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# STRUCTURAL ELUCIDATION, THERMAL STUDIES AND ANTIMICROBIAL SCREENING OF MIXED LIGAND COMPLEXES OF 4-BENZYLIMINO-2,3-DIMETHYL-1-PHENYLPYRAZAL-5-ONE AND L-LYSINE

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***ABSTRACT***

***The mixed ligand complexes involving Fe(II), Co(II) and Ni(II) ions, Schiff base 4-benzylimino-2,3-dimethyl-1-phenylpyrazal-5-one ($L_1$) and L-lysine (Lys) were synthesized. The complexes were characterized on the basis of their elemental analysis, conductivity measurements, FT-IR, MS,$^1$H-NMR and $^{13}$C-NMR spectral studies. All the synthesized complexes were subjected to simultaneous thermogravimetric analysis to study their decomposition mechanism and thermal stability. The mixed ligand complexes were screened against some strains of bacteria and fungi to study their antimicrobial activity. The complexes were found to be non-electrolytes and possessed octahedral geometry. The results showed that the metal complexes possessed better antimicrobial activity than the free ligands.***

***Keywords: mixed ligand, complexes, Schiff base, lysine, thermal studies, antimicrobial***

## INTRODUCTION

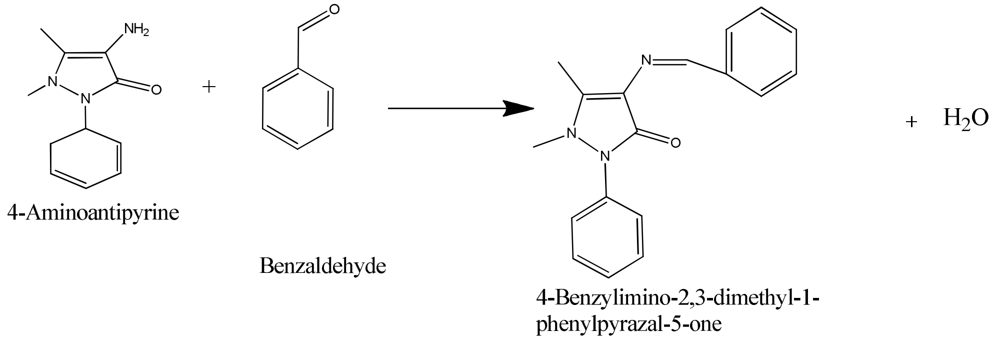
The derivatives of pyrazolone have received close attention for decades due to their interesting chemistry and pharmaceutical importance (Mahmoud *et al.,* 2004; Raman *et al.,* 2008;Theodore *et al.*, 2014).These classes of compounds have found use in analytical applications, catalysis, dye and extraction metallurgy (Shalim *et al.,* 2009; Anant and Devjani, 2011). The presence of electron-rich donor centres in these compounds is of paramount interest. Consequently, the compounds have been reported to form complexes with a wide range of transition metals with interesting chemical properties (Mohanan *et al.,* 2009; Suresh and Prakrash, 2010; Waleed and Israa, 2014). Schiff bases and their metal complexes have been noted for their stability, biological activity and potential applications in oxidative catalysis (Hughes, 1987; Freeman, 1973). Amino acids are basically O, N donor ligands with noted biological activity (Reddy and Reddy, 2000; Siham *et al.,* 2012).We are interested in examining thethermal stability, coordination mode, antibacterial and antifungal activities of mixed ligand complexes involving some transition metals, Schiff base 4-benzylimino-2,3-dimethyl-1-phenylpyrazal-5-one and an essential amino acid, L-lysine.

