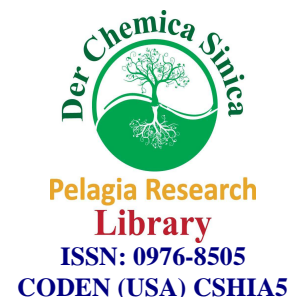




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Synthesis, characterization and antimicrobial studies of novel compound and its metal complexes derived from nicotinic acid

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ABSTRACT

A Schiff base of Nicotinic acid hydrazide was designed with the aim to develop novel antimicrobial agent of synthetic origin having broad spectrum of activity and high potency. The Schiff base (E)-N'-(thiophen-2-ylmethylene)nicotinohydrazide (TCNH), formed by the condensation of nicotinic acid hydrazide and thiophene-2-carboxaldehyde, was treated with Metal chlorides [Mn(II),Co(II),Cu(II) and Zn(II)] to prepare the complexes of TCNH. The chemical structure of TCNH was established by means of elemental, UV, IR, ^1H NMR, ^{13}C NMR and Mass Spectral studies. The metal complexes were characterized by elemental, UV, IR, Cyclic Voltammetry and ESR spectral studies. Further, TCNH and its metal complexes were tested against three pathogenic micro organism *Staphylococcus aureus* (S.aureus), *Escherichia coli* (E.coli) and *Aspergillus Niger* (A.niger). All the metal Complexes exhibit higher antibacterial activities than the free ligand

Keywords: Schiff base, Cyclic voltammetry, Overstone's theory, Ciprofloxacin, Amphotericine B

INTRODUCTION

The ligands containing N and O donor atoms possess a number of biological activities such as antimicrobial, antifungal, antiviral, anticonvulsant, anti-inflammatory, anti malarial etc. They are also used as powerful pesticides and insecticides apart from other biological applications [1]. These properties are enhanced when ligands form complexes with metals. The presence of donor atoms like N,O etc. play an important role in the formation of a stable chelate ring and this situation facilitates the complexation process [2]. Transition metal complexes of Schiff bases are some of them.

For example, Co(II) complex in dimethylformamide, pyridine and substituted pyridines proved to be involved in oxygen metabolism [3]. Copper complexes are good for liver functions, its level in blood and urine as influence in pregnancy disorders, nephritis hepatitis, leprosy, anemia and Leukemia in children [4].

Some of the more biologically active Schiff base are the derivatives of Hydrazine. Compare to the simple hydrazine Schiff bases, aryl or hetero aryl hydrazine Schiff base have additional donor sites. This introduces a wider range of biological properties of the compound [5].

A search through literature reveals that no work has been done on the synthesis of Schiff bases by the condensation of Thiophene-2-carbaldehyde and Nicotinic acid hydrazide.

Keeping the above facts in mind and in continuation of research work on transition metal (II) complexes with Schiff base, in the present paper we report the synthesis and characterization of Schiff base, *E*-*N'*-(thiophen-2-ylmethylene)nicotinohydrazide (TCNH) from the condensation of Thiophene-2-carbaldehyde and Nicotinic acid hydrazide and its Mn(II), Co(II), Cu(II) and Zn(II) complexes.

MATERIALS AND METHODS

All the chemicals and solvents used were of Merck and Sigma Alrich products (AR grade) and were used without further purification. The CHNS analyses were performed at STIC Cochin. FT-IR spectral measurements were made for the free ligand and its metal complexes using Perkin Elmer Spectrum -1 FT IR spectrometer. The homogeneity of the compounds was tested by TLC using silica G coated glass plates and visualized by iodine vapor. Melting points were determined on an open capillary and are uncorrected. ¹H NMR and ¹³C NMR spectra were recorded on 300 and 100 MHz Bruker Spectrometer respectively using DMSO-*d*₆ as the solvent and TMS as the internal reference. Mass spectrum was also recorded to conform the molecular mass of the ligand from JEOL GC Mate mass spectrometer.

IR spectra were recorded on Shimadzu FT-IR spectrometer in 4000-400cm⁻¹ region. UV spectrum was recorded using UV-Visible Perkin Elmer Model ranged from 200-1100 nm. The preliminary *in vitro* antimicrobial screening of the ligand and the metal complexes were carried out by Agar well diffusion technique using DMSO solution.

2.1. Synthesis of (*E*)-*N'*-(thiophen-2-ylmethylene)nicotinohydrazide

To the ethanolic solution of nicotinic acid hydrazide (3.4 g, 0.025 mol), taken in a round bottom flask, thiophene-2-carboxaldehyde (2.5 mL, 0.025 mol) in 1:1 molar ratio and few drops of sulphuric acid (pH 3-4) were added. The reaction mixture was kept over a magnetic stirrer and stirred well in an ice cold condition for 3 h. The colorless solid (Fig. 1) formed was filtered and washed several times with petroleum ether (40-60%). The crude solid obtained was dried and recrystallized using absolute alcohol. The recrystallized product was dried in a vacuum desiccators over fused Calcium Chloride. The purity of the ligand was checked by TLC.

