Thermal and Biological Estimate and Characterization of Cu(II) Complexes Based on Getifloxacin

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ABSTRACT:
Some newly heterochelates synthesized by reflux of different Coumarin derivative, Getifloxacin and transition metal. 1H, 13C, IR and ESI Mass confirm the formation of ligand. The heterochelates were characterized on the basis of different spectroscopic techniques like IR studies and elemental analysis while the geometry of complexes was octahedral which is confirmed by electronic spectra. The compounds were subjected to antimicrobial, antioxidant and anti-tubercular activity viewing using serial broth dilution method and Minimum Inhibitory Concentration (MIC) is determined.

Key words: Getifloxacin, biological facet, Coumarin derivative

1. INTRODUCTION
Coumarin and its derivatives represent one of the most active classes of compounds possessing a wide spectrum of biological activity [1-4]. Many of these compounds have proved to be active as antitumor [5], antibacterial [6], antifungal[7], anticoagulant[8] and antiinflammatory[9]. In addition, these compounds are used as additives to food and cosmetics[10], dispersed Fluorescent and laser[11]. Various analogues of 3-Substituted coumarins suchas 3-amino coumarins exhibit antimicrobial activity[12-13]. From the above line of reasoning we directed this paper toward synthesis of various coumarin derivatives of biological interest using 3-amino coumarin a key starting material. Coumarins are both naturally occurring as well as synthetic derivatives, and are having widespread applications as HIV protease inhibitors, anticoagulant, spasmyolytic and bacteriostatic agents.[14-15] However, the most widely reported activities for coumarin derivatives are their anti-inflammatory and anti-cancer sulfonamides, and 3-bromophenyl 6acetoxymethyl-2-oxo-2H-benzopyran-3carboxylate. Some of the medicinal compounds containing coumarin nucleus are Warfarin (vitamin K antagonists), Ensaclulin (antidementia agent), Umbelliferone or 7-hydroxycoumarin (find use in sunscreen creams and lotions), Psoralen (for psoriasis, eczema and vitiligo). activities. Coumarin derivatives with anticancer activities include aromatase inhibitors, carbonic anhydrase inhibitors, and steroid sulfatase inhibitors.
Coumarin in itself possess much of broad range of biological activities namely anticoagulation, antibiotic, antifungal, antipsoriasis, cytotoxic, anti-HIV, anti-inflammatory. Especially 7- hydroxycoumarin has antioxidant properties and cytostatic, antibacterial, antiviral, xanthine oxidase inhibitor, antihyperglycemic, cascin kinase 2 inhibitor activities, vasorelaxant, antitubercular. Recently, coumarin derivatives have been evaluated in the treatment of human immunodeficiency virus, due to their ability to inhibit human immunodeficiency virus integrase.

Levofloxacin is a broad spectrum antibiotic of the fluoroquinolone drug class, and the levo isomer of its predecessor ofloxacin. Its spectrum of activity includes most strains of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal, and abdominal infections, and atypical bacterial pathogens. Levofloxacin and other fluoroquinolones are valued for their broad spectrum of activity, excellent tissue penetration, and for their availability in both oral and intravenous formulations. Levofloxacin is used alone or in combination with other antibacterial drugs to treat certain bacterial infections including pneumonia, urinary tract infections, and abdominal infections. Levofloxacin is used to treat infections including: respiratory tract infections, cellulitis, urinary tract infections, prostatitis, anthrax, endocarditis, meningitis, pelvic inflammatory disease, traveler’s diarrhea, tuberculosis and plague. Levofloxacin plays an important role in professional medical society guidelines for the treatment of pneumonia, urinary tract infections, and abdominal infections. The Infectious Disease Society of America (IDSA).

The aim of this study was to prepare the mixed ligand complexes of Cu (II) using Livofloxacin with Dicoumarol derivatives and to determine their properties. In our previous reports, we have mentioned a series of fused coumarin derivatives and its transition metal complexes. In continuation of our preceding work, we describe here synthesis, characterization and spectroscopic features of new mixed ligand Cu (II) complexes along with antimicrobial, anti-oxidant and anti-tubercular activities.

