Synthesis, characterization and antimicrobial screening of a novel organylborate ligand, potassium hydro(phthalyl)(salicylyl)borate and its Co(II), Ni(II), and Cu(II) complexes

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Abstract A new organylborate ligand, potassium hydro (phthalyl) (salicylyl) borate and its Co(II), Ni(II), and Cu(II) complexes were synthesized. The compounds were characterized by elemental analysis, FTIR, 1H NMR, ESI MS, UV–Vis techniques, molar conductivity and magnetic data measurements. The spectroscopic data support a distorted square planar geometry around the Cu(II) ion, while the Co(II) and Ni(II) ions acquire a distorted octahedral geometry. These synthesised compounds were also tested for their in vitro antimicrobial activities against some bacterial and fungal strains to assess their inhibiting potential and the activities shown by these complexes were compared with standard drugs. Results showed that there is a marked increase in the antibacterial and antifungal activities of the cobalt(II) complex than the free ligand and other complexes when treated against the same microorganism at the same concentration.

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1. Introduction

The coordination chemistry of organylborates have found wide spread use in a variety of application as biocides (Woods, 1994), on account of their moderate to high level of biological activities, when coupled with some toxic organic and organo-metallic compounds (Schubert, 2003). Some borate esters of ortho hydroxomethyl phenol have been reported to perform well as preservative for wood. This system is of particular interest because the esters may be relatively resistant to hydrolysis. However, few reports are available about the anti fungal
properties of these borate esters in the absence of copper (Woodgate et al., 1999; Hedley et al., 2000). The organoboron complexes are used as source of radicals (Olivier and Renaud, 2001). This approach was adopted to take advantage of intrinsic ability of borate ions to form boron esters with acids, aldehydes, and phenols (Steinberg, 1964). This ester formation provides a means for the production of hydrostatic boron compounds (Humphrey et al., 2002). The bio essay results have shown that such compounds are good acaricide (Fang et al., 2001), with low mammalian toxicity (Fail et al., 1998).

Recently, it has been reported that metal complexes of organylborates such as Cu(I) phosphane complexes of triazolyl borate ligands are particularly effective against A549 carcinoma cells that are resistant to cis platin treatment (Cristina et al., 2006). Some mixed chelate copper-based drugs exhibit greater anti neoplastic potency than cis platin in in vitro and in vivo studies of a variety of tumor cell lines (De Vizcaya et al., 2004). On the other hand organo nickel and organo cobalt complexes have also shown high antimicrobial effects against micro organisms like Aspergillus fucum, Escherichia coli and Candida albicans (Anil and Devinder, 2007).

Some organylborates like poly(indazolyl) and poly(2-mercaptotriazolyl)borates (Soares et al., 2004) have excellent biological properties with transition metals, main group metals (Ruggiero et al., 1994) and organotin(IV) compounds (Pellei et al., 2005; Joshi et al., 2006). Boron plays several biochemical roles in animals, including humans and a boron-containing natural antibiotic, boromycin is known (Hutter, 1967; Dunitz, 1967). Medicinal applications of boron compounds include boron neutron capture therapy and drug delivery as some other boron compounds are promising in treating arthritis (Hunt et al., 1999). On the other hand aromatic acids like phthalic acid are used mainly in the form of anhydride to produce chemicals such as dyes, perfumes, and many other important compounds. Recently anti tumor action of phthalic acid mono-n-butyl ester cupric salt was reported (Yamamoto et al., 1990). Other aromatic acid like salicylic acid finds important application in the treatment of pharyngocoatosis (Dundas, 1907) and it also blocks UV B rays on the skin (Fetil et al., 2002).

Recognizing the above importance of organylborates, its metal complexes along with some important aromatic acids; we are particularly interested in making new chelating organoborates which contains extremely useful aromatic acids. These compounds when incorporated with transition metal ions would produce a broad spectrum of antimicrobial property. So here we report the synthesis and spectroscopic characterization of potassium salt of an organylborate ligand, potassium hydro(phthalyl)(salicyllyl)borate and its Co(II), Ni(II), and Cu(II) complexes. In addition the antibacterial and antifungal properties of the synthesized compounds are also reported.