## MATERIALS AND METHODS

The chemicals used for this study were purchased from Sigma-Aldrich Company and were used without further purification except ethanol that was re-distilled. Nutrient agar was procured from Tulip Diagnostics (P) Ltd (Belgium), sabouraud dextrose agar from Lab M Ltd (U.K), pure ciprofloxacin and fluconazole from Oxoide™, the test organisms: *E. coli,P. aeroginosa, S. aureus, B. subtilis, A, niger* and *C. albicans* were clinical isolates from University of Benin Teaching Hospital, Benin City, Edo State, Nigeria.The molar conductivity of the Schiff base ligand and mixed ligand complexes were determined in 10$^{-3}$M DMF using Inolab digital conductivity meter Cond 720 WTW Series while Stuart scientific model SMPS melting point apparatus was used for the determination of melting point. The elemental composition of the complexes was determined using LECO CHNS-932 elemental analyzer and Buck scientific model 210 VGP atomic absorption spectrometer. Perkin Elmer Universal ATR Spectrum 100 FT-IR, Water Synapt GR Electrospray Positive Mass Spectrometer, 400MHz Bruker Ultrashield NMR Spectrometer were used for determining the structures of the complexes. Thermal analyzer TGA/DSC Q600 was used to determine the thermal stability of the complexes as well as the enthalpy change (*ΔH*) for each decomposition step.

### Synthesis of 4-benzylimino-2,3-dimethyl-1-phenylpyrazal-5-one ($L_1$)

The Schiff base ligand was synthesized using: solid phase, green chemistry approach by grinding 1:1.2 moles of the respective aldehyde and amine in a mortar (Cave *et al.*, 2001; Imrie *et al.*, 2007; Suresh *et al.*, 2012; Omotade *et al.*, 2019). The reactions were monitored by IR spectroscopy and the products were crystallized and dried in vacuum.



Scheme 1: Synthesis of Schiff base ligand

### Synthesis of mixed ligand metal complexes

The mixed ligand metal complexes were synthesized by refluxing equimolar amounts (in ehanol) of Schiff base, metal(II) chloride, amino acid (L-lysine)and NaOH in an inert atmosphere of argon at 80°C for 4 hours. The resultant precipitates were washed with water, dried in vacuum and stored in sample containers over anhydrous $CaCl_2$in a dessicator (Reddy *et al.,* 2005; Nagar *et al.,* 2011; Atmaran and Kiran, 2011 and Kamini *et al.*,2012).

### Antimicrobial Properties

The antimicrobial activities of the free ligands and mixed ligand complexes were tested on four bacteria: *E. coli, P. aeroginosa, S. aureus, B. subtilis* and two fungi*: A. niger and C. albicans,* using agar-well diffusion method. Ciprofloxacin, an antibacterial agent and fluconazole, an antifungal agent were used as control. Nutrient agar and sabouraud agar were prepared according to manufacturer's recommendation while bacteria and fungi concentrations were diluted according to Macfarland's standard (Parez *et al.,* 1990). The resultant zones of inhibition were measured in millimeters.The minimum inhibitory concentrations (MICs) of the ligands and mixed ligand metal conplexes were determined using the agar-plate serial dilution method as reported by Van-Dyck *et al.*(1994).

## RESULTS AND DISCUSSION

The solvent-free synthesis of the Schiff base was completed in 15 minutes. Physico-chemical and analytical data of the ligand and mixed ligand complexes are shown in sections 1 and 2. The % elemental composition of the compounds showed that the ratio of metal to ligand to co-ligand was 1:1:1. The low molar conductance values (25.0-30.4 $\Omega^{-1}cm^2mol^{-1}$) of the metal complexes indicated that the complexes were non-electrolytes in nature (Waleed and Israa, 2014; Safia *et al.*, 2011).$NiL_1Lys$ is the most thermally stable of the complexes with temperature of incipient decomposition at 115°C.

### Section 1. Physico-chemical and analytical data of the Schiff base ligand, 4-benzylimino-2,3-dimethyl-1-phenylpyrazal-5-one ($L_1$).

