



Synthesis, Spectroscopic Characterization, Antioxidant Properties and Antimicrobial Activity of Transition Metal Complexes from Novel Schiff Base

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Benzoylpyridine and nitro benzoic acid hydrazides were used to examine novel Schiff base Co(II), Mn(II), Fe(II), and Cu(II) complexes. Elemental analysis, molar conductance, magnetic measurements and other methods that like FT-IR, UV-Vis and ¹H NMR studies have been used to deduce the nature of bonding and structure of these complexes. These studies revealed that the Co(II), Mn(II), Fe(II) and Cu(II) coordinated by four donor atoms in all the complexes. Free radical scavenging activity was determined according to the elimination of radicals from 2,2-diphenyl-1-

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picrylhydrazyl (DPPH). These compounds were compared to known antibiotics ciprofloxacin and fluconazole in their ability to inhibit the growth of pathogenic bacteria *Bacillus subtilis* (as gramme positive bacteria), *Pseudomonas sp.* (as gramme negative bacteria), and fungus *Rhizoctonia sp.*, *Alternaria sp.*, and *Fusarium oxysporum*. As an antibacterial and antioxidant agent, the complexes are more effective than the ligand. The copper (II) complex has been discovered to be the most effective antibacterial and antioxidant agent.

Keywords: Schiff bases; metal complexes; antimicrobial; antioxidant.

1. INTRODUCTION

Inorganic chemicals play an important part in biological processes, and it has been demonstrated that metal ions metabolism activates or biotransforms many organic molecules utilised in medicine [1]. Metals and ligands are studied in coordination chemistry, and metals are known to favour certain ligating atoms and adopt specific geometries [2]. They exhibit remarkable biological capabilities such as antimalarial, antiproliferative, antiviral, anticancer, antituberculosis, antibacterial, and antifungal activity against a variety of harmful microorganisms due to the presence of the azomethine group [3]. Metal ions have unique properties that are being exploited to generate new cancer, diabetic, anti-inflammatory, antipyretic, antileishmanial, and anticonvulsant drugs [4]. Under aerobic conditions, the generation of reactive oxygen species (ROS), such as OH and OOH radicals, poses a hazard to live cells. Under normal circumstances, it is effectively counterbalanced by the cells' robust enzymatic antioxidant defences. However, when the equilibrium between ROS production and clearance is disrupted, ROS accumulates excessively in the cells, resulting in oxidative stress [5-7]. As a result, some biocompatible antioxidants are required to reduce oxidative stress. Green chemistry refers to the use of chemicals that do not pollute the environment. Green chemistry involves materials, techniques, or practises that limit or eliminate the creation of pollutants or wastes in order to prevent pollution [8,9]. Heterocyclic chelating with nitrogen as medicinal agents, Schiff bases are gaining popularity [10]. Hydrazones and their transition metal complexes have a wide range of physiological and pharmacological effects, according to reports. They're utilised to cure neoplasia and thalassemia in plants, as a metal extraction agent in analytical chemistry, and as a catalyst in a range of hydrogenation, oxidation, carbonylation, and hydroformylation reactions [11,12]. In this paper, we describe our efforts to make a hydrazide-based Schiff base ligand (L_1)

via condensation of p-nitrobenzohydrazide and p-benzoyl pyridine. Mn(II), Fe(II), Co(II), and Cu(II) complexes were also produced when ligand (L_1) and chloride salts of Mn(II), Fe(II), Co(II), and Cu(II) interacted. The generated compounds were described using elemental analysis, molar conductance, and spectral data (IR, UV-Vis, 1H -NMR). The antibacterial and antifungal activity of the ligand (L_1) and its complexes was tested using the agar well diffusion method. According to a survey of the literature, no work has been done using lemon juice as an acid catalyst to synthesise the ligand (L_1) and its metal complexes.

