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Synthesis, Spectroscopic Studies and Biological Activity of Cu(II) Complex of Schiff Base Ligand 3-((2-(2,4-dinitrophenyl)hydrazineylidene)methyl)pyridine with Triphenylphosphine as Ancillary Ligand

***[1]Olufemi S. Odulaja, [2]Muritala A. Olusola, [1]Adejumoke M. Hashimi, [1]Taiwo A. Abiola, [1]Oyinlola O. Olugbile, [1]Taofeeq F. Adeleye, [1]Christiana A. Adeleye, [1]Adetutu V. Williams, [1]Christiana I. Ayangbesan and [1]Emmanuel O. Bamiro**

[1]Department of Chemical Sciences, Olabisi Onabanjo University, Ago-Iwoye, Nigeria.
[2]Department of Chemistry, Federal University of Medicine and Medical Sciences, Abeokuta, Nigeria

*Corresponding Author's Email: olufemi.odulaja@oouagoiwoye.edu.ng

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ABSTRACT

In this study we synthesized, characterized, and evaluated the antibacterial activity of a new mixed-ligand Cu(II) complex containing a Schiff base derived from 3-pyridinecarboxaldehyde and 2,4-dinitrophenylhydrazine, together with triphenylphosphine as ancillary ligand. The compound was characterized using Fourier Transform Infrared (FTIR) spectroscopy, UV-Visible spectroscopy, and magnetic susceptibility measurements. Antibacterial screening was carried out using agar well diffusion method against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. FTIR analysis confirmed coordination through the azomethine and pyridyl nitrogen atoms with characteristic bands at 1618 and 1584 cm^{-1}, while retaining nitro group absorptions at 1508 and 1325 cm^{-1}. The complex displayed an average inhibition zone of 26.25 mm, surpassing the standard drug Ofloxacin (23.50 mm) and showing significant enhancement over the free ligands and copper salt. Magnetic susceptibility measurements revealed paramagnetic behaviour. The synthesized heteroleptic Cu(II) complex demonstrated excellent broad-spectrum antibacterial activity, highlighting the synergistic effect of the Schiff base and triphenylphosphine ligands.

INTRODUCTION

Copper, a Group 11 element with the electronic configuration $[Ar]3d^{10}4s^1$, is a biologically essential transition metal that plays a pivotal role in numerous physiological and chemical processes. Unlike its group neighbours silver and gold, copper readily exists in two stable oxidation states namely the diamagnetic cuprous ion (Cu^+, d^{10}) and the paramagnetic cupric ion (Cu^{2+}, d^9). The Cu(II) state is particularly significant in coordination chemistry due to its stability in aqueous solution and its rich structural chemistry(Ghosh *et al.*, 2018). Cu(II) complexes typically adopt coordination numbers of 4, 5, or 6, exhibiting geometries such as square planar, tetrahedral, square pyramidal, and distorted octahedral (Mandal *et al.*, 2020). The versatility of copper's coordination geometry allows for the fine-tuning of ligand environments to optimize lipophilicity and bioavailability, making it a metal of choice for next-generation metallopharmaceuticals.

Schiff bases are a class of organic compounds containing an azomethine group (-C=N-). The azomethine nitrogen atom possesses a lone pair of electrons, enabling Schiff bases to act as excellent donor ligands in coordination chemistry. Schiff bases and their metal complexes have since gained immense attention due to their broad spectrum of biological activities. Literature abounds with reports of Schiff base complexes exhibiting antibacterial, antifungal, antiviral, antimalarial, anticancer, and anti-inflammatory properties (Dadgostar, 2019) In many instances, complexation with a metal ion enhances the biological activity of the ligand, a concept supported by Overtone's concept of cell permeability and Tweedy's chelation theory. The metal ion acts as a Lewis acid, polarizing the ligand and facilitating its permeation through the lipid layers of microbial cell membranes.

