



SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL STUDY OF NOVEL SCHIFF BASE LIGAND WITH SOME METAL ION Co(II), Ni(II), Cu(II) AND Zn(II)

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ABSTRACT

A new Schiff base ligand has been synthesized through the condensation of amino penicillanic acid with *o*-nitrobenzaldehyde dissolved in absolute ethanol in alkaline medium. Metal complexes of the Schiff base have been synthesized from chloride salt of Co(II), Ni(II), Cu(II) and Zn(II) metal in the alcoholic medium. Different analytical and diagnostic techniques UV-Vis spectrum, IR spectroscopy, mass spectrum, (H,C) NMR spectrophotometry, magnetic susceptibility studies and molar conductivity have been used in the investigation of prepared compound properties and their proposed stereochemistry. The effectiveness of inhibitory compounds prepared was against tested four types of bacteria (*Staphylococcus aureus*, *pseudomonas aeruginosa*, *Streptococcus facials* and *Proteus mirabilis*), which were isolated from different classes of ulcerative infections.

Key words: β -Lactam, Schiff base, Antibacterial, *o*-Nitrobenzaldehyde.

INTRODUCTION

β -Lactam antibiotics have been successfully used in the treatment of infectious diseases for many years and compounds containing β -Lactam ring are a class of important compounds in medicinal and pharmaceutical field. Since the discovery of β -Lactam antibiotics in 1907 by Scantest and its clinical introduction as an antibacterial agent in the early 1950s^{1,2} β -lactam antibiotics have remained the most popular drugs for treating bacterial infections of many diseases^{3,4}. Since that time, the biological applications of these compounds have attracted remarkable attention⁵, particularly the 6-aminopenicillanic acid have attracted more attention (Fig. 1). The 6-aminopenicillanic acid is the most widely used class of antimicrobial agents. This acid, has been shown to exert pronounced biological effects on various bacterial strains.

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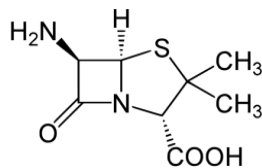


Fig. 1: 6-Aminopenicillanic acid

Schiff base compounds (azomethene) and its metal complexes have been found to exhibit biological activities. The biological applications of Schiff base and its complexes have attracted special attention. Some of Schiff-bases exhibited antibiotic, antiviral and antitumor activity because of their specific structure⁶. The widespread use of antibiotics has led to the proliferation of antibiotics resistant bacteria. Hence, a search has been on for synthesis of new derivatives of antibiotics and it has become an important task to cope with drug resistance problems.

EXPERIMENTAL

All chemicals used were reagent grade (Sigma Aldrich, B.D.H, C.D.H. and S.L. companies)

Instrumentation

The FT-IR spectra were recorded on 8400 FTIR Shimadzu spectrometer for ligand and complexes, UV-Visible spectra were recorded on Shimadzu 1800 series spectrometer in DMSO solvent, Molar conductance measurement in DMSO solvent using digital conductivity meter alpha-800, mass spectrum and the magnetic moment measurement was using Auto magnetic susceptibility balance Sherwood scientific. NMR spectra were recorded on ¹³C NMR and ¹H NMR spectrometer (Bruker Spectrometer, Germany operating at 400 MHz) with DMSO. The chemical shifts are reported in ppm relative to TMS (trimethylsilane).

Preparation of Schiff base ligand

Ligand was prepared by dissolving 6-aminopenicillanic acid (2.16 g, 0.01 mole) in 15 mL absolute ethanol (medium basic) and then of o-nitrobenzaldehyde (1.15 g, 0.01 mole) was added. The result out mixture was refluxed for 14 hrs, following its reaction using thin layer chromatograph. It was filtered, dried and repetitively recrystallized from absolute ethanol.

Preparation of complexes

For the preparation of complexes, a ligand (0.700 g, 0.002 mole) solution was mixed

with 0.001 mole of (0.170 g, 0.2379 g, 0.2377 g and 0.136 g) of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ and ZnCl_2 , separately in 15 mL of absolute ethanol. The mixture was refluxed for 4 h on a water bath. Then it was cooled to room temperature. The colored complexes got precipitated slowly, which was filtered, washed repeatedly with distilled water and ethanol. Now, the complexes were dried over anhydrous calcium chloride in desiccators. Table 1 shows chemical and physical properties of free ligand and its complexes.

