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Original article

Antimicrobial and SOD activities of novel transition metal ternary complexes of iminodiacetic acid containing α-diimine as auxiliary ligand

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ABSTRACT

Ternary complexes containing an α-diimine auxiliary ligand have been widely used as models for several mono and polynuclear metal enzymes. The present ternary complexes $[M(IDA)(Phen)H_2O]\cdot xH_2O$ ($x = 2$, 3 or 4) were prepared as novel antimicrobial agents employing reactions of $Cu(OAc)_2$ or MCl_2 (M = Co, Ni, Cr) with iminodiacetic acid (H_2IDA) in the presence of 1,10-phenanthroline (Phen), whose chemical structure and bonding were elucidated by IR, FAB-Mass, ^1H, ^{13}C NMR, EPR spectral and elemental analyses. The antimicrobial activities against *Escherichia coli* (K-12), *Bacillus subtilis* (MTCC 121), *Staphylococcus aureus* (IOA-SA-22), *Salmonella typhimurium* (MTCC 98), *Candida albicans*, *Aspergillus fumigatus* and *Penicillium marneffei* (isolates from Department of Microbiology, Faculty of Agricultural Science, AMU) were investigated and significant activities were obtained. The superoxide dismutase activity of the Cu(II) complex was assessed by NBT assay. The single crystal X-ray structure for $[Cu(IDA)(Phen)H_2O]\cdot 2H_2O$ indicates a triclinic unit cell in *P*-1 space group with structural parameters, $a = 6.745(5)$, $b = 10.551(5)$, $c = 11.414(5)$ Å, $\alpha = 95.770(5)$, $\beta = 91.396(5)$, $\gamma = 92.518(5)^\circ$ and presence of an extensive H-bonding and π–π stacking interactions which generate a supramolecular framework.

1. Introduction

The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens. In spite of a large number of antibiotics and chemotherapeutics available for medical use, at the same time the emergence of old and new antibiotic resistance created in the last decades revealed a substantial medical need for new classes of antimicrobial agents. There is a real perceived need for the discovery of new compounds endowed with antimicrobial activity, possibly acting through mechanism of action, which is distinct from those of well-known classes of antimicrobial agents to which many clinically relevant pathogens are now resistant.

The coordination chemistry of iminodiacetic acid (H_2IDA) has been a subject of continuous investigations due to its tridentate chelating behaviour towards metal ions resulting in complexes which show structural diversities [1,2]. In aqueous biological pH range (pH = 6–7), the carboxylic acid protons of H_2IDA dissociate to give iminodiacetato (IDA^{2-}) dianion which is quite reactive to transition metal ions forming metal iminodiacetate complexes [3]. Reactions of metal iminodiacetates with an α-diimine viz. 1,10-phenanthroline (Phen) or 2,2′-bipyridine (bipy) produce mixed-ligand complexes, some of which have been successfully exploited as model compounds to explain binding modes and structural features in several metalloenzymes [4]. Several mixed-ligand complexes have been found to exhibit superoxide dismutase activity [5,6], which is related to the flexibility of the geometric transformation around the metal centres [7]. Moreover, some of these complexes play a decisive role not only in activation of enzymes but also in the storage and transport of active substances through biological membranes [8]. Transition metal ternary complexes have received particular focus and have been employed in mapping protein surfaces [9], as probes for biological redox centres [10] and in protein capture for purifications [11].

The study of the structure of model ternary complexes provides informations about how biological systems achieve their specificity and stability, as well as strategies to improve these features for biotechnological applications. Ternary complexes of oxygen donor ligands and heteroaromatic N-bases have been of special interest as they can display exceptionally high stability [12]. X-ray structural data of the ternary complexes with iminodiacetato (IDA^{2-}) or its substituted analogues as primary ligand and N-donors as secondary ligand have revealed the influence of the secondary (auxiliary) ligand on the conformations of the primary

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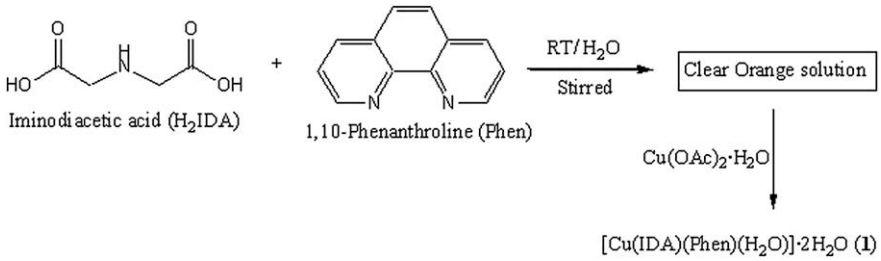