2. Experimental

2.1 Materials

All reagents were of analytical reagent (AR) grade purchased commercially from Spectro chem. Ltd., Mumbai-India and used without further purification. Solvents employed were distilled, purified and dried by standard procedures prior to use. Clioquinol was purchased from Agro Chemical Division, Atul Ltd., Valsad-India. The metal nitrates used were in hydrated form.

2.2 Physical measurements

All reactions were monitored by thin-layer chromatography (TLC on aluminium plates coated with silica gel 60 F254, 0.25 mm thickness, E. Merck, Mumbai-India) and detection of the components were measured under UV light or explore in iodine chamber. Carbon, hydrogen and nitrogen were estimated by elemental analyzer PerkinElmer, USA 2400-II CHN analyzer. Metal ion analyses was carry out by the dissolution of solid complex in hot concentrated nitric acid, further diluting with distilled water and filtered to remove the precipitated organic ligands. Remaining solution was neutralized with ammonia solution and the metal ions were titrated against EDTA. 1H and 13C NMR measurements were carried out on Advance-II 400 Bruker NMR spectrometer, SAIF, Chandigarh. The chemical shifts were measured with respect to TMS which used as internal standard and DMSO-d6 used as solvent. Infrared spectra of solids were recorded in the region 4000-400 cm⁻¹ on a Nicolet Impact 400D Fourier-Transform Infrared Spectrophotometer using KBr pellets. Melting point of the ligands and metal complexes were measured by open capillary tube method. Thermal decomposition (TG) analysis was obtained by a model Diamond TGA, PerkinElmer, U.S.A. The experiments were performed in N2 atmosphere at a heating rate of 20 °C min⁻¹ in the temperature range 30-800 °C.

2.3 Preparation of ligands

4-hydroxy-2H-chromen-2-one: 4-hydroxycoumarin was synthesized as reported method.

2.4 Synthesis of ligands (L1-L5)

General procedure for synthesis of the ligands (L) is shown in Scheme 1. The ligands were characterized using elemental analysis, FT-IR, Mass and NMR (1H & 13C) spectroscopy.
2.4.1 Synthesis of 6-chloro-3-(3-(3-chlorophenyl)acryloyl)-2H-chromen-2-one: (L')

Yield: 86%, m.p.: 164-165 °C. FT-IR (KBr, cm⁻¹): ν(C=O, α, β-unsaturated ketone) 1627, ν(C=O, lactone carbonyl of coumarin). ¹H NMR (DMSO-d⁶ 400 MHz) δ: 6.88 (1H, d, CH=CH- protons), 7.74-8.03 (7H, m, aromatic protons), 8.16 (1H, d, CH=CH- protons), 8.59 (1H, s, C=C-H). ¹³C NMR (DMSO-d⁶ 100 MHz) δ: 118.1, 119.8, 124.8, 125.5, 126.2, 126.7, 128.4, 130.1, 130.8, 133.6, 134.0, 134.7, 136.2, 142.6, (14 different types of aromatic carbons), 147.3(C=4), 152.6(C=9), 158.7(C=O, lactone carbonyl of coumarin), 182.9(C=O, α, β-unsaturated ketone). MS (ESI) m/z 326.03 [M+H]⁺, elemental analysis found (%): C, 62.63; H, 2.92; Calculated for C₁₉H₁₃ClO₃ (345.18): C, 62.49; H, 2.59.

2.4.2 Synthesis of 6-chloro-3-(3-(hydroxyphenyl)acryloyl)-2H-chromen-2-one: (L²)

Yield: 72%, m.p.: 167-169 °C. FT-IR (KBr, cm⁻¹): ν(C=O, α, β-unsaturated ketone) 1623, ν(C=O-H) 3426. ν(C=O, lactone carbonyl of coumarin) 1740. ¹H NMR (DMSO-d⁶ 400 MHz) δ: 6.77 (1H, d, CH=CH- protons), 6.92-8.14 (7H, m, aromatic protons), 8.22 (1H, d, CH=CH- protons), 8.56 (1H, s, C=C-H). 9.72 (1H, s, -OH). ¹³C NMR (DMSO-d⁶ 100 MHz) δ: 115.5, 117.9, 118.6, 120.2, 121.4, 124.8, 125.6, 130.2, 130.8, 134.5, 134.9, 135.4, 142.9, (13 different types of aromatic carbons), 147.2(C=4), 152.2(C=9), 158.4(C=16, carbon attach to phenolic OH), 160.5(C=O, lactone carbonyl of coumarin), 183.2(C=O, α, β-unsaturated ketone). MS (ESI) m/z 326.03 [M+H]⁺; elemental analysis found (%): C, 66.67; H, 3.39; Calculated for C₁₉H₁₃ClO₃ (326.73): C, 66.24; H, 3.21.