Table 1: Chemical and physical characterizations data for ligand and complexes

| Compound symbol | M. wt | M. p. (°C) | Color |
|------------------|--------|------------|-------------|
| L | 349.00 | 142-140 | Orange |
| Cu(L)_2 | 761.54 | 150-148 | Brown |
| Co(L)_2 | 756.69 | 117-115 | Green |
| Ni(L)_2 | 756.93 | 176-174 | Brown |
| Zn(L)_2 | 763.39 | 137-135 | Brown light |

RESULTS AND DISCUSSION

Infrared spectrum

The IR frequencies of free ligand and complexes are reported in Table 2. The IR spectra of ligand and all complexes show band at 1348 cm^{-1} due to $\nu(\text{R-NO}_2)$. This indicated that (NO_2) is not participating in coordination.

Table 2: FTIR data of Schiff base ligand and metal complexes

| Compd. | ν (OH) | ν (C-H) _{alph} | ν (C-H) _{arm.} | ν (C=O) Lactam | (-HC=N-) | ν (M-N) ν (M-O) | ν (R-NO ₂) |
|------------------|---------------|--------------------------------|--------------------------------|-----------------------|----------|----------------------------|-------------------------------|
| L | 3340 | 2974 | 3064 | 1720 | 1645 | - | 1348 |
| Cu(L)_2 | 3417 | 2978 | 3064 | 1728 | 1635 | 530 447 | 1348 |
| Co(L)_2 | 3431 | 2933 | 2980 | 1730 | 1635 | 557 443 | 1348 |
| Ni(L)_2 | 3417 | 2933 | 3080 | 1726 | 1635 | 555 447 | 1348 |
| Zn(L)_2 | 3421 | 2931 | 2976 | 1722 | 1639 | 570 443 | 1348 |

The spectrum of free ligand displays a band at 3340 cm^{-1} due to $\nu(\text{OH})$ of carboxylate group. This band is shifted to lower frequency in the spectra of all complexes. The bands at 1645 cm^{-1} and 1720 cm^{-1} were assigned to stretching vibration of $\nu(-\text{CH}=\text{N}-)$ azomethene and $\nu(\text{C}=\text{O})$ lactam groups, respectively. On the complexation, these bands have been shifted to higher frequency in the spectra of all complexes. The spectra of complexes recorded a new band in the range of $557\text{--}443\text{ cm}^{-1}$ for coordination with metal, the coordination occurred through the nitrogen atom of azomethene and two oxygen atom of $(\text{C}=\text{O})$ lactam and (OH) of carboxylate group⁷⁻⁹. Figs. 2 and 3 show spectra of free ligand and copper complex.