Ancillary ligands are those that stabilize the metal centre and tune its electronic and steric properties but are not the primary focus of the reactivity. PPh_3 acts as a σ-donor via

its phosphorus lone pair and significantly as a π-acceptor due to the presence of empty d-orbitals (or σ* orbitals) on the phosphorus atom, which can accept electron density from filled metal d-orbitals (Odulaja *et al.*, 2026). This π-backbonding capability helps to stabilize electron-rich metal centers. It can also distort the coordination geometry, potentially enhancing the biological activity by modifying the complex's shape and lipophilicity (Kumah *et al.*, 2021). The concept of mixed-ligand or heteroleptic complexes, combining a planar Schiff base with a bulky phosphine, is a powerful strategy in medicinal inorganic chemistry thus its synergistic combination often results in improved lipophilicity, facilitating transport across cell membranes. Several studies have demonstrated that Cu(II) complexes incorporating phosphine ligands exhibit superior cytotoxicity and antimicrobial activity compared to their phosphine-free counterparts The phosphine ligand can enhance the stability of the complex in physiological media and may also participate in secondary interactions with biological targets, such as DNA backbone phosphates(Amolegbe *et al.*, 2015). Hence, the integration of PPh_3 into the Cu(II)-Schiff base architecture is a deliberate design aimed at maximizing biological potency. In this study, PPh_3 is employed as an ancillary ligand alongside the primary Schiff base. Despite the extensive literature on Cu(II) Schiff base complexes, the specific combination of a Schiff base derived from 2,4-dinitrophenylhydrazine and 3-pyridinecarboxaldehyde has received limited attention, particularly regarding its coordination behaviour with Cu(II) in the presence of bulky phosphine ligands like triphenylphosphine. Most studies investigated them as analytical reagents or simple binary complexes, overlooking the potential synergistic effects of mixed-ligand engineering, there is also a paucity of information on structure activity relationship for the specific class of nitro-functionalized pyridine-hydrazone complexes (Yusuf *et al.*, 2021).

MATERIALS AND METHODS

Materials: 3-pyridinedicarboxaldehyde (C_6H_5NO, FW 107.11), 2,4-dinitrophenylhydrazine ($C_6H_6N_4O_4$; FW 198.14), triphenylphosphine ($C_{18}H_{15}P$; FW 262.5), copper(II) chloride dihydrate ($CuCl_2{\cdot}2H_2O$, FW 107.5), were purchased from sigma-Aldrich and other solvents commercially available were used as received.

Experimentals

Synthesis of 3-((2-(2,4-dinitrophenyl)hydrazineylidene)methyl)pyridine (L)

A solution of 3-pyridinecarboxaldehyde (0.98 mL, 10 mmol) was prepared by dissolving it completely in 10 mL of ethanol. 2,4-dinitrophenylhydrazine (1.9814 g, 10 mmol) was partially dissolved in 20 mL of ethanol. The two solutions A were mixed in a conical flask, followed by the addition of two drops of concentrated hydrochloric acid as a protonating catalyst. The reaction mixture was stirred at 200–203 rpm using a magnetic stirrer for 1 hour at room temperature. The resulting precipitate was filtered, air-dried, and washed twice with diethyl ether at 10-minute intervals. The product obtained was collected and stored as the Schiff base ligand.

Synthesis of Copper precursor, Cu(PPh$_3$)

Copper(II) chloride dihydrate ($CuCl_2{\cdot}2H_2O$) (3.4 g, 20 mmol) was partially dissolved in 20 mL of ethanol. In a separate round-bottom flask, (PPh_3) (12.6 g, 20 mmol) was dissolved in ethanol and heated under reflux at 70 °C with continuous stirring until complete dissolution of the ligand was achieved. The copper chloride solution was then added to the PPh3 solution under the same temperature conditions, and the mixture was maintained under reflux. A milky solution was initially observed, which gradually turned yellow upon continued stirring as the temperature was reduced to 60 °C, indicating formation of the copper complex. The reaction mixture was quenched in an ice–salt bath to promote crystallization. The product d was filtered, washed, dried, and stored as chlorido(triphenylphosphine) copper(II) complex (Odulaja *et al.*, 2024; Oladipo *et al.*, 2023).