2.4.3 Synthesis of 6-chloro-3-(3-(4-hydroxyphenyl)acryloyl)-2H-chromen-2-one: (L³)

Yield: 74%, m.p.: 158-160 °C. FT-IR (KBr, cm⁻¹): ν(C=O, α, β-unsaturated ketone) 1617, ν(C=O, lactone carbonyl of coumarin) 1734, ν(C=O-H) 3426. ¹H NMR (DMSO-d⁶ 400 MHz) δ: 6.88 (1H, d, CH=CH- protons), 7.22 (2H, d, CH=CH- protons), 7.56 (2H, d, CH=CH- protons), 7.48-8.11 (3H, m, three aromatic protons), 8.20 (1H, d, CH=CH- protons), 8.58 (1H, s, C=C-H), 9.76 (1H, s, -OH). ¹³C NMR (DMSO-d⁶ 100 MHz) δ: 114.9, 115.8, 118.1, 119.5, 124.2, 125.5, 127.3, 130.9, 134.7, 134.4, 142.6 (11 different types of aromatic carbons), 147.7(C=4), 152.8(C=9), 157.9(C-17, carbon attach to phenolic -OH), 159.2 (C=O, lactone carbonyl of coumarin), 183.4 (C=O, α, β-unsaturated ketone). MS (ESI) m/z 326.03 [M+H]⁺; elemental analysis found (%): C, 58.09; H, 2.78; Calculated for C₁₉H₁₄ClO₄ (326.73): C, 58.24; H, 2.99.

2.4.4 Synthesis of 6-chloro-3-(3-(4-nitrophenyl)acryloyl)-2H-chromen-2-one: (L⁴)

Yield: 75%, m.p.: 158-160 °C. FT-IR (KBr, cm⁻¹): ν(C=O, α, β-unsaturated ketone) 1625, ν(C=O, lactone carbonyl of coumarin) 1740. ¹H NMR (DMSO-d⁶, 400 MHz) δ: 6.86 (1H, d, CH=CH- protons), 7.10-8.05 (7H, m, Ar-H), 8.26 (1H, d, CH=CH- protons); 8.57(1H, s, C=C-H). ¹³C NMR (100 MHz, DMSO-d⁶) δ: 114.2, 116.5, 118.7, 124.4, 125.9, 126.5, 127.7, 129.8, 130.9, 134.2, 146.4 (11 different types of aromatic carbons), 147.6 (C=4), 148.9(C=16, carbon attach to NO₂), 154.6(C=9), 159.8(C=O, lactone carbonyl of coumarin), 189.5(C=O, α, β-unsaturated ketone). MS (ESI) m/z 355.20 [M+H]⁺; elemental analysis found (%): C, 60.77; H, 2.83; N, 3.94; Calculated for C₁₉H₁₂ClO₄ (455.73): C, 60.02; H, 2.52; N, 3.63.

2.4.5 Synthesis of 6-chloro-3-(3-(3-methoxyphenyl)acryloyl)-2H-chromen-2-one: (L⁵)

Yield: 76%, m.p.: 168-169 °C. FTIR (KBr, cm⁻¹): ν(C=O, α, β-unsaturated ketone) 1621, ν(C=O, lactone carbonyl of coumarin) 1743, (C-O-C, asymmetric) 1249, (C-O-C, symmetric) 1,044, (aromatic C=C & C-H Stretching) 3017, 3014. ¹H NMR (DMSO-d⁶, 400 MHz) δ: 6.83(1H, d, CH=CH- protons), 7.07-8.05 (7H, m, Ar-H), 8.25 (1H, d, CH=CH- protons); 3.79 (3H, s, -OCH₃); 8.58(1H, s, C=C-H). ¹³C NMR (100 MHz, DMSO-d⁶) δ: 55.7 (C-17, OCH₃), δ: 114.1, 116.3, 118.5, 124.9, 125.7, 126.9, 127.1, 129.5, 130.6, 134.8, 146.8 (11 different types of aromatic carbons), 147.8(C=4), 154.4(C=9), 159.2(C=O, lactone carbonyl of coumarin), 160.4(C=16), 189.5(C=O, α, β-unsaturated ketone). MS (ESI) m/z 340.05 [M+H]⁺; elemental analysis found (%): C, 60.67; H, 3.85; Calculated for C₁₉H₁₃ClO₄ (340.76): C, 60.24; H, 3.40.


