SYNTHESIS, CHARACTERIZATION, DOCKING AND ANTIMICROBIAL ACTIVITY STUDIES OF BINUCLEAR Co(II) AND Ni(II) COMPLEXES OF BIS AROYLHYDRAZONE AND PHENANTHROLINE

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ABSTRACT. Schiff base (HL) N1,N2-bis(4-(methylthio)benzaldehyde)-5-nitrobenzene-1,3-dihydrazide (HL) has been prepared from condensation of 4-(methyl thio)benzaldehyde with 5-nitrobenzene-1,3-dihydrazide. Binucleated mixed ligand complexes of nickel(II) ([Ni(L)(dmphen)]Cl2, [Ni(L)(phen)]Cl2) and cobalt(II) ([Co2(HL)(dmphen)]Cl2, [Co2(HL)(phen)]Cl2) complexes have been synthesized from Schiff base (HL) and 1,10-phenanthroline/2,9-dimethyl-1,10-phenanthroline. The synthesized compounds have been characterized by elemental analysis, 1H-NMR, 13C-NMR, FT-IR, UV-Visible, magnetic moment, SEM, powder X-ray diffraction and molar conductivity measurements. Further, the Schiff base and its metal complexes have been investigated for fluorescence activity and molecular docking studies. In addition, Schiff base and its metal complexes were screened for antimicrobial activity against bacteria: Escherichia coli, Bacillus subtilis and fungi: Sclerotium rolfsii and Macrophomina phaseolina.

KEY WORDS: Growth inhibitors, Ternary complex, Tuberculosis, Fluorescence activity, Crystal system

INTRODUCTION

Schiff bases have been playing vital role in coordination chemistry, mainly due to their characteristic properties in formation of variety of complexes with most of transition metal ions in different oxidation states [1]. These Schiff base transition metal complexes, can kibosh the enzymatic activity in the field of medicinal biology. The transition metal complexes of nickel and cobalt metal ions have received the overwhelming attention in recent years, due to their important magnetic, catalytic and biological properties [2, 3]. Cobalt plays a vital role in biological system and exhibits various biological properties such as, antioxidant, antiviral, antitumor/antiproliferative, antimicrobial and anticancer activity [4, 5]. Hydrazones are one of the most important categories of Schiff bases and exhibit the activities in the treatment of several diseases such as tuberculosis, iron overload and in many enzymes inhibition. Many hydrazone compounds act as anticancer drugs by binding to DNA and can cause the blockage to the cancer cell division and finally lead to the cancer cell death [6]. The transition metal complexes with hydrazones show potent antimicrobial activity and have excellent DNA intercalative binding nature [7]. In the field of hydrazone chemistry, the study of bis(aryl)hydrazone complexes is of interest due to their complexes can yields the supra molecular coordinated polymers and binuclear complexes. The brief survey of literature revealed that only a few of these compounds related to bis (acyl/aryl) hydrazones were reported [8]. DNA interactions and antimicrobial studies of binary and ternary metal complexes of isoazole and benzothiazole Schiff bases were reported earlier from our laboratory [9]. In view of the above facts herein we reported the synthesis, spectroscopic characterization, bio-molecule interaction (docking) and antimicrobial activity of binuclear Ni(II) and Co(II) mixed ligand complexes. These Co(II) and Ni(II) complexes are obtained from bis aroylhydrazone Schiff base and 1,10-phenanthroline/2,9-dimethyl-1,10-phenanthroline.