Scheme 1. Synthetic procedure of complex (**1**).

iminodiacetato ligands. Studies of such complexes have also explained the observed changes in the protein conformations at the metal–protein centres in the absence as well as in the presence of appropriate substrates [13]. The structure of the ternary complexes bearing the composition M/IDA/N-heterocycle in the mole ratio 1/1/2 displays a hexa coordinate elongated octahedral $(4+1+1)$ geometry where IDA is in a fac-tridentate conformation [14]. Complexes having the composition M/IDA/N-heterocycle in 1/1/1 mole ratio, invariably adopt a five coordinate square pyramidal $(4+1)$ coordination geometry, albeight the possibility of the rarely observed mer-tridentate [15] chelation giving an elongated six coordinate octahedral $(4+1+1$ or $4+2)$ coordination geometry, as observed in the present case, may not be ruled out.

Prompted by the biological activities of the transition metal ternary complexes, it was contemplated to design syntheses of mixed-ligand metal iminodiacetate complexes, M/IDA/Phen (1/1/1 mole ratio) and to pursue in vitro antimicrobial and SOD activities. It was considered worthwhile to investigate if there exists a correlation between the rare mer-tridentate conformation of the IDA^{2-} with the overall biological activities of the complexes.

2. Chemistry

Metal salts (Merck), 1,10-phenanthroline (Merck) and iminodiacetic acid (Aldrich) were used for the synthesis. Ultrapure Milli Q water (18.3 lX) was used for all experiments. The commercial solvents were distilled and then used for the preparation of complexes. Ternary complexes $[Cu(IDA)(Phen)(H_2O)] \cdot 2H_2O$ (**1**), $[Co(IDA)(Phen)(H_2O)] \cdot 4H_2O$ (**2**), $[Ni(IDA)(Phen)(H_2O)] \cdot 3H_2O$ (**3**) and $[Cr(IDA)(Phen)(H_2O)] \cdot 4H_2O$ (**4**) were synthesized by the stoichiometric reaction of copper(II) acetate [15] or MCl_2 (M = Co, Ni and Cr) with iminodiacetic acid (H_2IDA) in the presence of 1,10-phenanthroline (Phen) in water at room temperature. The reaction sequences are outlined in Schemes 1 and 2. The recrystallization of the copper complex in ethanol produced cubic crystals suitable for X-ray crystallography.

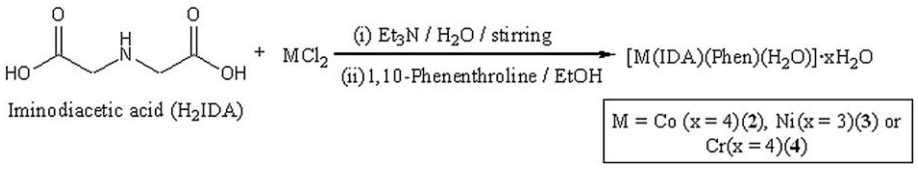
2.1. Crystallographic data collection and structure analysis

A light blue cubic crystal of copper complex was mounted on a glass fibre and all measurements were performed on BRUKER SMART APEX CCD diffractometer with graphite monochromator using Mo Kα ($\lambda = 0.71069$ Å) radiation. Cell constants and an orientation matrix for data collection were obtained from a least-square refinement [16] using the setting angles of 21 carefully centered reflections in the range of $2.51 < 2\theta < 25.99°$. Hydrogen atoms were refined isotropically. A summary of data collection and structure refinement is given in Table 1.