Colour: Yellow. Yield: 97%. Mol. Wt.: 291. Melting point: 185°C. Selected FT-IR ($cm^{-1}$): 3038$\nu(CH_{Ar})$, 1647$\nu(C{=}O_L)$, 1564$\nu(C{=}N)$. $^1H$-NMR (δppm): 9.58 (s, HC=N), 7.36-7.82 (m, Ar-H), 3.18 (s, $H_3C$-N), 2.46 (s, C-$CH_3$). $^{13}C$-NMR(δppm): 159.57 (C=O), 154.31 (C=N), 124.57-137.49 (Ar-C), 35.30 ($CH_3$-N). Anal. Found (%): C, 74.32; H, 5.79; N, 14.34; O, 5.55. Calc. (%) C, 74.23; H, 5.84; N, 14.43; O, 5.50. *ΔH*: +111.67J/g

### Section 2.Physico-chemical and analytical data of the mixed ligand complexes.

$FeL_1Lys$

Colour: Dark-brown. Yield: 94%. Mol. Wt.: 528. Decomp. Temp.: 105°C. Molar cond.($\Omega^{-1}cm^2mol^{-1}$): 25.0 Selected FT-IR ($cm^{-1}$): 3368$\nu(N\text{-}H_{AH})$, 3035$\nu(OH)$,2937$\nu(CH_{Ar})$,1611$\nu(C{=}O_L)$, 1566 $\nu(C{=}N)$, 1647$\nu(COO_{AH})$, 427$\nu(M\text{-}O)$, 387$\nu(M\text{-}N)$. Anal. Found: (%) C, 54.42; H, 6.46; N, 13.30; O, 15.22; Fe, 10.60 Calc. (%) C, 54.56; H, 6.44; N, 13.26; O, 15.15; Fe, 10.59. *ΔH*: +455.58 J/g.

$CoL_1Lys$

Colour: Light brown. Yield: 77%. Mol. Wt.: 531. Decomp. Temp.: 105°C. Molar cond.($\Omega^{-1}cm^2mol^{-1}$): 30.2 Selected FT-IR ($cm^{-1}$): 3372$\nu(N\text{-}H_{AH})$, 3038$\nu(OH)$,2942$\nu(CH_{Ar})$,1595$\nu(C{=}O_L)$, 1565 $\nu(C{=}N)$ 1647$\nu(COO_{AH})$, 428$\nu(M\text{-}O)$, 386$\nu(M\text{-}N)$. Anal. Found (%): C, 54.23; H, 6.42; N, 13.16; O, 15.14; Co, 11.05 Calc. (%) C, 54.25; H, 6.40; N, 13.19; O, 15.07; Co, 11.09. *ΔH*: +660.00J/g

$NiL_1Lys$

Colour: Green. Yield: 85%. Mol. Wt.: 530.7. Decomp. Temp.: 115°C Molar cond.($\Omega^{-1}cm^2mol^{-1}$): 30.4. Selected FT-IR ($cm^{-1}$): 3265 $\nu$(N-H$_{AH}$), 3049 $\nu$(OH), 2944 $\nu$(CH$_{Ar}$), 1597 $\nu$(C=O$_L$), 1563 $\nu$(C=N) 1647 $\nu$(COO$_{AH}$), 447 $\nu$(M-O), 428 $\nu$(M-N). Anal. Found (%): C, 54.21; H, 6.51; N, 13.15; O, 15.05; Ni, 11.08. Calc. (%) C, 54.27 H, 6.41; N, 13.19; O, 15.07; Ni, 11.06. *ΔH*: +159.48 J/g

**FT-IR**

From Sections 1 and 2, $L_1$ showed absorption at 3038$cm^{-1}$, 1647$cm^{-1}$ 1564$cm^{-1}$ etc., which were sharp and intense peaks. The mixed ligand complexes showed absorption bands at 3265-3372$cm^{-1}$ for N-H$_{str}$,3035-3049$cm^{-1}$$H_2O$/OH, 2937-2944$cm^{-1}$for C-H$_{str}$ of aromatic ring frequencies, 1566-1597$cm^{-1}$ for C=O of Schiff base moiety, 1647$cm^{-1}$ for COO$^-$ of L-Lys moiety, 1563-1566$cm^{-1}$ for C=N$_{str}$ frequencies, 427-447$cm^{-1}$ for M-O and 386-428$cm^{-1}$ for M-N bands (Fayad *et al.,* 2012). The bands were intense bands. The shifts in the frequencies of C=N, C=O and COO$^-$in the mixed ligand complexes, in comparison to the Schiff base, were due to $\pi$-bonding interactions between metal and ligands. The absorption bands of $NiL_1Lys$ is shown in Fig. 1.