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EXPERIMENTAL

Material and methods

Metal salts, [CoCl$_2$.6H$_2$O], [NiCl$_2$.6H$_2$O] and hydrazine hydrochloride purchased from Merck, 5-nitrobenzene-1,3-dioic acid, 4-(methylthio) benzaldehyde, 2,9-dimethyl-1,10-phenanthroline and 1,10-phenanthroline were purchased from Sigma Aldrich. Atomic absorption spectrophotometer (Perkinelmer-500) used to determine the metal ratio of the complexes. NMR spectra of the ligands were recorded on Bruker 400 MHz NMR instrument using TMS (tetramethyl silane) as internal standard. IR spectra of the compounds were recorded on Shimadzu FT-IR 8400S spectrometer in the range 4000–200 cm$^{-1}$ using KBr pellet. The UV–Vis spectra recorded on Shimadzu UV-2600 in DMSO in the wavelength range 200-800 nm. Vario micro elemental analyzer (USA) was used to determine the percentage composition of C, H, N and S of the compounds. Powder XRD of complexes was recorded by Shimadzu diffractometer. The morphology of compounds was studied by ZEISS-SEM-EDX packed scanning electron microscope. The fluorescent study carried out by Shimadzu fluorescent instrument using DMSO as the solvent at room temperature and magnetic moments measurements done on Gouy balance model No.7550. The docking study was carried out using Hex 8.0 software.

Synthesis of ligand (HL)

Hot methanolic solution of 5-nitrobenzene-1,3-dioic acid (1 mmol) was added to hot methanolic solution of hydrazine (2 mmol) and the resulting mixture was stirred at 60-80 °C temperature. The reaction mixture was poured on crushed ice, the precipitate formed was filtered and washed with hot methanol. Then the washed solid product (5-nitrobenzene-1,3-dihydrazide) (1 mmol) was stirred with 4-(methylthio)benzaldehyde (2 mmol) for 5-7 h at 60-80 °C that precipitated the yellow color solid ligand (bis(4-(methylthio)benzylidene)-5-nitrobenzene-1,3-dihydrazide) filtered and recrystallized in methanol. The synthesis of ligand is shown in Scheme 1.

![Scheme 1. Synthesis of ligand (HL).](image)

Synthesis of metal complexes

The present mixed ligand complexes were prepared by mixing of metal to ligands (metal:(HL): dmphen/phen) ratio at 2:1:2. Hot mixture of methanol and DMF solution of ligand (1 mmol), hot methanol solution of metal chloride MCl$_2$.6H$_2$O (2 mmol) and methanol solution of 1,10-phenanthroline/2,9-dimethyl-1,10-phenanthroline (2 mmol) were mixed together with constant stirring. The mixture refluxed for 6-7 h at 60-80 °C on water bath. The contents were cooled and allowed for precipitation. The precipitated complexes were collected by filtration and washed with petroleum ether and methanol.

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Antimicrobial activity

The stock solution prepared by dissolving the compounds 10 mg in 2 mL of DMSO and the solution was serially diluted to 25, 50, 75 and 100 µg/mL. The prepared nutrient agar media and activated bacterial culture (Escherichia coli and Bacillus subtilis) was mixed together and poured and spread on Mueller-Hinton agar plate with cotton swab the bacterial colonies was streaked onto the surface of the agar four times in the different directions by rotating the plate each time to ensure the bacterial distribution evenly on agar medium. In addition, around the agar should also be swabbed with bacterial colonies. A well of 6 mm diameter was punched off into agar medium with sterile cork borer and filled with 100 µL of different concentration of stock solution by using micropipette in each well in aseptic condition. Plates were then kept in a refrigerator for 30 min and further incubated in an incubator at 37 ºC for 24 h. The standard antibacterial drug Streptomycin was used for comparison under similar conditions. The antibacterial activity was evaluated by measuring the zone of inhibition at different concentration of the compound. The experiment was done in triplicate and the mean diameter of the inhibition zone was calculated.

Anti fungal activity was carried out using two fungi (S. rolfsii and M. phaseolina). Potato dextrose agar media was prepared and the fungal plugs were placed in the center of the plate then incubated for 24 h and the compounds were loaded in the wells surrounding the plug and the zone of inhibition was measured after 72 h. Endofil used as a standard drug for comparison with same conditions in antifungal screening.

RESULTS AND DISCUSSION

Chemistry

The elemental composition and physical properties of all synthesized compounds are given in Table 1. The synthesized ligand and its metal complexes have been studied for fluorescence, antimicrobial activity and docking studies.