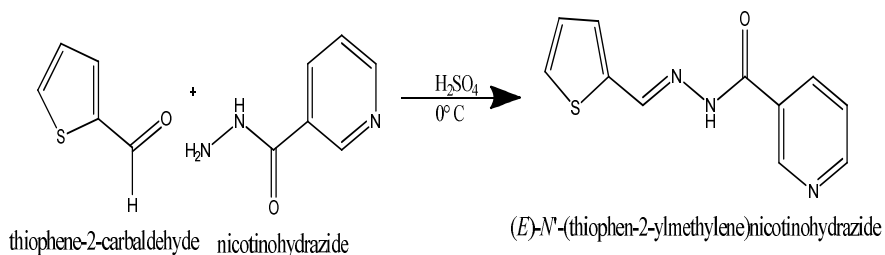


Fig 1. Synthesis of (*E*)-*N'*-(thiophen-2-ylmethylene)nicotinohydrazide

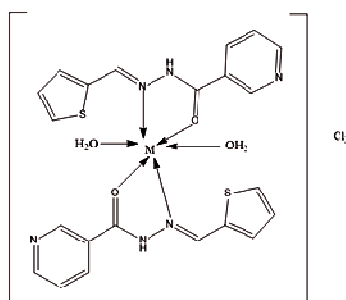


Fig 2. Proposed Structure of the metal complex

2.2. Synthesis of metal complexes

Metal(II) chloride was added to the calculated amount of the ligand (TCNH) in 1:2 molar ratio using methanol as solvent. The reaction mixture was taken in a round bottom flask and kept over a magnetic stirrer with hot plate and stirred under 100°C. After 2h, the product separated as a solid (Fig. 2) was washed, filtered and dried in a vacuum

desiccators over fused calcium chloride. The same procedure was followed to prepare all the complexes. All the complexes were solids, stable in air and soluble in DMSO and DMF (But not other common organic solvents).

2.3. BIOLOGICAL ACTIVITIES

2.3.1. Antibacterial activity (in vitro)

The synthesized Schiff base TCNH and its metal complexes were screened in vitro for their antibacterial activity against a gram-positive (*S.aureus*) and a gram negative (*E.coli*) bacterial strains by the agar-well diffusion method [6]. The wells (6mm in diameter) were dug in the media with the help of a sterile metallic borer with centers at least 24 mm. Bacterial inocula containing approximately 10^5 - 10^6 CFU/ml was spread on the surface of the nutrient agar with the help of a sterilized cot buds. The recommended concentration of the test sample (100 mg/mL in DMSO) was introduced in the respective wells. Other wells supplemented with DMSO and standard antibacterial drug Ciprofloxacin. The plates were incubated at 37°C for 24 h. The activity was determined by measuring the diameter (mm) of zones showing complete inhibition. In order to clarify any participating role of DMSO in the bacterial screening, separate studies were carried out with the solutions alone of DMSO and they showed no activity against any bacterial strains.

2.3.2. Antifungal activity (in vitro)

Antifungal activity of the ligand and its complexes was studied against a fungal strains (*A.niger*). It was also done by the same antibacterial activity procedure corresponding with antifungal drug Amphotericin B as standard.

RESULTS AND DISCUSSION

The elemental analyses data (Table 1) confirm the proposed Molecular formulae $C_{11}H_9N_3OS$ for the synthesized ligand (E)-N'-(thiophen-2-ylmethylene)nicotinohydrazide (TCNH)

The IR spectrum of the TCNH shows characteristic bands due to the $\nu(NH)$, $\nu(CH)$, $\nu(C=O)$ and $\nu(C=N)$ functional groups. The frequencies of the above functional groups are showed in the range of 3408, 2848, 1674, 1147cm^{-1} respectively [7]. The UV spectrum of TCNH exhibited an intense absorption peak at 33333cm^{-1} assigned to $n \rightarrow \pi^*$ transition of azomethine linkage [8].

The results of elemental analyses show 1:2 (metal : Ligand) stoichiometry for all the complexes which confirm the suggested the general formula $[ML_2(H_2O)_2]Cl_2$. The analytical data of ligand and the complexes are given in Table 1. The presence of Chloride ion is evident from $AgNO_3$ test. The high molar conductance of the chelates in DMF (Table 2), supports the electrolytic nature of metal complexes [9].