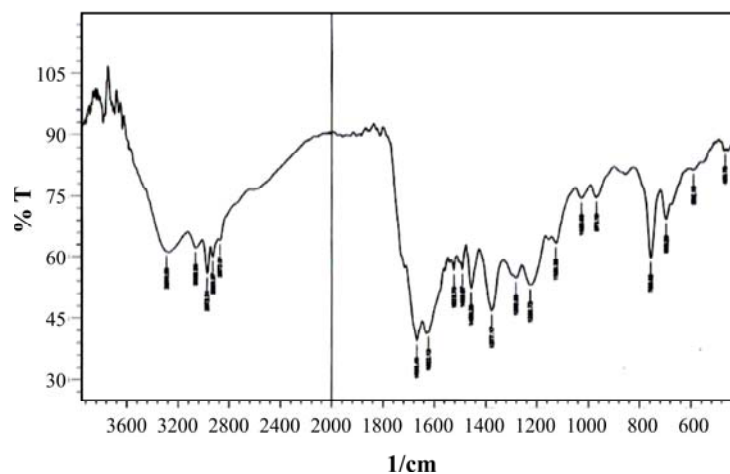


Fig. 2: FTIR Spectra of ligand

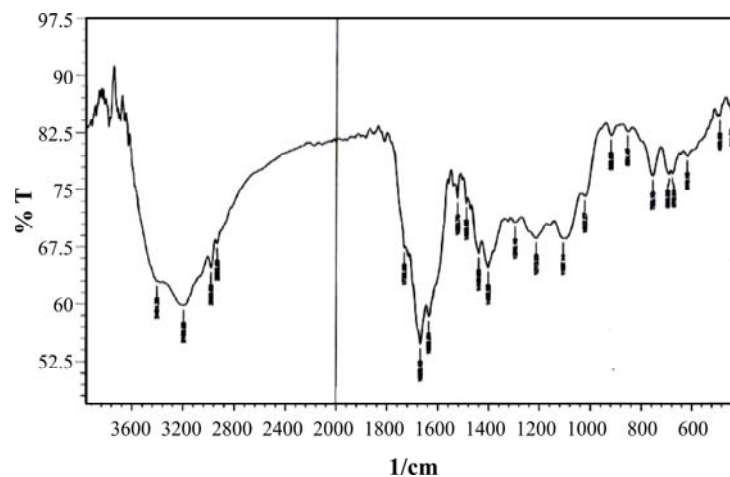


Fig. 3: FTIR Spectra of copper (II) complex

Electronic spectra

The electronic spectrum of the ligand and complexes have been measured in DMSO solution between 200-1100 nm at room temperature. The UV-Vis data of ligand and complexes are reported in Table 3 and UV-Vis spectrum of the Schiff base ligand in Fig. 4. In the spectrum of the Schiff base free ligand, the absorption band observed at 270 nm and 322 nm, which are assigned to π - π^* and n - π^* transition, respectively. In the UV-Vis spectrum of complexes, two peaks are exhibited due to the (C.T)¹⁰⁻¹².

Table 3: Electronic spectra data of Schiff base ligand and metal complexes

| Complexes | Absorption band (nm; λ_{\max}) | Assignment |
|--------------------|---|---------------------------------|
| L | 270, 322 | n - π^* , π - π^* |
| Cu(L) ₂ | 458, 334 | C.T. |
| Co(L) ₂ | 455, 333 | C.T. |
| Ni(L) ₂ | 405, 374 | C.T. |
| Zn(L) ₂ | 580, 362 | C.T. |

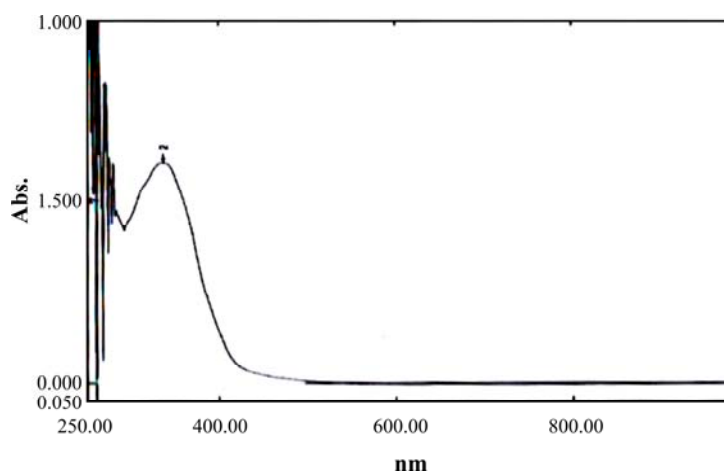


Fig. 4: Electronic spectra of Schiff base ligand

Mass spectrum

The mass spectrum of Schiff base ligand is shown in Fig. 5. The spectrum exhibit a peak representing the parent ion ($M + 1$) centered at 351. The proposed fragmentation pathways include breaking β -lactam cycle of β -lactam and carboxyl group in addition to

breaking the azomethene group, the general pattern of fragmentation are summarized in Scheme 1¹³⁻¹⁵.

