SYNTHESIS, CHARACTERIZATION, AND BIOLOGICAL EVALUATION OF SOME 3D-METAL COMPLEXES OF MACROCYCLIC LIGAND DERIVED FROM SUBSTITUTED CARBOHYDRAZONE AND THIOSEMICARBAZIDE MOIETY

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ABSTRACT

The present paper deals with the synthesis and characterization of transition metal complexes of Schiff base derived from condensation of substituted carbohydrazone with thiosemicarbazide moiety in 1:1 molar ratio. Using this tridentate ligand, complexes of Manganese (II), Cobalt (II), Nickel (II), Copper (II) and Zinc (II) complexes with general formula ML2 have been synthesized. The synthesized complexes were characterized by several techniques using molar conductance, elemental analysis, magnetic susceptibility, FT-IR spectroscopy, electronic spectra and mass spectra. The elemental analysis data suggest the stoichiometry to be 1:2 [M:L]. All the complexes are non-electrolytic in nature as suggested by molar conductance measurements. Infrared spectral data indicate the coordination between the ligand and the central metal ion through sulfur and azomethine nitrogen atoms. Spectral studies suggest tetrahedral geometry for Mn(II), Ni(II) complexes, square pyramidal geometry for Cu(II) complex and distorted octahedral geometry for Co(II) and Zn (II) complexes. The pure drug, synthesized ligand, and metal complexes were screened for their anti-bacterial and anti-oxidant activities against gram positive and gram negative bacteria like E.Coli, S. aureus, P. aerogenosa, B. Subtilis species and ascorbic acid. It was observed that Schiff base and its metal complexes shows enhanced biological activity as compared to ligand or metal salts.

KEYWORDS: Schiff base, Thiosemicarbazide moiety, Metal Complexes, substituted carbohydrazone, molar conductance, fluorescence spectroscopy, Invitro microbial activity and antioxidant activity.
INTRODUCTION
Coordination complexes are gaining importance in recent years especially in the designing of long acting drugs in metabolism. The metal complexes from tri dentate ligands have often been studied recently because of their technical applications\textsuperscript{[1,2]} and applications in enhancement of drug action.\textsuperscript{[3,4]} Transition metals are essential for normal functioning of living organism and are, therefore, of great interest as potential drugs.\textsuperscript{[5]} The coordination chemistry of nitrogen donor ligands is an active area of research. A great deal of attention in this area has been focused on the complexes formed by 3d metals with tridentate ligands using both sulfur and nitrogen atoms.\textsuperscript{[6,7]} Macrocyclic species based on transition metal compounds and multi-dentate ligands is an interesting field in chemistry and has been the subject of extensive research due to their potential applications in building block macrocyclic-based chemistry and environmental chemistry and biomedical. The intense interest in synthetic macro-cycles and their metal complexes depends on the fact that they mimic naturally occurring macrocyclic molecules in their structural and functional features due to rich chemical properties.\textsuperscript{[8,9,10]} Physical and chemical properties of macrocyclic complexes are increased thermochemical and kinetic stability of the complexes with respect to their dissociation which is due to lesser liability and larger association constants than the complexes with homologous open chain chelating ligands. The application of aza-macrocyclic precursors in the synthesis of transition metal macrocyclic complexes ligands mainly from their use as models for protein metal binding sites in biological systems\textsuperscript{[11,12,13,14]} and as selective complexing agents for metal ions.\textsuperscript{[15,16,17,18]} Macrocyclic complexes of transition metal ions have received great attention due to their biological activities, including antiviral, anticarcinogenic,\textsuperscript{[19]} antifertile,\textsuperscript{[20]} antibacterial and antifungal.\textsuperscript{[21]} Macrocyclic nickel complexes have been reported as DNA recognition and oxidation while macrocyclic Copper complexes find use in DNA binding and cleavage.\textsuperscript{[22]} Some macrocyclic complexes of lanthanides eg Cd3+ are used as MRI contrast enhancing agents.\textsuperscript{[23,24]} In the present study synthesis, spectroscopic characterization and pharmacological evaluation of novel macrocyclic transition metal complexes have been carried out. It will help in understanding the chelated behavior of Schiff base with transition metal ions and enlighten more on the biological application of chelated complexes of transition metal and it has been observed that the overall value of nitrogen and sulfur containing organic fragment is improved when they are binding with metal ion.\textsuperscript{[25-26]} The study of structural and binding features of various Schiff base complexes can play an important role in better understanding of the complex biological process. Schiff bases derived from salicylaldehyde are well known for their interesting
ligational properties and exclusive applications in different fields.\textsuperscript{[27,28,29]} It is well known from the literature that Schiff bases derived from thiosemicarbazide have a strong ability to form metal complexes.\textsuperscript{[30]} The interaction of these donor ligands and metal ions gives complexes of different geometries, and literature survey reveals that these complexes are potentially more biologically active. Thus, in recent years Schiff bases and their metal complexes have attained much attraction because of their extensive biological activities.\textsuperscript{[31,32]} Keeping the above fact in our mind and in continuation of our earlier work on transition metal complexes with Schiff bases,\textsuperscript{[33,34]} macrocyclic Schiff base ligand derived from substituted carbohydrazone and thiosemicarbazide moiety has been synthesized. In the present paper, the synthesis and characterization of the ligand and its complexes with Manganese (II), Cobalt (II), Nickel (II), Copper (II) and Zinc (II) are being reported.