Synthesis of Copper(II)–Schiff Base–Triphenylphosphine Mixed Ligand Complex

The synthesized chlorido(triphenylphosphine)copper(II) complex (2 mmol) was dissolved in 10 mL of ethanol in a conical flask. To this solution, 2 mmol of the Schiff base ligand was added. The reaction mixture was stirred at room temperature for 2 hours using a magnetic stirrer to facilitate coordination of the ligand to the metal center. The resulting mixture was filtered, and the solid product obtained was washed with ethanol, air-dried, and further washed with diethyl ether. The final product was collected and stored as the copper(II) mixed ligand complex (Adeleke *et al.*, 2023; Busari *et al.*, 2026).

Characterisation Techniques for Metal Complexes

FTIR Spectroscopy: Spectra were recorded as KBr pellets over the range 4000 to 650 cm^{-1} using an Agilent Technologies FTIR spectrometer. Pellets were prepared by grinding approximately 2 mg of sample with 100 mg of dried KBr and pressing under vacuum. Central Lab, Bowen University, Iwo, Osun State

UV-Vis Spectroscopy: Spectra were recorded from 200 to 800 nm in DMSO solution at a concentration of approximately 1×10^{-4} mol/L using a UV-Visible spectrophotometer with 1 cm quartz cuvettes.

Magnetic Susceptibility: Magnetic susceptibility was determined at room temperature by the Gouy balance method at Jabio Laboratory Services, Kwara State University, Molete.

Antimicrobial Assay: In vitro antimicrobial activity was determined by the agar disc diffusion method. Test organisms were *Escherichia coli* (EC), *Staphylococcus aureus* (SA), *Klebsiella pneumoniae* (KP) and *Pseudomonas aeruginosa* (PA). All compounds were dissolved in DMSO at a concentration of 1 mg/mL. Filter paper discs (6 mm, Whatman) were impregnated with 20 microlitres of each solution and placed on Mueller Hinton agar plates that had been inoculated with the appropriate test organism. Ofloxacin served as the positive antibacterial control and DMSO served as the negative control. All plates were incubated at 37 °C for 24 hours. Zones of inhibition were measured in triplicate and the mean values recorded (Benzerka *et al.*, 2012).