3. Results and discussion

3.1. Spectra

In the present work, a series of four new complexes were synthesized. Schemes 1 and 2 illustrate the way used for the preparation of target compounds. The structure of the compounds was elucidated by IR, ^1H, ^{13}C NMR, mass spectral data and elemental analyses. In the IR spectra of the compounds ν(C=N) and ν(C=C) str. vib. bands were observed in the 1580–1427 cm^{-1} region. The presence of a band of medium intensity appearing at ~420 cm^{-1} is consistent with the formation of M–N bond(s) in the molecule. A well separated strong intensity absorption band near 1625 cm^{-1} and 1303 cm^{-1} is assignable to ν_{asym}(COO) str. and ν_{sym}(COO) str. vib., respectively. The separation of the ν_{asym} and ν_{sym} (COO) str. vib. [$\Delta\nu = \nu_{asym}(COO) - \nu_{sym}(COO)$ ~300 cm^{-1}] is very large indicating a strong bidentate chelation from the anionic COO^- group of IDA^{2-} moiety to the metal ion in all the complexes. The observed negative shift in ν(C–N) str. vib. (~100 cm^{-1}) relative to that observed in free uncoordinated H_2IDA moiety further corroborates the additional coordination from the iminic nitrogen of the iminodiacetate moiety. The presence of coordinated water molecule as well as the lattice water was also indicated as a broad band in the 3500–3200 cm^{-1} region. The ^1H NMR spectra of the complexes recorded in D_2O showed a singlet at 3.40–3.52 ppm attributable to $-CH_2-$ protons. Iminic –NH– proton in the complexes resonates as a singlet at about 10.22–11.10 ppm. The aromatic protons characteristic of phenanthroline appeared as multiplets in 7.4–8.8 ppm. In ^{13}C NMR spectra, the resonance signals due to carbons of the >C=O and $-CH_2-$ groups appeared near 176 ppm and 43 ppm, respectively. The resonance peaks characteristic of aromatic ring carbons appear in the 120–139 ppm region. However, a sharp signal observed at 150 ppm is assignable to the α-diimine (Phen) carbon bonded to hetrocyclic nitrogen. The X-band EPR spectra of the complex (**1**) recorded at room temperature as well as liquid



Scheme 2. Synthetic procedure of complexes (**2**–**4**).

Table 1
Crystal data and refinement parameters.

CCDC deposit No.	681969
Empirical formula	$C_{16}H_{16}CuN_3O_7$
Formula weight	425.87
Crystal system	Triclinic
Colour/Shape	Light blue/cubic
Space group	*P*-1
Unit cell dimensions	*a* (Å) – 6.745(5); *b* (Å) – 10.551(5); *c* (Å) – 11.414(5); *α* (°) – 95.770(5); *β* (°) – 91.396(5); *γ* (°) – 92.518(5)
V (Å)^3	807.1(8)
Crystal size (mm)	0.20 × 0.15 × 0.10
Z	2
T (K)	293(2)
λ (Mo K*α*) (Å)	0.71069
θ Range (°)	2.51–25.99
Limiting indices	$-8 \le h \le 8$; $-12 \le k \le 12$; $-14 \le l \le 14$
Structure solution	Direct method (SHELXL97)
Total reflections	3080
Reflections gt.	2775
No. of refined parameters	260
Goodness of fit on F^2	1.09
Final R indices [$1 > 2\sigma(I)$]	$R_1 = 0.0494$, $wR_2 = 0.1330$
R indices (all data)	$R_1 = 0.0542$, $wR_2 = 0.1382$

Important bond lengths are: Cu–O3 = 1.980(3), Cu–N2 = 2.010(3), Cu–N1 = 2.029(3), Cu–N3 = 2.043(3), Cu–O1 = 2.313(3), Cu–O5 = 2.362(3).

nitrogen temperature were identical exhibiting an anisotropic behaviour with $g_{\|} = 2.32$ and $g_{\perp} = 2.13$. The observed anisotropy in the spectroscopic Lande parameter ($g_{\|} > g_{\perp} > 2.0$) indicates an axial distortion around Cu(II) (d^9) system which arises due to a strong Jahn–Teller distortion. FAB-Mass spectra of the complexes showed M + 1 peaks, in agreement with their molecular formulae.

3.2. Single crystal X-ray diffraction studies

The crystal data with structure refinements are given in Table 1. The molecular structure and packing diagrams are shown in Figs. 1 and 2, respectively. The structure consists of a discrete monomeric unit in which Cu(II) ion acquires an un-symmetrically elongated hexagonal coordination geometry. The iminodiacetate (IDA) acts as a tridentate chelating ligand with one carboxylato–O(O3) and imino–N(N3) centres as part of the equatorial coordination basal plane whose other donor centres are the two N-atoms (N1 and N2) of the coordinated heterocycle (Phen). However, the second carboxylato–O(O1) of IDA and the coordinated H_2O–O(O5) form the