**Experimental**

All the chemicals used were of AR/GR grade and purchased from E-Merck (USA). Chemicals were used without any purification. Elemental analyses were carried out on model 240 PerkinElmer elemental analyzer at CDRI, Lucknow. Metal contents were determined gravimetrically using standard methods.\textsuperscript{[35]} Conductivity measurements were made in anhydrous DMF on a Systronics model 305 (India) Conductivity Bridge. Magnetic susceptibility measurements of the complexes in the solid state were determined by vibrating sample magnetometer at Centre for Advance Technology, Indore at room temperature. The electronic spectra of the metal complexes in DMF were recorded on a Perkin-Elmer UV WinLab Spectrophotometer at ITM University, Gwalior. The infrared spectra of the ligand and complexes were recorded in KBr pellets using Perkin-Elmer FT-IR spectrophotometer in the range 4000–400 cm\(^{-1}\) at ITM University, Gwalior India. The melting points of the ligand and complexes were recorded in open capillaries on a capillary melting point apparatus. The antibacterial activities of both the ligand and their complexes were tested in vitro for growth inhibitory against E.Coli, S. aureus, P. aerogenosa, B. Subtilis species using gentamycin antibiotic as positive control. The antifungal activities of both the ligands and their complexes were tested in vitro for growth inhibitory against Aspergillus niger and Aspergillus flavus by agar growth food poison technique\textsuperscript{[36]} at different concentrations compared with Grisofulvin as appositive control. The antioxidant activities of both the ligands and their complexes were tested in vitro via Hydrogen peroxide method using ascorbic acid as positive control.
Synthesis of Macrocyclic Ligand
The equimolar ratio of substituted carbohydrazone (0.502 gm; 2 m mol) and thiosemicarbaxide (0.183 g; 2 mmol) was taken in absolute alcohol and refluxed for 3 hours. Peach colour crystals of macrocyclic Schiff base was formed in the reaction mixture and was filtered and washed thoroughly with 50% methanol-water mixture, dried over vacuum, and weighed. Melting point of Schiff base was recorded.

Synthesis of Complexes
For the synthesis of complexes, 0.006 M ligand solution was prepared in 50% acetone-water solvent and refluxed for four hours with 0.003 M solution of metal salts separately. The refluxed solutions were kept for some days. Solid crystalline compounds appeared in the solution, which were filtered, washed with 50% acetone-water mixture, dried, and weighed. Melting points of the complexes were recorded.

In vitro Antioxidant Activity
A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). Samples (100 µg/ml) in DMSO were added to a H2O2 solution (0.6 ml, 40 mM). Absorbance of H2O2 at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without H2O2. In this experiment, ascorbic acid used as a standard substance.

The percentage of H2O2 scavenging by ligand and metal complexes was calculated as follows-

% Scavenged [H2O2] = [(Ac – As) / Ac] × 100

Where Ac is the absorbance of the control and As is the absorbance in the presence of samples.