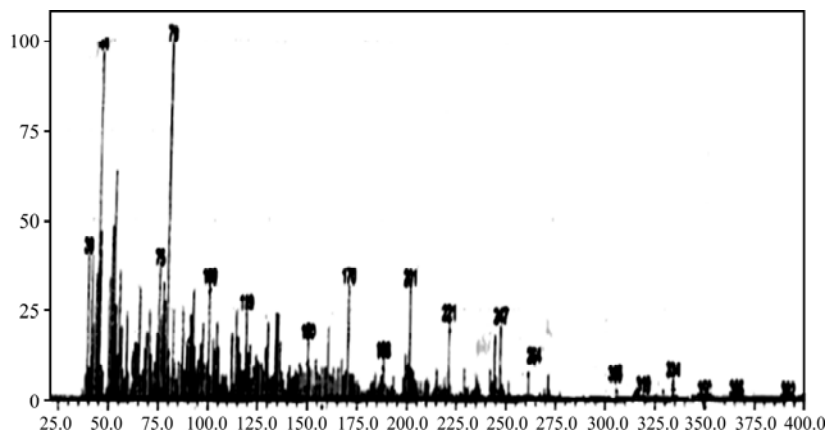
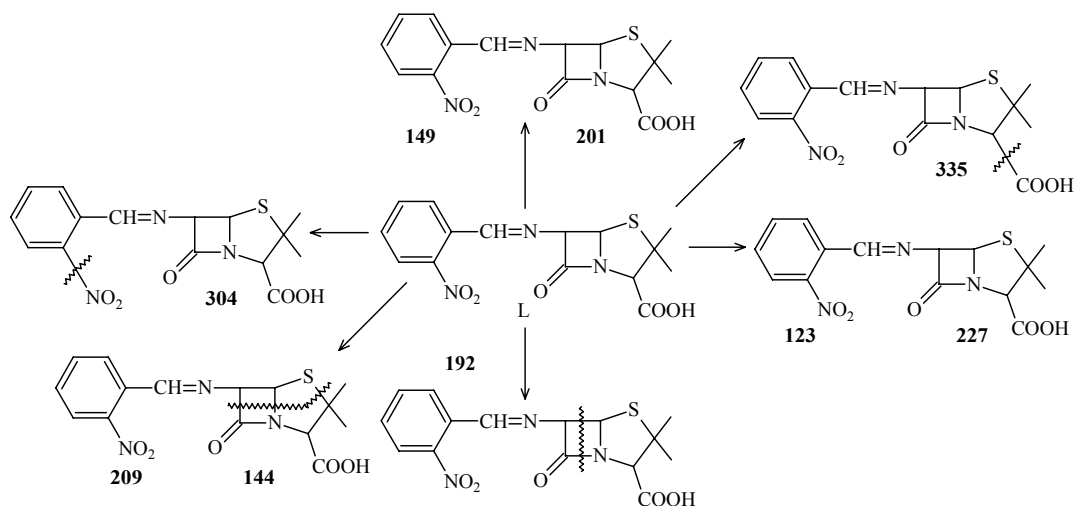


Fig. 5: Mass spectrum of Schiff base ligand



Scheme 1: Fragmentation pattern of ligand

Proton and carbon nuclear magnetic resonance spectra of the Schiff base ligand

¹H NMR spectrum of Schiff base ligand in DMSO-d₆ is given in Fig. 6. The signal obtained at (δ 8.5) ppm was assigned for singlet due one proton of -CH=N linkage in the ligand. A single peak attributed to hydroxyl group, which appeared at (δ 9.2) ppm. The signal obtained in range (δ 7.1-7.6) ppm was assigned for doublet, due to one proton of aromatic ring and single peaks attributed to methyl groups appearing at (δ 2.45) ppm. The ¹³C NMR

spectrum of Schiff base ligand was also recorded in DMSO- d_6 Fig. 7 showed the azomethene carbon peak at (δ 167) ppm. The carboxylic (C=O) group peak showed at (δ 171) ppm and the aromatic carbon peak was observed at (δ 115) ppm¹⁶⁻²⁰.

