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Synthesis, spectral aspects and biological activities of 5-hydroxy-2-nitrobenzaldehydethiosemicarbazone and their Mn(II), Co(II) and Ni(II) complexes

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ABSTRACT

The ligation behavior of 5-hydroxy-2-nitrobenzaldehydethiosemicarbazone (L) and its Mn(II), Co(II) and Ni(II) complexes has been synthesized and characterized by Elemental analysis, Electronic, FT-IR, Raman, EPR spectral techniques and Cyclic voltammetric study. The ligand (L) belongs to triclinic system with *P*1 space group. The IR spectral data of ligand indicate the taking part of sulphur and azomethine nitrogen in coordination to the central metal ion. The electronic, FT-IR and EPR spectral studies reveals all the complexes are distorted octahedral geometry. The synthesized ligand and its metal(II) complexes were tested for their antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method and antibacterial activity against *E.coli, Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi* and *Klebsiella pneumonia*. The antibacterial and antioxidant activities of the ligand and its metal complexes had shown moderate to good activity, among all the compounds Ni(II) complex had shown more activity.

INTRODUCTION

Thiosemicarbazones belongs to a large group of thiourea derivatives, it is an important structural motif that has been potential to display chemical functionality in biologically active molecules (Lobana *et al.*, 2009). The recent review (Narayana *et al.,* 2012) discussed the analytical and biological applications of thio and phenyl thiosemicarbazones, but mainly focused on the analytical applications (Wood *et al.,* 2008). Thiosemicarbazones usually act as chelating ligands with transition metal ions, bonding through the sulphur and hydrazine nitrogen atoms. Schiff bases derived from thiosemicarbazide and their metal complexes are of great significance for their pharmacological properties such as antibacterial, antifungal, antitumoral, antiviral and anticancer (Patil *et al.*, 2010; Sharma *et al.*, 2010; Al-Amiery *et al.*, 2011; Wiecek *et al.*, 2010; Ferraz *et al.*, 2009; Graminha *et al.*, 2008; Raja *et al.*, 2010). Manganese and Cobalt are essential trace elements, forming the active sites of a number of metalloproteins; Mn shows many oxidation states (Sreekanth *et al.*, 2006). Cobalt-60 is used as an efficient radioactive tracer and an anticancer treatment agent in medicine, cobalt such as vitamin B_{12} are imported for biological activities (Praveen Kumar *et al.*, 2007).

Now we reported the synthesis and spectral characterization of spectral aspects by using CHN analyzer, FT-IR, Raman, EPR, Electronic absorption spectra, and cyclic voltammetric techniques for the ligand and its Mn(II), Co(II) and Ni(II) complexes. Further, the ligand characterized by NMR and HRMS techniques. Hence above synthesized compounds were screened for their antimicrobial and antioxidant activities.

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EXPERIMENTAL

Chemicals

All the chemicals used in the study were of analytical grade unless reported. The organic chemicals 5-hydroxy-2-nitrobenzaldehyde was purchased from AVRA synthesis, thiosemicarbazide and nickel chloride were purchased from Sd-Fine chemicals. Mn(II), Co(II) chlorides and organic solvents were used of GR grade without further purification.

Physical measurements

Elemental analyses (CHN) was performed by using Flash EA 1112 series. IR spectra were recorded as KBr pellets in the 4000-400 cm^{-1} region using a Thermo scientific Nicolet 380 spectrophotometer. Shimadzu 3600 UV-Vis spectrophotometer equipped with quartz cell is used for the absorbance studies. The NMR spectrum of L was recorded on a Bruker(400 MHz) spectrometer at 300°K, using DMSO-d^6 as a solvent and tetramethylsilane(TMS) as an internal reference compound. A mass spectrum of the ligand was recorded in ESI mode using Bruker HRMS at University of Hyderabad.