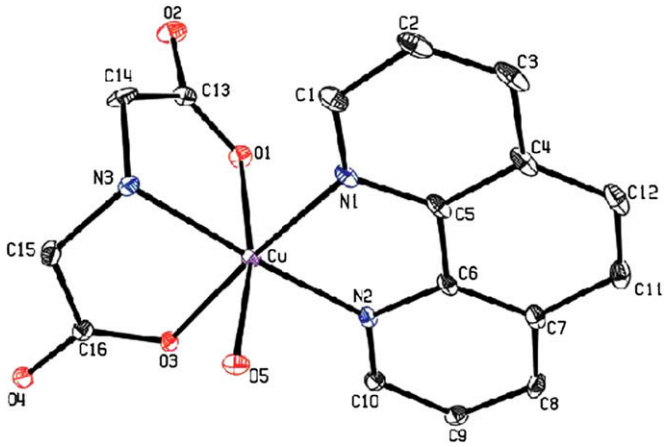


Fig 1. ORTEP view of the copper complex with 50% probability of thermal ellipsoids. (Hydrogen atoms and lattice water molecules have been omitted for clarity).

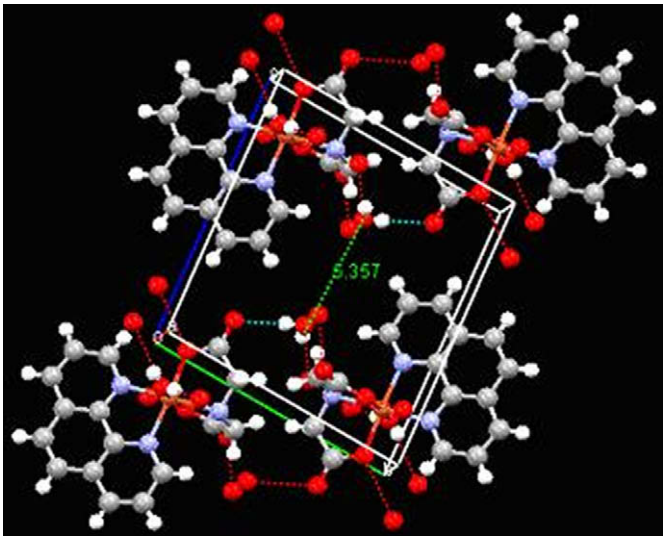


Fig 2. Packing diagram of the complex (**1**).

axial bonds. The axial Cu–O1 and Cu–O5 bond lengths are much longer than the Cu–O3 and Cu–N bonds of the basal plane due to Jahn–Teller distortion forming a 4 + 1+1 type coordination geometry with longitudinal tetragonal distortions (axial elongation). The average deviation between the apical and basal Cu–O bond distance is ~0.357 Å and the extent of tetragonality (*T*) defined [15] as the ratio of the mean in-plane bond distance to the mean of larger distance is ~0.87. The magnitude of *T* for the complex is rather small compared to the reported [17] values (*T* = 0.91–0.96) for a gross axially distorted octahedral structure in the presence of Jahn–Teller distortion. Each discrete unit is joined together through a wide spread H-bonds and π–π interactions to constitute a supramolecular network [Fig. 3(a) and (b)]. It is apparent from Fig. 3(a) that the water molecules act as bridges through H-bonds between each monomeric unit to generate a stable polymeric structure. The observed H-bond distance [*d*(O···H–O) = 1.975 Å] is shorter compared to the reported [15] value [cf. *d*(O···H–O) = 2.728 Å]. The observed centroid(π)–centroid(π) distance, i.e., *d*(π–π) = 3.678 Å, for the copper complex indicates an effective participation of the side-on ring–ring π–π interactions between the phenanthroline rings of the neighbouring layers [Fig. 3(b)] in the stacking or molecular recognition process [14].

4. Pharmacology

All compounds were evaluated for their antimicrobial properties. MICs were recorded as minimum concentration of the compound, which inhibits the growth of tested microorganism. Complexes (**1**) and (**2**) showed good activities while (**3**) and (**4**) were found to be less active or inactive against some of the bacterial and fungal stains (Tables 2 and 4). The copper complex (**1**) also exhibited superoxide dismutase (SOD) activity.