In vitro Antibacterial activity
Antibacterial activities of the complexes were tested against using muller Hinton agar medium.[37-38] The sterilized (autoclaved at 121 °C for 15 min) medium (40-50 °C) was poured into the petri dishes to give a depth of 3-4 mm and allowed to solidify. The suspension of the microorganism streaked on plates. The paper discs were placed on the solidified medium. The plates were incubated for 1 hr. at room temperature and incubated at 37 °C for 24 hrs 39.
RESULTS AND DISCUSSION

The analytical and physical data of ligand & their transition metal complexes are given in Table 1. All these complexes are analyzed for 1:2 stoichiometry of the type ML2. On the basis of these characterizations it has been found that all the complexes are non-hygroscopic, stable at room temperature, insoluble in water, but fairly soluble in DMSO. The molar conductance values of these complexes are too low to account for their electrolytic behavior.\textsuperscript{[40,41]}
Table 1: Analytical and physical data of transition metal complexes of macrocyclic Schiff base ligand derived from substituted carbohydrazone and thiosemicarbazide moiety.

<table>
<thead>
<tr>
<th>Molecular Formula</th>
<th>Color</th>
<th>Yield</th>
<th>M.P. (°C)</th>
<th>Mol.wt.</th>
<th>Molar conductance Ω⁻¹cm²mol⁻¹</th>
<th>Analysis (%) Found (Calcd.)</th>
<th>λ max</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Mn(C₃H₃₀N₁₀S₂)₂]Cl₂</td>
<td>Yellow</td>
<td>42</td>
<td>180-190</td>
<td>787.9</td>
<td>14.4</td>
<td>49.8/50.69</td>
<td>395 nm</td>
</tr>
<tr>
<td>[Ni(C₃H₃₀N₁₀S₂)₂]Cl₂</td>
<td>Green</td>
<td>30</td>
<td>175-180</td>
<td>791.9</td>
<td>16.5</td>
<td>52.8/53.89</td>
<td>365 nm</td>
</tr>
<tr>
<td>[Co(C₃H₃₀N₁₀S₂)₂]Cl₂</td>
<td>Dust</td>
<td>33</td>
<td>230-235</td>
<td>791.7</td>
<td>8.5</td>
<td>49.27/49.53</td>
<td>400 nm</td>
</tr>
<tr>
<td>[Cu(C₃H₃₀N₁₀S₂)₂]Cl₂</td>
<td>Brown</td>
<td>25</td>
<td>215-220</td>
<td>796.5</td>
<td>8.7</td>
<td>49.42/49.56</td>
<td>310 nm</td>
</tr>
<tr>
<td>[Zn(C₃H₃₀N₁₀S₂)₂]Cl₂</td>
<td>Cream</td>
<td>40</td>
<td>320</td>
<td>798.4</td>
<td>30.3</td>
<td>52.10/52.86</td>
<td>360 nm</td>
</tr>
</tbody>
</table>
Spectral Studies of Ligand and Its Complexes

IR Spectra

The IR spectra of the complexes indicate that the ligand behaves as tridentate and coordinates with metals via azomethine nitrogen and sulphur groups. The shift of spectra to lower wave number by 30–40 cm\(^{-1}\) in the complexes indicates that these groups are involved in complexation.\(^{[42,43]}\) IR Spectral data of free ligand and its metal complexes reveal the involvement of co-ordination sites in chelation (Table 2). In the free ligand, a characterization band observed at 1613 cm\(^{-1}\) is assigned to azomethine V (-CH=N-) group, which shifted to lower wavelength (1649 cm\(^{-1}\)) in all the metal complexes, indicating the co-ordination through N atom of azomethine group with metal ion.\(^{[25]}\) In the spectrum of Schiff base, a broad band observed at 3266.55 cm\(^{-1}\) due to thiol group disappeared in metal complexes which indicates the deprotonation and co-ordination through S atom to the metal ion. This is further supported by two new non ligand bands observed in the region 310-360 cm\(^{-1}\) and 453-563 cm\(^{-1}\) in metal complexes, which has been assigned to (M-S) and (M-N) group respectively. In addition to this, some new bands were observed in metal complexes, that is bands ~ 1625.72 cm\(^{-1}\) ad 1531.35 cm\(^{-1}\). These bands represents the asymmetric and symmetric sketch of thio group and confirm the co-ordination of thio group in tridentate mode fashion. The bands for modes appeared in the range of 580 cm\(^{-1}\)–615 cm\(^{-1}\) in all the complexes.\(^{[44]}\) The presence of sharp band in the region 503–514 cm\(^{-1}\) in the spectra of all the complexes assigned to mode\(^{[45]}\) further support the involvement of azomethine nitrogen atom in coordination.