Table 1. Elemental analysis, molecular weight and melting point of the TCNH and its complexes

Compounds	Mol. Formula	Mol. Weight	Melt. Point	Elemental Analysis % Found (% Calc.)				
				C	H	N	O	S
TCNH	$C_{11}H_9N_3OS$	231	156	57.10 (57.13)	3.80 (3.92)	18.12 (18.17)	6.88 (6.92)	13.78 (13.86)
Mn-TCNH	$C_{22}H_{22}Cl_2MnN_6O_4S_4$	623	198	42.30 (42.32)	3.58 (3.55)	13.46 (13.46)	10.20 (10.25)	10.15 (10.27)
Co-TCNH	$C_{22}H_{22}Cl_2CoN_6O_4S_4$	627	288	42.08 (42.05)	3.50 (3.53)	13.30 (13.37)	10.15 (10.18)	10.28 (10.21)
Cu-TCNH	$C_{22}H_{22}Cl_2CuN_6O_4S_4$	631	244	41.70 (41.74)	3.55 (3.50)	13.20 (13.28)	10.08 (10.11)	10.05 (10.13)
Zn-TCNH	$C_{22}H_{22}Cl_2ZnN_6O_4S_4$	632	296	41.56 (41.62)	3.44 (3.49)	13.22 (13.24)	10.29 (10.08)	10.30 (10.10)

Table 2. Molar conductivity of the complexes

Molar Conductance $\Omega^{-1} \text{mol}^{-1} \text{cm}^2$	Mn-TCNH	Co-TCNH	Cu-TCNH	Zn-TCNH
	218	95	108	144

3.1. IR Spectra

In order to study the binding mode of the TCNH to the metal ion in the complexes, the IR spectrum of the free TCNH was compared with the spectra of the complexes. The IR spectrum of free ligand showed characteristic band at 3408cm^{-1} (Table 3) can be attributed to $\nu(-N-H)$ [10]. A sharp band appeared at 1674cm^{-1} can be attributed to

the $\nu(\text{-C=O})$ of the ligand, has been found shifted to $1658\text{-}1647\text{cm}^{-1}$ in the spectra of the complexes indicate the involvement of oxygen atom of carbonyl group in binding with metal ions [11]. The band appeared at 1292cm^{-1} due to $\nu(\text{-C=N})$ of azomethine moiety of the ligand is shifted to lower frequency by nearly $10\text{-}48\text{cm}^{-1}$ in the complexes suggest that the coordination is through N atom of azomethine (-CH=N-) linkage[12]. On the basis of the above evidences, it is concluded that the ligand acts as a neutral bidentate manner in all the complexes.

In the far-IR region all the complexes exhibit bands around $572\text{-}505$ and $440\text{-}418\text{cm}^{-1}$ which are assignable to $\nu(\text{M-O})$ [13] and $\nu(\text{M-N})$ [14] respectively. Due to the larger dipole moment change in the vibration of $\nu(\text{M-O})$ band in comparison to that in $\nu(\text{M-N})$ band, the band due to $\nu(\text{M-O})$ usually appear at higher frequency region which are sharp and stronger than $\nu(\text{M-N})$.

Table 3. Characteristic IR bands(cm^{-1}) of the TCNH and its metal complexes

Compounds	$\nu(\text{NH})$	$\nu(\text{CH})$	$\nu(\text{C=O})$	$\nu(\text{C-O})$	$\nu(\text{CN})$	$\nu(\text{M-N})$	$\nu(\text{M-O})$
TCNH	3408	2848	1674	1597	1147	-	-
Mn-TCNH	3238	2848	1647	1566	1093	505	426
Co-TCNH	3375	2860	1658	1525	1105	570	440
Cu-TCNH	3400	2852	1654	1525	1055	572	418
Zn-TCNH	3234	2852	1653	1583	1126	497	426