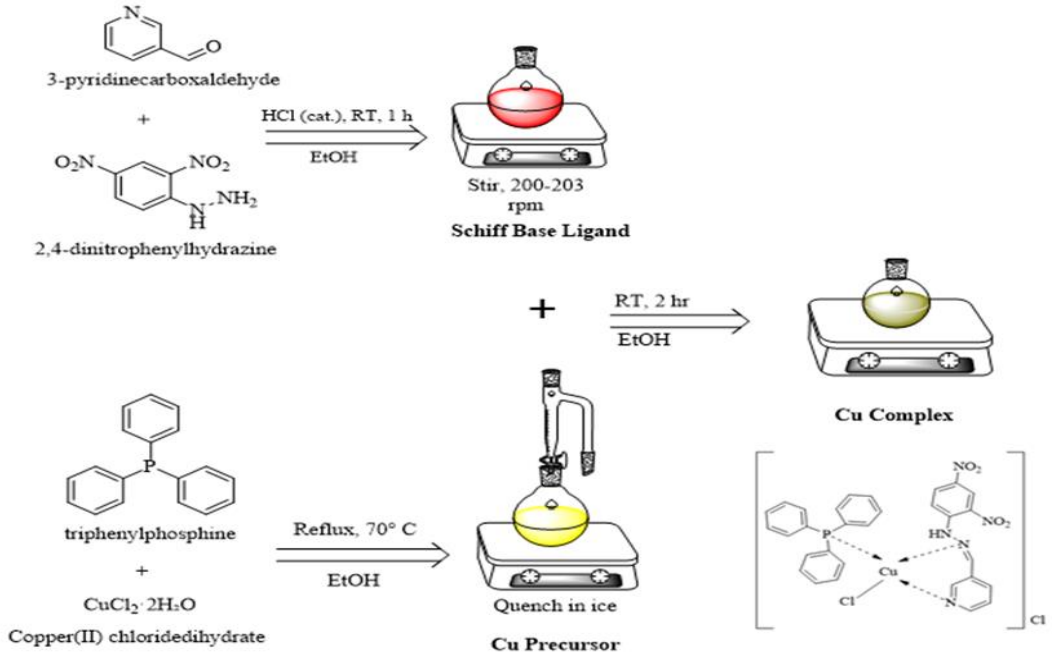

Figure 1: Schematic diagram of the synthesis procedure

RESULTS AND DISCUSSION

FTIR Spectroscopy

In the FTIR spectrum of the Cu(PPh_3) precursor complex (Figure 2), fingerprint-region bands are the dominant species which is in line with the expected composition of a simple phosphine-copper adduct. The strong absorptions at 1597.33, 1475.32 and 1386.55 cm^{-1} are mainly due to the aryl skeletal C=C vibrations of the triphenylphosphine rings. Additionally, typical P-C(aryl) stretching modes are represented by middle-intensity bands at 1157.50, 1109.88 and 1006.42 cm^{-1} (Nakamoto, 2009). The sharp peaks at 768.17 and 715.46 cm^{-1} in the bottom region are the conclusive out-of-plane C-H bending vibrations of mono-substituted phenyl rings.

In the FTIR spectrum of the complex C1, a very weak, but separate, absorption at 3047 cm^{-1} is attributed to the aromatic C-H stretch vibrations of the rings of the pyridine and dinitrophenyl rings and the remainder of the PPh_3 that is retained. More importantly, the overlapping azomethine (νC=N) and pyridyl ring vibrations are attributed to the appearance of the absorption bands at 1618 and 1584 cm^{-1}. The change of these bands and the change in structure in comparison with the usual free hydrazone ligands indicate the presence of the azomethine nitrogen and/or pyridyl nitrogen coordinated to the copper center (Fudulu *et al.*, 2020). In addition, the existence of sharp asymmetric and symmetric NO_2 vibrations at1508 and 1325 cm^{-1} respectively, indicates the presence of the 2,4-dinitrophenylhydrazone group. In the meantime, the occurrence of absorptions at 1088, 1027, 997, 831, 742, and 690 cm^{-1} are consistent with the occurrence of the P-C(aryl) stretching and out-of-plane phenyl C -H bending modes, which indeed confirms the presence of the triphenylphosphine ancillary. It is noted that direct Cu–N or Cu–P stretching vibrations are not strictly identified here, since they are normally found in the far-infrared spectrum (below 500 cm^{-1}) which borders the measuring threshold, and which is often inherently weak. The occurrence of both important azomethine, nitro and phosphine bands at the same time and the slight modulation of the bands are strong evidence in favour of the successful synthesis of the mixed-ligand system.