2. MATERIALS AND METHODS

2.1 Experimental and Instrumentation

Chemically pure and analytical reagent grade reagents were obtained for all commercial reagents. Before being utilised in the normal procedures, the solvents were dried and distilled. Unless otherwise noted, metal salts and benzoylpyridine were acquired from Sigma Aldrich and utilised without additional purification. Sigma-Aldrich Chemical Co. provided the DPPH (1,1-diphenyl-2-picrylhydrazyl). On a Nicolet IS 50 Thermo Fisher scientific FT-IR spectrometer, the infrared spectra of the synthesised ligand and its metal complexes were analysed using the potassium bromide (KBr) disc method, which included dissolving 2 mg of sample in 100 mg of KBr. The infrared spectra of the synthesised compounds were acquired in the 4000-400 cm^{-1} region. A UV-1900 Shimadzu spectrophotometer was used to collect UV-visible spectra in the range of 260-400 nm. An EUTECH PC 700 conductivity metre was used to measure the molar conductance of the complexes in DMSO solution. 1H NMR spectra of the ligand and complexes were obtained using TMS as an internal standard and DMSO as a solvent on a Bruker Avance II 400 MHz NMR spectrometer. The CHN elemental analysis was performed using a Carlo-Erba 1106 elemental analyzer. A melting point instrument and a little amount of

material in a capillary tube closed at one end were used to determine the melting points of the compounds. The magnetic susceptibilities measurements on powder form of the complexes with magnetic susceptibility balance (Mk1) of Sherwood Scientific Corporation, UK using $MgSO_4$ as a celebrant at room temperature. Solvents were evaporated using Heidolph rota evaporator. As bacteria, *Bacillus subtilis* and *Pseudomonas sp.* were used, while fungal isolates included *Rhizoctonia sp.*, *Alternaria sp.*, and *Fusarium oxysporum*.

2.2 Synthesis of the Ligand (L₁)

The Schiff base ligand was prepared using an equimolar mixture of p-nitrobenzohydrazide (10 mmol) and p-benzoyl pyridine (10 mmol) in 30 mL of anhydrous methanol. After adding a few drops of lemon juice (lemon extract was made by pressing the lemons in a beaker, it was filtered using muslin cloths to obtain clean lemon juice) to the finished product, it was refluxed for 5 hours. TLC was used to track the reaction's progress. The reaction mixture was left overnight after it was done. The resulting yellowish-brown substance was washed in methanol and dried in a desiccator on $CaCl_2$.

2.3 Synthesis of Metal Complexes (M-L₁)

The generated ligand, L₁ (1 mmol), was refluxed with sodium salt and methanol for 3 hours. The metal complexes were produced by adding a hot methanolic ligand solution dropwise after refluxing respective metal salts $MCl_2 \cdot nH_2O$ (0.5 mmol) with methanol. After being refluxed for 6 hours with constant stirring on a stirrer, the reaction mixture was left at room temperature overnight. The metal complexes were filtered, washed in methanol, and dried in a desiccator over calcium chloride.

2.4 Antimicrobial Activity

Using the agar well diffusion method [13], the antibacterial and antifungal properties of synthetic Schiff base and its Mn(II), Fe(II), Co(II), and Cu(II) metal complexes were examined. Nutrient agar for bacteria and Potato dextrose agar (PDA) for fungi were employed as culture medium in the antimicrobial experiment. *Bacillus subtilis* and *Pseudomonas sp.* bacteria, as well as the fungi *Rhizoctonia sp.*, *Alternaria sp.*, and *Fusarium oxysporum*, were used to investigate antibacterial and antifungal activities in DMSO solvent at concentrations of 250, 500, 750, and

1000 ppm. The activities were measured (diameter of the zone of inhibition) and compared to the antibacterial and antifungal activity of reference standards, ciprofloxacin and fluconazole, respectively.

2.5 Antioxidant Assay

For the assessment of antioxidant properties of substances over a short time frame, the scavenging of stable DPPH radical is commonly used. Because of the significant delocalization of the unpaired electron, using a stable DPPH radical offers the benefit of being less impacted by side reactions such enzyme inhibition and metal chelation (Fu et al., 2003). A significant decrease in absorption was found as the concentration of the test compounds increased, showing that the test compounds have high antioxidant capabilities. The following equation was used to compute the % scavenging activity of compounds:

$$\% \text{ DPPH}^*_{sc} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where,

A_{control} is the absorbance of the control,
 A_{sample} is the absorbance of the sample.

3. RESULTS AND DISCUSSION

3.1 Physical Properties, Molar Conductance and Elemental Analysis

The Schiff base ligand and its metal complexes were non-hygroscopic, coloured, and air stable. In DMF, DMSO, and $CHCl_3$, the Schiff base ligand and its metal (II) complexes were soluble, but not in water, hexane, or CCl_4 . Methanol and ethanol were just slightly soluble, but DMF, DMSO, and $CHCl_3$ were completely soluble. The ligand (L₁) has a melting temperature of 126°C, while metal complexes have a melting temperature range of 200-220°C. The greater melting point of the ligand and complexes is credited with their stability [14]. Non-electrolytes were defined as ligand and metal complexes with low molar conductances in the range of 12-25 Sm^2mol^{-1} [15]. Table 1 reveals that all of the metal complexes have a 1:2 stoichiometry, and the analytical data of the synthesised compound fits well with the calculated values from the molecular formula assigned to them.