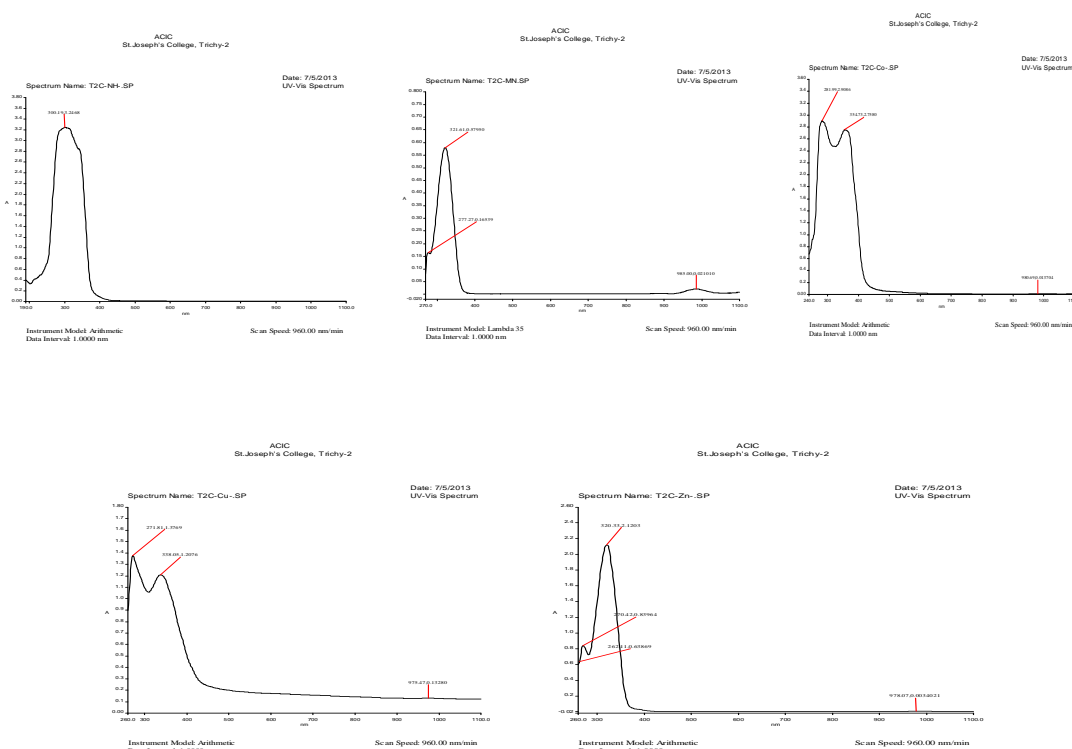


Fig 3. UV- Spectra of the ligand and its complexes

3.2. Electronic absorption spectra and Magnetic measurements

The electronic absorption spectra are often very helpful in the evaluation of results furnished by other methods of structural investigation. The electronic spectral measurements were used for assigning the stereochemistries of metal ions in the complexes based on the position and number of d-d transitions peaks. The electronic absorption spectra of TCNH and its Mn(II), Co(II), Cu(II) and Zn(II) complexes were recorded at room temperature using DMSO as solvent (Fig.3, Table 4). The electronic spectrum of manganese complex displays a broad band at 36100cm^{-1} well defined shoulder around 31055cm^{-1} and a broad peak shows at 10150cm^{-1} attributed to ${}^6\text{A}_{1g} \rightarrow {}^4\text{E}_{1g}$, ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{2g}$ and

${}^6A_{1g} \rightarrow {}^4T_{1g}(G)$ transition respectively which are strongly favor a high spin octahedral geometry around the central metal ion [15]. This is further supported by the magnetic susceptibility value of 5.89 BM.

The cobalt complex is paramagnetic and its transitions suggest octahedral geometry. The electronic absorption spectrum of the cobalt complex shows two d-d bands one at 35460cm^{-1} assigned as $n \rightarrow \pi^*$ transition and another at 28248cm^{-1} also assigned as $n \rightarrow \pi^*$, which are indicating octahedral geometry [16]. This is further confirmed by its magnetic susceptibility value (3.94 BM). Copper complex shows magnetic moment corresponding to one unpaired electron (1.70 BM.), which confirm the outer orbital high spin complex. Electronic spectrum of the copper complex displays bands at 36764cm^{-1} and 29585cm^{-1} . These bands may be assigned to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions respectively. The electronic transition observed from Zn complex suggests the octahedral geometry. Three peaks are predicted in their electronic spectra, namely, $\pi \rightarrow \pi^*$, $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ found at 38160cm^{-1} , 37030cm^{-1} and 31250cm^{-1} respectively for the zinc complex. These are conforming the octahedral arrangement Zn(II) complex.

Table 4. Magnetic Susceptibility and electronic transition of TCNH and its metal complexes

Compounds	Magnetic Moment B.M	Absorption Maxima		Transition Assignment	Geometry
		Nm	cm^{-1}		
TCNH	-	300	33333	$n \rightarrow \pi^*$	---
Mn-TCNH	5.89	277 321 985	36100 31055 10150	${}^6A_{1g} \rightarrow {}^4E_{1g}$ ${}^6A_{1g} \rightarrow {}^4T_{2g}(P)$ ${}^6A_{1g} \rightarrow {}^4T_{2g}(G)$	Octahedral
Co-TCNH	3.94	282 355 980	35460 28248 10200	$n \rightarrow \pi^*$ $n \rightarrow \pi^*$ ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)$	Octahedral
Cu-TCNH	2.78	272 338	36764 29585	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	Octahedral
Zn-TCNH	1.90	262 270 320	38160 37030 31250	$\pi \rightarrow \pi^*$ $\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	Octahedral

