	$g_3$
Cu(L) <sub>2</sub>	2.50	2.26	1.9313	-	-	-

**Figure 5. EPR spectrum of the Cu(II) complex.**



### UV-Vis spectra

Ligand exhibited bands in the region 246, 309 nm which may be attributed to  $\Pi-\Pi^*$  transitions of the thiophene ring and  $n-\Pi^*$  transitions of azomethine moiety respectively. In addition, a broad band at 410nm is due to the  $n-\Pi^*$  transition which is overlapping with the intermolecular CT from the phenyl ring to the azomethine ring [22]. In metal complexes, these transitions were found to be shifted to lower or higher energy region compared to the free ligand transitions confirming the coordination of the ligand to metal ions.

Electronic spectra of the Cu(II) complex displayed bands at 206, 252, 379 and 417nm which may be assigned to  $\Pi-\Pi^*$  transition of thiophene ring,  $L \rightarrow Cu$  charge transfer,  $n-\Pi^*$  transition of azomethine group and d-d transitions respectively.

Electronic spectra of Fe (III) complex displayed bands at 213, 367, 441nm. The first two bands are assigned to  $\Pi-\Pi^*$ ,  $n-\Pi^*$  transitions of thiophene ring and azomethine groups respectively. The band at 441nm can be assigned to the d-d transitions.

#### TG-DTA

The Thermo-gravimetry analysis (TG-DTA) of the complexes are carried out in the temperature range 0-800 °C with a sample heating rate 10 °C/min in a static nitrogen atmosphere. During the heating of the metal complexes, the TG-curves undergone a series of thermal changes associated with a weight loss of the samples. Cu(II) complex showed three steps of decomposition. First stage is observed at a temperature range of 50-110 °C which involves the loss of water molecules. This dehydration process is accompanied by an exothermic peak and a small endothermic peak centered at about 60 °C and 100 °C respectively. Second stage occurred with in the temperature range of 110 to 210 °C. This process is accompanied by a broad exothermic peak centered at about 210 °C. Third stage of decomposition is observed in the temperature range 210 to 360 °C, leaving the metal oxide as the decomposition residue. This process is further confirmed by the presence of a broad exothermic peak centered at 370 °C.

Fe(III) chelate showed two decomposition steps within the temperature range 50-250 °C. The first step of decomposition observed in the temperature range 50-120 °C corresponds to the loss of chloride ion. The second step occurred at 200-240 °C corresponds to the removal of the organic part of the ligand leaving metal oxide as residue. The above degradation process was further confirmed by the presence of two exo-effects centered at 50 and 150 °C respectively.

#### Result of Antibacterial activities

Table 4 shows the mean of inhibition zone of the ligand and mixed ligand complexes, which are tested at different concentrations of 0.5, 1 and 2 mg against several species of human pathogenic bacteria. The high dose of Cu(II) complex i.e 2mg has shown good antibacterial activity against E. coli, E. aerogenes and Staphylococcus aureus which was known as a resistant to most commercial antibiotic.

The high antibacterial activity of the metal complexes compared to Schiff base ligand can be explained interms of chelation theory [23]. Chelation reduces the polarity of the metal ion to a considerable extent due to the partial sharing of its positive charge with the donor groups and possible  $\Pi$ -electron delocalization over the whole chelate ring. Reduction in polarity in turn increases the lipophilic character of the chelate and the interaction between the metal ion and the lipid is favoured. This may result in breaking down of the permeability barrier of the cell and interference with the normal cell processes [24].

Table 4 .Antimicrobial activity of ligand and its metal complexes

compound	E.coli					E.aerogenes					S.aureus				
	NC	PC	0.5	1.0	2.0	NC	PC	0.5	1.0	2.0	NC	PC	0.5	1.0	2.0
Ligand	0.2	27	-	-	-	0.3	27	-	-	-	0.2	27	-	-	-
Fe(L) <sub>2</sub>	0.2	26	11	16	17	0.2	26	12	13	12	0.2	27	13	12	19
Cu(L) <sub>2</sub>	0.1	25	12	19	22	0.1	26	11	11	14	0.2	28	14	16	19

NC = Negative control (DMSO)

PC = Positive control (Tetracycline)

#### CONCLUSION

A Schiff base ligand, derived from the condensation of 5-methyl 2-thiophene carboxaldehyde and 4-amino 3-hydroxy benzoic acid was synthesized and characterized. The metal complexes with Cu(II) and Fe(III) ions were prepared. From the IR spectra, it is concluded that ligand is tridentate binding to the metal ions; Cu(II) and Fe(III) through azomethine N, phenolic OH and thiophene S. The biological activity revealed that Cu(II) complex exhibited maximum inhibition against all the three bacteria used, which was almost greater than the standard antibiotic used. The chelation induced significant changes in the biological activity of the ligand.

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