



## Synthesis, Characterization and Biological Evaluation of Fe(III) and Cu(II) Complexes with 2,4-Dinitrophenyl hydrazine and Thiocyanate Ions

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The metal complexes of Fe(III) and Cu(II) were prepared by using 2,4-dinitrophenyl hydrazine (DNPH) and thiocyanate (SCN) with stirrer refluxed for about 6 h. The prepared Fe(III) and Cu(II) complexes were characterized by elemental analysis, molar conductance, magnetic susceptibility and electronic spectrum, FT-IR spectral studies. The result suggested the octahedral geometry for Fe(III) and Cu(II) complexes. Powder X-ray diffraction indicate the crystalline nature of the metal complexes. The antimicrobial activities of the Fe(III) and Cu(II) complexes were tested with various micro organisms by disc diffusion method. The antimicrobial results indicate that the metal complexes are highly active with compared to the free ligand. The *in vitro* antioxidant activity of the free ligand and its metal complexes was assayed by radical scavenging activity (DPPH). The result proposed that Fe (III) and Cu(II) complexes exhibited strong antioxidant activity than that of the ligand.

**Keywords:** Transition metal(II) complexes, 2,4-Dinitrophenyl hydrazine, Thiocyanate, Antimicrobial, Antioxidant.

### INTRODUCTION

Synthesis of metals-ligand coordinated complexes play an important role in many biological systems [1-4]. It was observed that metal ions have considerable effect on the antimicrobial activity of antibiotics [5-9]. Metals have played a significant role in biological systems over the years. 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 [10-12].

The 2,4-dinitrophenylhydrazine has a crucial role for biological systems and constituent in various biomedical, pharmaceutical products and in toxicology [13]. The 2,4-dinitrophenylhydrazine is an analogues of hydrazine and an important class of drugs [14]. The oxygen and nitrogen may be involved in coordination providing a useful model for bioinorganic processes. These complexes are also of interest due to their pharmaceutical applications and biological activities [15]. Thiocyanate group can be present as anion or monodentate ligand and can coordinate with sulfur, nitrogen and oxygen atoms. The strong

coordination ability of thiocyanate group and a variety of bonding modes are responsible for the existence of the number of coordination compounds [16].

Aim of the present work devoted to the synthesis of Fe(III) and Cu(II) complexes of 2,4-dinitrophenyl hydrazine and thiocyanate ion. The synthesized metal complexes were characterized by various analytical and spectral techniques like elemental analysis, magnetic moment measurement, molar conductance measurement, FTIR, UV-visible and PXRD. *in vitro* Antimicrobial activity of synthesized metal complexes and free ligand against various pathogenic bacteria and fungal species were also screened.

### EXPERIMENTAL

All the chemicals were obtained from commercial sources and used without further purifications. The chemicals *viz.*, KSCN, FeCl<sub>3</sub>·4H<sub>2</sub>O and CuCl<sub>2</sub>·2H<sub>2</sub>O were procured from Merck, while 2,4-dinitrophenyl hydrazine was purchased from Sigma Aldrich and solvents *viz.*, DMF, DMSO, methanol and ethanol used as a AR grade.

The elemental analysis was carried out Elementar Vario EL III CHNS analyzer. The electrical conductivity measure-

ments were carried out in DMF medium ( $10^{-3}$  M) using a digital conductivity bridge (Equip-Tronics, EQ-660A) at 30 °C. The FT-IR spectra were recorded using Agilent spectrophotometer with the range of 4000-400  $\text{cm}^{-1}$  based on KBr pellet technique. The UV-visible spectra were recorded on Jasco V-630 wavelength ranging from 200-800 nm with optical resolution 1 nm. X-ray diffraction meter (XRD-600, Shimadzu, model - PW3050/60) using  $\text{CuK}\alpha_1 = 1.5406 \text{ \AA}$ ,  $\text{K}\alpha_2 1.5444$ . Melting points were determined in open capillary tubes in a electrical melting point apparatus, The magnetic susceptibility measurements were obtained using Gouy's balance magnetic susceptibility model MsB-MK1. The pharmacological studies of 2,4-dinitrophenylhydrazine and the metal complexes.

**Synthesis of metal complexes:** The metal complexes were synthesized by DMF solution of 2,4-dinitrophenyl hydrazine (0.002 M) with metal chloride (0.001 M) in ethanol medium. The mixture was stirred reflection for about 1 h. Afterwards potassium thiocyanate (0.002 M) was dissolved in deionized water added and the whole reaction mixture was stirrer refluxed for about 6 h. The obtained coloured metal complexes were filtered and washed with several times in ethanol solution and dried under vacuum desiccators over anhydrous  $\text{CaCl}_2$ .