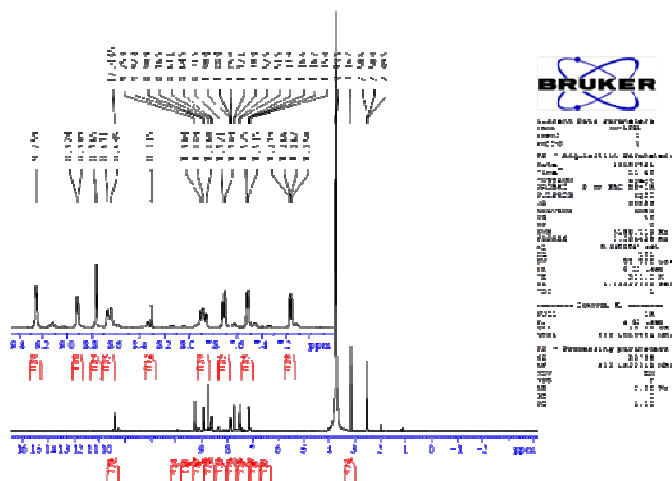
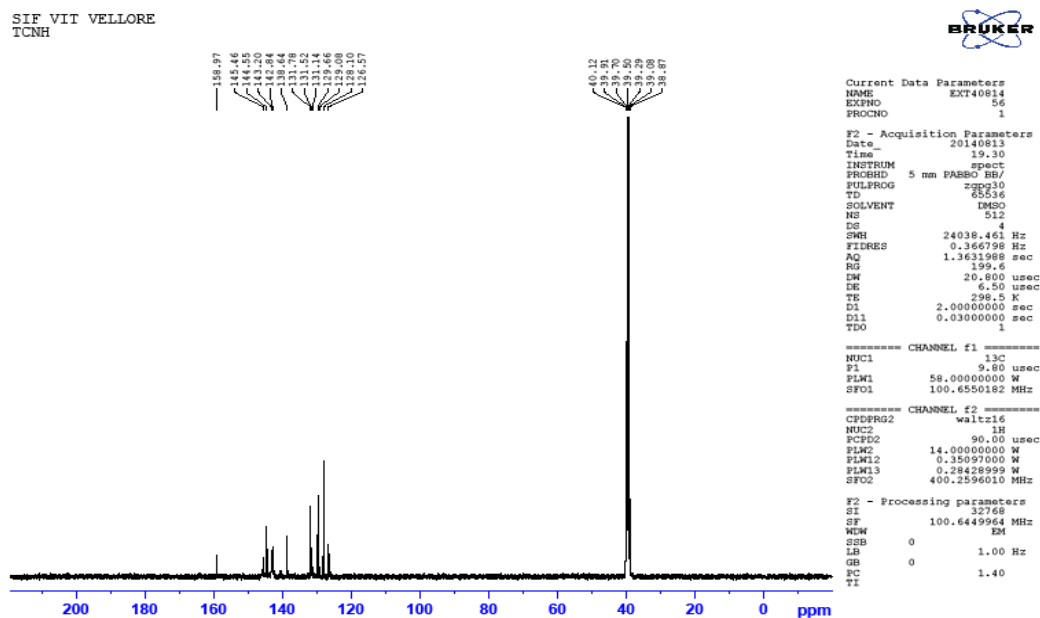


Fig 4. ${}^1\text{H}$ NMR of TCNH

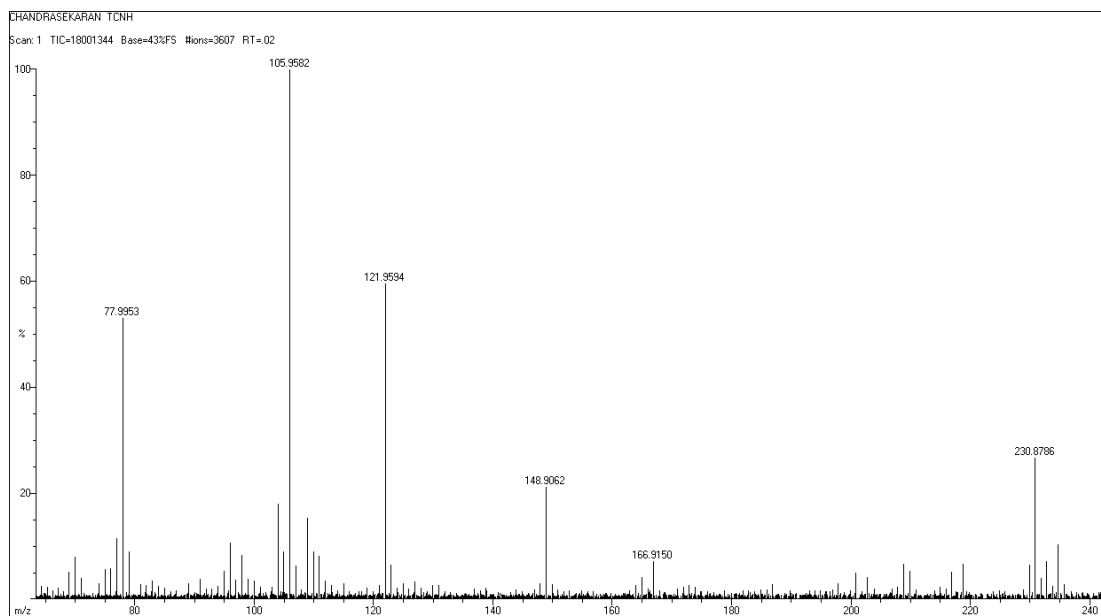
3.3. ${}^1\text{H}$ NMR and ${}^{13}\text{C}$ NMR spectrum