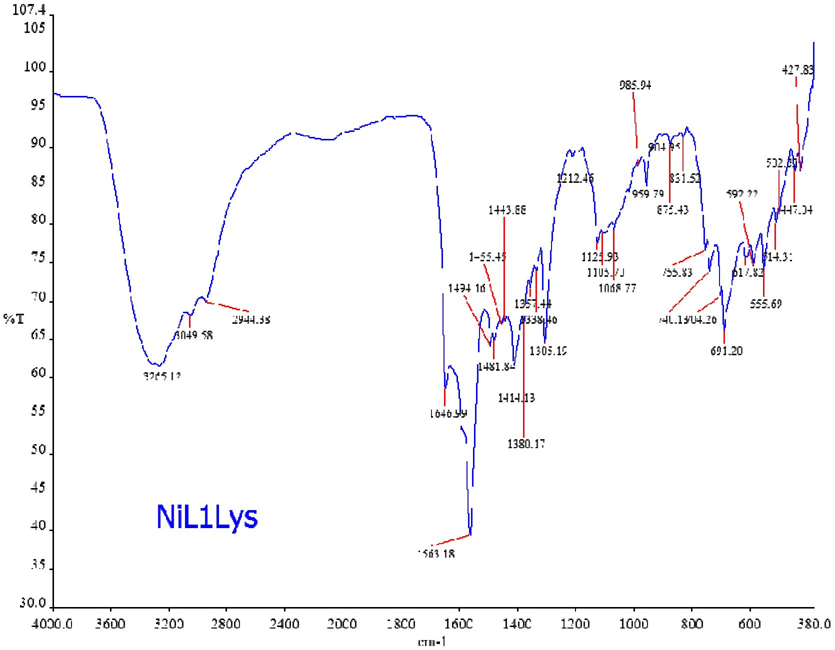
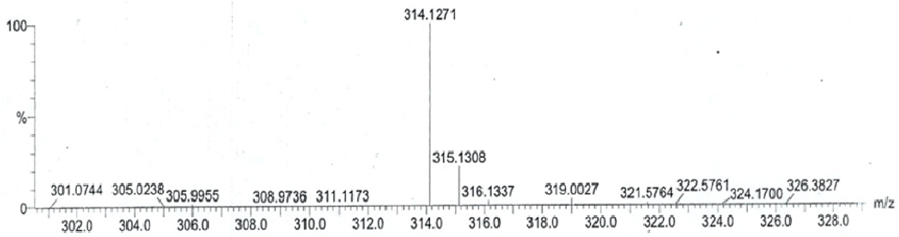


Fig. 1: FT-IR spectrum of $NiL_1Lys$ complex

**Mass Spectrum**

The mass spectrum of $L_1$ is shown in Fig. 2. The actual mass was 314.1271 for the experimental mass and 314.1269 for the calculated mass. The formula of $L_1$as indicated on the elemental composition report was $C_{18}$-$H_{17}N_3O$ with molecular weight of 291 units(i.e 314-23 sodium correction). The relative abundance of the ligand was 100%.