**Antimicrobial activity:** The metal complexes and free ligand have been screened for their antimicrobial activity by using different species of pathogenic bacteria/fungi's, such as, gram positive: *Bacillus subtilis*, *Staphylococcus aureus* and *Candida albicans*, Gram negative: *Escherichia coli*, *Pseudomonas aeruginosa* and *Aspergillus niger* using disc diffusion method. Each of the metal complexes and free ligand is dissolved in DMSO and the solutions of the concentrations (30, 60 and 90  $\mu\text{g/mL}$ ) were prepared separately. The paper discs soaked in the desired concentration of the complexes and free ligand solutions were placed aseptically in the Petri dishes containing nutrient agar media seeded with each bacterial/fungi serums separately. The Petri dishes were incubated at 37 °C for 24 h for bacteria and 48 h for fungi then the inhibition zones were measured.

#### Antioxidant activity

**DPPH assay:** The free radical scavenging activity of the DNPH and its Fe(III) and Cu(II) complexes test samples was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method [17]. Different concentrations of ligand and its metal complexes (50, 100 and 150  $\mu\text{g/mL}$ ) of tested compound. To the tested sample solutions in DMF, 2 mL methanolic solution of DPPH (0.1 mM) was added to these tubes. The solution was allowed to stand for 30 min inhibition. The control of DPPH was determined as above without the test samples. The absorbance of ligand and metal complexes of solutions were measured at 517 nm. The reduction of DPPH was calculated relative

to the measured absorbance of the DPPH control. The percentage of Scavenging activity was calculated by using the following equation [18]:

$$\text{DPPH scavenging ability (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

## RESULTS AND DISCUSSION

**Microanalysis:** The elemental analysis data of the complexes (Table-1) show the formation of the complexes in the ratio of 1:2 for [M:L] is corresponding to a [Metal:DNPH:SCN]. It is found that the calculated values are in agreement with the found values.

**Molar conductance:** The complexes are dissolved in DMF and the molar conductivity values of  $10^{-3}$  M solutions at  $25 \pm 2$  °C are measured and the values are exhibited in Table-1. It is concluded from the results that the mixed ligand DNPH complexes have the molar conductivity values in the ranges of 12.9 and 15.8  $\Omega^{-1} \text{ mol}^{-1} \text{ cm}^2$  complex which indicates the nonionic nature of these complexes and they are considered as non-electrolytes. From the conductivity measurements, it is inferred that the thiocyanate ions are coordinated to metal ions [19].

**FTIR spectra analysis of metal complexes and ligands:** The FTIR spectra of the free ligand and its metal complexes were carried out in the range of 4000-400  $\text{cm}^{-1}$ . The spectra of the metal complexes and ligand (Fig. 1). FT IR spectra of the ligand showed a sharp peak at 3324 and 1635  $\text{cm}^{-1}$  assigned the NH stretching vibration on the free ligand. In these metal complexes strong adsorption band with attributed NH group of 2,4-dinitrophenyl hydrazine, which renders to attribute to the involvement of coordinate. The stretching vibration of the DNPH nitrogen  $\nu(\text{C-N})$  was observed intense band at 1282  $\text{cm}^{-1}$  in the free ligand. 2,4-Dinitrophenyl hydrazine  $\nu(\text{N-H})$  in the free ligand were shifted to higher frequency in the metal complexes assigned to the impregnations through nitrogen atom, So that higher frequency of  $\nu(\text{C-N})$  band was shifted to lower frequencies indicating that involvement of nitrogen coordinate to the metal ion [20,21]. This is further supported by the appearance of new band at (581-551  $\text{cm}^{-1}$ ) formed due to nitrogen to metal stretching vibration of the metal complexes. The band at 1319  $\text{cm}^{-1}$  in the ligand is still in the same position in the complexes indicating the non-involvement of the  $\text{NO}_2$  in coordination. The FTIR spectrum of thiocyanate complexes showed a high intensity  $\nu(\text{C-N})$  band in 2152-2117 and 2076-2050  $\text{cm}^{-1}$  that frequency provides the formation of coordination from nitrogen to metal ions bonded to  $\text{NCS}^-$  in the isothiocyanated mode [22]. The FTIR spectra indicate that behaves as DNPH and SCN the coordination sites being aromatic primary and secondary amine and thiocyanate nitrogen atom involve the coordination sides give the (Table-2).