${}^1\text{H}$ NMR spectrum of the ligand TCNH was recorded in DMSO-d_6 (300 MHz) (Fig. 4). The ligand shows a singlet at δ 12.4 is assigned to enolic OH proton. The signals appeared in the region δ 7.1 and 8.6-8.9 are due to nicotinic

acid ring protons. A signal at δ :9.2 is assigned to azomethine (-CH=N-) proton. δ 7.1 (q,1H), 8.6(d,1H), 8.7(s,1H) and 8.9(d,1H) are assigned to pyridine ring protons where as signals at δ 7.5(d,1H),7.7(d,1H) and 7.9(t,1H) assigned to thiophene ring protons. ^{13}C NMR spectrum of the ligand TCNH was recorded in DMSO- d_6 (100MHz) (Fig. 5). δ C=O (159), pyridine ring (145, 142, 138, 131, 129), thiophene ring (144, 131, 129,128) and CH=N (126)

Fig 5. ^{13}C NMR of TCNH

3.4. Mass Spectrum



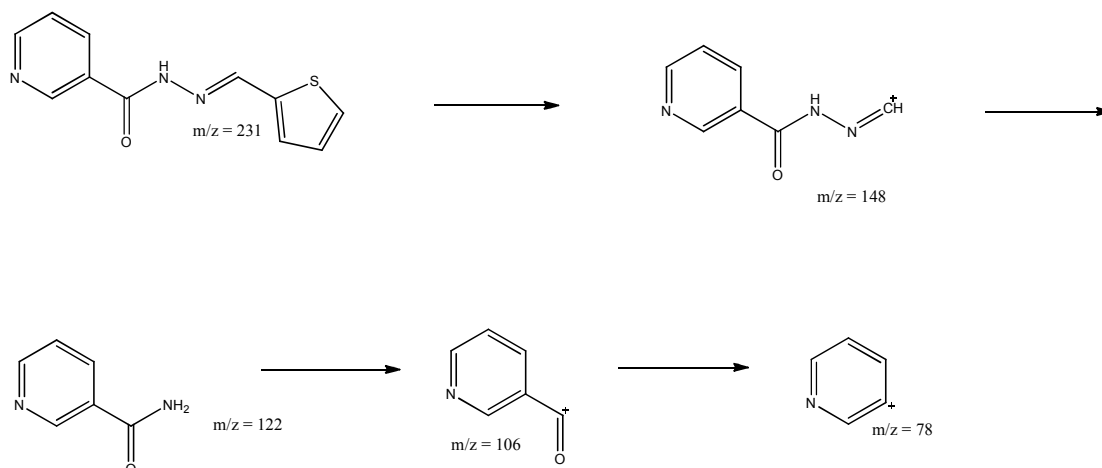


Fig 6. Mass Spectrum and fragmentation pattern

The mass spectrum of TCNH obtained on electro ionization mode shows a molecular ion peak $m/z=231$, (Fig.6) which confirms the assigned molecular mass to the Schiff base. There upon on fragmentation it records intense signals at $m/z=148$, 121 and 106 are due to the removal of Thiophene ring, NCH and NH groups respectively. Finally the signal at $m/z=78$ is due to the presence C_5H_4N cation after the removal of CO.

3.5. Cyclic Voltametry

Cyclic Voltammogram of the Mn(II) and Cu(II) complexes in DMSO solution using NH_4Cl as supporting electrolyte (potential range for Mn = +1.0 to -1.5V and Cu(II) = +0.6 to -0.6 V) shows a well defined redox process corresponding to the formation of Mn(II)/Mn(0) couple at $E_{pa} = -0.985V$ and the cathodic peak $E_{pc} = +0.506V$. This couple is found to be reversible with $E_p = -1.49V$ and the ratio of cathodic to anodic currents (i_{pc}/i_{pa} 1) almost equal to one, corresponding to a simple one electron process. For the copper complex, one reversible process occurs at +0.506 to -0.985V versus NHE ($E_p = -0.218V$) respectively attributed to Cu(II)/Cu(I) process. One reversible wave is clearly visible in Fig. 8. The CV data's are presented in Table 5.