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Minimum:                                   -1.5
Maximum:                   5.0      5.0    100.0

Mass       Calc. Mass      mDa      PPM    DBE      i-FIT      i-FIT (Norm)  Formula

314.1271   314.1269        0.2      0.6    11.5     665.7      0.0           C18  H17  N3  O  Na
```

Fig. 2: Mass spectrum of Schiff base $L_1$.

## NMR

The NMR spectra data in Sections 1 and 2 show the chemical shifts of relevant environments. In the $^1$H-NMR spectrum of the Schiff base ligand (Fig. 3), the peak at δ2.46ppm represents the alkyl group $CH_3$(Raman *et al.,* 2007), the $CH_3$-N band was shifted to lower field and appeared at δ3.18ppm due to the presence of nitrogen group that de-shielded the electrons (Theodore *et al.,* 2014). Within the region δ7.36-7.82ppm, there were some peaks which showed the presence of aromatic protons. The HC=N peak appeared at δ9.58ppm in $L_1$. In L-Lys, the $\alpha$-$NH_2$ and ε-$NH_3^+$ peaks appeared at chemical shifts 8.41ppm and 7.52ppm respectively. (Teoder, 2003).

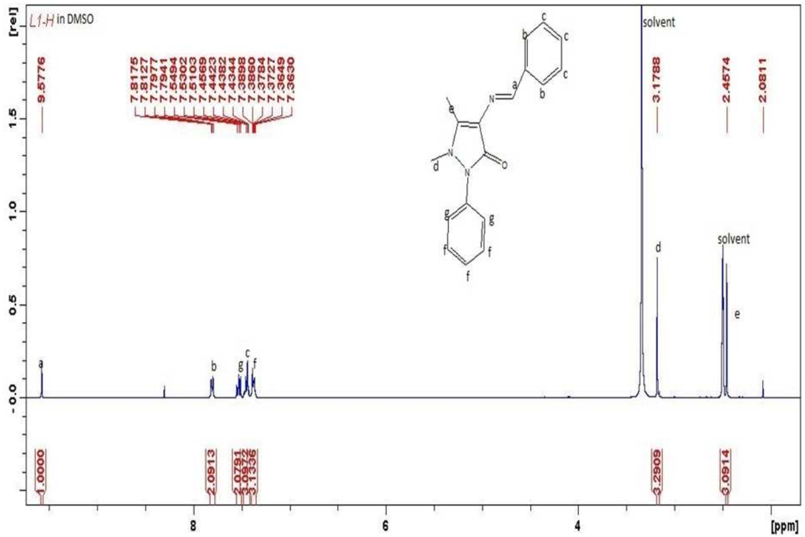


Fig. 3:$^1$H-NMR spectrum of Schiff base ligand

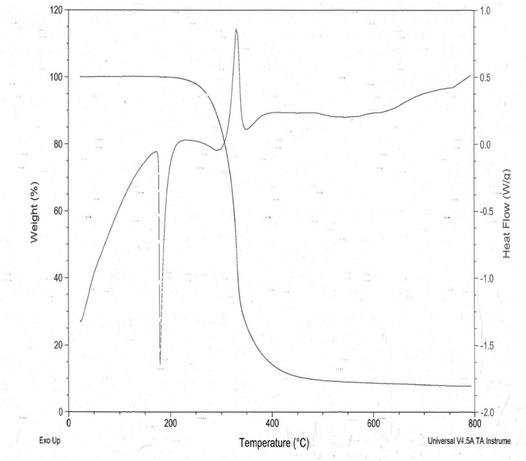
## Thermal studies

The results of TGA-DSC analysis of the Schiff base ligand and mixed ligand metal complexes are in good agreement with the suggested formulae of the complexes from elemental analysis data.

As shown in Fig. 4, $L_1$ ($C_{18}H_{17}N_3O$) exhibited a one-step decomposition process from 185-450°C. The weight loss of 100% was accompanied by an endothermic peak at 330°C with *ΔH* being +130.75 J/g. The exothermic peak at 180°C was due to a crystalline melt, indicating the melting point of the ligand with *ΔH*at -19.08J/g.The stability temperature is 185°C.

$FeL_1$Lys exhibited four steps of decomposition process from 105-785°C. The first stage loss of 23.18% which was accompanied by an endothermic peak at 180°C with ΔH = +89.04J/g was due to the loss of two coordinated water molecules and amide. The second stage loss of 11.22% was accompanied by an endothermic peak at 245°C with ΔH = +173.16J/g and was due to loss of amino acid fraction. The third stage of decomposition showed a loss of 23.38%, a fraction of the Schiff base ligand and was accompanied by an endothermic peak at 425°C and ΔH of +122.76J/g. In the fourth stage, there was a loss of 14.69% due to the loss of an aromatic ring of the Schiff base moiety. This stage was accompanied by an exothermic peak at 650°C with ΔH = +70.62J/g. The stability temperature of $FeL_1$Lys was found to be 105°C.In the thermogram