$^1$H-NMR

$^1$H-NMR spectrum of Schiff base, showed a sharp singlet peak at δ 9.91 ppm is due to ketonic form of N-H protons and enolic -OH proton signal is not detected in ligand. This indicates that the ligand (HL) is in ketonic form. The aromatic protons signal appeared in the range of δ 7.43-8.78 ppm and the thio methyl (SCH$_3$) signals appeared as strong singlet at δ 2.55 ppm.

$^{13}$C-NMR

$^{13}$C-NMR spectrum of ligand, the signals observed at δ 13.8 ppm, δ 147.3 ppm, δ 164.7 ppm and δ 191.9 ppm are corresponding to CH$_3$, C=N, C=O and C-NO$_2$ groups of hydrazone, respectively, and the signals of aromatic carbons appeared in the range of δ 125.1-134.9 ppm.

Molar conductivity measurement

The synthesized complexes are subjected to solubility test in water and it is found to be nickel(II) complexes are soluble and cobalt(II) are complexes insoluble. That, suggests the chloride ions are present in outer sphere of Ni(II) complexes and inner sphere of Co(II) complexes [10]. The molar conductance values of the complexes measured at room temperature in DMF solution with 0.001 M concentration and the values are given in Table 1. The conductivity of nickel(II) complexes are falls in the range 180-220 Ω$^{-1}$ cm$^2$ mol$^{-1}$, it indicates that the electrolytic nature of chloride ions are in outer sphere. Whereas in the case of cobalt(II) complexes the molar conductivity is fall in
the range 18-26 Ω⁻¹ cm² mol⁻¹ indicates the chloride ions are in inner sphere and are non-electrolytic in nature [11].

Table 1. Molar conductivity and elemental composition data of ligand and its metal complexes.

| Compound                  | Molar conductance (Ω⁻¹ cm² mol⁻¹) | Melting point (°C) | Found (Calculated) % |
|---------------------------|-----------------------------------|---------------------|-----------------------|
| C₂H₅NiOsS₆ (HL)           | -                                 | 310                 | C 56.79 (56.69) H 4.17 (4.07) N 13.80 (13.58) S 12.63 (12.60) Metal - |
| C₂H₆Cl₂NiOsS₆ [Ni(L)dmphen]Cl₂ | 180                              | >300                | C 56.19 (56.15) H 4.05 (4.03) N 11.34 (11.33) S 5.70 (5.77) Metal 10.50 (10.55) |
| C₄H₅Cl₂NiOsS₆ [Ni(L)phen]Cl₂ | 220                              | >300                | C 54.35 (54.58) H 3.48 (3.53) N 11.96 (11.93) S 6.08 (6.07) Metal 11.31 (11.11) |
| C₂H₆Cl₂Co₂OsS₆ [Co(L)phen]Cl₂ | 18                               | >300                | C 56.46 (56.67) H 4.06 (4.00) N 10.64 (10.63) S 5.44 (5.41) Metal 10.01 (9.94) |
| C₄H₅Cl₂Co₂OsS₆ [Co(L)phen]Cl₂ | 26                               | >300                | C 51.33 (51.36) H 3.38 (3.31) N 11.16 (11.36) S 5.67 (5.61) Metal 10.43 (10.56) |