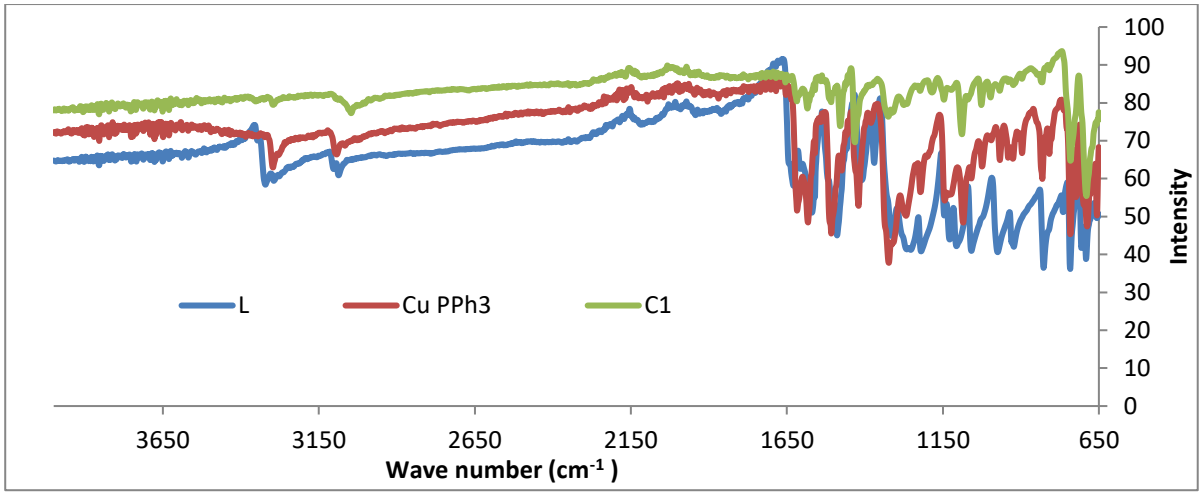

Figure 2: FTIR spectrum of ligand L, copper precursor and complex C1

Table 1: Summary of key FTIR absorption bands (cm^{-1}) and their tentative assignments

Tentative Assignment	Cu(PPh_3)	L	CuC1
ν(C–H) aromatic	3200, 3036	3220,3056	3047
ν(C=N)azomethine/pyridyl	----	1625, 1576	1618,1584
ν(C=C) aryl skeletal	1597,1475	1414	1479, 1431
ν(NO_2)asymmetric/ symmetric	----	1505,1302	1508 / 1325
ν(P–C) aryl	1157,1109	-----	1146, 1088, 1027
δ(C–H) out-of-plane phenyl	768,715	739	742, 690

UV–Visible Spectroscopy

The electronic absorption spectra of ligand L and its corresponding Cu(II) complex C1 recorded in ethanol solution (Figure 3). All data are summarized in Table 1. The free ligand exhibited one principal and a shoulder absorption and a shoulder band. The higher-energy band (340 nm) assigned to π→π* transitions within the aromatic framework and azomethine chromophore and the lower-energy shoulder band (410) nm is attributed to n→π* transitions associated with the imine and pyridine nitrogen lone pairs (Abu-DiefMohamed, 2015).

Upon coordination to Cu(II), the absorption profile of C1 showed a hypochromic redshift, with the higher-energy π→π* band shifting to 268 nm. This bathochromic shift indicates increased conjugation or altered electronic distribution following metal complexation, possibly involving metal-to-ligand charge transfer (MLCT) contributions. Notably, the longer -wavelength band at 410 nm observed in free L persists in the complex, suggesting that the n→π* transition is retained or enhanced upon coordination (Moreno-Regino *et al.*, 2026).

Electronic absorption spectrum of the Cu(PPh_3) precursor (Figure 3) is marked by strong absorptions in the upper-energy ultraviolet range (around 296 nm), It is believed that these properties are due to the intra-ligand π-π* transitions of ligands centered on the phenyl rings of the phosphine ligand. The tail or shoulder at long wavelengths (347-370 nm) is relatively weak and could be attributed to an n→π* transition or a more intense metal to ligand charge transfer (MLCT) band(Ghaneian *et al.*, 2015) .