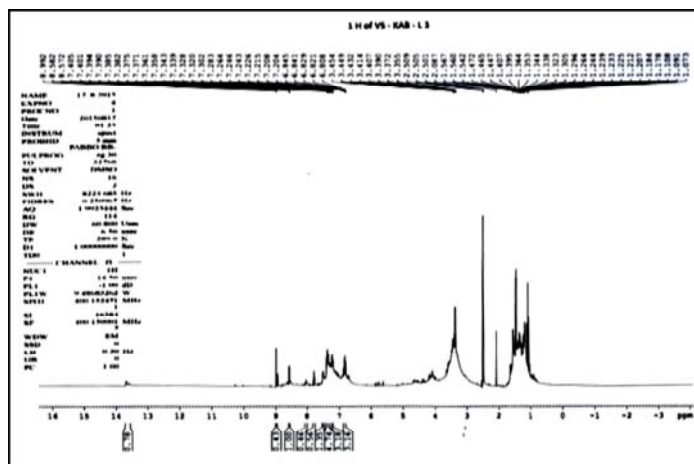


Fig. 6: ^1H -NMR spectrum of ligand

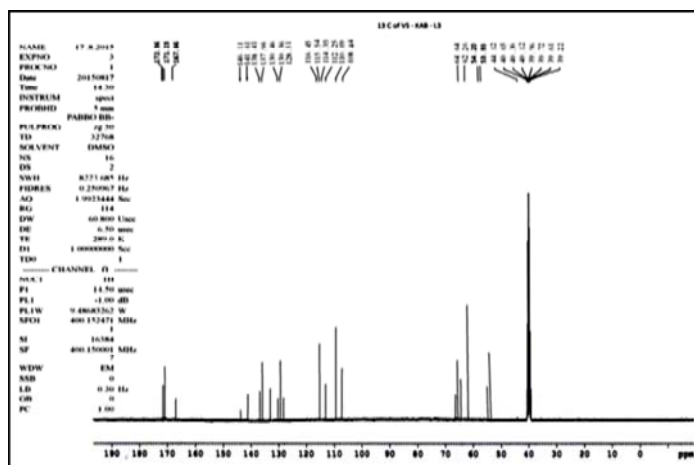


Fig. 7: ^{13}C -NMR Spectrum of ligand

Magnetic susceptibility and molar conductivity for coordination complexes

The magnetic properties of complexes were measured and compared with the literature²¹⁻²³, which suggests geometrical shape of these complexes to be of the octahedral shape as for molar conductivity of every complexes recipe is ionic neutral. Table 4 illustrates the magnetic properties and molar conductivity results for all the complexes.

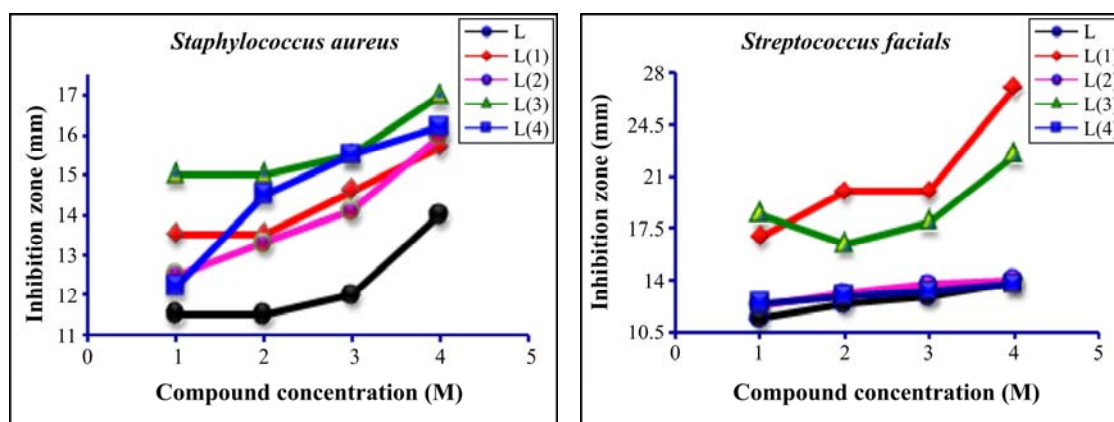
Table 4: Magnetic moments and molar conductivity data of Schiff base ligand and metal complexes

| Complexes | μ eff (M.B) | Molar conductivity | Shape |
|---------------------|-----------------|--------------------|-------------|
| Cu (L) ₂ | 1.75 | 7.1 | Octahedral |
| Co (L) ₂ | 4.2 | 6.2 | Octahedral |
| Ni (L) ₂ | 3.1 | 7.4 | Octahedral |
| Zn (L) ₂ | - | 5.6 | Diamagnetic |

Antibacterial activity

The prepared complexes of ligand with Cu, Co, Ni and Zn were studied for their biological activity against four different types of bacteria (*Staphylococcus aureus*, *pseudomonas aeruginosa*, *Streptococcus facials* and *Proteus mirabilis*), at the concentrations of 1×10^{-6} , 1×10^{-5} , 1×10^{-4} , 1×10^{-3} M and compared with the inhibitory effect of the original ligand toward same types of bacteria. These bacteria were isolated from the most active plantation of numerous type of ulcers (grades III and II burn ulcers, diabetes foot ulcer, in addition to bed ulcers).