Synthesis of ligand (L)

The ligand was synthesized by slightly modifying the previously reported procedure (Ferrari *et al.*, 2000), an ethanolic solution of thiosemicarbazide (0.015 M) was added to a ethanolic solution of 5-hydroxy-2-nitrobenzaldehyde (0.015 M). The mixture was refluxed for 3-4 h. During which period a yellow color precipitate was formed. The reaction was cooled to room temperature and the solid compound was filtered, precipitate was collected. The single crystal X-ray structural analysis of the ligand compound HNBATSC ($C_8H_8N_4O_3S$) crystallizes in the triclinic lattice with space group *P*1 symmetry with unit cell dimensions: a = 7.1328(13) Å, b = 8.0738(15) Å, c = 17.868(3) Å and α = 102.142 (16)°, β = 94.325(15)°, γ = 95.212 (15)° and V = 997.1 (3) Å^3 (Sivasankar Reddy *et al.*, 2014). The schematic representation of synthesis is depicted as **Scheme 1**.

Synthesis of complexes

A mixture of hot methanolic solution of free ligand (0.002 M) and hot methanolic solution of (0.001 M) of the metal chloride solutions ($MnCl_2.4H_2O/CoCl_2.6H_2O/ NiCl_2.6H_2O$) was refluxed for 4 h. Precipitate was observed, the resulting solutions are allowed to stand at room temperature and upon slow evaporation gives cream, red and brown colored crystals of Mn(II), Co(II) and Ni(II) complexes, respectively. Then filter and washed with methanol and ether, dried under vacuum P_4O_{10}. The purity of the complexes was checked by TLC. Elemental and chemical analysis data are shown in **Table 1**.

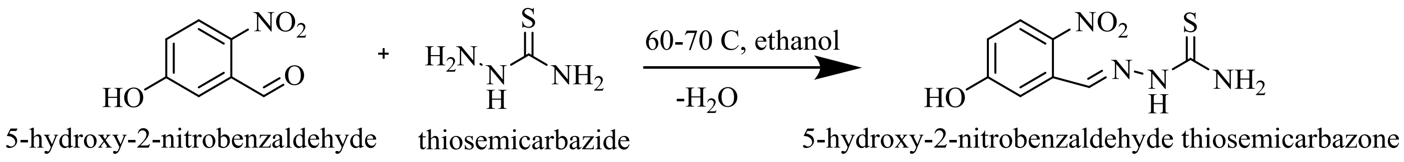
Antibacterial activity

The ligand and its metal complexes were screened against two Gram-positive of *Staphylococcus aureus* and *Bacillus subtilis* and three Gram-negative of *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumonia* bacteria using agar-well diffusion method (Raman *et al.*, 2003). To each agar well 25, 50, 100 and 150 µL of the compound reconstituted in DMSO in concentration of 1.0 mg/ml is added.

DMSO is used as a negative control and streptomycin used as positive control. All the plates were incubated at 37°C for 24 h, and observed for the zone of inhibition. The diameter of zone of inhibition was calculated in millimeters. The well diameter was deducted from the zone diameter to get the actual zone of inhibition diameter. All the experiments were done twice independently for each of the compounds.

Antioxidant activity

DPPH radical scavenging activity of the ligand and its metal complexes were determined by using DPPH according to the literature (Baraca *et al.*, 2001). The principle for the reduction of DPPH free radicals are that the antioxidants reacts with stable free radical DPPH and it converts in to 1,1-diphenyl-2-picrylhydrazine. The ability to scavenge the stable free radical DPPH is measured by decrease in the absorbance at 517 nm.



Scheme 1.

Table 1: Analytical data for the ligand and its Mn(II), Co(II) and Ni(II) complexes.