Table 1. Analytical data and physical properties of ligand and their metal complexes

Entry	Compounds	Colour	Melting point (°C)	Molar conductivity $S\ m^2\ mol^{-1}$	Elemental analysis % (found calc.)		
					C (%)	H (%)	N (%)
L ₁	C ₁₉ H ₁₄ N ₄ O ₃	Yellow Brown	124-126	23	65.06 (65.89)	4.14 (4.07)	16.11 (16.17)
Co-L ₁	[Co(C ₁₉ H ₁₄ N ₄ O ₃) ₂ .Cl ₂]	Cream	200-202	16	59.90 (60.72)	3.95 (3.76)	13.78 (14.91)
Cu-L ₁	[Cu(C ₁₉ H ₁₄ N ₄ O ₃) ₂ .Cl ₂]	Green	212-214	13	59.39 (60.35)	3.92 (3.73)	13.61 (14.82)
Fe-L ₁	[Fe(C ₁₉ H ₁₄ N ₄ O ₃) ₂ .Cl ₂]	Orange	218-220	18	60.20 (60.97)	3.48 (3.77)	14.68 (14.97)
Mn-L ₁	[Mn(C ₁₉ H ₁₄ N ₄ O ₃) ₂ .Cl ₂]	Off-white	212-214	17	60.30 (61.05)	3.35 (3.78)	14.83 (14.99)

3.2 Magnetic Behaviour

At ambient temperature, all of the metal complexes Mn(II), Fe(II), Co(II), and Cu(II) have magnetic moments in the range of 5.88, 3.50, 2.33, and 1.81B.M., indicating the existence of unpaired electrons and paramagnetic in nature [16].

3.3 Spectral Characterization

In the IR spectra of the ligand, the N-H stretching vibration at $3660\ cm^{-1}$, C=O at $1667\ cm^{-1}$, and C=N at $1598\ cm^{-1}$ created a prominent absorption band. The azomethine group's band fell by $2-20\ cm^{-1}$, showing that electrons on the nitrogen of the azomethine group were involved in the formation of the coordinate bond with the central metal atom [17]. The appearance of additional bands at $508-518\ cm^{-1}$ and $530-550\ cm^{-1}$ revealed the nature of the metal-ligand relationship, which illustrates (M-N) and (M-O) bonding in the ligand. The formation of new bands at lower wave numbers was attributed to metal-ligand bonding [18]. In the IR spectra of complexes, the absence of absorption lines owing to carbonyl and NH groups suggested that the ligand was bound to the metal in the enolic state.

The electronic spectra of the ligand revealed a band at 270 nm that is attributed π to π^* transitions of the phenyl ring, which shifts to a lower wavelength upon complexation and appears at 249-266 nm in the complexes. The C=N chromophore was identified in the spectra of the Schiff base ligand at 315 nm, which emerges due to the n to π^* transition, which shifts to a higher wavelength upon complexation, and the matching bands were found at 345-375 nm. The shift is caused by the polarisation of the C=N bond caused by metal-ligand interaction [19].

The presence of an azomethine proton was revealed by a singlet at 9.975 ppm in the ¹H-NMR spectra of the synthesised Schiff base ligand L₁. Protons present at the carbon close to the N-atom of the pyridine ring correlate to the signal at 8.64 and 7.98 ppm in the spectrum. The benzylideneimino protons caused a doublet at 7.8 ppm. The proton magnetic resonance spectra of metal complexes display all of the predicted signals. When comparing NMR spectra of metal complexes with free ligand, the elimination of the azomethine proton signal was identified, which is due to azomethine nitrogen coordination to metal ions. The signals corresponding to pyridine and aromatic protons in Co, Cu, Fe, and Mn complexes change shape and location from 8.64 in the ligand to 8.28, 8.295, 8.298 and 8.98 ppm, respectively, due to complexation [16]. The electronic spectra and FT-IR spectra of the Schiff base and its metal (II) complexes is shown in Table 2 and Table 3, respectively.