Fig. 8 illustrates the effect of ligand and its complexes the biological activity of *Staphylococcus aureus*, *pseudomonas aeruginosa*, *Streptococcus facials* and *Proteus mirabilis* bacteria, respectively. The inhibitory effect of the prepared complexes were significantly higher than their original ligand, when they had been tested on the four types of bacteria. *Proteus mirabilis* bacteria illustrated the most responsibility at the ligand and its complexes were tested in the plantation of this bacteria.



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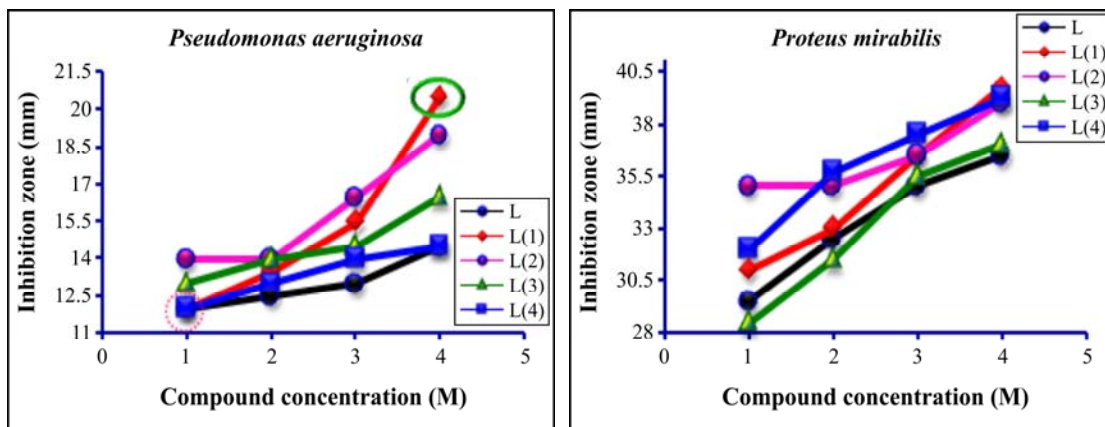


Fig. 8: Effect of original ligand (L) and its four complexes in the biological activity of the different types of isolated bacteria

The complex of nickel was recorded to show clear inhibitory effect in comparison to other complexes and original ligand at the lowest tested concentration (1×10^{-6} M) in the biological activity of *Staphylococcus aureus* (Table 5 and Fig. 8).

Table 5: Vital effect of the ligand and complexes in the biological activity of *Staphylococcus aureus*

| Concentration (M) | Compound symbol | | | | |
|--------------------|-----------------|------|------|------|------|
| | L | L(1) | L(2) | L(3) | L(4) |
| 1×10^{-6} | - | + | - | ++ | - |
| 1×10^{-5} | - | + | + | ++ | ++ |
| 1×10^{-4} | - | ++ | ++ | +++ | +++ |
| 1×10^{-3} | + | +++ | +++ | +++ | +++ |

- = <13 mm, + = ≥ 14 mm, ++ = 14.1-15 mm, +++ = > 15.1 . (L(1): Complex of Cu(II), L(2): Complex of Co(II), L(3): Complex of Ni(II), and L(4): Complex of Zn(II))

Although the original ligand has weak effect in the activity of *Streptococcus faecalis*, but its complexes of the copper and nickel demonstrated respectable inhibitory ability, while the complexes of cobalt and zinc failed to illustrate the same effect against same type of bacteria (Table 6 and Fig. 8).