Table 2: IR frequencies in cm\(^{-1}\) of Schiff base ligand and its metal complexes.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>-(N=CH)</th>
<th>-(C-S)</th>
<th>-(M-S)</th>
<th>-M-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand(HL)</td>
<td>1563.54</td>
<td>1587</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[Mn(<em>{34}H</em>{30}N_{10}S_{22})]Cl(_2)</td>
<td>1562.52</td>
<td>1586</td>
<td>460</td>
<td>469</td>
</tr>
<tr>
<td>[Ni(C(<em>{34}H</em>{30}N_{10}S_{22})]Cl(_2)</td>
<td>1564.49</td>
<td>1570</td>
<td>438</td>
<td>482</td>
</tr>
<tr>
<td>[Co(C(<em>{34}H</em>{30}N_{10}S_{22})]Cl(_2)</td>
<td>1560.47</td>
<td>1549</td>
<td>468</td>
<td>491</td>
</tr>
<tr>
<td>[Cu(C(<em>{34}H</em>{30}N_{10}S_{22})]Cl(_2)</td>
<td>1557.52</td>
<td>1545</td>
<td>459</td>
<td>446</td>
</tr>
<tr>
<td>[Zn(C(<em>{34}H</em>{30}N_{10}S_{22})]Cl(_2)</td>
<td>1555.51</td>
<td>1555</td>
<td>460</td>
<td>496</td>
</tr>
</tbody>
</table>
Figure 1: IR Spectra of Macrocyclic Schiff base ligand derived from substituted carbohydrazone and thiosemicarbazide moiety.

Figure 2: IR Spectra of transition metal complex derived from substituted carbohydrazone and thiosemicarbazide moiety.

$^1$H-NMR Spectra
To elucidate the structure of Schiff base and its metal complexes, $^1$H-NMR spectra were recorded in DMSO-d$_6$. In Schiff base, peak appears at 9.22ppm due to azomethine proton, which deshielded in the spectra of Cu (II) complex, indicating the complexation through azomethine nitrogen atom. In Schiff base, signal that appeared at 13.66 ppm was not observed in the spectra of Cu (II) complexes, further supporting the complexation through S atom of the thiol group. The peak that appeared at 2.18 ppm (due to –CH$_3$ group) present in ligand did not change in the spectra of metal complexes. The $^1$H-NMR spectrum of ligand exhibits signal at S9.263 ( S, 1H, -CH=N-), 8.012 (d, 2H, Ar-H), 7.558 (t, 2H, Ar-H), 2.168 (S, 3H, -CH$_3$), 13.65(br-s, 1H, -SH). The $^1$H-NMR spectrum of Cu(II) H$_2$O exhibits signals at
9.72(S, 2H, -CH=N-), 8.016(d, 4H, Ar-H), 2.165(S, 6H, -CH3). The signals due to aromatic protons (m, 15 H, ArH) have resonated as multiplets I the region 7.1-7.9 ppm.