It is interesting to note that there is no d-d transition band that is resolved, suggesting a purely d^9 copper configuration, or a strongly obscured weak transition. The observed bathochromic (red) shift of the ligand coupled with the secondary absorption at 410 nm could point to a more efficient conjugation of the new mixed-ligand structure (Ghaneian *et al.*, 2015), as well as a strong donor-acceptor interaction of the charge-transfer of the electron-withdrawing dinitrophenylhydrazine ligand Importantly, as with the precursor, the Cu-mixed-ligand complex spectrum does not show a clearly defined, broad absorption within the 600-900 nm visible window that is usually taken to be the characteristic of a standard mononuclear Cu(II) d^9 geometry(Iaroshenko, 2019). This lack implies that UV- Vis data itself cannot definitively determine the coordination geometry. However, when combined with the magnetic susceptibility result, it is the ligand-centered transitions and charge-transfers results that dominate, suggesting a highly perturbed, effectively spin-paired or even reduced copper environment. This interpretation is however to be approached with caution pending verification by additional methods like the EPR or single crystal analysis.

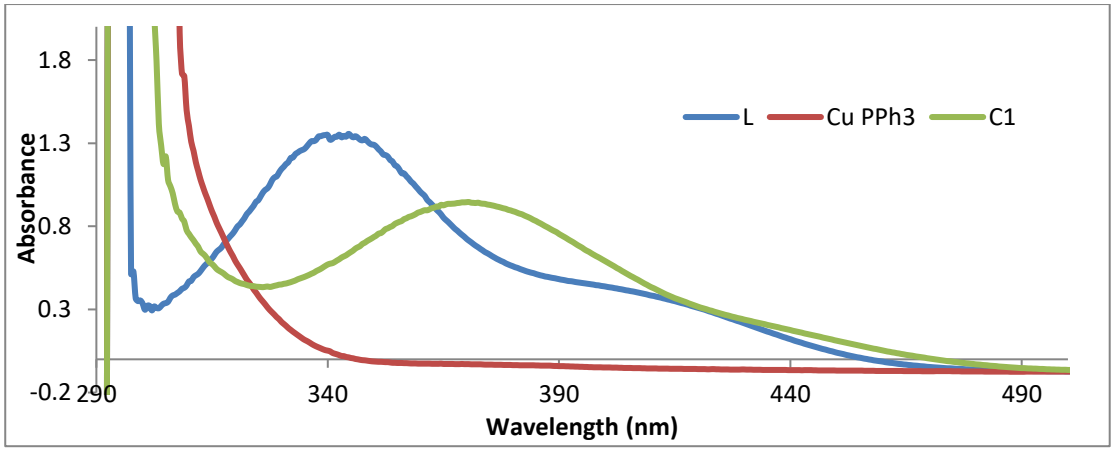

Figure 3: UV–Visible spectrum of ligand L, copper precursor and complex C1

Magnetic Susceptibility

Room-temperature magnetic susceptibility measurements wase conducted to probe the electronic spin state of the metal centre in the Cu-mixed-ligand complex. The mass susceptibility (Xg) of the complex experimentally is -0.0000012. A negative value is a pointer of a weak, overall diamagnetic response in the presence of the applied external magnetic field. A pure, commonplace, mononuclear, d^9, electronic configuration of Cu(II) complex has one unpaired electron and is generally paramagnetic with a magnetic moment of about 1.732.2 Bohr magnetons (Fetoh *et al.*, 2019). The noted diamagnetic nature of the complex is an interesting deviation of this pattern. It can however exist either with a Cu(I) d^{10} closed shell structure due to potential reduction of the ligands in situ or with a Cu(II) state where the spin-pairing/antiferromagnetic interactions between pairs of copper atoms are extreme in a dimeric or polymeric structure. This observation is directly associated with the UV Vis results where the typical Cu(II) d-d absorption band was not observed conspicuously. As a result, although the bulk data show that the complex is effectively spin-paired or diamagnetic-coordinated, therefore an affirmative assignment of final oxidation state or nuclearity can only be with advanced studies on Electron Paramagnetic Resonance (EPR) X-ray crystallography.