2. Material and methods

2.1. General

Potassium borohydride (Across Organics), phthalic acid (MERCK), salicylic acid (MERCK) were purchased and used as received. The solvents were purchased from E. Merck (India Ltd.). All solvents were degassed with dry nitrogen prior to use. Samples for microanalysis were dried in vacuum to constant weight. All syntheses were carried out under a nitrogen atmosphere. Elemental analyses were performed by a Perkin Elmer 2400 CHNOS elemental analyzer. IR spectra were recorded on KBr pellets using a Perkin Elmer 1620 FT IR spectrophotometer. Far IR spectra were recorded on CsI pellets using a JASCO FT IR 550 spectrophotometer. 1H NMR spectra were recorded using a Bruker DPX-300 MHz spectrometer operating at room temperature with DMSO-d$_6$ as solvent. The chemical shift ($\delta$) are reported in parts per million (ppm) using tetramethylsilane as internal standard. Positive and negative ESI mass spectra were recorded on a Micro mass Quattro II triple quadrupole mass spectrometer using chloroform and methanol as solvent. Magnetic data measurements were carried out at a microanalysis lab by Gouy’s method at room temperature. Melting point was recorded on a Metrex melting point apparatus. Electronic spectra were taken on Spectronic 20 D$^+$ spectrophotometer in DMSO solvent. The molar conductance measurements were carried out using a Tacussel CD810 conductivity meter at 25 °C using 1 x 10$^{-3}$ M solution in DMSO.

2.2. Synthesis

2.2.1. Synthesis of potassium hydro(phthalyl)(salicyllyl)borate, ([KLa])

Phthalic acid (1.66 g, 10 mmol) and salicylic acid (1.38 g, 10 mmol), were dissolved in 10 ml of ethanol separately. The two solutions were poured into a schlenk flask containing potassium borohydride, (0.54 g, 10 mmol) together with a magnetic stirring bar. The flask was connected to a condenser and placed in an oil bath. The other opening of the condenser was fitted with a gas collecting device. The solution mixture was heated very slowly up to 70 °C where upon the evolution of H$_2$ gas commences. It was heated until 30 mmol equiv of H$_2$ gas was evolved. Then it was allowed to cool at room temperature and the precipitate was filtered. The precipitate was then washed several times with small portions of acetone followed by diethyl ether. The solid residue was dried in a vacuum desiccator to constant weight under reduced pressure to leave the desired compound as a colorless powder which was recrystallised from methanol.

1H NMR (300 MHz, $\delta$ ppm from TMS in DMSO-d$_6$, 300 k): 8.11–7.45 (m, 4H, Ar), 6.91–6.86 (m, 4H, Ar), 4.2 (s, 1H, O–H), 3.06 (br s, 1H, B–H). ESI MS (m/z) 352 [M]+, 353 [M + 1]$^+$.

2.2.2. Synthesis of [Co(HB(C$_6$H$_4$O$_4$)(C$_7$H$_3$O$_3$)Cl(H$_2$O)$_2$]$z$ complex

To a solution of CoCl$_2$·6H$_2$O (0.237 g, 1 mmol) dissolved in 20 ml methanol was added dropwise a solution of the ligand (0.352 g, 1 mmol) in 20 ml methanol with continuous stirring. The resulting purple solution was stirred for 6 h and the solution was concentrated to 30 ml. It was then allowed to stand overnight in a refrigerator. A purple product separates out, which was isolated by filtration under vacuum. It was washed thoroughly with hexane and dried in vacuo over fused CaCl$_2$ which was recrystallised from methanol.

1H NMR (300 MHz, $\delta$ ppm from TMS in DMSO-d$_6$, 300 k): 8.68–7.58 (m, 4H, Ar), 7.05–6.95 (m, 4H, Ar), 4.23 (s, 1H, O–H), 3.07 (br s, 1H, B–H). ESI MS (m/z) 444 [M]$^+$. 

446 [M + 2]+. Molar conductance, \( A_m (\Omega^{-1} \text{ cm}^{-1} \text{ mol}^{-1}, 10^{-3} \) DMSO, r.t.): 25. \( \mu_{\text{eff}} \) (rt, BM): 4.86.

2.2.3. Synthesis of \([\text{Ni}\{\text{HB(C}_8\text{H}_4\text{O}_4)(\text{C}_7\text{H}_5\text{O}_3)\text{Cl(H}_2\text{O})_2\}\] complex

The procedure followed was similar to that for the cobalt(II) complex except NiCl2•6H2O used instead of CoCl2•6H2O. A light green product was obtained.