Table 3: 1H-NMR peaks of transition metal complex derived from substituted carbohydrazone and thiosemicarbazide moiety.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compounds</th>
<th>δ - CH3</th>
<th>δ - CH2</th>
<th>δ - NH</th>
<th>δ - C6 H5</th>
<th>δ - HC=N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ligand</td>
<td>2.12-2.26</td>
<td>3.16</td>
<td>3.65</td>
<td>6.64-6.86</td>
<td>11.86</td>
</tr>
<tr>
<td>1.</td>
<td>[Mn(C34H30N10S2)2Cl2]</td>
<td>2.13-2.23</td>
<td>3.15</td>
<td>4.17</td>
<td>6.53-7.94</td>
<td>12.1</td>
</tr>
<tr>
<td>2.</td>
<td>[Ni(C34H30N10S2)2Cl2]</td>
<td>2.50</td>
<td>3.15</td>
<td>3.62-3.75</td>
<td>6.9-7.1</td>
<td>11.3</td>
</tr>
<tr>
<td>3.</td>
<td>[Co(C34H30N10S2)2Cl2]</td>
<td>1.02-1.05</td>
<td>2.25-2.65</td>
<td>3.41-3.47</td>
<td>8.1-8.17</td>
<td>11.6</td>
</tr>
<tr>
<td>4.</td>
<td>[Cu(C34H30N10S2)2]Cl</td>
<td>2.49-2.50</td>
<td>3.14</td>
<td>5.85</td>
<td>7.0-7.9</td>
<td>11.74</td>
</tr>
<tr>
<td>5.</td>
<td>[Zn(C34H30N10S2)2]Cl2</td>
<td>2.50-2.51</td>
<td>3.12</td>
<td>3.79</td>
<td>7.01-7.73</td>
<td>11.54</td>
</tr>
</tbody>
</table>

Electronic Spectra and Magnetic Moment Measurements

To understand the nature of the M-L bond, the electronic spectral data of the complexes were calculated in DMF. Co(II) complexes exhibited two absorption bands, which fall in the range of 10,498 – 19,694 cm⁻¹ and 10,914 – 20,288 cm⁻¹ attributed to 4T1g(F) – 4T1g(F) V1 and 4T1g(F) – 4T1g(P) V2 transitions respectively. V3 was not observed but it could be calculated by using the relation V3= V1 + 10Dq. By using Band – Fitting equation, the ligand field parameter (D4, β) have been calculated. The calculated value of crystal field splitting every (D0) was 1168.4 and 1220 cm⁻¹. These values are well within the range reported for octahedral complexes. The value of Racah parameter (B) was less than free ion value (947 cm⁻¹) indicating the orbital overlap and delocalisation of d- electron on the ligand. The nephelauxetic ratio (β) was less than one, which reveals the partial covalent nature of metal ligand bonds. The magnetic moment data of Co (II) complex indicate the presence of three unpaired electrons. The magnetic moment values were found in the range of 4.6 – 5.3 B.M.) which is in the expected range (4.3 – 4.2 B.M.) of octahedral complexes. Ni(II) complex exhibited three absorption band in the region 9681- 10,800 cm⁻¹ (V1), 16,629- 17,897 cm⁻¹ (V2) and 23,864- 24,929 cm⁻¹ (V3) attributed to 3A2g(F) – 3T2g(F) (v1), 3A2g(F) – 3T1g(F) (v2) and 3A2g(F) – 3T2g(P) (v3) transitions respectively. The ligand field parameter (D4, B, β) have also been calculated for Ni(II) complex. These parameter indicate octahedral arrangement around the Ni(II) complex and suggest the partial covalent nature of M-L bond. The observed magnetic moment values were found in the range of 3.4- 3.6 B.M. which is in the usual range of reported octahedral complexes. For Cu(II) complex, a band observed in the region of 18,688- 19,962 cm⁻¹ assigned to 2B1g – 2A1g indicates the square planar geometry of the copper complex.
Photoluminescence Spectra

The photoluminescence spectra of Schiff base and its Mn (II), Cu (II), Ni (II), Co (II) and Zn (II) complexes were recorded on DMF with an excitation wavelength of 265 nm (fig 5). The most enhancement in fluorescent intensity of metal complexes was observed in the case of Zn (II) complexes, with emission band observed at 497nm as they are difficult to oxidize or reduce due to their stable d$^{10}$ configuration.\cite{30} The emission bands for Mn (II), Co (II), Ni (II) and Cu (II) were observed at 473nm, 410nm, and 405nm respectively. A weak fluorescent emission band at 390nm was observed for ligand. The enhancement are in the order of Zn (II) > Cu (II) > Co (II) > Ni (II) > Mn (II) and Schiff base. The enhancement in
the fluorescent intensity of metal complexes show that Schiff base is one of the good chelating agents.\cite{31} Thus, Schiff base and metal complexes are fluorescent in nature and they open a way for the photochemical application of the complexes.\cite{32}