**IR spectra**

The characteristic IR frequencies of ligand and its metal complexes are presented in Table 2. In free ligand, the stretching frequency bands appeared at 1719 and 1587 cm⁻¹ due to vC=O and vC=N bands respectively [12]. In metal(II) complexes vOH is disappeared and the broad band vN-H shifting takes place in cobalt(II) complexes. The bands at 1719 cm⁻¹ due to vC=O and 1587 cm⁻¹ due to vC=N of free ligand are shifted to the range 1724-1736 cm⁻¹ and 1584-1634 cm⁻¹, respectively. It bespeaks the coordination of ligand to the metal ion through the keto group by the delocalization of electrons [13]. In nickel(II) complexes, the broad bands of ligand at 3470 and 3084 cm⁻¹ due to vOH and vN-H, respectively, are disappeared; this indicates the ligand binds to the metal ion through ionization of enolic proton. The intense aromatic vibrational bands of free phenothroline appeared at 738, 853 cm⁻¹ and pyridyl vC=N vibrations appeared at 1421 cm⁻¹. On complexation, these peaks are observed in the range 729-785 cm⁻¹ and 1030-1152 cm⁻¹, respectively [14]. In metal complexes, the observed low intense vibrational bands at low frequency region in the range of 351-395 cm⁻¹ represents the metal-nitrogen bonding (M-N) and the bands observed in the range from 432-584 cm⁻¹ represents the metal-oxygen bonding (M-O) [15, 16].

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

| IR spectral data (cm⁻¹) |
|------------------------|
| Compound               | V-O-H | V-N-H | V-C=O | V-C=N | V=O     |
|                        |       |       |       |       |         |
| Ligand(HL)             | 3470  | 3084  | 1719  | 1587  | -       |
| [Ni(L)dmphen]Cl₂       | -     | -     | 1624  | -     | -       |
| [Ni(L)phen]Cl₂         | -     | -     | 1597  | 1082,785 | -  |
| [Co(L)phen]Cl₂         | -     | 3065  | 1736  | 1584  | -       |
| [Co(L)phen]Cl₂         | -     | 3079  | 1724  | 1634  | 1088,783 | -  |
Electronic spectra

The electronic absorption spectra of ligand and its metal complexes are recorded in DMSO at room temperature in the range 200-800 nm. The electronic spectrum of free ligand (HL) showed two bands at 281 and 354 nm, these are assigned to π-π* and n-π* transitions, respectively. In addition to ligand bands, in all complexes the ligand to metal charge transfer bands are observed in the range 347-373 nm. The complex \([\text{Ni}_2(L)(\text{dmphen})_2]\)Cl₂ showed d-d band at 574 nm, assigned as \(^1A_{1g} \rightarrow ^1A_{2g}\) transition which refers to the square planar geometry around the Ni(II) ion \([17]\). In complex \([\text{Ni}_2(L)(\text{phen})_2]\)Cl₂, the weak d-d transition bands are not observed probably because the bands are lost in the low energy tail of intense charge transfer band \([18, 19]\), whereas, in cobalt complexes \([\text{Co}_2(\text{HL})(\text{dmphen})_2]\)Cl₂, \([\text{Co}_2(\text{HL})(\text{phen})_2]\)Cl₂ the d-d transitions ascertained at 640 and 617 nm, respectively. These bands are assigned as a \(^4T_{1g}(F) \rightarrow ^4A_{2g}(F)\) transition \([20]\), usually the octahedral complexes contain three bands, but here only one band is appeared.

Magnetic moments

The magnetic moments of all the metal complexes measured at room temperature. The magnetic moment values of complexes \([\text{Co}_2(\text{HL})(\text{dmphen})_2]\)Cl₂, \([\text{Co}_2(\text{HL})(\text{phen})_2]\)Cl₂ found to be 5.3 and 5.17 BM, respectively, these values are slightly higher than the theoretical values of mono nucleated cobalt complexes and lower than the expected di nucleated cobalt complexes. These magnetic moment values attribute the di nuclear nature of complexes and it may be occurred due to the weak anti ferromagnetic intra molecular coupling interaction between the two metal ions \([18, 21]\). Whereas nickel complexes are diamagnetic in nature, it suggests the square planar geometry around nickel(II) ion \([22, 23]\). Based on above spectral data, the proposed structures of metal complexes are shown in Scheme 2.