\(^{1}H\) NMR (300 MHz, \( \delta \) ppm from TMS in DMSO-d6, 300 k): 8.55–7.54 (m, 4H, Ar), 6.98–6.89 (m, 4H, Ar), 4.22 (s, 1H, O–H), 3.08 (br s, 1H, B–H) ESI MS (m/z) 443 [M]+, 445 [M + 2]+. Molar conductance, \( A_m (\Omega^{-1} \text{ cm}^{-1} \text{ mol}^{-1}, 10^{-3} \) DMSO, rt): 29. \( \mu_{\text{eff}} \) (rt, BM): 2.90.

2.2.4. Synthesis of \([\text{Cu}\{\text{HB(C}_8\text{H}_4\text{O}_4)(\text{C}_7\text{H}_5\text{O}_3)\text{Cl(H}_2\text{O})\}\] complex

For the synthesis of copper complex same procedure was followed as in the above case except CuCl2•2H2O was used as the metal chloride. A blue product was obtained.

\(^{1}H\) NMR (300 MHz, \( \delta \) ppm from TMS in DMSO-d6, 300 k): 8.26–7.57 (m, 4H, Ar), 6.98–6.89 (m, 4H, Ar), 4.22 (s, 1H, O–H), 3.1 (br s, 1H, B–H). ESI MS (m/z) 430 M+, 432 [M + 2]+. Molar conductance, \( A_m (\Omega^{-1} \text{ cm}^{-1} \text{ mol}^{-1}, 10^{-3} \) DMSO, rt): 31. \( \mu_{\text{eff}} \) (rt, BM): 1.83.

2.3. Antimicrobial screening

2.3.1. Strains, media, growth conditions, and experimental protocols

The aim of this study was to find the effect of the given ligand and its complexes on various bacterial and fungal species that are responsible for causing various infectious diseases. The antimicrobial activity was first done by disc diffusion assay (Resistotyping) in which the ligand and its complexes were treated with different fungal and bacterial species. The second study was done for more accuracy which involved the growth curve studies (Turbidometric measurement) by using conventional spectrophotometer. All of the fungal and bacterial strains used in this study were obtained from IIIM formerly called Regional Research Laboratory, Jammu, India.

The disc diffusion method (Bawer and Kirby, 1966) was used to measure the antimicrobial activity of the ligand and its complexes, and were then placed on the surface of agar plate. As the compound diffused into the medium, it produced a gradient of compound concentration. For control studies sterilized disc of same diameter were dipped in distilled water and then placed on the surface of agar plate. The plates were then incubated at 37 °C for 24 h for bacteria and 72 h for fungi. After suitable incubation the activities were determined by measuring the width of the inhibition zone (mm) around the disk.

Second study (growth curve) was performed only for cobalt complex that showed highest biological activity against one of the fungal strains (Candida glabrata ATCC 90030). In case of growth curve studies five flasks (capacity 250 ml), each containing 50 ml autoclaved YEPD media were taken, one flask containing 30 ml YEPD media and 50 μl primary culture was kept as control. Various concentrations of cobalt complex were prepared and added to different flasks, and incubated at 37 °C and 150 rpm. At different intervals of time (i.e., after every 2 h) sample were collected for experimental purposes. For analyzing the growth curve, O.D. was measured at 595 nm at the interval of 2 h up to 20 h. Antimicrobial activities were performed in triplicate and the average was taken as the final reading. Error limits are also indicated in the respective tables.

3. Results and discussion

The physical properties and analytical data of the ligand, and its metal complexes (Tables 1 and 2) support their proposed structure. The molar conductivity (\( A_m \)) of the metal complexes measured in 1 × 10^{-3} \text{ mol L}^{-1} DMSO at room temperature shows values of 25, 29 and 31 \( \Omega^{-1} \text{ cm}^{-1} \text{ mol}^{-1} \) for the cobalt(II), nickel(II) and copper(II) complexes, respectively. These values indicate that they are non-electrolyte species (Geary and Coord, 1971). Elemental analysis shows that the composition of the complexes is in agreement with their formulation.