S.No	Compounds	Color	M.Wt.	M.P(°C)	Yield (%)	Elemental analyses data (%) (calculated)		
						C	H	N
1	Ligand	Yellow	240.2391	240	84	40.36(40.00)	3.41(3.36)	23.45(23.32)
2	Mn(II)	Cream	637.3993	>300	68	33.98(33.92)	3.66(3.64)	17.60(17.58)
3	Co(II)	Red	641.3944	>300	65	33.74(33.71)	3.62(3.61)	17.51(17.47)
4	Ni(II)	Brown	641.1546	>300	58	33.81(33.72)	3.63(3.62)	9.18(9.15)

Table 2: Assignments of the characteristic FT-IR (cm^{-1}) and Raman (cm^{-1}) spectral bands of the ligands and their Mn (II), Co(II) and Ni (II) complexes.

Ligand		**Mn(II)**		**Co(II)**		**Ni(II)**		**Assignments**
FT-IR	**Raman**	**FT-IR**	**Raman**	**FT-IR**	**Raman**	**FT-IR**	**Raman**	
3224	-	3398	-	3414	-	3401	-	υsyNH
1598	1602	1567	1570	1543	1564	1620	1585	υC=N + υC=C
1305	1324	1226	1235	1225	1241	1371	1360	υNH + υasyC=S
848	850	827	824	829	827	770	782	υsyC=S + δNH
	-	462	477	454	478	463	472	M-N

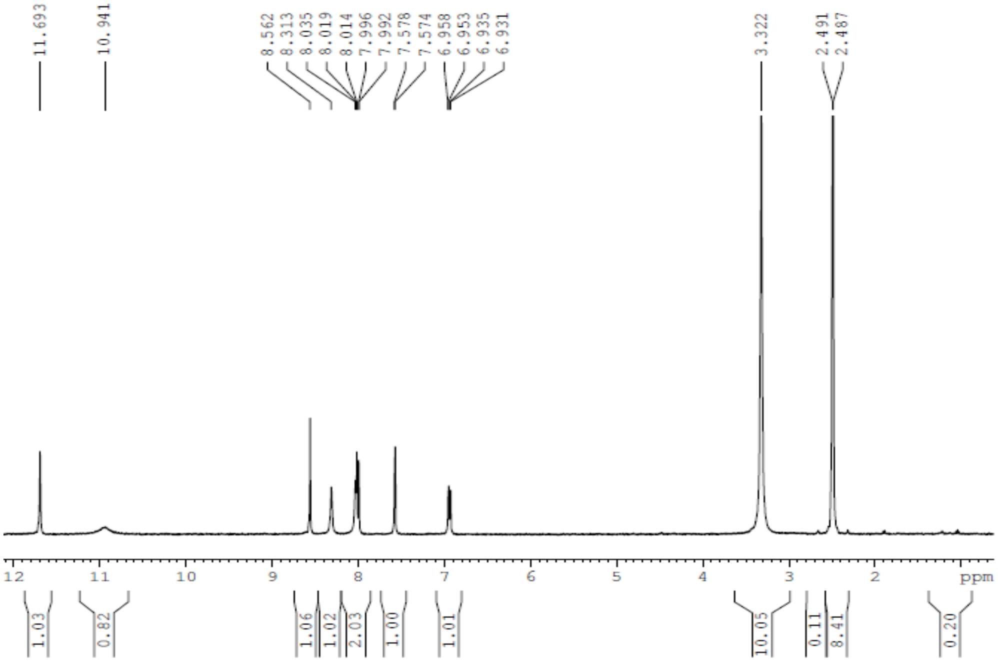


Fig. 1: ^{1}H NMR spectrum of the ligand.