3.4 Antimicrobial Activity

Biologically, the metal complexes were more effective than the parent ligand against the tested bacterial and fungal species. The ligand was found to be more efficient against gram-positive bacteria (*Bacillus*) than gram-negative bacteria (*Streptococcus*) in terms of antibacterial activity (*Pseudomonas*). The order of antibacterial activity of the substances among the metal complexes as shown in Fig. 1 and Cu and Mn complexes exhibit the most antibacterial action. With the exception of the copper complex, which was effective against all three fungal strains tested, neither the ligand nor the metal complexes showed promising antifungal activity. Fig. 1 visually depicts Cu-L1 action against the three fungus species.

Table 2. Infrared spectral data for ligand L₁ and its metal complexes with Mn, Co, Fe and Cu

Entry	v(N-H)	v(C=N)	v(C=C)	v(N-N)	v(NO ₂)	v(M-N)	v(M-O)
L ₁	3172	1598	1521.53	1131	1356	-	-
Co-L ₁	3166	1579	1520	1134	1342	517	532
Cu-L ₁	3167.57	1587	1521.31	1132	1343	510	545
Fe-L ₁	3167.49	1581	1520.06	1134	1343	516	540
Mn-L ₁	3046	1596	1516	1128	1341	508	534

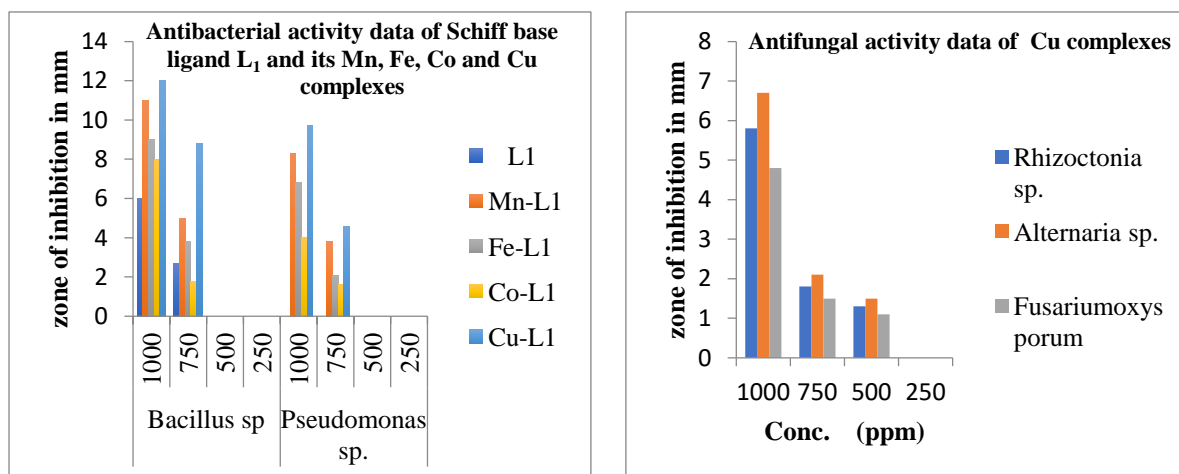


Fig. 1. Antimicrobial activity of Schiff base ligand and metal complexes

Table 3. Electronic spectral data for ligand L₁ and its metal complexes with Mn, Co, Fe and Cu

Entry	Compounds	λ_{max} (nm)	Band assignment
L ₁	C ₁₉ H ₁₄ N ₄ O ₃	270	$\pi \rightarrow \pi^*$
		315	$n \rightarrow \pi^*$
Co-L ₁	[Co(C ₁₉ H ₁₄ N ₄ O ₃).Cl ₂]	260	$\pi \rightarrow \pi^*$
		349	$n \rightarrow \pi^*$
Cu-L ₁	[Cu(C ₁₉ H ₁₄ N ₄ O ₃).Cl ₂]	249	$\pi \rightarrow \pi^*$
		365	$n \rightarrow \pi^*$
Fe-L ₁	[Fe(C ₁₉ H ₁₄ N ₄ O ₃).Cl ₂]	254	$\pi \rightarrow \pi^*$
		359	$n \rightarrow \pi^*$
Mn-L ₁	[Mn(C ₁₉ H ₁₄ N ₄ O ₃).Cl ₂]	266	$\pi \rightarrow \pi^*$
		370	$n \rightarrow \pi^*$

The heterocyclic ring contained in the molecular structure of the metal complexes is responsible for these interpretations. Chelation hypothesis can explain the increased lipophilic character of metal complexes and the increased activity of metal chelates [20]. The lipid membrane that surrounds the cell, according to Overton's idea of cell permeability, favours the passage of only lipid soluble molecules. Due to ligand orbital overlap and partial sharing of positive charge of metal ion with donor groups of the Schiff base ligand, the polarity of the metal ion is lowered to a higher extent by chelation

[21,22]. As a result of the enhanced delocalization of electrons across the entire chelates ring, the complex's lipophilicity rises. This increased lipophilicity allows the complexes to penetrate the lipid barrier and inhibit the metal binding sites on microorganism enzymes [23]. These metal complexes also disrupt the cell's respiration mechanism, preventing protein synthesis. Because protein synthesis is inhibited, bacterial cell wall production is impossible, resulting in cell death and ultimately limiting the organism's ability to proliferate [24].