Fig.1 ¹H-NMR spectrum of L4

Fig.2 IR spectrum of L4

Scheme 2. General procedure for synthesis of complexe (C)

2.5 Synthesis of metal complexes

[Cu(L)(LF)(H₂O)]OH₂·2H₂O (C¹-C⁵)

The Coumarin derivative (0.01 mol) was dissolved in water(25 ml) by gradually adding aqueous solution of Cu(NO₃)₂·6H₂O (0.01 mol, 25 ml) and then was slowly added to an ethanolic solution of Getifloxacin (0.01 mol, 25 ml). The pH was adjusted to 4.5-6.0 with diluted NH₃OH solution. Furthermore, the mixture was heated under reflux for 5-8 h and then heated over a steam bath to evaporate up to half of the volume. The reaction mixture was kept overnight at room temperature. A fine amorphous powder was obtained by filtration and dried in air. The complexes comprise high melting points (above 300 °C) and insoluble in common organic solvents and partially soluble in DMSO. Complexes C¹-C⁵ was prepared according to same method. The synthetic protocol of complexes is shown in scheme 2.

Table 1 Analytical and physical parameters of complexe

<table>
<thead>
<tr>
<th>Compound</th>
<th>C</th>
<th>H</th>
<th>N</th>
<th>Metal(II)</th>
<th>M. p. (oC)</th>
<th>Yield (%)</th>
<th>Mol. Wt.</th>
<th>µeff (B. M.)</th>
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<td>C¹</td>
<td>47.29(47.67)</td>
<td>3.41(3.72)</td>
<td>5.13(5.23)</td>
<td>&gt;3</td>
<td>61</td>
<td>109</td>
<td>1.82</td>
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<tr>
<td>C¹</td>
<td>49.99(49.99)</td>
<td>3.79(4.17)</td>
<td>6.19(6.19)</td>
<td>50</td>
<td>9.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C²</td>
<td>48.57(48.81)</td>
<td>3.54(4.97)</td>
<td>6.39(6.39)</td>
<td>50</td>
<td>3.2</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>C³</td>
<td>48.63(48.89)</td>
<td>3.64(4.02)</td>
<td>6.45(6.45)</td>
<td>50</td>
<td>3.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C³</td>
<td>47.29(47.65)</td>
<td>3.76(5.26)</td>
<td>5.95(5.95)</td>
<td>50</td>
<td>2.1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.6 Antimicrobial activity

All the ATCC culture was collected from institute of microbial technology, Bangalore. 2% Luria broth solution was prepared in distilled water while, pH of the solution was adjusted to 7.4±0.2 at room temperature and sterilized by autoclaving at 15 lb pressure for 25 min. The tested bacterial and fungal strains were prepared in luria broth and incubated at 37 °C and 200 rpm in an orbital incubator for overnight. Sample solutions were prepared in DMSO for concentration 200, 150, 100, 50, 25, 12 and 6µg/mL. The standard drug solution of Streptomycin (antibacterial drug) and Nystatin (antifungal drug) were prepared in DMSO. Serial broth micro dilution was adopted as a reference method. 10 µl solution of test compound was inoculated in 5 mL luria broth for each concentration respectively and additionally one test tubes was kept as control. Each of
the test tubes was inoculated with a suspension of standard microorganism to be tested and incubated at 35 °C for 24 h. At the end of the incubation period, the tubes were examined for the turbidity. Turbidity in the test tubes indicated that microorganism growth has not inhibited by the antibiotic contained in the medium at the test concentration. The antimicrobial activity tests were run in triplicate.