4.1. Antibacterial studies

The newly prepared compounds were screened for their antibacterial activity against *Escherichia coli* (K-12), *Bacillus subtilis* (MTCC-121), *Staphylococcus aureus* (IOA-SA-22) and *Salmonella typhimurium* (MTCC-98) bacterial stains by disc diffusion method [18,19]. The discs measuring 5 mm in diameter were prepared from Whatman no. 1 filter paper sterilized by dry heat at 140 °C for 1 h. The sterile discs previously soaked in a concentration of the test

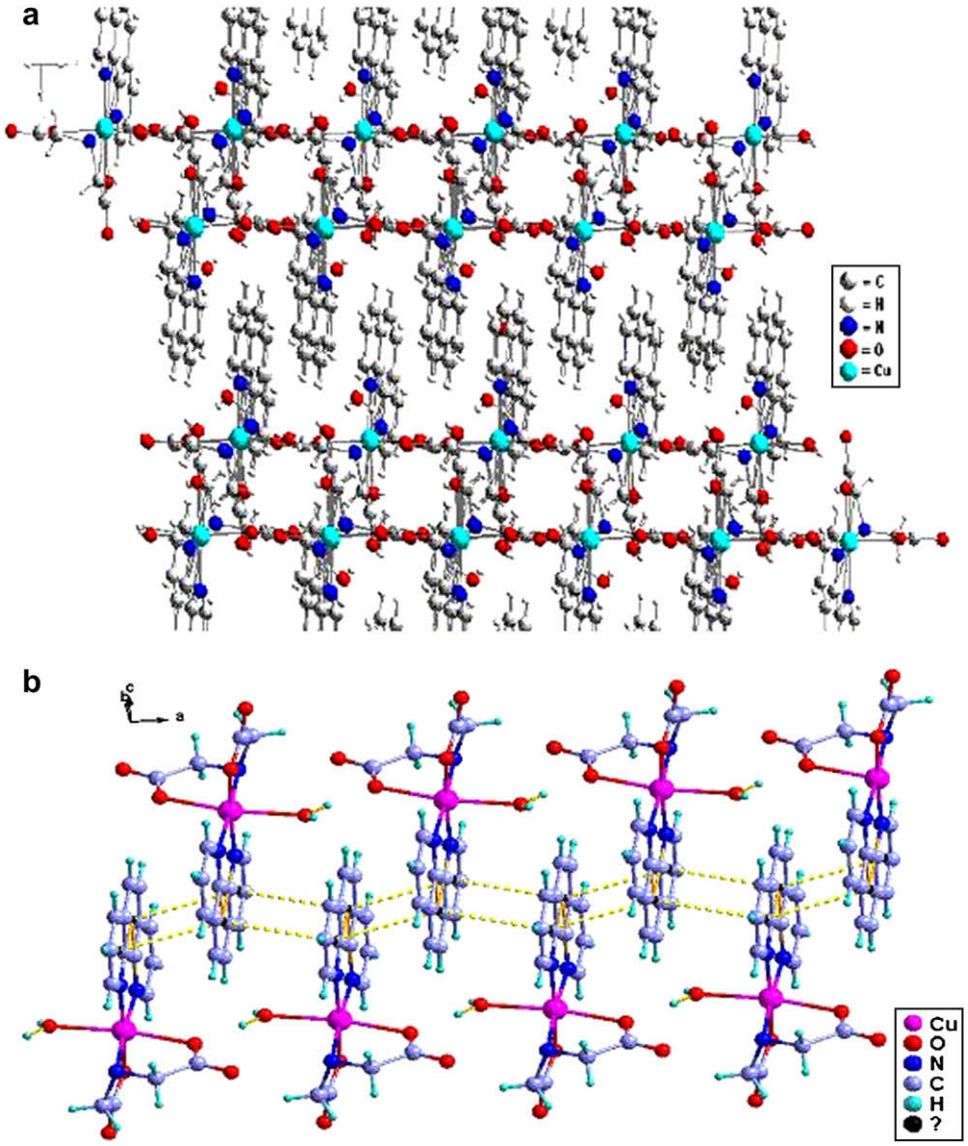


Fig 3. (a) A supramolecular network of the complex (**1**) constructed from H-bonding. (b) Two layers of the complex (**1**) showing π–π interactions between phenanthroline rings [$d(\pi-\pi) = 3.673$ Å] and forming a supramolecular framework.

compounds were placed in a nutrient agar medium. The plates were inverted and kept in an incubator at 30 ± 1 °C. The inhibition zone thus formed was measured (in mm) after 24 h (Table 2). The screening was performed at two different concentrations, i.e., 1 μg/ml and 100 μg/ml and ciprofloxacin was used as the standard.