![Figure 5: The photoluminescence spectra of transition metal complex derived from substituted carbohydrazone and thiosemicarbazide moiety.](image)

**ESR Spectra**

The ESR spectra of Cu (L) Cl\(_2\).H\(_2\)O complex was studied which provided useful information of metal ion environment in complexe and also indicated the anisotropic behaviour of Cu (II) complex. The g values observed for cu (L) Cl\(_2\).H\(_2\)O \(g_1\) = 2.14, \(g_2\) = 2.07, \(g_a\) = 2.09, \(G = 2.04\). The axial geometry pattern for Cu (II) ion has been observed, which is confirmed by two \(g_1\) value which are more than 2.0.

**In vitro- anti bacterial activity**

All metal complexes were also evaluated for their potential antibacterial activity against *B. subtilis* (*MTCC 1134*), *S. aureus* (*MTCC 3160*), *E. coli* (*MTCC 50*) and *P. aeruginosa* (*MTCC 1034*). Tables 4 highlight the antibacterial activity of complexes (1-7) against *B. subtilis, S. aureus, E. coli and P. aeruginosa* as observed by disc-diffusion method. The high antibacterial activity of compounds may be due to coordination and chelation which tend to make compounds act as powerful and potent bacteriostatic agents, thus inhibiting the growth of the bacteria. In a compound, the positive charge on the metal is partially shared with the donor atoms present in the ligands and there may be delocalization of \(\pi\) electrons over the whole chelate. The increased activity of the metal compounds can be explained on the basis of chelation theory. The result of the this series revealed that the all compounds contain
significant antibacterial activity against two gram positive bacteria and two gram positive bacteria. All compounds exhibit higher antibacterial activity against the P. aurogenosa bacteria. All the compounds showed the less antibacterial activity in compared to standard antibiotic drug gentamycin.

Table 4: Invitro antibacterial activities of all transition metal complexes against gram positive and gram negative bacteria.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compounds</th>
<th>Zone of inhibition (in mm) and Concentration 10mg/mL of various strains</th>
<th>E.Coli</th>
<th>P. aerogenosa</th>
<th>B. subtilis</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ligand(L)</td>
<td></td>
<td>7</td>
<td>8</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>2.</td>
<td>[Mn(C34H30N10S2)2Cl2]</td>
<td></td>
<td>13</td>
<td>22</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>3.</td>
<td>[Ni(C34H30N10S2)2Cl2]</td>
<td></td>
<td>14</td>
<td>25</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>4.</td>
<td>[Co(C34H30N10S2)2Cl2]</td>
<td></td>
<td>9</td>
<td>18</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>5.</td>
<td>[Cu(C34H30N10S2)2]Cl2</td>
<td></td>
<td>10</td>
<td>16</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>6.</td>
<td>[Zn(C34H30N10S2)2]Cl2</td>
<td></td>
<td>8</td>
<td>10</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>7.</td>
<td>Gentamycin (Antibiotic)</td>
<td></td>
<td>18</td>
<td>28</td>
<td>20</td>
<td>22</td>
</tr>
</tbody>
</table>

Mode of Action: The chelation theory accounts for the increased activity of the metal complexes. The chelation reduces the polarity of the metal atom mainly because of partial sharing of its positive charge with the donor groups and possible π-electron delocalisation within the whole chelating ring. The chelation increases the lipophilic nature of the central atom which subsequently favours its permeation through the lipid layer of the cell membrane. The degradative enzymes produced by the microorganism are important in host infection. For food deterioration and break down of organic matter. The enzyme production is here intended to mean both synthesis of the enzyme by the microorganisms and activity of the enzyme in the medium after it is produced. Since the metal complexes inhibit the growth of microorganism it is assumed that the production of enzyme is being affected and hence the
microorganism is unable to utilize the food for itself or the intake of nutrients in suitable forms decreases and consequently the growth of microorganism is arrested, while higher concentration proves fatal. The higher concentration destroys the enzyme mechanism by blocking any of the metabolism path way and due to the lack of availability of proper food, the organism dies. The results of biological activity have been compared with the conventional antibiotic gentamycin used as standard drug.