2.7 Anti-tubercular activity

Test compounds were evaluated for in vitro anti-tubercular activity. The MICs were determined and interpreted for M. tuberculosis H37Rv according to the procedure of the approved micro dilution reference method of antimicrobial susceptibility testing [27]. Compounds were taken at concentrations of 100, 50, 25 and 12 µg/mL in DMSO, 1.0 ml of each concentration was used for the study. To this, 9.0 ml of Lowenstein-Jensen medium was added. A sweep from M. tuberculosis H37Rv strain culture was discharged with the help of nichrome wire loop with a 3 mm external diameter into a vial containing 4 ml of sterile distilled water. The vial was shaken for 5 min. Then using nichrome wire loop suspension was inoculated on the surface of each of Lowenstein-Jensen medium containing the test compounds. Further test media was incubated for four weeks at 37 °C. Readings were taken at the end of fourth week. The appearance of turbidity was considered as bacterial growth and indicates resistance to the compound. Test compounds were compared to reference drugs Isoniazid (MIC = 0.025µg/mL), Streptomycin (MIC = 6.25µg/mL) and Ethambutol (MIC = 20µg/mL). Lowenstein-Jensen medium containing standard drugs as well as DMSO was inoculated with M. tuberculosis H37Rv strain. The anti-tubercular activity tests were run in triplicate.

2.8 Antioxidant studies

Ferric reducing antioxidant power (FRAP) was determine using an adapted method [28]. The antioxidant potentials of the compounds were examine by their reducing power of the TPTZ-Fe(III) complex to TPTZ-Fe(II) complex for the total antioxidant capacity of tested samples, This method was employed because of its simple, fast and also results can be obtain was reproducible. Initially following solutions were prepared, A) acetate buffer, 300 mM pH 3.6 (3.1g sodium acetate trihydrate and 16 ml conc. acetic acid per L of buffer solution), B) 10 mM 2,4,6-tripyridyl-s-triazine in 40 mM HCl, C) 20 mM FeCl₃•6H₂O in distilled water, D) 1mM of ascorbic acid dissolved in 100 mL distilled water. FRAP working solution was prepared by mixing the above (A), (B) and (C) solutions in the ratio of 10:1:1 respectively. A mixture of 40.0 µL, 0.5 mM sample solution and 1.2 mL FRAP reagent was incubated at 37 °C for 15 min. The working solution was necessary to use as freshly prepared. The ascorbic acid was used as a standard antioxidant compound and results were expressed with compared to ascorbic acid.

3. RESULT AND DISCUSSION

The synthesized Cu(II) complexes were characterized by elemental analysis, FTIR and mass spectra, The metal ion in their complexes were determined after mineralization. The metal content in chemical analysis was estimated by complexometrically[29], while geometry of the complexes was confirmed from electronic spectra, magnetic moment and thermal properties However, ligands and its complexes have been screened for their in vitro antitubercular and antimicrobial activities.

3.1 Elemental analysis

The analytical and physiochemical data of the complexes are summarized in Table 1. The experimental data were in very good agreement with the calculated ones. The complexes were colored, insoluble in water and commonly organic solvents while soluble in DMSO as well as stable in air. The structure of the complexes is assumed according to the chemical reaction as shown below;

\[ \text{Cu(NO}_3\text{)}_2 \cdot 3 \text{H}_2\text{O} + \text{L} + \text{LF} \quad \text{-------->}[\text{Cu}(\text{L})(\text{LF})(\text{H}_2\text{O})_x] \cdot x \text{H}_2\text{O} + 2\text{HNO}_3 + n \text{H}_2\text{O} \]

(Where \text{L = L}_1^1, L_2^2, L_3^3, \text{L}_4^4; x = 1, 0, 2, 0, 1 and n = 2, 3, 1, 3, 2)