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The nutrient broth, which contained logarithmic serially twofold diluted amount of test compounds and controls was inoculated within approximately 5×10^5 c.f.u. of actively dividing bacteria cells. The cultures were incubated for 24 h at 37 °C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC). To obtain the minimum bacterial concentration (MBC), 0.1 ml volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted after 18–24 h of incubation at 35 °C. MBC was defined as the lowest drug concentration at which 99.9% of the inoculum was killed. The

Table 2
Zone of inhibition (mm) of the complexes.

Complex	*Escherichia coli*		*Bacillus subtilis*		*Staphylococcus aureus*		*Salmonella typhimurium*	
Conc. (μg/ml)	1	100	1	100	1	100	1	100
(**1**)	33	10	30	19	35	17	29	13
(**2**)	18	13	17	14	9	7	16	14
(**3**)	12	7	13	11	0	0	0	0
(**4**)	0	0	19	15	14	5	0	0

Table 3
MIC and MBC results of the complexes.

Complex	*Escherichia coli*		*Bacillus subtilis*		*Staphylococcus aureus*		*Salmonella typhimurium*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
(**1**)	6.25	12.5	6.25	6.25	25	12.5	12.5	25
(**2**)	12.5	6.25	12.5	6.25	6.25	6.25	6.25	12.5
(**3**)	6.25	12.5	6.25	6.25	0	0	0	0
(**4**)	0	0	0	0	12.5	6.25	6.25	6.25
Standard	6.25	12.5	6.25	12.5	6.25	12.5	12.5	12.5

Ciprofloxacin is used as the standard. MIC (μg/ml) = minimum inhibitory concentration, i.e., the lowest concentration to completely inhibit bacterial growth; MBC (μg/ml) = minimum bactericidal concentration, i.e., the lowest concentration to completely kill bacteria.

Table 5
MIC and MFC results of the complexes.

Complex	*Candida albicans*		*Aspergillus fumingatus*		*Penicillium marneffei*	
	MIC	MFC	MIC	MFC	MIC	MFC
(**1**)	6.25	6.25	6.25	12.5	6.25	12.5
(**2**)	6.25	12.5	6.25	6.25	6.25	6.25
(**3**)	6.25	6.25	0	0	6.25	12.5
(**4**)	6.25	6.25	6.25	12.5	0	0
Standard	6.25	12.5	6.25	12.5	6.25	12.5

Greseofulvin is used as the standard. MIC (μg/ml) = minimum inhibitory concentration, i.e., the lowest concentration to completely inhibit fungal growth; MFC (μg/ml) = minimum fungicidal concentration, i.e., the lowest concentration to completely kill fungus.

minimum inhibitory concentration and minimum bacterial concentration are given in Table 3. The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition.

Copper complex showed good inhibition against all bacteria at 1 μg/ml concentration. Cobalt complex showed slightly less activity than that of copper complex. Nickel complex was found to be inactive against *S. aureus* and *S. typhimurium* while chromium complex showed no activity towards *B. subtilis* and *S. typhimurium*.

4.2. Antifungal studies

The newly prepared complexes were screened for their antifungal activity against *Candida albicans*, *Aspergillus fumigatus* and *Penicillium marneffei* by agar diffusion method [20,21]. Sabouraud's agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting pH to 5.7. Normal saline water was used to make suspension spore of fungal stain lawning. A loopful of particular fungal stain was transferred to 3 ml saline to get suspension of corresponding species. Twenty milliliters of agar media was poured into each petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37 °C for 1 h. Using an agar punch, wells were made and each well was labeled. A control was also prepared in triplicate and maintained at 37 °C for 3–4 days. The fungal activity of each compound was compared with Greseofulvin as standard drug. Inhibition zones were measured and compared with controls. The fungal zones of inhibition are given in Table 4. The cultures were incubated for 48 h at 35 °C and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentration (MIC). To obtain minimum fungicidal concentration (MFC), 0.1 ml volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted as the lowest drug concentration at which 99% of the inoculum was killed. The minimum inhibitory concentration and minimum fungicidal concentration are given in Table 5. The antifungal screening data showed that only copper and cobalt complexes exhibited good activity. Nickel and chromium complexes are inactive towards *Aspergillus fumingatus* and *P. marneffei* respectively.