RESULTS AND DISCUSSION

Infrared and Raman spectra

The FT-IR and Raman spectral assignments of ligand and their metal complexes are listed in **Table 2**. The bonding sites of the thiosemicarbazones to the metal ion have been deduced by comparing the spectra of the complexes with the spectrum of the ligand. IR spectrum of the free ligand is compared with the spectra of the metal complexes shows many characteristic absorption bands. In the IR spectrum of ligand ν(N-H) band at 3224 cm^{-1} is present, the stretching frequency of the C=N function was observed at 1598 cm^{-1} in the IR, and 1602 cm^{-1} in Raman spectrum of the ligand band. For the same compound, one more important stretching band at 848 cm^{-1} in IR and 850 cm^{-1} in Raman, that can be attributed for the stretching vibrations of the thione function (C=S) of the free ligand. The NH band was disappeared upon the complexation in all complexes. While the stretching vibrations of C=N in Mn(II), Co(II) and Ni(II) complexes showed bands at 1567, 1543 ,1620 cm^{-1} in IR and 1570, 1564, 1585 cm^{-1} in Raman, respectively. The reduce in frequency of this band in the spectra of the complexes is an confirmation for the coordination *via* the azomethine nitrogen atoms (Kovala Demertzi *et al.*, 2000). A similar pattern of red shift was observed with the stretching frequency of C=S bond show bands at 827, 829, 770 cm^{-1} and same bands are observed at 824, 827, 782 cm^{-1} in FT-IR and Raman respectively. These bands were shifted to lower frequencies, this indicates the coordination of sulfur atom (Kovala Demertzi *et al.*, 2001).

NMR spectra

^{1}H NMR **(Fig. 1)** and ^{13}C NMR (400 MHz, δ (ppm)) spectrum of ligand in DMSO-d$_6$ exhibit following signals: δ 11.69 (s, 1H, OH); δ 10.94 (s, 1H, ^{2}NH); δ 8.56 (-CH=); δ 8.31 (s, 1H, ^{4}NH) δ 6.9-8.3 (m, aromatic) (Bindu et al., 1999)[18]. ^{13}C NMR: δ 140 (C=S); δ 179 (C=N); δ 114-140 (aromatic). The electronic impact mass spectrum of ligand **(Fig. 2)**, owed a molecular ion peak at m/z = 241.0396 corresponding to species ($C_8H_8N_4O_3S$) which confirms the proposed structure of the formulae.

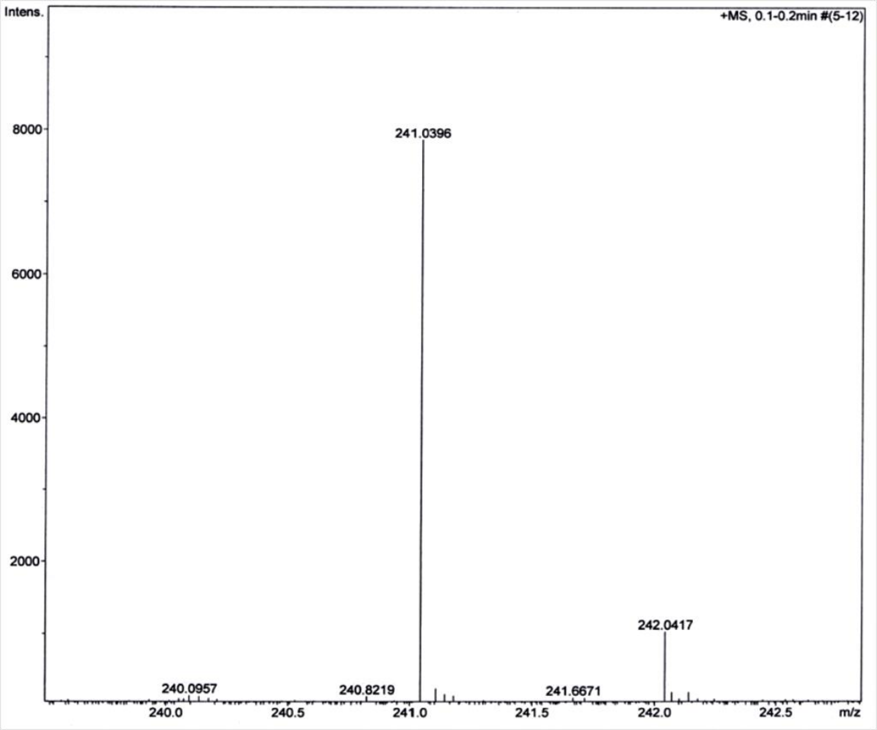


Fig. 2: HRMS spectrum of the ligand.