Table 5. CV data

Compounds	$E_{red1/2}$ (V)	$E_{ox1/2}$ (V)	E_p (V)
Mn-TCNH	+0.506	-0.985	-1.49
Cu-TCNH	+0.056	-0.162	-0.218

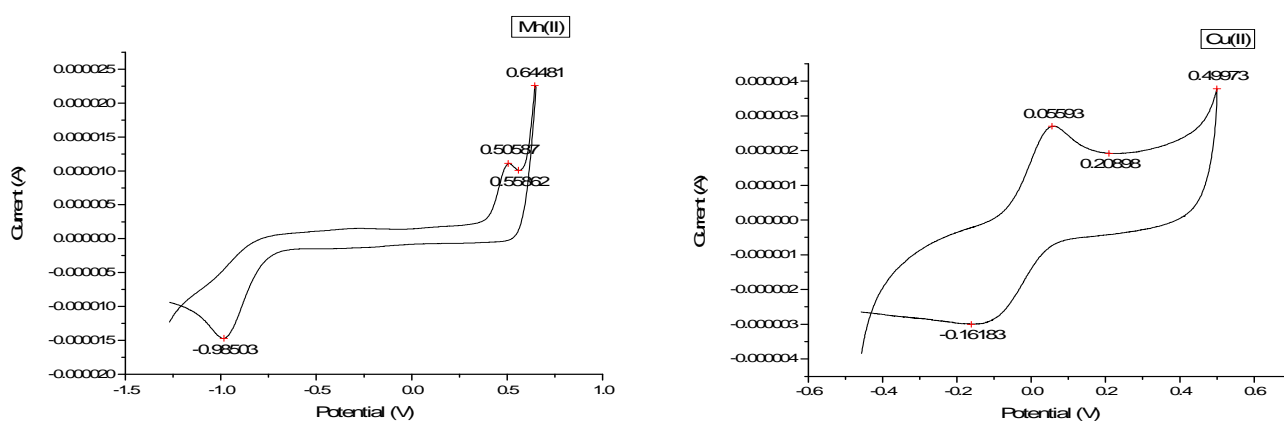


Fig 8. Cyclic Voltammogram of Mn(II) and Cu(II) complexes

3.6. ESR Spectrum

ESR spectral studies of TCNH-Cu were recorded at room temperature (300 K). The ESR spectral analysis of spectrum (Fig 9) showed that $g_{\parallel} = 2.21$ and $g_{\perp} = 2.01$. Therefore $g_{\parallel} > g_{\perp} > 2.0023$. These observations indicate that the unpaired electron is localized in $d_x^2-d_y^2$ orbital of the Cu(II) ion and hence the system is axially symmetric. Thus the octahedral elongated geometry is confirmed for the aforesaid complex.

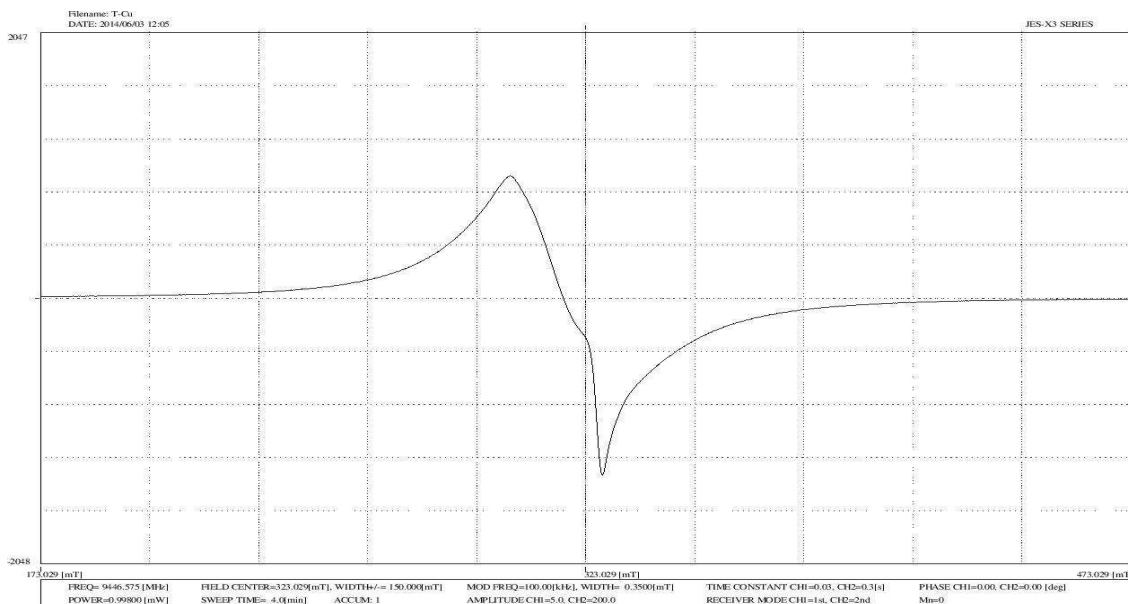


Fig 9. ESR Spectrum of TCNH

3.7. Antimicrobial Studies

The main objective of the production and synthesis of any antimicrobial compound is to inhibit the casual microbe without any side effect on the patients. The antimicrobial activity and its metal complexes were done by well diffusion method (Fig.10). The tested in-vitro against *S.aureus* and *E. coli* bacteria by agar compound were tested at concentration 100 $\mu\text{g mm/ml}$ in DMSO and compared with known antibiotics viz Ciproflaxin. For fungal activity, compounds were screened *in vitro* against *A. Niger* with Nutrient Agar medium (Table 5).