Antbacterial Activity: A thorough examination of the antibacterial study (Figure 4) shows that the Cu-mixed-ligand complex (C1) has an outstanding, broad-spectrum antibacterial effect, notably, the complex recorded an average inhibition zone of 26.25 mm, which was slightly higher than the standard clinical drug Ofloxacin (23.50 mm) over the panel used. It was 1 mm superior to Ofloxacin on an organism-by-organism basis against *E. coli*, 2 mm against *S. aureus*, and 4 mm against *K. pneumoniae* and *P. aeruginosa*. Most significantly, the mixed-ligand complex had an astonishing increase in activity compared to its respective uncoordinated components. The activity of the complex was +20 mm higher against *E. coli* than against simple $CuCl_2$ salt; the same organisms, *S. aureus*, +17 mm, *K. pneumoniae*, +20 mm, and *P. aeruginosa*, +15 mm. The fact that the free Schiff base L was completely inactive with the widely-known-resistant P. aeruginosa (0 mm) is especially noteworthy, whereas the incorporation of the CuC1 complex provided a potent 26 mm zone of inhibition. This very strong synergistic action may be interpreted as a chelation-enhanced lipophilicity and enhanced membrane-penetrating ability following coordination (Zoubi *et al.*, 2017)The interaction of the ligand with the metal center and the ligand is known to reduce the effective polarity of the metal ion due to partial charge transfer with donor atoms, thereby enhancing the lipophilic nature of the resultant complex. This would make diffusion through lipid-rich microbial membranes easier and result in increased access to intracellular targets, which, probably, would be enhanced by the hydrophobic triphenylphosphine co-ligand(Hassan *et al.*, 2019; Ngece *et al.*, 2025).

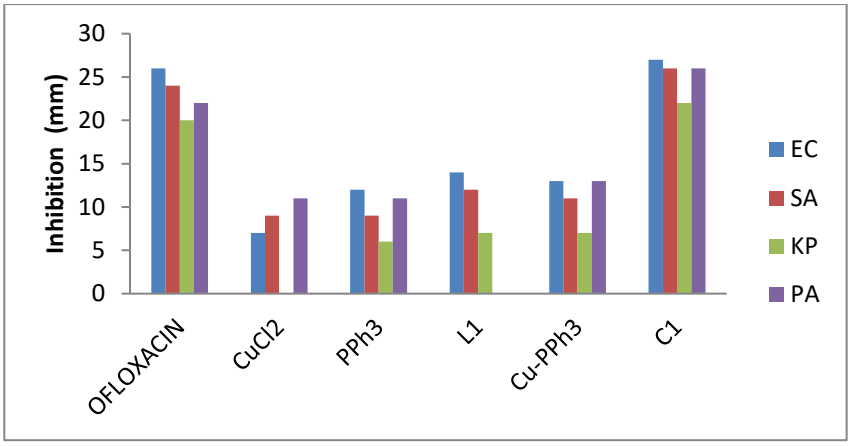

Figure 4: Antimicrobial Analysis of the Complex and its precursors

CONCLUSION

The current research has been able to synthesize and assess a new mixed-ligand copper complex that contains both a 2, 4- dinitrophenylhydrazine 3-pyridinecarboxaldehyde derived Schiff base and a triphenylphosphine ancillary ligand. The coordination of the ligands, characterized by typical changes in the azomethine and pyridyl functionalities and the preservation of diagnostic phosphine and nitro bands, were substantively evidenced by the combined characterization using Fourier Transform Infrared (FTIR) spectroscopy and UV- visible spectroscopy. Interestingly, the bulk magnetic susceptibility measurements and the lack of characteristic low-energy d-d transitions in the UV -Vis spectrum implied that the complex is in a diamagnetic or effectively spin-paired environment, and the simple mononuclear Cu(II) species assumption is challenged. The rational assembly of these ligands around a copper center, which is proven to be a highly potent antibacterial agent, was demonstrated by biological tests without any doubts. The complex showed broad-spectrum activity that not only was vastly superior to its uncoordinated constituents, but also was slightly superior to standard drug Ofloxacin, especially against resistant strains like *P. aeruginosa*. Finally, the results confirm the heteroleptic complexation approach as a viable avenue to bioactivity increase of metal-based drugs.

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