Electronic absorption spectra

Electronic absorption spectrum **(Fig. 3)** of Mn(II) complex exhibit four weak intensity bands at 18621, 22123, 28409 and 33112 cm^{-1}. These bands may be assigned to the transitions: $^{6}A_{1g} \rightarrow {}^{4}T_{1g}$, $^{6}A_{1g} \rightarrow {}^{4}E_{g}$ (G), $^{6}A_{1g} \rightarrow {}^{4}T_{2g}$ (D) and $^{4}T_{1g} \leftarrow {}^{4}A_{2g}$, respectively. These bands are characteristics of distorted octahedral geometry (Chandra *et al.*, 2009). The electronic spectra of cobalt(II) complex exhibit absorption bands at 14880, 19047 and 28325 cm^{-1}. These bands may be $^{4}T_{1g} \rightarrow {}^{4}A_{2g}$ and $^{4}T_{1g}(F) \rightarrow {}^{4}T_{1g}(P)$ respectively. The third band may be due to charge transfer. The position of above bands indicates that these complexes have distorted octahedral geometry (Chandra *et al.*, 2004).

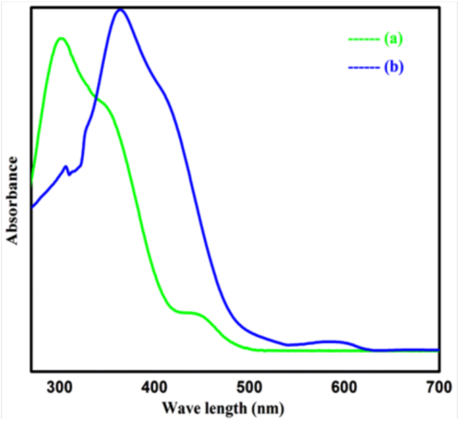


Fig. 3. Electronic spectra of ligand(a) and Co(II) (b) complex.

The ground state of the regular octahedral Ni(II) complexes is $^{3}A_{2g}$ and no singlet levels arising from "d" configuration. Ni(II) complexes of electronic spectrum display bands at 18282, 28325 cm^{-1} (Chandra *et al.*, 2005) assigned to $^{3}A_{2g}$ (F) $\rightarrow {}^{3}T_{1g}$ (P) and $^{3}A_{2g}$ (F) $\rightarrow {}^{3}T_{1g}$ (P). These transitions indicate distorted octahedral geometry of arrangement.

Electron paramagnetic resonance (EPR) spectrum

The X-band EPR spectrum of Mn(II) complex is obtained at room temperature for the polycrystalline sample and is shown in **Fig. 4**. The EPR spectrum exhibits a broad signal without fine or hyperfine structure.

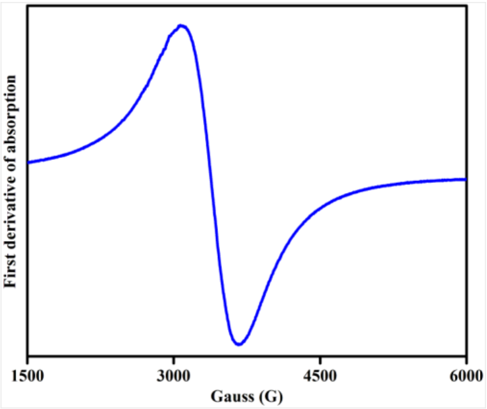


Fig. 4. X-band EPR spectrum of Mn(II) complex

The effective g value is found to be 2.04. This signal suggests that dipolar interactions between manganese ions exist. Mn(II) ions belongs to 3d^{5} electron configuration for ^{55}Mn nucleus, the nuclear spin I = 5/2 and hence EPR spectrum of Mn(II) complex at room temperature exhibits a characteristic six line hyperfine splitting. If the symmetry around Mn(II) is distorted due to complexation, the resonances become anisotropic and a randomly oriented sample may exhibit a broad line (Batista *et al.*, 2010). The observed broad signal in the present work suggests that the symmetry around Mn(II) ion in the complex is distorted one.