TABLE-1  
ANALYTICAL DATA OF METAL COMPLEXES

Complexes	m. w.	Colour	Elemental analysis (%): Found (calcd.)						$\Lambda_m (\Omega^{-1} \text{ cm}^2 \text{ mol}^{-1})$
			C	H	N	O	S	M	
[Fe(DNPH) <sub>2</sub> (SCN) <sub>2</sub> ]	568.27	Blood red	29.59 (29.52)	2.13 (2.10)	24.65 (24.59)	22.52 (22.46)	11.28 (11.23)	9.83 (9.75)	12.9
[Cu(DNPH) <sub>2</sub> (SCN) <sub>2</sub> ]	575.97	Yellow	29.19 (29.12)	2.1 (2.2)	24.32 (24.27)	22.22 (22.11)	11.3 (10.98)	11.03 (10.95)	15.8

TABLE-2  
SELECTED IR BANDS (cm<sup>-1</sup>) OF LIGAND AND ITS METAL COMPLEXES

Compound	$\nu(\text{N-H})$	$\nu(\text{N-H})$	$\nu(\text{C-N})$	$\nu(\text{NO}_2)$	$\nu(\text{SCN})$	$\nu(\text{N-M})$
DNPH	3324	1635	1282	1319	–	–
[Fe(DNPH) <sub>2</sub> (SCN) <sub>2</sub> ]	3364	1647	1259	1320	2117, 2050	581
[Cu(DNPH) <sub>2</sub> (SCN) <sub>2</sub> ]	3288	1618	1261	1320	2152, 2076	551