Table 4
Zones of inhibition (mm) of the complexes.

Complex	*Candida albicans*	*Aspergillus fumingatus*	*Penicillium marneffei*
(**1**)	28	29	19
(**2**)	18	14	16
(**3**)	13	0	9
(**4**)	15	11	0

4.3. Superoxide dismutase activity

Out of the synthesized complexes, only copper complex (**1**) was found to be active towards superoxide dismutase. The SOD activity of the complex was investigated by NBT assay. Several complexes containing transition metal [22] are known to give good SOD activity, although, their structures are totally unrelated with native enzyme [23]. Herein we report the SOD activity measured at pH 7.8. The chromophore concentration required yield 50% inhibition of the reduction of NBT (IC_{50}) value of the present complex (i.e., 62 μmol/dm^3) which is higher than the value exhibited by the native enzyme ($IC_{50} = 0.04$ μmol/dm^3). This higher value may be correlated to the presence of a mer-conformation of the primary (IDA^{2-}) ligand as well as the greater ligand field crowding by the α-diimine ligand (phen) over the central metal ion. A greater interaction between superoxide ion and Cu(II) in mixed-ligand complex is due to strong axial bond [24], which results in an increased catalytic activity.

5. Conclusion

Mixed-ligand ternary transition metal (i.e., Cu, Co, Ni and Cr) complexes bearing iminodiacetic acid ligand and 1,10-phenanthroline co-ligand, were synthesized and characterized from spectral methods. The complexes usually adopt a distorted octahedral geometry around the metal ion. Single crystal X-ray and EPR studies of $[Cu(IDA)(Phen)H_2O]\cdot 2H_2O$ confirm an elongated hexa coordinate (4 + 1 + 1) geometry with mer-tridentate conformation of the primary iminodiacetate (IDA^{2-}) ligand. Among the synthesized ternary complexes, copper and cobalt complexes showed remarkable antibacterial and antifungal activities while nickel and chromium complexes showed these activities up to less extent. The copper complex was found to show superoxide dismutase (SOD) activity.

6. Experimental protocols

6.1. Chemistry

Melting points were determined by open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Shimadzu FT-IR 157 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury Plus 300 spectrometer using TMS as an internal standard. The X-band EPR spectra were taken at room temperature as well as liquid nitrogen temperature on a Varian E-112 spectrometer operating at 9.1 GHz. The crystal structure was performed on BRUKER SMART APEX CCD diffractometer with graphite monochromator using Mo Kα ($\lambda = 0.71069$ Å) radiation and solved by direct method using the program SHELXL97 [16]. The mass spectra were recorded on an

FAB-mass spectrometer operating at 70 eV. The purity of the complexes was checked by thin layer chromatography (TLC) on silica gel plate using a mixture of petroleum ether and ethyl acetate.

6.2. General procedure for the synthesis of the complexes

6.2.1. Synthesis of the complex $[Cu(IDA)(Phen)(H_2O)]\cdot 2H_2O$ (**1**)

Equimolecular mixture of iminodiacetic acid (5 mmol) and 1,10-phenanthroline (5 mmol) in H_2O (15 ml) was stirred for 1 h at room temperature producing an orange coloured homogeneous clear solution. To this solution was added aqueous solution of $Cu(OAc)_2\cdot H_2O$ (5 mmol) affording an immediate blue coloured solid (**1**), which was then recrystallized in ethanol giving light blue cubic crystals in a good yield.

6.2.2. Synthesis of the complex $[Co(IDA)(Phen)(H_2O)]\cdot 4H_2O$ (**2**)

The reaction of iminodiacetic acid (5 mmol) with $CoCl_2\cdot 6H_2O$ (5 mmol) was carried out in H_2O (15 ml) in the presence of triethylamine (Et_3N) with overnight stirring producing a pink coloured solid. The aqueous solution of this intermediate product was further reacted with 1,10-phenanthroline (5 mmol) in ethanol (5 ml), which resulted in the formation of an immediate orange coloured solid (**2**).

6.2.3. Synthesis of the complex $[Ni(IDA)(Phen)(H_2O)]\cdot 3H_2O$ (**3**)

Iminodiacetic acid (5 mmol) was reacted with $NiCl_2\cdot 6H_2O$ (5 mmol) in H_2O (15 ml) in the presence of triethylamine. The solution mixture was then refluxed for 4 h resulting in a light green clear solution. To this solution, 1,10-phenanthroline (5 mmol) was added in batches producing a green coloured solid (**3**) after 1/2 h stirring.

