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SYNTHESIS, CHARACTERIZATION, ANTI-OXIDANT ACTIVITIES OF SCHIFF BASE, ITS METAL COMPLEXES AND ITS ASSESSMENT AS AN ANTIMICROBIAL AGENT AGAINST DIFFERENT PATHOGENIC MICROBES

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ABSTRACT

A novelSchiff base ligand, (Z)-3-((2-(4-methyl-2,5-dinitrophenyl) hydrazineylidene)methyl)pyridine]was synthesized via the condensation of 2,4-dinitrophenylhydrazine with pyridine-3-aldehyde. Transition metal chloride salts of copper (Cu) and cobalt (Co), both in the (II) oxidation state, were employed to form coordination complexes with the synthesized Schiff base. The metal-to-ligand ratio for all complexes was determined to be 1:2, with the general molecular formula represented as ML_2 $M(C_{13}H_{11}N_5O_4)_2)$, where M denotes either Cu (II) or Co (II) and L denotes Schiff base ligand. In the present casethe molecular formula of the Schiff base ligand was$C_{13}H_{11}N_5O_4$.

The synthesized ligand and its metal complexes were characterized using Fourier-transform infrared spectroscopy (FTIR) and proton nuclear magnetic resonance (^{1}H NMR) to confirm their structural composition. Biological activities including antimicrobial and antioxidant properties were evaluated to assess the potential applications of these compounds in pharmaceutical and biomedical fields.

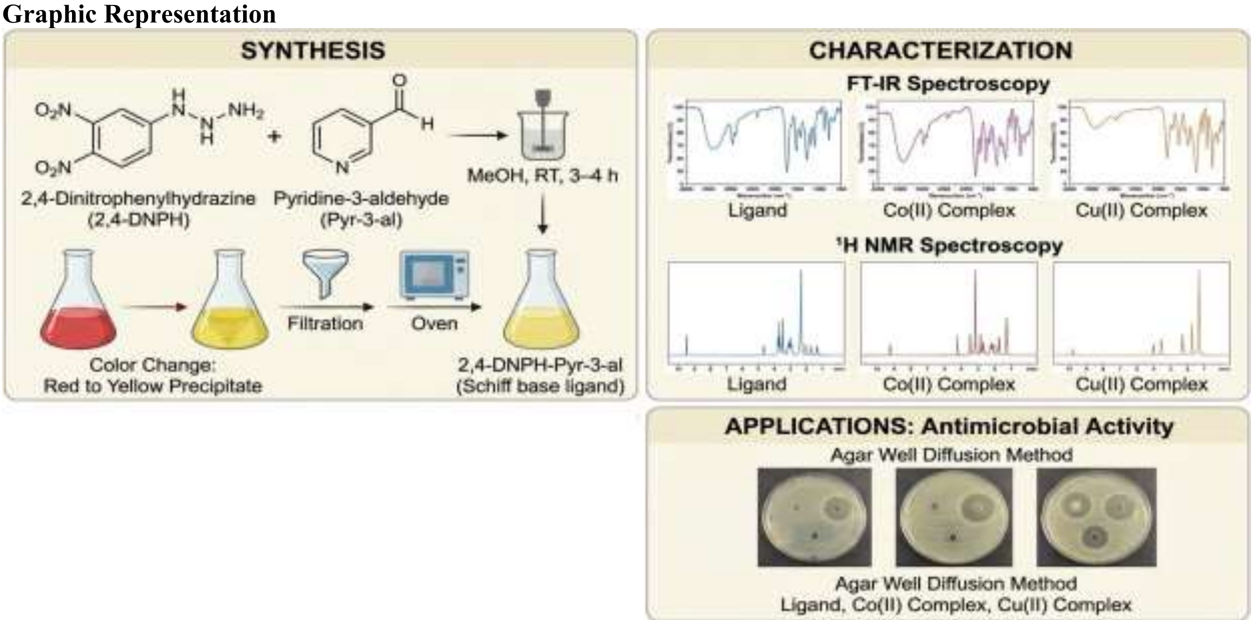
Four bacterial strains namely:*E. coli, E. bacelisa, B.Subtilis, A. bacter, S.typhi* and *C. bacter*were used to determine the antibacterial efficiency of the Schiff base ligand and its ML_2 complexes. Antibacterial activity was assessed using the disk diffusion method, and the results indicated notable inhibitory effects. Similarly, antifungal activity was evaluated using four fungal strains*Candida albicans, Aspergillus niger, Aspergillus flavus*, and *Trichoderma viride*. The results demonstrated impressive antifungal activity for the synthesized complexes, indicating their potential as antifungal agents.