3.2 FT-IR spectra

The coordination sites of ligand are elucidated using IR. The IR band assignments of dicoumarol derivatives and its complexes are included in Table 2. The IR data of free ligands and its metal complexes were carried out within the IR range 4000-400 cm⁻¹. The IR spectra of the dicoumarol derivatives show weak bands at ~3127-3054 cm⁻¹ and ~1331-1337 cm⁻¹, corresponding to ν(O–H) and ν(C–OH) respectively. On complexation O–H peak has vanished, indicates deprotonation of O–H proton. The
ν(C=O) of lactone rings observed at ~1647 and 1655 cm⁻¹ in free ligand is shifted to lower frequencies (~12-14 cm⁻¹ and 40-50 cm⁻¹) due to complex formation, and further supported by shifting of ν(C–C), ν(C–O), and ν(C–O–C) stretch frequencies to higher values. Two bands at ~1613 and ~1563 cm⁻¹ were assigned to stretching vibration of conjugate double bonding in the free ligand. The H–O–H bending mode occurring about ~1602 cm⁻¹ has not been observed because of the presence of strong absorbing group like methine group (–CH=). It is difficult to resolve both these bands. A broad band at ~3424-3453 cm⁻¹ observed in the complex was due to the ν(O–H) characteristic peak of a coordinated water molecule. The bands at ~1732 cm⁻¹ and ~1250 cm⁻¹ attributed to the stretching vibrations ν(C=O)carboxylic and ν(C=O)carboxylic respectively, of the carboxylic moiety (~COOH) of livoofloxacin, have been shifted in the range ~1584-1603 cm⁻¹ and ~1361-1382 cm⁻¹ assigned as antisymmetric, ν(C=O)asym, and symmetric, ν(C=O)sym, stretching vibrations of the carboxylato group, respectively. The difference Δ=[ν(C=O)sym−ν(C=O)sym], a useful characteristic tool for determining the coordination mode of the carboxylato ligands, reaches a value of ~204–222 cm⁻¹ indicative of a monodentate coordination mode. Whereas ν(C=O)p is shifted from ~1623–1644 cm⁻¹ upon bonding. The overall changes of the IR spectrum suggest that livoofloxacin and ligands are coordinated to the copper via the ketone oxygen and carboxylato oxygen. These changes in the IR spectra suggest that livoofloxacin is coordinated to metal via pyridone and one carboxylato oxygen atoms. These data are further supported by ν(Cu–O) which appear at ~519 cm⁻¹[30-32].

![Fig. 3. FT-IR spectrum of complex C4](image)

<table>
<thead>
<tr>
<th>Comp</th>
<th>ν(O–H) s</th>
<th>ν(C=O) cm⁻¹</th>
<th>ν(C=C) cm⁻¹</th>
<th>u(C=C), u(C=O), u(C–O–C) cm⁻¹</th>
<th>u(COO)sy</th>
<th>u(COO)asy</th>
<th>u(C=O) of pyridone</th>
<th>u(Cu–O)²</th>
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<tr>
<td>C¹</td>
<td>3432</td>
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<td>1587</td>
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<td>1585</td>
<td>1638</td>
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s = strong, w = weak, br = broad

### 3.3 Electronic spectra

The Cu(II), Ni(II), Co(II), and Mn(II) complexes show magnetic moments of 1.82, 3.14, 3.86 and 5.93 B.M. respectively which is characteristic of mononuclear, Cu(II) (d⁹, 1 unpaired electron) octahedral, Ni(II) (d⁸, 2 unpaired electrons), Co(II) (d⁷, 3 unpaired electrons), and Mn(II) (d⁶, 5 unpaired electrons) complexes[33].

The electronic spectral data of the complexes in DMF are shown in Table 3. The Cu(II) complexes display three prominent bands. Low intensity broad band in the region 16,930-17,880 cm⁻¹ was assigned as 10 Dq band corresponding to ²E₉→³T₂g transition [34]. In addition, there was a high intensity band in the region 22,910-27,200 cm⁻¹. This band is due to symmetry forbidden ligand → metal charge transfer transition [35]. The band above 27,200 cm⁻¹ was assigned as ligand band. Therefore distorted octahedral geometry around Cu(II) ion was suggested on the basis of electronic spectra [36]. (Fig. 4).

![Fig. 4. Electronics Spectrum of complex Cu(II)](image)

### 3.4 Antioxidant studies

Antioxidant power was specifically the ability of transfer a single electron for compound and antioxidant capacity of complexes C¹-C⁵ was determined by a FRAP method.
The FRAP results was expressed as an equivalent of standard antioxidant ascorbic acid (mmol/100 g of dried compound). FRAP values indicate that all the compounds have a ferric reducing antioxidant power. The compounds C₁ and C² showed relatively high antioxidant activity while compound C₅ shows poor antioxidant power (Table 4). However, none of the compounds have been found to show excellent activity with compared to standard ascorbic acid.