X-Ray powder diffraction study

The crystals size of synthesized compounds is inadequate to study the single crystal X-ray crystallography. Therefore, we used the powder X-ray diffraction technique to solve the mystified crystal systems of these compounds. The XRD patterns of the synthesized compounds scanned in the range of 10-80° at a wavelength of 1.5406 Å and their representative diffractograms are shown in Figure 1. The XRD pattern of the ligand and complexes with respect to major peaks having relative intensity greater than 10% indexed using software programme Expo-2014 \([24-28]\). The above indexed results yielded the unit cell parameters, unit cell volumes and crystal systems. The assigned crystal systems of ligand (HL) and complexes \([\text{Ni}_2(L)(\text{dmphen})_2]\)Cl₂ and
[Ni₂(L)(phen)₂]Cl₂ are orthorhombic, triclinic and triclinic, respectively and their corresponding crystal data presented in Table 3.

The number of molecules per unit cell are calculated by using Eq. (1) [29] and are found to be 2, 4 and 4 for ligand (L), complexes; [Ni₂(L)(dmphen)₂]Cl₂ and [Ni₂(L)(phen)₂]Cl₂, respectively.

\[ n = \frac{dN}{V} \]

(1)

where, \( n \) = number of molecules per unit cell, \( d \) is calculated density, \( N \) is Avogadro number, \( V \) is unit cell volume and \( M \) is molecular weight of complex.

The crystal sizes of ligand (L), complexes [Ni₂(L)(dmphen)₂]Cl₂ and [Ni₂(L)(phen)₂]Cl₂ are calculated by using Debye-Scherrer’s equation, Eq. (2) [29] and found to be 9.49, 12.77 and 66.02 nm, respectively.

\[ D = \frac{k\lambda}{\beta \cos \theta} \]

(2)

where \( D \) is the particle diameter in angstroms, \( k \) is a coefficient and is equal to 0.89, \( \beta \) is the full width at half maximum (FWHM), \( \theta \) is the Bragg diffraction angle and \( \lambda \) is the wavelength of X-rays.

![Figure 1. Powder X-ray diffraction pattern of A for ligand (HL), B for complex [Ni₂(L)(dmphen)₂]Cl₂ and C for complex [Ni₂(L)(phen)₂]Cl₂.](image)

Table 3. Powder X-ray crystallographic data of ligand (HL), complexes.
Synthesis of binuclear Co(II) and Ni(II) complexes of bis aroylhydrazone and phenanthroline

| Crystal system | Orthorhomic | Triclinic | Triclinic |
|----------------|-------------|-----------|-----------|
| a Å            | 10.54       | 9.20      | 12.376    |
| b Å            | 20.13       | 8.596     | 12.182    |
| c Å            | 4.16        | 7.25      | 8.4602    |
| α deg          | 90          | 101.47    | 92.32     |
| β deg          | 90          | 102.496   | 96.25     |
| γ deg          | 90          | 93.51     | 117.04    |
| V Å³           | 871.11      | 545.7     | 1123.4    |
| Z              | 2           | 4         | 4         |
| 20 range       | 10-80°      | 10-70°    | 10-80°    |
| μ(CuKα), cm⁻¹  | 1.543       | 1.543     | 1.543     |
| hkl indexed range | 0 ≤ h ≤ 3, 0 ≤ k ≤ 4, 0 ≤ l ≤ 2 | 0 ≤ h ≤ 2, -4 ≤ k ≤ -3, -2 ≤ l ≤ 2 | 0 ≤ h ≤ 4, -4 ≤ k ≤ -3, -2 ≤ l ≤ 2 |
| k/l % Indexed  | >=10        | >=10      | >=10      |

**Scanning electron microscopic analysis**

The scanning electron microscopy (SEM) used to determine the surface morphology of the synthesized compounds. From the micrographs, surface morphology changes was observed in the complexes compared to the ligand (HL), complex [Ni(L)(dmphen)]Cl₂ and [Ni(L)(phen)]Cl₂ appeared with irregular shape are shown in Figure 2. SEM micrographs revealed that upon complexation the surface morphology of ligand changed with changing the metal ion in their coordination.