Additionally, the antioxidant activity of the synthesized compounds was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The results suggested that the antioxidant properties of the synthesized samples were remarkable, further highlighting the potential of these compounds for use in biological applications.

In conclusion, the synthesized Schiff base ligand and its metal complexes with Cu(II) and Co(II) exhibit promising antibacterial, antifungal, and antioxidant properties, making them suitable candidates for further exploration in biomedical and pharmaceutical applications.

KEYWORDS; Anti-microbial, anti-oxidant, Schiff base, FTIR, ^{1}HNMR

Graphic Representation



1. INTRODUCTION

The condensation of two or more compounds to obtain novel products with potential biological activity is a common approach in drug design. Among these, Schiff bases have garnered significant attention due to their ease of synthesis from primary amines and aldehydes or ketones through condensation reactions under well-defined conditions. Schiff bases are particularly important because of their ability to stabilize metal ions in various oxidation states, making them excellent candidates for catalyzing reactions and serving a wide range of industrial and biological applications[1]. The formation of stable complexes is attributed to the lone pair of electrons on the nitrogen atom of the azomethine (-N=CH-) group, which plays a crucial role in bonding and influencing the structure of these complexes. As a result, Schiff base metal complexes are extensively studied in fields such as bioinorganic chemistry, supramolecular chemistry, and biological sciences. These complexes exhibit a variety of biological activities, including antimicrobial, anticancer, and antitumor properties[2].

In recent years, researchers have increasingly focused on the synthesis of ligand-metal complexes due to their promising biological potential and emerging applications in the pharmaceutical industry. Ligands and their metal complexes have become central to contemporary biological studieswith new complexes often demonstrating significant biological efficacy. Many ligands and their complexes have been synthesized[3] and evaluated for their various properties, including reversible oxygen-binding efficiency, catalytic activity in olefin hydrogenation, photochromic behavior, and complexation with toxic metals[4]. Ligands exhibit a strong ability to form stable complexes, particularly with transition metal ions, due to their chelating properties. In coordination chemistry, Schiff bases serve as key ligands for forming metal complexes[5], thereby expanding the frontiers of the field. Ligand-metal complexes have been widely studied for their biological applications[6], showing potential as antimicrobial, antiviral, antitumor, and antifungal agents[7].

Schiff bases, as ligands containing the azomethine group (-CH=N-), are typically synthesized via the condensation of carbonyl compounds with primary amines[8]. The resulting ligands coordinate with metal ions primarily through the nitrogen atom of the azomethine group, contributing to their utility in coordination chemistry[9]. Because of this, ligands served as a cornerstone for the development of modern coordination chemistry and have a wide range of applications. Most Metal complex of ligands have shown application as biological, antimicrobial, antiviral, antitumor and antifungal activity[1, 10-14]compounds, and flexibility in design[15], making them ideal for tailoring properties for specific applications. Along with other organic components used, ligands hold brilliant synchronization features, relatively simple preparation procedures, structural resemblances with natural biological compounds, and the mock variety and elasticity that enables design of suitable structural properties[16].

In the present study Schiff base ligands were synthesized from 2,4-dinitrophenylhydrazine and pyridine-3-aldehyde using a modified protocol, followed by the formation of their novel metal complexes with copper (Cu) and cobalt (Co) ionsin (II) oxidation state. The synthesized compounds were characterized through spectroscopic techniques, including FTIR and ^{1}H NMR, to confirm their structures. The antifungal, antibacterial, and ant oxidative activities of the ligands and their metal complexes were also investigated, with promising results obtained for their biological properties.

2. MATERIALS AND METHODS

2.1. Materials

The reagents and solvents used in the research work were of analytical grade and did not go through to any additional purification processes. The compounds used in the present study are 2, 4-Dinitrophenylhydrazine, Pyridine-3-al, sodium hydroxide, n-hexane, ethyl acetate, chloroform, DMSO, methanol, DMF, ethanol, and DPPH, as well as $CoCl_2.6H_2O$ and $CuCl_2.2H_2O$.