Cyclic voltammetric studies

Thiosemicarbazone metal complexes of electrochemical properties with sulphur donor atoms have been studied in order to ensure spectral and structural changes associated electron transfer (Bond *et al.*, 1984). The cyclic voltammograms of the synthesized ligand and its Mn(II), Co(II) and Ni(II) complexes of 0.1 mM are carried in 1 M KCl at 25 ± 0.1^{o}C with 0.1 mM of tetrabutyl ammonium hexafluoro phosphate (TBAHFP) as an electrolyte. The redox properties of the ligand and their complexes were performed by using a conventional three-electrode system and at a scan rate of 50 mV/S by using glassy carbon electrode (GCE). The cyclic voltammogram of substituted thiosemicarbazone was investigated at the potential range (-1000 to 1500 mV). The ligand and metal complexes of the cyclic voltammograms were shown in Fig. 5. The ligand related voltammogram contain two redox peak currents and potentials found to be 0.1049, -0.6044 and 0.0530, -0.8370 V with respectively. The Co(II), Ni(II) and Mn(II) complexes voltammograms were shows the potential differences between two anodic peaks and cathodic peak potential. These potentials are shifted towards negative direction for Co(II), Ni(II) and Mn(II) complexes ($\Delta Epa = Epa_1 - Epa_2$) 0.0731, 0.0807 and 0.0519 V potentials differences with respectively and in cathodic peak potential are also shifted towards negative direction with 0.6044, 0.3359 and 0.0227 V potentials differences with respectively. Coordination of the ligands shifts redox potential of the ligands to negative potentials due to the electron deficient character of the metal centre and also the obtained potentials differences shows the formation of ligand to metal linkage were observed (Chikate *et al.*, 2005).

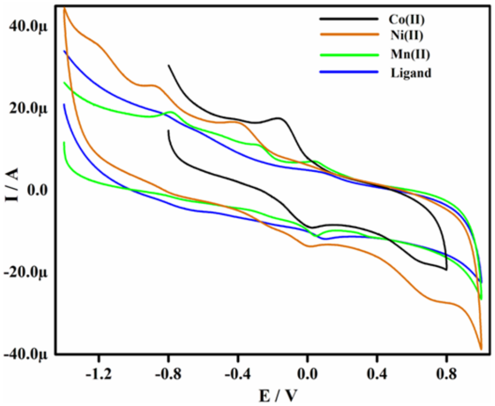


Fig. 5: Cyclic voltammogram of ligand and its metal complexes.

BIOLOGICAL SCREENING

Antibacterial activity

Ligand(L) and its Mn(II), Co(II) and Ni(II) complexes are screened against gram positive and gram negative bacteria. These are the complexes show more activity compared to the parent ligand, but less than the standard antibiotic of streptomycin. The ligand did not show any activity against the *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Klebsiella pneumonia* and except for *E.coli*, whereas Mn, Co and Ni complexes are inhibited the growth of organisms, respectively. Among the three complexes, Ni showing more active against all the bacterial isolates in all concentrations than the Mn(II) and Co(II) complexes. And where as Co complex showing the more activity for *Bacillus subtilis* and *Salmonellatyphi* with increasing of concentration. The reason for greater activity of complexes can be explained by Chelation theory (Naz et al., 2005). From the chelation theory, the polarity of the metal ion is found to be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups especially with sulphur and nitrogen donors. Finally, the growth of antibacterial acitvity for all compounds following in the order of Ni(II) > Co(II) > Mn(II) > ligand.