**Fluorescence emission spectra**

The fluorescence properties of ligand (HL) and its metal complexes were studied at room temperature in 10⁻⁴ M DMSO solution (Figure 3). The Excitation and emission slit widths are set at 10 nm with a scan speed of 500 nm/min. Generally azo Schiff base compounds exhibit fluorescence due to intra ligand π-π* transitions [30]. The ligand and its metal complexes excitation observed at 370 nm and emission peaks observed at 442, 439, 440, 436 and 438 nm for ligand (HL), complexes; [Ni(L)(dmphen)]Cl₂, [Ni(L)(phen)]Cl₂, [Co₂(HL)(dmphen)]Cl₂ and [Co₂(HL)(phen)]Cl₂ respectively. The emission intensity of metal complexes found to be lower than the free ligand. Thus, the fluorescence emission intensity of ligand decreased on complex formation with transition metal ions is due to decrease in electron density on ligand (L) [31, 32]. The emission maxima of all the synthesized compounds are in the following order ligand (HL) > [Ni₂(L)(phen)]Cl₂ > [Ni₂(L)(dmphen)]Cl₂ > [Co₂(HL)(phen)]Cl₂ > [Co₂(HL)(dmphen)]Cl₂.
Docking study

The crystal structural models of proteins, obtained from protein data bank and the binding efficacy of synthesized compounds with proteins structures (B. subtilis and S. rolfsii) are predicted by...
generating the $E_{\text{Total}}$ values using HEX 8.0 software [33, 34]. The predicted results of ligand and its metal complexes on proteins are presented in Table 4 and shown in Figure 4. The metal complexes showed the lower $E_{\text{Total}}$ values compared to ligand (HL), which brought out the fact of high binding affinity of metal complexes with proteins. The ligand (HL) exhibits the minimum $E_{\text{Total}}$ value on B. subtilis (-352 kJ/mol) compared to S. rolfsii proteins, it demonstrates the stable association of ligand-B. subtilis due to the presence of H-bonds. The complex [Co$_2$(L)(phen)$_2$Cl$_4$] has the highest relative binding affinity with $E_{\text{Total}}$ Value -443 and -402 kJ/mol on B. subtilis and S. rolfsii proteins, respectively. The remaining synthesized complexes also show the lower $E_{\text{Total}}$ values than ligand on B. subtilis.

Table 4. Docking results of ligand (HL) and its metal complexes against microbial proteins (Bacillus and S. rolfsii).

| Compound                  | Total binding energy, kJ/mol | Bacillus (PDB: 2VAM) | S. rolfsii (PDB: 4YLD) |
|---------------------------|-----------------------------|----------------------|------------------------|
| Ligand (HL)               | -352.0                      | -294.05              |
| [Ni$_2$(L)(dmphen)$_2$]Cl$_2$ | 415.18                     | -369.03              |
| [Ni$_2$(L)(phen)$_2$]Cl$_2$ | -408.09                     | -359.03              |
| [Co$_2$(HL)(dmphen)$_2$]Cl$_4$ | -427.8                      | -393.62              |
| [Co$_2$(HL)(phen)$_2$]Cl$_4$ | -442.8                      | -402.15              |

Figure 4. Molecular docked model of (a) ligand (HL) and (b) [Co$_2$(HL)(phen)$_2$]Cl$_4$ complex with Bacillus (PDB: 2VAM).