Reacton scheme:

\[
\begin{align*}
\text{KBH}_4 + \text{C}_8\text{H}_4\text{O}_4 + \text{C}_7\text{H}_5\text{O}_3 \rightarrow & \text{K[H[B(C}_8\text{H}_4\text{O}_4)]} + \text{H}_2 \\
\text{KL + MCl}_2 \rightarrow & \text{MCl}_3 + \text{KL} \\
\text{KL + CuCl}_2 \rightarrow & \text{CuCl}_3 + \text{H}_2 \\
\text{K[H[B(C}_8\text{H}_4\text{O}_4)]Cl(H}_2\text{O}) & \rightarrow \text{K[HB(C}_8\text{H}_4\text{O}_4)}(\text{C}_7\text{H}_5\text{O}_3)\text{Cl(H}_2\text{O})_2 \rightarrow \text{Cu[H[B(C}_8\text{H}_4\text{O}_4)]Cl(H}_2\text{O}) & \rightarrow \text{CuCl}_3 + \text{H}_2 \\
\text{M} & \rightarrow \text{MCl}_3 + \text{KL} \\
\end{align*}
\]

Table 1  IR Spectral data of the ligand and its metal complexes (cm^{-1}).

<table>
<thead>
<tr>
<th>Compound</th>
<th>v(C–H)</th>
<th>v(O–H)</th>
<th>v(B–H)</th>
<th>v(C=O)</th>
<th>v(B–O)</th>
<th>v(C–O)</th>
<th>v(M–O)</th>
<th>v(M–Cl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand, KL</td>
<td>2918</td>
<td>3440</td>
<td>2484</td>
<td>1673</td>
<td>1323</td>
<td>1138</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[CoKL(H_2O)]_2</td>
<td>2890</td>
<td>3405</td>
<td>2530</td>
<td>1610</td>
<td>1335</td>
<td>1138</td>
<td>475</td>
<td>330</td>
</tr>
<tr>
<td>[NiKL(H_2O)]_2</td>
<td>2905</td>
<td>3395</td>
<td>2533</td>
<td>1612</td>
<td>1335</td>
<td>1137</td>
<td>469</td>
<td>342</td>
</tr>
<tr>
<td>[CuKL(H_2O)]_2</td>
<td>2925</td>
<td>3415</td>
<td>2535</td>
<td>1614</td>
<td>1330</td>
<td>1140</td>
<td>483</td>
<td>310</td>
</tr>
</tbody>
</table>

Ligand, KL = K[HB(C8H4O4)(C7H5O3)].
3.1. Infrared spectra

IR spectral bands of the ligand and its metal complexes suggest the formation of desired complexes and support their structure (Figs. 1–3). The spectrum of ligand showed the absence of characteristic carboxylic group O–H absorption band at 2500–3100 cm\(^{-1}\), indicating deprotonation of phthalic acid and salicylic acid to make a B–O bond. The absorption at 1323 cm\(^{-1}\) in the spectra of the ligand may be assigned to \(\nu_{\text{sym}}(B–O)\) vibration (Werner and Brien, 1955), confirming the formation of ligand. Broad featureless band around 3405, 3395 and 3415 cm\(^{-1}\) in the cobalt, nickel and copper complex, respectively, shows the presence of water molecules in the metal complexes. It supports the presence of coordinated water molecule in the metal complexes. A signal at 1138 cm\(^{-1}\) is seen in the spectra of the ligand and it may be assigned to the \(\nu(C–O)\) stretching vibration in the ligand. Broad featureless band around 3405, 3395 and 3415 cm\(^{-1}\) in the cobalt, nickel and copper complex, respectively, shows the presence of water molecules in the metal complexes. It supports the presence of coordinated water molecule in the metal complexes. A signal at 1138 cm\(^{-1}\) is seen in the spectra of the ligand and it may be assigned to the phenolic \(\nu(C–O)\) stretching vibration. This signal shows no significant shifting in the cobalt, nickel and copper complex. This shows the non-participation of the phenolic oxygen during coordination to the metal atom. The \(\nu(C–O)\) stretching vibration in the ligand is seen around 1673 cm\(^{-1}\). This vibration shows significant shifts in the metal complexes and is seen at 1610–1614 cm\(^{-1}\) range in all the complexes. It clearly indicates the formation of a bond between the \(\nu(C–O)\) oxygen and the metal atom in each respective metal complex. This is further supported by the presence of signals at 475, 469 and 483 cm\(^{-1}\) which are assigned to the \(\nu(CO–O)\), \(\nu(Ni–O)\) and \(\nu(Cu–O)\) in the metal complexes. Signals that are seen at 330, 342 and 310 cm\(^{-1}\) in the spectra of the metal complexes are indicative of the presence of Co–Cl, Ni–Cl and Cu–Cl vibration in the respective complexes (Nakamoto, 1977).