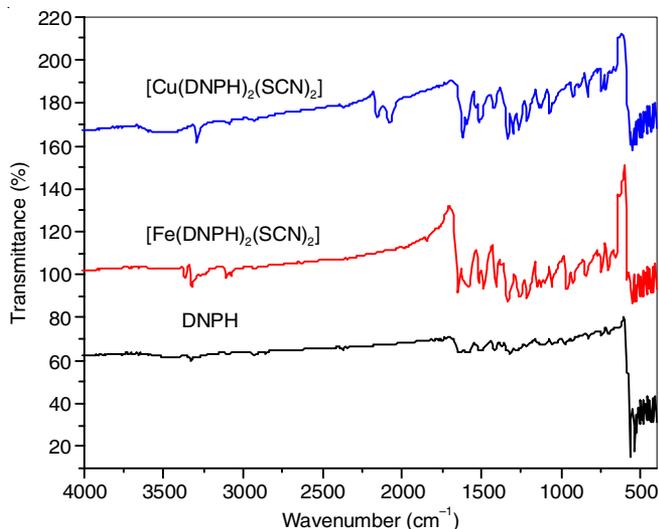


Fig. 1. FT-IR spectra of the metal complexes and free ligand

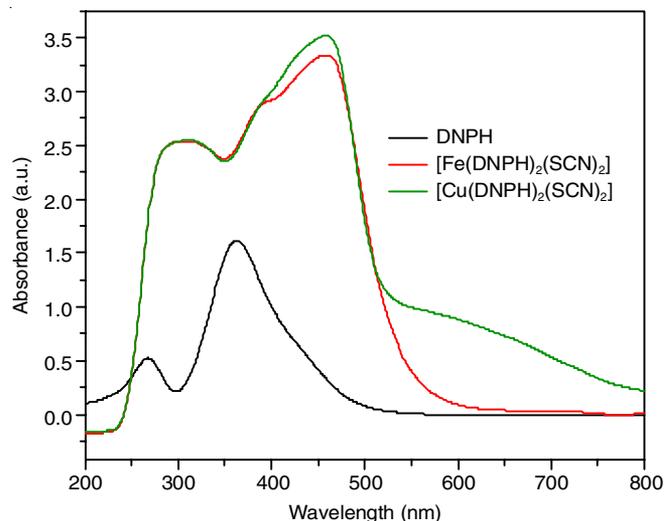


Fig. 2. Electronic spectra of free ligand and its metal complexes

**Electronic spectra and magnetic moments:** The electronic spectral data of the 2,4-dinitrophenyl hydrazine (DNPH) and its metal complexes in DMF is given in Table-3. The electronic spectrum of 2,4-dinitrophenyl hydrazine and complexes are given in (Table-3) and (Fig. 2). The ligand shows two strong peaks at 267 and 363 nm. The two strong bands at 267 and 363 nm are attributed to  $\pi$ - $\pi^*$  and  $n$ - $\pi^*$  transitions respectively. In the metal complexes this band is shifted to a longer wavelength with increasing intensity. This shift may be attributed to the donation of lone pair of electrons of nitrogen atom of the 2,4-dinitrophenyl hydrazine to the metal ion (M-N). The electronic spectrum of the Fe(III) complex exhibits three absorption at 308, 388 and 458 nm, which are assigned to  $\pi$ - $\pi^*$ , LMCT and  ${}^6A_{1g} \rightarrow {}^4T_{1g}$  transitions respectively, suggesting an octahedral geometry. The observed magnetic moment value of 5.91 BM is in accordance with the high spin six coordinated octahedral arrangement around the iron complexes [23]. The electronic spectrum of the Cu(II) complex exhibits three absorption at 301, 457 and 621 nm, which are assigned to  $\pi$ - $\pi^*$ ,  ${}^2B_{1g} \rightarrow {}^2B_{2g}$  and  ${}^2E_g \rightarrow {}^2T_g$  transitions respectively, suggesting

TABLE-3  
ELECTRONIC SPECTRA OF COMPLEXES  
AND MAGNETIC SUSCEPTIBILITY

Complex	$\lambda_{\text{max}}$ (nm)	Band assignments	$\mu_{\text{eff}}$	Geometry
DNPH	267	$\pi$ - $\pi^*$	–	–
	363	$n$ - $\pi^*$		
[Fe(DNPH) <sub>2</sub> (SCN) <sub>2</sub> ]	308	$\pi$ - $\pi^*$	5.91	Octahedral
	395	LMCT		
	458	${}^6A_{1g} \rightarrow {}^4T_{1g}$		
[Cu(DNPH) <sub>2</sub> (SCN) <sub>2</sub> ]	301	$\pi$ - $\pi^*$	1.9	Octahedral
	457	${}^2B_{1g} \rightarrow {}^2B_{2g}$		
	621	${}^2E_g \rightarrow {}^2T_g$		

an octahedral geometry. The observed magnetic moment value of 1.9 BM is in accordance with the six coordinated octahedral arrangement around the copper complexes [24].

**X-ray diffraction analysis:** The X-ray powder diffraction analysis of the complexes was performed in order to determine the structural information of polycrystalline material as it was difficult to isolate suitable single crystal for X-ray crystallography. The powder diffraction analysis of metal complexes well defined crystalline peaks (Fig. 3) while Fe(III) and Cu(II) complexes were found to be amorphous in nature. The indexing and calculations of unit cell parameters were performed using XPERT PRO software and scattering angles ( $2\theta$ ) corresponding to each reflection, inter-planar spacing ( $d$ ) along with Miller's indices and lattice constants were evaluated which corresponds to cubic crystal system for Fe(III) and Cu(II) complexes. The average crystallite size for the above mentioned complexes was calculated using Debye Scherrer's formula:

$$D = \frac{0.9\lambda}{\beta \cos \theta}$$

where constant 0.9 is the shape factor,  $\lambda$  is the x-ray wavelength of  $\text{CuK}\alpha$  radiation (1.5406 Å),  $\theta$  is the Bragg diffraction angle and  $\beta$  is the full width at half maximum (FWHM). The experimental average crystallite sizes of Fe(III) and Cu(II) complexes were found to be 43.3, 88.2, nm respectively.

**Microbial activities:** The synthesized Fe(III) and Cu(II) complexes of the antibacterial and antifungal activities results are given in Table-4. Against all organisms, the ligand is moderately active and the complexes are more active than the ligand. The Fe(III) complex is highly active against the bacterial and fungal species compared with other complexes. The activity increases with increase in concentration of complexes. It is suggested that the complexes having antimicrobial activity may act either by killing the microbe or by inhibiting multiplication