6.2.4. Synthesis of the complex $[Cr(IDA)(Phen)(H_2O)]\cdot 4H_2O$ (**4**)

Iminodiacetic acid (5 mmol) in H_2O (15 ml) was reacted with $CrCl_2$ (5 mmol) in the presence of triethylamine in an inert atmosphere. The solution was stirred overnight and then refluxed for 2 h to give a reddish brown solution. To this solution, 1,10-phenanthroline (5 mmol) in ethanol was added giving a green coloured solid (**4**) after 1/2 h stirring.

Complex (**1**): yield 70%, m.p. 210 °C, IR (KBr, υ cm^{-1}): 1625 (COO), 422 (Cu-N), 1580–1430 (C=C and C=N), 3431 (H_2O). ^1H NMR (δ ppm, D_2O) (300 MHz): 3.49 (2H, s, CH_2), 10.22 (1H, s, iminic NH), 7.6–8.8 (8H, m, aromatic protons), 2.1 (2H, s, coordinated H_2O). ^{13}C NMR (δ ppm, D_2O) (75 MHz): 37, 176, 121, 136, 139, 129, 127. MS (FAB) m/z: 426 [M + 1]. Anal calc. for $C_{16}H_{16}CuN_3O_7$: C 45.13, H 3.79, N 9.8, Found: C 45.08, H 3.37, N 9.4.

Complex (**2**): yield 65%, m.p. 215 °C, IR (KBr, υ cm^{-1}): 1628 (COO), 419 (Co-N), 1578–1435 (C=C and C=N), 3425 (H_2O). ^1H NMR (δ ppm, D_2O) (300 MHz): 3.48 (2H, s, CH_2), 10.49 (1H, s, iminic NH), 7.4–8.6 (8H, m, aromatic protons), 2.0 (2H, s, coordinated H_2O). ^{13}C NMR (δ ppm, D_2O) (75 MHz): 35, 174, 122, 136, 137, 129, 127. MS (FAB) m/z: 461 [M + 1]. Anal calc. for $C_{16}H_{23}CoN_3O_9$: C 41.75, H 5.04, N 9.13, Found: C 41.65, H 5.1, N 9.18.

Complex (**3**): yield 62%, m.p. 218 °C, IR (KBr, υ cm^{-1}): 1620 (COO), 421 (Ni-N), 1575–1430 (C=C and C=N), 3430 (H_2O). ^1H NMR (δ ppm, D_2O) (300 MHz): 3.51 (2H, s, CH_2), 11.10 (1H, s, iminic NH), 7.5–8.7 (8H, m, aromatic protons), 2.3 (2H, s, coordinated H_2O). ^{13}C NMR (δ ppm, D_2O) (75 MHz): 36, 177, 120, 134, 138, 128, 126. MS (FAB) m/z: 443 [M + 1]. Anal calc. for $C_{16}H_{21}NiN_3O_8$: C 43.57, H 4.79, N 9.51, Found: C 43.1, H 4.35, N 9.24.

Complex (**4**): yield 69%, m.p. 205 °C, IR (KBr, υ cm^{-1}): 1622 (COO), 418 (Cr-N), 1577–1427 (C=C and C=N), 3428 (H_2O). ^1H NMR (δ ppm, D_2O) (300 MHz): 3.52 (2H, s, CH_2), 10.37 (1H, s, iminic NH), 7.6–8.6 (8H, m, aromatic protons), 2.1 (2H, s, coordinated H_2O). ^{13}C NMR (δ ppm, D_2O) (75 MHz): 34, 175, 123, 133, 137, 130, 128. MS (FAB) m/z: 450 [M + 1]. Anal calc. for $C_{16}H_{19}CrN_3O_9$: C 42.77, H 4.26, N 9.35, Found: C 42.73, H 4.38, N 9.24.

6.3. Microbial analyses

Antibacterial screening was performed by disc diffusion method and ciprofloxacin was used as the standard. Antifungal screening was done by agar diffusion method and Greseofulvin was used as standard. The stains used were *E. coli* (K-12), *B. subtilis* (MTCC-121), *S. aureus* (IOA-SA-22), *S. typhimurium* (MTCC-98), *C. albicans*, *A. fumigatus* and *P. marneffei*. The SOD activity of the complex was investigated by NBT assay at pH 7.8.

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