Microbial screening

For in-vitro antimicrobial activity, the synthesized compounds have been tested by well diffusion method on bacteria; Escherichia coli (E. coli), Bacillus subtilis (B. subtilis) and fungi; Sclerotium rolfsii (S. rolfsii) and Macrophomina phaseolina (M. phaseolina). The zone of inhibition values of investigated compounds and standard drugs such as Streptomycin and Endofil are summarized in Table 5. The values revealed that the metal complexes have higher antimicrobial activity than free ligand [35-40] and the inhibition level is increased with increasing the concentration. In the present investigation all synthesized compounds showed the remarkable inhibition on B. subtilis.
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(gram +ve) bacteria. Among these, \([\text{Ni}_2(L)(\text{dmphen})_2]\text{Cl}_2\) and \([\text{Co}_2(\text{HL})(\text{phen})\text{Cl}_4]\) compounds showed the excellent growth inhibition on \(B.\ subtilis\). While on \(E.\ coli\) (gram -ve) only \([\text{Co}_2(\text{HL})(\text{phen})\text{Cl}_4]\) exhibited the inhibitory activity with values 75 and 100 µg/mL and rest of the compounds showed the moderate activity. In the case of anti-fungal activity, only cobalt complexes have the activity at the concentration of 75 and 100 µg/mL due to other factors such as solubility, dipole moment, stereo chemistry, size, coordination sites, geometry of complexes, concentration and hydrophobicity also influence the antimicrobial potency of the complexes and the remaining compounds not having any inhibitory activity on fungi even at higher concentration. On the other hand, the ligand (HL) showed the low growth inhibition only on \(Bacillus\) with values 75 and 100 µg/mL.

Table 5. Antimicrobial activity of ligand and its metal complexes (zone of inhibition).

| Compound                  | MIC (µg/mL) | Zone of inhibition, cm |
|---------------------------|-------------|------------------------|
|                           | B. subtilis | E. coli | S. rolfsii | M. phaseolina |
| Ligand (HL)               |             |          |            |              |
| 25                        | R           | R        | R          | R            |
| 50                        | R           | R        | R          | R            |
| 75                        | 0.1         | R        | R          | R            |
| 100                       | 0.1         | R        | R          | R            |
| \([\text{Ni}_2(L)(\text{dmphen})_2]\text{Cl}_2\) | 25          | 0.3      | R          | R            |
|                           | 50          | 0.5      | R          | R            |
|                           | 75          | 0.7      | R          | R            |
|                           | 100         | 0.9      | 0.3        | R            |
| \([\text{Ni}_2(L)(\text{phen})\text{Cl}_4]\) | 25          | R        | R          | R            |
|                           | 50          | R        | R          | R            |
|                           | 75          | 0.2      | R          | R            |
|                           | 100         | 0.5      | 0.2        | R            |
| \([\text{Co}_2(\text{HL})(\text{dmphen})\text{Cl}_4]\) | 25          | 0.1      | R          | R            |
|                           | 50          | 0.3      | R          | R            |
|                           | 75          | 0.3      | 0.1        | 0.4          |
|                           | 100         | 0.7      | 0.2        | 0.4          |
| \([\text{Co}_2(\text{HL})(\text{phen})\text{Cl}_4]\) | 25          | 0.5      | R          | R            |
|                           | 50          | 0.6      | R          | R            |
|                           | 75          | 0.7      | 0.8        | 0.4          |
|                           | 100         | 0.7      | 0.8        | 0.4          |
| Streptomycin              | 100         | 1.3      | 1.3        | R            |
| Endofil                   | 100         | R        | 1.5        | 1.5          |

\(R\) = Resistant.

**CONCLUSION**

Ligand and its of bimetallic mixed ligand complexes of Ni(II) and Co(II) have been successfully synthesized and characterized by different analytical and spectral techniques. The molar conductivity data revealed that, the Ni(II) complexes are electrolytes and the Co(II) complexes are non-electrolytes. The powder X-ray diffraction studies indicated that orthorhombic and triclinic crystal structures for ligand and Ni(II) complexes, respectively. In fluorescence spectra, the decrease in intensity of metal complexes compared with ligand, confirms the formation of metal complexes. The docking study of ligand and its metal complexes supported that, all compounds have the minimum \(E_{total}\) values on binding with \(B.\ subtilis\) protein. The anti microbial evaluation revealed that all synthesized compounds showed good activity against \(B.\ subtilis\) gram (+ve) bacteria. On fungi, only Co(II) complexes showed very good inhibitory activity at the concentration of 75 and 100 µg/mL.
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