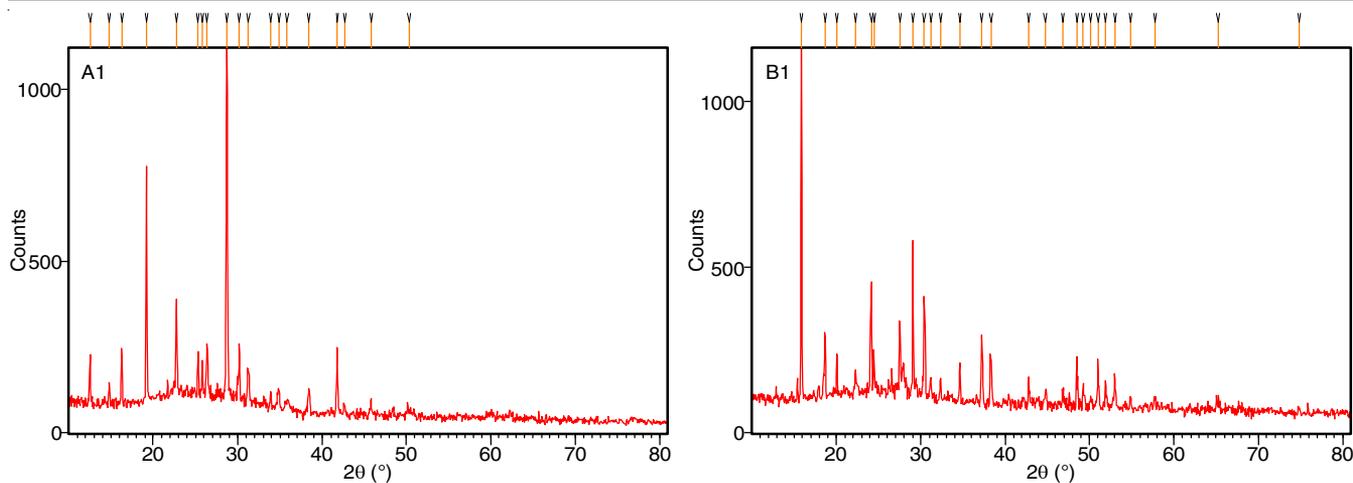


Fig. 3. X-ray diffraction patterns of Fe and Cu complexes

TABLE-4  
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF COMPLEXES AND FREE LIGAND

Organism name	[Fe(DNPH) <sub>2</sub> (SCN) <sub>2</sub> ]			[Cu(DNPH) <sub>2</sub> (SCN) <sub>2</sub> ]			DNPH		
	30 μg/mL	60 μg/mL	90 μg/mL	30 μg/mL	60 μg/mL	90 μg/mL	30 μg/mL	60 μg/mL	90 μg/mL
<i>Bacillus subtilis</i>	15	17	23	10	12	22	–	2	5
<i>Staphylococcus aureus</i>	10	15	20	13	17	26	1	3	6
<i>Pseudomonas aeruginosa</i>	11	13	16	10	14	19	–	1	2
<i>Escherichia coli</i>	12	19	25	11	16	20	–	–	3
<i>Aspergillus niger</i>	8	10	20	10	15	23	5	10	12
<i>Candida albicans</i>	7	11	19	14	18	21	–	8	10

of the microbe by blocking their active sites [25]. The activity order of the synthesized complexes is as follows: Cu > Fe > L (Fig. 4).

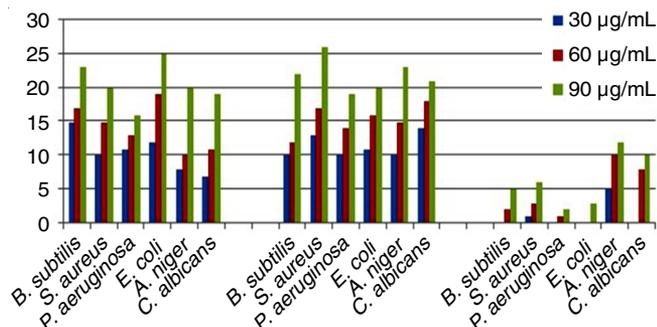


Fig. 4. Microbial activity of metal complexes and free ligand with different concentration and various pathogenic bacteria/fungi's

**in vitro Antioxidant activity:** The results of radical scavenging effect of the compounds at different concentrations (50, 100 and 150 μg/mL) are shown in Table-5 as well as in concentration dependent curve in Fig. 5. It is evident from the results that free ligand, showed remarkably less potent activity

TABLE-5  
% SCAVENGING ACTIVITY OF THE COMPLEXES AND FREE LIGAND

Complex	50 μg/mL	100 μg/mL	150 μg/mL
DNPH	50.57	52.24	59.60
[Fe(DNPH) <sub>2</sub> (SCN) <sub>2</sub> ]	62.78	65.41	86.34
[Cu(DNPH) <sub>2</sub> (SCN) <sub>2</sub> ]	55.31	56.61	60.62