3.2. \(^1H\) NMR spectra

\(^1H\) NMR support and provide additional information about the ligand and its transition metal complexes. In the spectrum of the ligand, absence of signal in the range 9–12 ppm indicates deprotonation of –COOH protons from phthalic acid and salicylic acid to make a B–O bond. The multiplets in the range 6.86–6.91 (4H) and 7.45–8.11 ppm (4H) correspond to the aromatic ring protons of the salicylyl group and phthalyl group in the ligand, respectively. These proton signals undergo downfield shifting in all the metal complexes of the ligand, because of the paramagnetic effect of metal(II) ions and hence support the coordination of the ligand towards the metal ions.
In the cobalt complex the aromatic ring protons are seen to be shifted in the range of 6.95–7.05 (4H) and 8.38–8.91 ppm (4H) as a broad multiplet. The nickel complex shows aromatic ring protons at the range 6.93–7.01 (4H) and 8.39–9.05 ppm (4H) while the copper complex shows ring protons at 6.89–6.98 (4H) and 7.57–8.36 ppm (4H), respectively, as broad multiplets. The B–H protons and aromatic phenolic O–H protons show insignificant shifting in the complexes.

3.3. Electron spray ionization mass spectra (ESI MS)

The mass spectrum of the ligand supports its proposed formulation. It reveals the molecular ion peak \( m/z \) at 352, consistent with the molecular weight of the ligand (Fig. 4). A peak at \( m/z \) 353 corresponds to the \([M+1]^+\) species. Other fragments were observed at \( m/z \) 230, 121, 77 corresponding to the fragments KC₈H₅BO₅, C₆H₂O₆, and C₆H₅⁺, respectively. The mass spectra of the cobalt(II) and nickel(II) complex show molecular ion peak \( m/z \) at 444 and 443 consistent with the molecular weight of the complexes. The \([M+2]^+\) peaks were also seen in these complexes, possibly due to the presence of isotopic chlorine. The cobalt complex shows peaks corresponding to fragment ions at \( m/z \) 425, 424, 389, 387, 164, 121, and 77 which also confirm the structure of the cobalt complex. The detailed spectra are shown in Table 3. The nickel complex shows fragment ions at \( m/z \) 425, 423, 387, 176, 164, 121, 93 and 77 which also supports the proposed structure of the nickel complex. The copper(II) complex shows a weak molecular ion peak at \( m/z \) 430, perhaps due to the higher instability of the Cu(II) complex.

![Figure 4: ESI-mass spectra of ligand.](image)

Table 3  Mass spectral data of the ligand and its complexes.

<table>
<thead>
<tr>
<th>Ligand/complexes</th>
<th>( m/z )</th>
<th>Intensity (%)</th>
<th>Fragment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand, K[HB(C₈H₄O₄)(C₇H₅O₃)]</td>
<td>352</td>
<td>5</td>
<td>( M^+ )</td>
</tr>
<tr>
<td></td>
<td>353</td>
<td>3</td>
<td>([M+1]^+)</td>
</tr>
<tr>
<td></td>
<td>230</td>
<td>55</td>
<td>KC₈H₅BO₅</td>
</tr>
<tr>
<td></td>
<td>163</td>
<td>8</td>
<td>C₆H₂O₆</td>
</tr>
<tr>
<td></td>
<td>121</td>
<td>100</td>
<td>C₆H₅O₂</td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>40</td>
<td>C₆H₅</td>
</tr>
<tr>
<td>[CoL(H₂O)₂Cl]</td>
<td>444</td>
<td>50</td>
<td>( M^+ )</td>
</tr>
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<td></td>
<td>446</td>
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<td>([M+2]^+)</td>
</tr>
<tr>
<td></td>
<td>425</td>
<td>60</td>
<td>([Co{HB(C₈H₄O₄)(C₇H₅O₃)}(H₂O)ÆCl}]</td>
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<tr>
<td></td>
<td>424</td>
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<td>389</td>
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<td>([Co{HB(C₈H₄O₄)(C₇H₅O₃)}(H₂O)ÆCl}]</td>
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<td>12</td>
<td>( M^+ )</td>
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<td></td>
<td>445</td>
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<tr>
<td></td>
<td>425</td>
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<td>([Ni{HB(C₈H₄O₄)(C₇H₅O₃)}(H₂O)ÆCl}]</td>
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<td>([Ni{HB(C₈H₄O₄)(C₇H₅O₃)}(OH)ÆCl}]</td>
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<td>9</td>
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<tr>
<td></td>
<td>412</td>
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<td>([Cu{HB(C₈H₄O₄)(C₇H₅O₃)}(Cl}]</td>
</tr>
<tr>
<td></td>
<td>164</td>
<td>6</td>
<td>C₆H₅O₄</td>
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</tr>
<tr>
<td></td>
<td>77</td>
<td>25</td>
<td>C₆H₅</td>
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</table>