Antioxidant activity

5-Hydroxy-2-nitrobenzaldehydethiosemicarbazone and its metal complexes are tested for *in vitro* antioxidant activity at 37°C by DPPH free radical scavenging assay method (Sivasankar Reddy *et al.*, 2015). DPPH is a stable free radical used for studying radical scavenging activity in chemical analysis (Bukhari *et al.*, 2009). The DPPH radical shows a strong absorption band at 517 nm in visible spectrum due to the presence of an odd electron. A radical scavenging antioxidant reacts with DPPH stable free radical and converts it into 1,1-diphenyl-2-picrylhydazine as shown in **Fig. 6**.

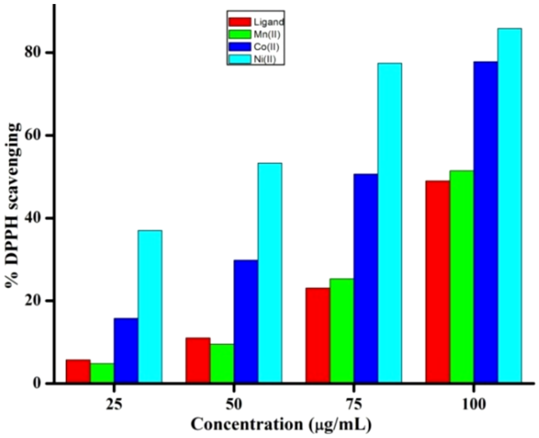


Fig. 6: DPPH scavenging activity of ligand and its Mn(II), Co(II) and Ni(II) complexes.

The change in the absorbance produced in this reaction is used to measure antioxidant properties. All compound solutions are prepared at different concentrations in DMSO solvent. 1 ml of every sample solution having different concentrations and 4 ml of 0.1 mM DPPH solution are taken in different test tubes and the mixture is shaken vigorously for 2 min, after these tubes are incubated for 30 min at room temperature (37°C). The absorbance is measured at 517 nm against blank samples lacking scavenger. The percent of DPPH radical scavenging effect is calculated using the following equation: All the compounds are show good antioxidant activity against DPPH method especially.

$$\text{DPPH}^{\cdot}\ \text{Scavenging effect (\%)} = [(A_0 - A_1)/A_0] \times 100$$

A_0 is the absorbance of the control and A_1 is the absorbance of the test solution.

Table 3: Anti bacterial activity of ligand and their Mn(II), Co(II) and Ni(II) complexes measured in mm.

Bacterial species	**Compound**	**Zone of inhibition in mm**				
		Concentration in µg/ml				
		25	**50**	**100**	**150**	**30 (std.)**
Escherichia coli	Ligand	-	4	7	10	31
	Mn(II)	4	7	9	12	31
	Co(II)	-	3	4	6	31
	Ni(II)	4	7	11	14	30
Klebsiella pneumonia	Ligand	-	-	-	-	30
	Mn(II)	-	3	6	9	31
	Co(II)	-	4	8	11	30
	Ni(II)	3	4	7	11	32
Salmonella typhi	Ligand	-	-	-	-	32
	Mn(II)	-	-	4	7	32
	Co(II)	-	3	7	11	31
	Ni(II)	4	6	9	14	31
Bacillus cerus	Ligand	-	-	-	-	30
	Mn(II)	2	5	6	8	30
	Co(II)	-	3	9	13	31
	Ni(II)	5	7	10	14	31
Staphylococcus aureus	Ligand	-	-	-	-	32
	Mn(II)	2	4	6	9	32
	Co(II)	2	4	7	10	30
	Ni(II)	3	5	8	12	31

Std. Streptomycin; Standard error: ± 0.3 mm

CONCLUSIONS

Syntheses of Mn(II), Co(II) and Ni(II) complexes with the ligand of 5-hydroxy-2-nitro-benzaldehydethiosemicarbazone, and were characterized by different spectral methods. Infrared and electronic spectral studies were revealed a distorted octahedral geometry for all the metal complexes. The antibacterial and antioxidant activities of the ligand and its metal complexes were shows good activity, among the all compounds Ni(II) complex showing more activity.

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