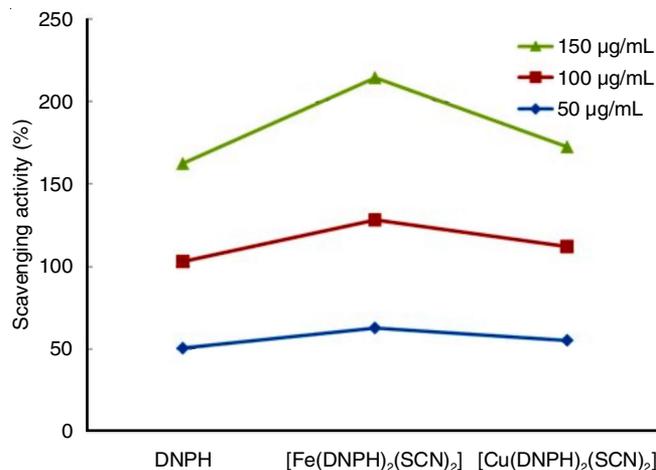


Fig. 5. % of scavenging activity of ligand and its metal complexes

than metal complexes, which is mainly due to the chelation of organic ligand with the metal ion. Among the examined complexes, Fe(III) complexes exhibited best scavenging activity whereas Cu(II) complexes have shown moderate activity in comparison to free ligand. The difference in the activity of the metal complexes may be ascribed due to the coordination environment and the redox properties. In general, the redox properties of the complex depend on several factors such as chelate ring size, axial ligation, degree of unsaturation in the chelate ring. The comparative assessment of the antioxidant activity data on free ligand and its metal complexes suggest that have better antioxidant activity as compared to free ligand (Fig. 6) which warrant them further *in vivo* experiments for pharmacological purposes.

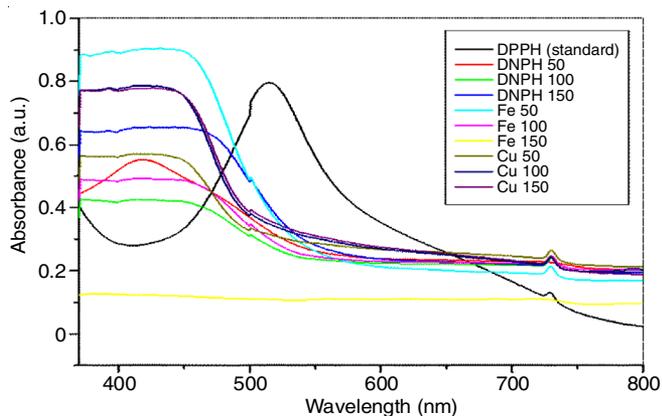


Fig. 6. UV-visible absorption spectra of free radical scavenging activity of metal complexes and free ligand with different concentrations

## Conclusion

Mixed ligand complexes of Fe(III) and Cu(II) with 2,4-dinitrophenyl hydrazine and thiocyanate were synthesized. The structure of the complexes was confirmed by the elemental analyses, IR, UV-visible, magnetic moment, molar conductance. The molar conductance values show that the complexes are non-electrolytes. Spectral and magnetic moment suggest to octahedral geometry (Fig. 7) for the mixed ligand complexes. PXRD analysis shows the crystalline nature of the metal complexes. The comparative study of *in vitro* antibacterial and antioxidant activities was carried out which revealed that metal complexes exhibited more potential activities as compared to free ligand.

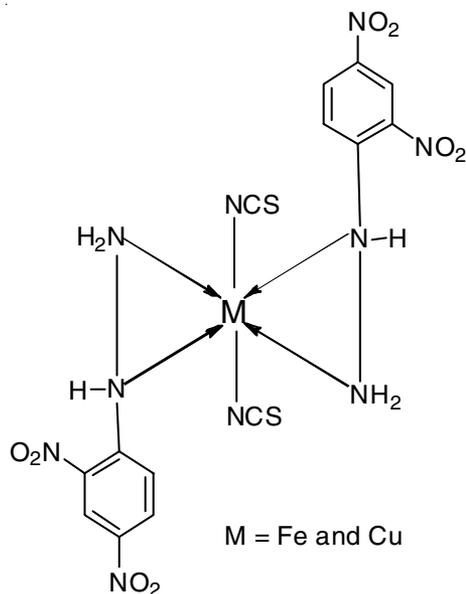


Fig. 7. Schematic structure of octahedral geometry of metal complexes

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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