L = [HB(C₈H₄O₄)(C₇H₅O₃)]⁻.
and 770 nm can be assigned to LMCT, $3A_2g$ display the characteristic pattern for distorted octahedral nickel(II) complex. The electronic spectra of the Ni(II) complex may be assigned to LMCT transition. These bands firmly suggest a distorted octahedral geometry around the cobalt(II) ion. Further Jahn Teller effect will also influence the electronic spectra of the Co(II) complex exhibits absorption in the range 521 and 667 nm which may be assigned to $4T_{1d}(F)\rightarrow4T_{2g}(P)$ and $4T_{1d}(F)\rightarrow4A_{2g}(F)$ transitions, respectively (Lever, 1984). It also shows a band near 350 nm which may be assigned to LMCT transition. These bands firmly suggest a distorted octahedral geometry around the cobalt(II) ion in the complex. The electronic spectra of the Ni(II) complex display the characteristic pattern for distorted octahedral nickel(II) compounds. The appearance of bands at 416, 580, 692 and 770 nm can be assigned to LMCT, $3A_2\rightarrow3T_{2g}$, $3A_2\rightarrow4T_1(F)$ and $3A_2\rightarrow4T_1(P)$ transitions (Landry-Hum et al., 2001). As both the cobalt(II) and nickel(II) ions are attached to different coordinating groups with different coordinating powers like H$_2$O molecule, chloride ion and the anionic ligand containing large benzene ring, we cannot expect a completely regular octahedral geometry of both the metal complexes. Further Jahn Teller effect will also influence the distortion from normal octahedral environment. Therefore there is a distorted octahedral geometry of both the cobalt(II) and nickel(II) complexes. The copper(II) complex show a d-d broad and a weak band centered at 680 nm which is attributed to $^3B_{1g}\rightarrow^2A_{1g}$ transition (Lever, 1984). It also displays a band near 400 nm which may be assigned to LMCT transition. This electronic spectrum is consistent with the degree of distortion from the square planar geometry.

3.4. Electronic absorption spectra

The electronic spectra of the Co(II) complex exhibits absorption in the range 521 and 667 nm which may be assigned to $4T_{1d}(F)\rightarrow4T_{1d}(P)$ and $4T_{1d}(F)\rightarrow4A_{2g}(F)$ transitions, respectively (Lever, 1984). It also shows a band near 350 nm which may be assigned to LMCT transition. These bands firmly suggest a distorted octahedral geometry around the cobalt(II) ion. Further Jahn Teller effect will also influence the electronic spectra of the Co(II) complex may be expected to have a distorted octahedral configuration. The Ni(II) complex has a magnetic moment value of 2.90 BM and it supports a distorted octahedral configuration (Figgis, 1976). It is reported that, six-coordinated Ni(II) complexes exhibit magnetic moments in the 2.5–3.5 BM range (Cotton and Wilkinson, 1988). The magnetic moment value of the copper complex is 1.83 BM corresponding to the presence of one unpaired electron and it supports a square planar geometry (Sonmez, 2001) though with some distortions.