of $CoL_1$Lys (Fig. 5), there were three stages of decomposition between 105-790°C. The first decomposition showed weight loss of 20.76% in the range 105-260°C, was attributed to the loss of two molecules of coordination water and carboxylic acid which was accompanied by an endothermic peak at 180°C with ΔH = +166.98J/g. The second step showed a weight loss of 33.13% in the range of 260-370°C, which was due to the loss of a fraction of both ligands. This decomposition stage was accompanied by an endothermic peak at 270°C with ΔH = +260.22J/g. The third stage of decomposition was within a temperature range of 370-790°C. The weight loss of 23.75% was due to loss of Schiff base aromatic ring and the imine fraction of the complex. This stage was accompanied by an endothermic peak at 395°C with ΔH = +232.80J/g. The stability temperature of the complex is 105°C. $NiL_1$Lys exhibited four stages of decomposition from 115-790°C. The first step showed a loss of 12.40% which was accompanied by an exothermic peak at 185°C with ΔH = +24.96J/g. The loss was due to loss of two coordinated water molecules and two methyl groups of the Schiff base moiety.

The second stage decomposition, with a loss of 26.40% was due to a loss of a phenyl ring, $CO_2$ and ammonium molecules from both ligands. This step was accompanied by an endothermic peak at 265$^oC$ with $\Delta H = +109.50 J/g$. The third step showed a loss of aromatic imine fraction, $N_2H_4$ and $CH_4$ from the ligands with 28.31% loss. This was accompanied by an exothermic peak at $\Delta H = +33.72 J/g$ at 350$^oC$. The fourth stage exhibited a loss of 11.17% and was accompanied by an exothermic peak at 555$^oC$ and $\Delta H = -8.70 J/g$. The stability temperature is 115$^oC$. There was presence of coordinated water molecules in FeL$_1$Lys,CoL$_1$Lys and NiL$_1$Lys. The water molecules were observed between temperatures>150$^oC$, hence they are coordinated water molecules (Mashaly *et al.,* 1999; Mashaly, 2002). TheSchiff base ligand appeared to be more thermally stable than the complexes.



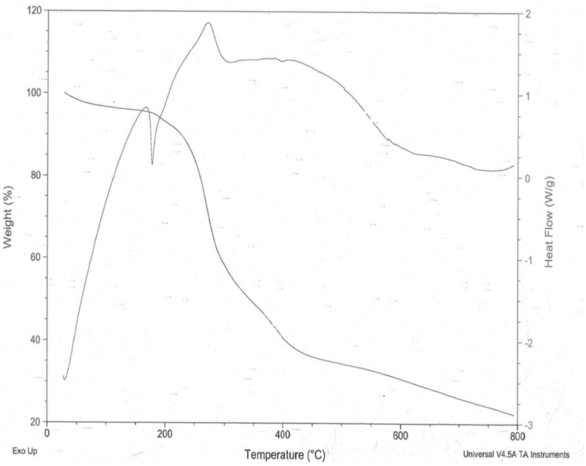


Fig 4: TGA/DSC thermogram of L$_1$Fig. 5: TGA/DSC thermogram of CoL$_1$Lys

**Antimicrobial Properties**

The results of antibacterial and antifungal screening (Tables 1 and 2) show that all the complexes exhibited better activity when compared to the ligand, co-ligand and the standard drugs. The enhanced activity of the ligands upon complexation is in good agreement with Tweedy's chelation theory(Tweedy, 1964) which emphasizes that on complexation, the polarity of the metal ion