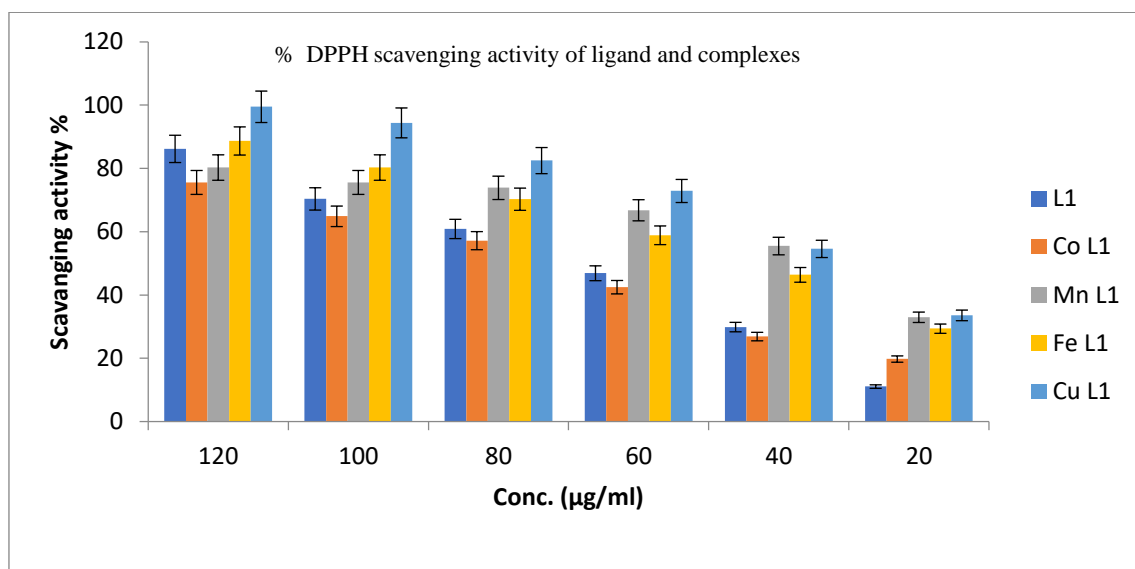


Fig. 2. % DPPH scavenging activity of ligand and complexes

3.5 Antioxidant Assay

The capacity of metal complexes to scavenge free radicals of DPPH, which has been routinely employed to test antioxidant activity, was used to determine their antioxidant activity. Because of the significant delocalization of the unpaired electron, using a stable DPPH radical offers the benefit of being less impacted by side reactions such as enzyme inhibition and metal chelation [25]. To become a stable diamagnetic radical, DPPH takes an electron or hydrogen [26-28]. DPPH has a significant absorption band at 517 nm due to its unpaired electron, giving it a deep violet colour. The absorption decreases as the electron pairs up in the presence of the ligand (L_1) and its complex, resulting in a yellow colour, and this decolorization is stoichiometry in terms of the number of electrons taken up. A significant decrease in absorption was found as the concentration of the test compounds increased, showing that the test compounds have high antioxidant capabilities. In Fig. 2, the values of DPPH radical scavenging activities of the ligand and complex are shown, with the Schiff base ligand showing the lowest value compared to the metal complex.

4. CONCLUSION

Mn(II), Fe(II), Co(II), and Cu(II) Schiff base complexes derived from p-nitrobenzohydrazide and p-benzoyl pyridine are stable, non-hygroscopic, and non-electrolytic. The Schiff base is a neutral bidentate ligand connected to the central metal ion by the azomethine. The

metal (II) complexes have an octahedral geometry. The complexes are organised in the following sequence and exhibit stronger biological activity than the free Schiff base ligand: Cu (II) > Mn (II) > Fe (II) > Co (II). This shows that metal chelation has a significant impact on the antibacterial activity of the organic ligand.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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