**In vitro Antioxidant Assay (H$_2$O$_2$ free radical scavenging activity)**

Transition metal complexes have been suggested as promising agents for the diagnosis and treatment of different diseases$^{[36,37,38]}$. All metal complexes showed significant free radical scavenging action against peroxide induced release of free radicals at varying concentrations (200-µg/ml). In this experiment, ascorbic acid was used as a standard compound. The % scavenging is shown in the table. In addition, some complexes have been suggested as a potential SOD mimics, mainly because of their highly thermodynamic stability$^{[39]}$.

**Table 5: Invitro Antioxidant Activity of transition metal complexes derived from substituted carbohydrazone and thiosemicarbazide moiety.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Complexes</th>
<th>% Scavenging of triplicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ligand (L)</td>
<td>33.18 36.96 37.69 39.69 42.68</td>
</tr>
<tr>
<td>2.</td>
<td>[Mn(C$<em>{34}$H$</em>{30}$N$<em>{10}$S$</em>{2}$)$<em>{2}$Cl]$</em>{2}$</td>
<td>34.16 35.96 36.14 36.95 36.94</td>
</tr>
<tr>
<td>3.</td>
<td>[Ni(C$<em>{34}$H$</em>{30}$N$<em>{10}$S$</em>{2}$)$<em>{2}$Cl]$</em>{2}$</td>
<td>38.18 37.18 37.46 37.96 38.10</td>
</tr>
<tr>
<td>4.</td>
<td>[Co(C$<em>{34}$H$</em>{30}$N$<em>{10}$S$</em>{2}$)$<em>{2}$Cl]$</em>{2}$</td>
<td>42.52 44.55 45.32 46.32 48.72</td>
</tr>
<tr>
<td>5.</td>
<td>[Cu(C$<em>{34}$H$</em>{30}$N$<em>{10}$S$</em>{2}$)$<em>{2}$Cl]$</em>{2}$</td>
<td>50.61 53.85 54.68 56.32 58.68</td>
</tr>
<tr>
<td>6.</td>
<td>[Zn(C$<em>{34}$H$</em>{30}$N$<em>{10}$S$</em>{2}$)$<em>{2}$Cl]$</em>{2}$</td>
<td>28.22 31.85 33.65 36.65 40.62</td>
</tr>
<tr>
<td>7.</td>
<td>Ascorbic acid (standard)</td>
<td>54.61 63.82 69.69 70.46 71.68</td>
</tr>
</tbody>
</table>

Among the examined compound Schiff base ligand (L) and its Co (II), Cu (II), and Ni (II) complexes have exhibited a good free radical activity, whereas Mn (II), Zn (II) complexes have shown moderate activity. All these compounds exhibit significant antioxidant activity but less than control ascorbic acid. Further, the synthesized compounds scavenged the H$_2$O$_2$ radical in a condition dependent manner.

**CONCLUSION**

The outcome of the above results confirms the stoichiometry of the complexes to be 1:2 [M : L] as indicated by elemental analysis and conductometric measurements. IR spectra suggest that the ligand behaves as tridentate and coordinates to the central metal ion through
azomethine nitrogen and sulphur group. This has been further confirmed on the basis of NMR spectral studies. Mass spectra further support the above stoichiometry on the basis of respective molecular masses and fragmentation patterns. Thus, on the basis of above physicochemical and spectral studies, the assigned structures for the metal complexes are shown in Figures. The complexes are found to have higher biological activities as compared to the respective ligand and the parent drug that, somehow, justifies the purpose of the research work. The present work will be further extended to the synthesis of metal complexes using other biologically active metals and evaluation of their biological activity.

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REFERENCES


39. Kumar K and Tweedle M.F.; Macrocyclic polyaminocarboxylate complexes of lanthanides as magnetic resonance imaging contrast agents, pure and applied chemistry, 1993; 65(3): 515-520.