3.5. Magnetic data measurements

An indication of the most probable geometric configuration of the synthesized metal complexes is their magnetic moment values. The magnetic moment of the Co(II) complex is 4.86 BM which supports a six-coordinated distorted octahedral arrangement (Konig, 1971). This value is within the expected range of 4.7–5.2 BM for six-coordinated cobalt(II) complexes (Badiger et al., 1986). Hence the Co(II) complex may be expected to have a distorted octahedral configuration. The Ni(II) complex has a magnetic moment value of 2.90 BM and it supports a distorted octahedral configuration (Figgis, 1976). It is reported that, six-coordinated Ni(II) complexes exhibit magnetic moments in the 2.5–3.5 BM range (Cotton and Wilkinson, 1988). The magnetic moment value of the copper complex is 1.83 BM corresponding to the presence of one unpaired electron and it supports a square planar geometry (Sonmez, 2001) though with some distortions.

3.6. Bacterial and fungicidal activity

Experimental results have shown that the complexes show significant and matching biological activities as that of the standard antifungal drugs.

Antibacterial activity (in vitro) of the ligand and its complexes were studied against two Gram positive and two Gram negative bacteria at a single concentration (1000 µg/ml). The results, summarized in Table 4, suggest that ligand is less active against the tested pathogens, but its complexes show significant antimicrobial activity. The degree of inhibition varied with the nature of the compound. The highest zone of inhibition i.e., 33, 31 and 29 mm were measured in Staphylococcus aureus, Bacillus subtilis and E. coli when treated with [CoLCl(H$_2$O)$_2$], [NiLCl(H$_2$O)$_2$] and [CuLCl(H$_2$O)] complexes, respectively. The cobalt(II) complex showed highest zone of inhibition (33 mm) against S. aureus, it was noticed that all the three complexes showed same activity when treated against Salmonella typhi. In case of B. subtilis the antibacterial property of nickel complex was equal to the standard drug. Comparison between antibacterial activities of ligand, its complexes and standard drug is shown in Fig. 6.

For antifungal activity (Table 5) the highest inhibitory zone, i.e., 35, 30 and 32 mm were measured in [CoLCl(H$_2$O)$_2$], [NiLCl(H$_2$O)$_2$] and [CuLCl(H$_2$O)] complexes when treated

![Figure 5](image)

ESI-mass spectra of [CuLCl(H$_2$O)].

(Fig. 5). A weak peak at $m/z$ 432 corresponds to the [M + 2]$^+$ peak possibly due to the presence of isotopic chlorine in the copper complex (Kemp, 1975). Other peaks were observed at $m/z$ 412, 164, 121, 93 and 77 which corresponds to different fragments that supports the structure of the copper complex.

Table 4 Antibacterial activity screening data for the ligand and its metal complexes in terms of diameter of zone of inhibition $^a$ (mm) 1000 µg/mL. The data represents (mean ± SD) of three experiments.

<table>
<thead>
<tr>
<th>Name of bacteria (gram –ve)</th>
<th>Ligand, KL</th>
<th>[CoLCl(H$_2$O)$_2$]</th>
<th>[NiLCl(H$_2$O)$_2$]</th>
<th>[CuLCl(H$_2$O)]</th>
<th>Kanamycin $^b$ (1000 µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli (gram –ve)</td>
<td>09 (±0.5)</td>
<td>21 (±1.0)</td>
<td>17 (±1.5)</td>
<td>29 (±1.2)</td>
<td>33 (±0.5)</td>
</tr>
<tr>
<td>S. typhi (gram –ve)</td>
<td>06 (±1.0)</td>
<td>23 (±0.5)</td>
<td>23 (±1.0)</td>
<td>23 (±1.5)</td>
<td>26 (±1.0)</td>
</tr>
<tr>
<td>B. subtilis (gram +ve)</td>
<td>10 (±0.0)</td>
<td>28 (±0.5)</td>
<td>31 (±1.0)</td>
<td>19 (±1.5)</td>
<td>31 (±1.0)</td>
</tr>
<tr>
<td>S. aureus (gram +ve)</td>
<td>18 (±1.5)</td>
<td>33 (±1.2)</td>
<td>27 (±1.0)</td>
<td>21 (±1.0)</td>
<td>36 (±0.5)</td>
</tr>
<tr>
<td>DMSO$^c$</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

$^a$ 25–40 mm significantly active, 12–25 mm moderately active, <12 less active.

$^b$ Standard drug (positive control).