Table 3 Electronic spectral data of the complexes

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Transition band observed (cm⁻¹)</th>
<th>μeff</th>
<th>B.M</th>
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<td>C₁</td>
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</table>

3.5 Anti-tubercular activity

The anti- tubercular activities of all the synthesized compounds were assessed against M. tuberculosis H37RV at 25, 50 and 100 µg/mL. The Minimum Inhibitory Concentrations of compounds compared with Isoniazid, Streptomycin and Ethambutol, the standard drugs and are summarized in Table 4. Ligands show inhibition at concentration 100µg/mL. Complexes C¹ also exhibits activity at 50µg/mL concentration while C² and C⁴ complexes have shown enhancement in activity with MIC of 25µg/mL. None of the tested compounds have the inhibition more than standards.

Table 4 Antimicrobial, Anti-tubercular and antioxidant results of compounds

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Minimal Inhibition Concentration a (µg/mL) of microorganisms</th>
<th>Antioxidant Activity b FRAP value (mmol/100g)</th>
<th>Anti-tubercular activity c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bacteria</td>
<td>S.P.</td>
<td>B.S.</td>
</tr>
<tr>
<td>L¹</td>
<td>100</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>L²</td>
<td>50</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>L³</td>
<td>100</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>L⁴</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>L⁵</td>
<td>100</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>C²</td>
<td>6.25</td>
<td>25</td>
<td>12.5</td>
</tr>
<tr>
<td>C³</td>
<td>25</td>
<td>12.5</td>
<td>25</td>
</tr>
<tr>
<td>C⁴</td>
<td>6.25</td>
<td>25</td>
<td>12.5</td>
</tr>
<tr>
<td>C⁵</td>
<td>12.5</td>
<td>6.25</td>
<td>12.5</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>0.025</td>
<td>0.025</td>
<td>0.020</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Flucanazole</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
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<tr>
<td>Ascorbic acid</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

3.6 Antimicrobial bioassay

All the synthesized ligands and complexes were evaluated for their antibacterial and antifungal studies. The antibacterial and antifungal tests were carried out using the serial broth dilution method. The in vitro antimicrobial activities of the investigated compounds were screened against the bacterial species SA, BS, EC, PA and fungal species CA, AN and AC. The minimum inhibitory concentration (MIC) values of the compounds are summarized in Table 4.

A relative study for MIC values of the ligands and their complexes signify that complexes display higher antimicrobial activity than the free ligands. In present investigation, the antimicrobial activity of the ligands may be due to the heteroaromatic residues. Compounds
containing C=N group have improved antimicrobial activity than C=C group. The growth of certain microorganisms takes place even in the absence of oxygen. The results were compared with those of the standard drug. All the metal complexes were more potent bactericides than the ligand. C1 complex was much less microbially active than the other complexes. From Table 4, it can be seen that the highest inhibition of growth occurred on C1 complex against the microorganism, while C2, C4 and C3 shows enhance activity than C1 but less potent than C5. There was a marked increase in the bacterial activities of the Cu(II) complex as compared with the free ligand under test, which is in agreement with antibacterial properties of a range of Cu(II) complexes evaluated against several pathogenic bacteria. The fungal strains used to demonstrate the antifungal potency of the synthesized compounds were Candida albicans (ATCC 66027) and Aspergillus niger (ATCC 64958). The results of inhibition are compared with standard antifungal drug Fluconazole (Table 4).

4. CONCLUSIONS

Here Newly the synthesised heterochelates from biological active Ligand (L) and livoofoxacin. The structures of the ligand were investigated and confirmed by the elemental analysis, FT-IR, 1H-NMR, 13C-NMR and mass spectral studies. Octahedral geometry were all Metal(II) complexes dispense on the basis of electronic. All Cu(II) complexes tested by in vitro antimicrobial, antitubercular and antioxidant activity which shows fine results with an enhancement of activity on complexation with metal ions. This enhancement in the activity may be due to increased lipophilicity of the complexes. In review, the antimicrobial testing results reveal that complexes possess higher activity compared to parent ligand.

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