Table 6: Vital effect of the ligand and complexes in the biological activity of *Streptococcus faecalis*

| Concentration (M) | Compound symbol | | | | |
|----------------------|-----------------|------|------|------|------|
| | L | L(1) | L(2) | L(3) | L(4) |
| 1×10^{-6} | - | +++ | - | +++ | - |
| 1×10^{-5} | - | +++ | + | +++ | - |
| 1×10^{-4} | - | +++ | + | +++ | + |
| 1×10^{-3} | + | +++ | ++ | +++ | + |

- = <13 mm, + = ≥14 mm, ++ = 14.1-15 mm, +++ = >15.1. (L(1): Complex of Cu(II), L(2): Complex of Co(II), L(3): Complex of Ni(II), and L(4): Complex of Zn(II)

The complexes of copper demonstrated the highest inhibition ability for the activity of *Pseudomonas aeruginosa* followed by cobalt and then nickel complexes at the greatest exam concentration (1×10^{-3} M), while the ability of zinc complex didn't exceed the inhibition ability of original ligand at same concentrations (Table 7 and Fig. 8).

Table 7: Vital effect of ligand and complexes in the biological activity of bacterial *Pseudomonas aeruginosa*

| Concentration (M) | Compound symbol | | | | |
|----------------------|-----------------|------|------|------|------|
| | L | L(1) | L(2) | L(3) | L(4) |
| 1×10^{-6} | - | - | + | - | - |
| 1×10^{-5} | - | + | + | + | - |
| 1×10^{-4} | - | +++ | +++ | ++ | + |
| 1×10^{-3} | ++ | +++ | +++ | +++ | ++ |

- = <13 mm, + = ≥14 mm, ++ = 14.1-15 mm, +++ = >15.1. (L(1): Complex of Cu(II), L(2): Complex of Co(II), L(3): Complex of Ni(II), and L(4): Complex of Zn(II)

Fig. 8 shows the complex of copper has the highest inhibition ability among the four complexes, followed by zinc and cobalt complexes, where the results of nickel complex has the same range of inhibitory affect for the original ligand, especially at the lowest concentration (Table 8).

Table 8: Vital effect of the ligand and complexes in the biological activity of proteus mirabilis

| Concentration (M) | Compound symbol | | | | |
|----------------------|-----------------|------|------|------|------|
| | L | L(1) | L(2) | L(3) | L(4) |
| 1×10^{-6} | + | ++ | ++ | + | ++ |
| 1×10^{-5} | + | ++ | ++ | ++ | +++ |
| 1×10^{-4} | ++ | +++ | +++ | +++ | +++ |
| 1×10^{-3} | +++ | +++ | +++ | +++ | +++ |

- = 20-25 mm, + = 26-30 mm, ++ = 30-35mm, +++ = >35.1. (L(1): Complex of Cu(II), L(2): Complex of Co(II), L(3): Complex of Ni(II), and L(4): Complex of Zn(II))

Results of the present study agreed with several previous studies²⁴⁻²⁶.

CONCLUSION

Novel Schiff base ligand has been synthesised by condensation of o-nitrobenzaldehyde with 6-amino penicillanic acid. Its metal complexes have been synthesised with Co(II), Ni(II), Cu(II), and Zn(II) ion in (1:2) molar ratio of metal ion to ligand, etc. They have been characterized by FT-IR, UV-Vis, ¹H-NMR, ¹³C-NMR, elemental analysis. The results of these investigations support the suggested structure of the metal complexes. Molar conductance values and magnetic susceptibility data suggests the Schiff base acts as single negative charge bidentate ligand and forms complexes through its oxygen atom of one (COO⁻) group, oxygen atom of carboxylic group (C=O) for β- lactam and nitrogen atom of azomethene group (-C=N-). The metal ion displays the coordination number 6 in these complexes suggesting [M(L)₂] (octahedral) composition of the coordination compounds. The octahedral geometry of metal complex as given in Fig. 9.

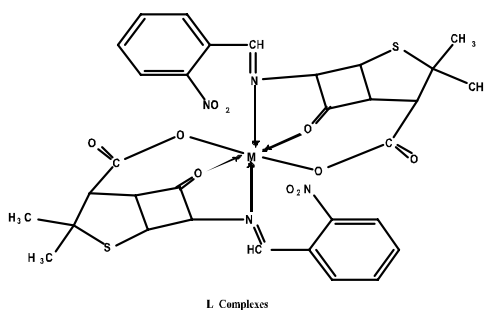


Fig. 9: The octahedral geometry of metal complex where M = Cu(II), Co(II), Ni(II), and Zn(II)

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