Fig 10 Zone of inhibition

From the table (Table 6), it is clear that the inhibition by Zinc complex is higher than that of the ligand. In the case of antimicrobial studies it was observed that, the ligand is moderately active towards *S.aureus* and less active towards *E.coli*. Comparison activities of the ligand and its metal complexes showed that the Zinc complex is approximately found to be more active than the ligand against *E.coli* and activity of ligand against *S.aureus* is found to be increased after chelation. The Mn(II) and Co(II) complexes show the less activity comparable to either ligand or Zn(II) complex. The activity of these complexes follow the order $\text{Zn} > \text{Cu} > \text{Co} > \text{Mn}$. This high antibacterial activity of ligand and its metal complexes can be attributed on the basis of Overtone's concepts and Tweedy's concepts. According to these concepts, chelation reduces considerably the polarity of the metal ion mainly because of partial sharing of its positive charge with donor groups and possible π - electron delocalization of electron over the

chelate ring. This increases the lipophilic character of the chelate which favors its permeation through lipid layers of fungus membranes. In the case of antifungal activity, Zn(II) and Cu(II) complexes are more fungi toxic than the free ligand (Fig. 11). Based on the above results it is come to know that the Zn(II) complex shows good antibacterial and antifungal activity.

Table 6. Disc diffusion method results of TCNH, metal complexes and standard drugs (diameter of the zone of inhibition (mm)) at 100 μ l mm/ml

Organism	Concentration of DMSO extract added and Zone of inhibition (100 μ l mm/ml)		
	<i>S.aureus</i>	<i>E.coli</i>	<i>A.niger</i>
Control	30	40	20
TCNH	30	30	27
Cu-TCNH	20	22	21
Mn-TCNH	15	-	13
Co-TCNH	16	-	11
Zn-TCNH	38	40	31

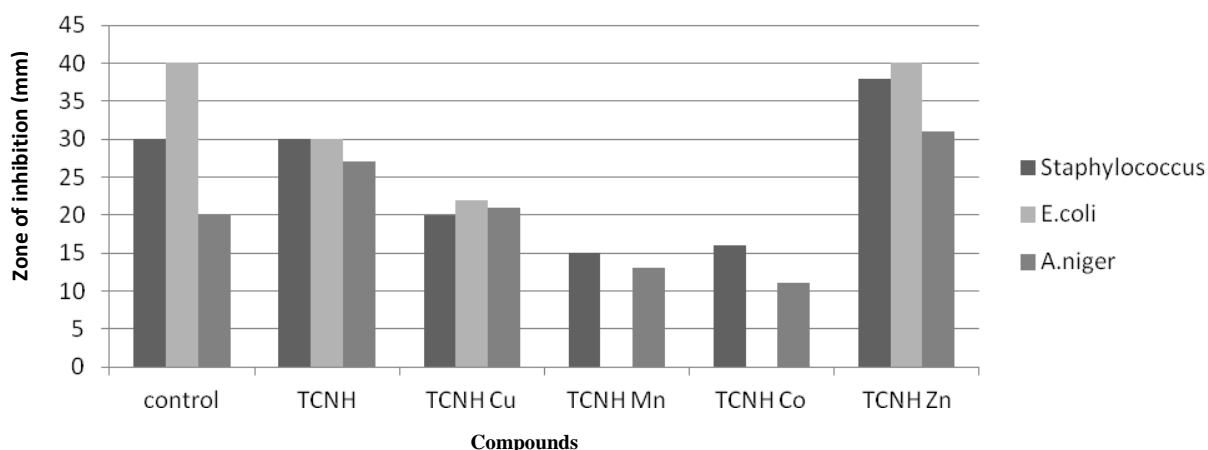


Fig 11. Comparison of antibacterial and anti fungal activity of ligand TCNH and its metal complexes

CONCLUSION

In this paper the synthesis of the Schiff base ligand TCNH derived from condensation of Thiophene-2-carbaldehyde with Nicotinic acid hydrazide and its Mn(II), Co(II), Cu(II) and Zn(II) complexes have been described. The Schiff base coordinates with metal ions through its N and O indicates the bidentate nature of the ligand. This is supported by IR data. On the basis of ESR, electronic and magnetic data, octahedral geometry is assigned to all the complexes. The high molar conductance of the complexes supports the electrolytic nature. The ligand and its metal complexes show significant antimicrobial activities. In particular the Zn(II) complex is found to be more effective than the free ligand and the standard drug. Additionally some metal complexes possess antibacterial and antifungal activity against selected bacteria and fungi more than the free ligand which is in accordance with the fact that the chelating of metal to ligand increases the microbial activity of the molecule. The results show that Zn(II) and Cu(II) complexes of the ligand possess more antibacterial activity.

Acknowledgement

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