$^c$ Solvent (negative control).
against *Candida glabrata, Candida tropicalis* and *Candida albicans* at the single concentration of 1000 µg/mL. Here in case of fungi the highest zone of inhibition (35 mm) was shown by cobalt(II) complex when treated against *C. glabrata* which proves that the cobalt(II) complex is having the highest antimicrobial activity both in case of tested bacteria as well as in case of tested fungi. Further the comparison between antifungal activities of ligand its complexes and standard drug is given in Fig. 7. The result showed that, in case of control disc no zone of inhibition was observed. Hence we can effectively conclude that whole of the antimicrobial effect is due to the different concentration of the metal complexes and its ligand used in this study. In case of growth curve studies the effect of increasing concentrations of cobalt complex on the growth of *Candida glabrata* has been studied as shown in Fig. 8. The absorbance obtained for the growth control showed that the culture reached the stationary growth phase after 12–14 h showing a normal growth pattern. The curve depicts a lag phase in the initial phase of growth, active log phase and stationary phase. Growth decreases in the presence of different concentrations of cobalt complex, the inhibition increases with increasing concentrations. At concentration of 700 µg/ml the growth pattern has changed, the lag phase is extended by 10 h, the stationary phase has not reached the same level of cell growth as in case of control and at 1000 µg/ml there is total inhibition of growth shown by a flat line. Growth pattern of *Candida glabrata* in presence of cobalt complex is depicted in Table 6.

**Table 5** Antifungal activity screening data for the ligand and its metal complexes in terms of diameter of zone of inhibitiona (mm) 1000 µg/mL. The data represents (mean ± S.D) of three experiments.

<table>
<thead>
<tr>
<th>Name of fungi</th>
<th>Ligand, KL</th>
<th>[CoLCl(H2O)2]</th>
<th>[NiLCl(H2O)2]</th>
<th>[CuLCl(H2O)]</th>
<th>Fluconazole b (1000 µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em> 10261</td>
<td>09 (±0.2)</td>
<td>22 (±1.7)</td>
<td>22 (±1.2)</td>
<td>32 (±1.5)</td>
<td>37 (±0.9)</td>
</tr>
<tr>
<td><em>C. tropicalis</em> 750</td>
<td>08 (±0.8)</td>
<td>23 (±0.0)</td>
<td>30 (±0.0)</td>
<td>18 (±1.2)</td>
<td>34 (±0.2)</td>
</tr>
<tr>
<td><em>C. glabrata</em> 90030</td>
<td>11 (±0.8)</td>
<td>35 (±1.0)</td>
<td>25 (±0.5)</td>
<td>19 (±0.5)</td>
<td>40 (±0.2)</td>
</tr>
<tr>
<td>DMSOc</td>
<td>––</td>
<td>––</td>
<td>––</td>
<td>––</td>
<td>––</td>
</tr>
</tbody>
</table>

a 25–40 mm significantly active, 12–25 mm moderately active, <12 less active.
b Standard drug (positive control).
c Solvent (negative control).
4. Conclusion

The present investigation was undertaken to synthesize a ligand and its metal derivatives and screen them for their antimicrobial activities. Their structures were confirmed by spectral and elemental analyses. The spectroscopic data support a distorted square planar geometry around the Cu(II) ion, while the Co(II) and Ni(II) ions acquire a distorted octahedral geometry. Further their anticandidial and antibacterial activities were evaluated by disc diffusion assay, and growth curve studies. The most important thing noticed in this study was that the metal complexes have higher activity as compared to parent ligand against the same microorganism. Such increased activity of the metal complexes may be due to chelation of the metal ion in the complex (Mishra and Singh, 1996), which enhances the lipophilic character favoring its permeation through the lipid layer of cell membrane thus causing the metal complex to cross the membrane more effectively and hence increasing the activity of these complexes. Besides this many other factors such as dipole movement, stability constant, molar conductivity, solubility and magnetic moment influenced by metal complexes may be the possible reasons for increasing the antimicrobial activity of the complexes (Chohan et al., 2002). The variation in the activity of different metal complexes against different microorganisms depends on either the impermeability of cells of the microbes or differences in ribosomes in microbial cells (Lawrence et al, 1980). All of the fungal species used in this study are responsible for causing candidiasis, a disease that varies from superficial mucosal to life threatening systemic disorders. As far as our results are concerned the metal complexes are more potent bactericides and fungicides than the free ligand. So these metal complexes may be explored in future as an option for decreasing pathogenic potential of infecting bacterial and fungal species.

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