will be reduced to a great extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups (Kralova *et al.,* 2004; Parekh *et al.,* 2005). This also increases the delocalization of the π-electrons over the whole chelate ring and enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of the microorganisms (Vaghasiya *et al.,* 2004). Thus this enhanced lipophilicity makes the metal complexes to inhibit the growth of the microbes than the free ligand. The minimum inhibitory concentration of the mixed ligand complexes are presented on on Table 3.

Table 1: Antibacterial activity of ligands and mixed ligand complexes (mm).

| **Compound** | ***E. Coli*** | ***P. aeroginosa*** | ***S. aureus*** | ***B. subtilis*** |
|---|---|---|---|---|
| L$_1$ | 10 | 9 | 8 | 11 |
| L-Lys | 0 | 0 | 0 | 0 |
| FeL$_1$Lys | 28 | 21 | 16 | 19 |
| CoL$_1$Lys | 28 | 24 | 26 | 12 |
| NiL$_1$Lys | 29 | 21 | 18 | 24 |
| CuL$_1$Lys | 29 | 28 | 26 | 35 |
| Ciprofloxacin (standard) | 27 | 18 | 0 | 26 |

Table 2: Antifungal activity of ligands and mixed ligand complexes (mm).

| Compound | A. niger | B. albicans |
|---|---|---|
| $L_I$ | 6 | 4 |
| L-Lys | 0 | 0 |
| $FeL_1Lys$ | 10 | 19 |
| $CoL_1Lys$ | 13 | 19 |
| $NiL_1Lys$ | 14 | 15 |
| Fluconazole (control) | 12 | 18 |

Table 3: The minimum inhibitory concentration of the Schiff base and mixed ligand complexes (mg/mL).

| **Compound** | ***E. Coli*** | ***P. aeroginosa*** | ***S. aureus*** | ***B. subtilis*** | ***A. niger*** | ***C. albicans*** |
|---|---|---|---|---|---|---|
| $L_1$ | 0.5 | 0.25 | 0.5 | 0.25 | 0.5 | 1 |
| $FeL_1Lys$ | 0.25 | 0.25 | 0.5 | 0.25 | 0.5 | 0.5 |
| $CoL_1Lys$ | 0.5 | 0.5 | 0.25 | 0.25 | 0.5 | 0.5 |
| $NiL_1Lys$ | 0.25 | 0.25 | 0.25 | 0.5 | 1 | 0.5 |

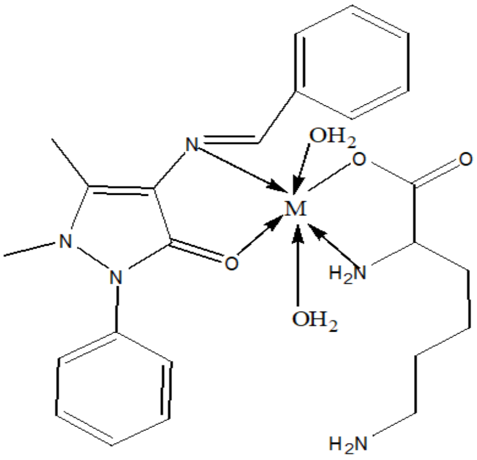


Fig. 6: Proposed structure for the mixed ligand complexes (where M= Fe, Co,Ni)

## CONCLUSION

Mixed ligand complexes having NN and NO donor sites have been synthesized from 4-benzyl-imino-2,3-dimethyl-1-phenylpyrazal-5-one and L-lysine. The spectra data showed that the Schiff base ligand and amino acid coordinated as bidentate ligands. The analytical data showed that the metal:ligand:co-ligand ratio is 1:1:1 in all mixed ligand complexes studied. From the spectra, analytical and thermal data, octahedral geometry has been proposed for the complexes (Fig. 6). The metal complexes showed better antimicrobial activity when compared to the free ligands and control drugs.

**Conflict of interest**

The authors declare that there is no conflict of interest concerning this work.

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