2.2. Instruments

The Stuart SMP20 Melting Point Apparatus was used to determine the melting points of the Schiff base ligand and its co-ordination compounds. The IR spectra of the synthesized Schiff base and its metal complexes were determined using an 8400 FT-IR SHIMADZU spectrometer. KBr discs were used to determine IR spectra within the 400-4000 range. 1H-NMR and C-13 NMR spectra were obtained using a Bruker Avance III HD NMR spectrometer operating at 400Hz and 100 Hz, respectively. The samples were dissolved in DMSO-d6 solvent for 1H-NMR and C-13 NMR spectral analysis.

2.3. Synthesis of 2, 4-Dinitrophenylhydrazine – Pyridine-3-aldehyde Schiff Base

Ligand was synthesized using the procedure given in the literature (Shalauriel al, 2018 Silverstain and Bassler, 1981) by condensing 2, 4-Dinitrophenylhydrazine (3.61g, 20 mmol) and Pyridine-3-aldehyde (2.14, 20mmol) in 15ml of methanol. The stirring of the mixed reactants was carried out for five hours at normal temperature and the progress of reaction was tracked by caring out thin layer chromatographic technique (TLC) at different time intervals.Theligand precipitateswere orange yellow solidswhich ensure thecompletion of reaction. To obtain the purified product, the solid precipitate was washed thoroughly with ethanol through filtration process. The purifiedproduct was confirmed from melting point and thin. The Ligand wasshowing solubility in dimethyl sulfoxide and dimethylformamide. The structure of the presently synthesized ligand is shown in scheme 1.

2.4. General Procedure for the Synthesis of Schiff Base Metal Complexes

A methanolic solution of the Schiff base was prepared by adding 0.602 g of Schiff base (2 mmol) to 20 mL of methanol. By dissolving 1 mmol of each salt in methanol, a 10 mL solution of metal chloride compounds was generated. The ligand solution was magnetically stirred before the salt solution was gradually added. The continuous stirring was maintained at normal temperature for five hours. The metal complexes formed in a variety of colors. The complexes' precipitates were filtered, washed, and dried.

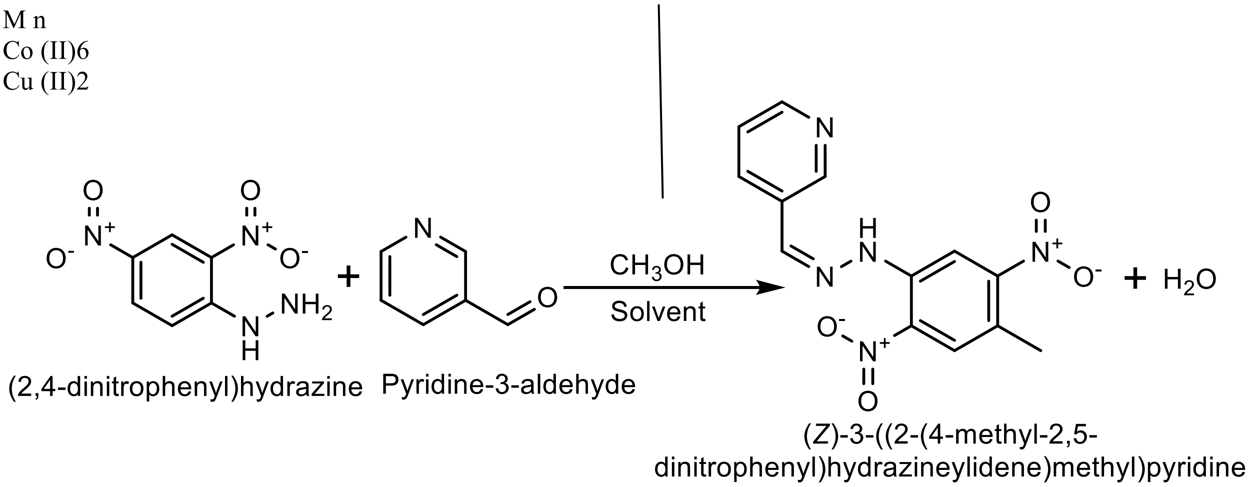
$MCl_2 \cdot nH_2O + HL \rightarrow ML_2 + HCl + nH_2O$

Where

M n

Co (II)6

Cu (II)2



Scheme 1. Structure of Ligand

2.5. Antimicrobial Activity

The Disc Diffusion method was used to conduct an antimicrobial assay of the Schiff base ligand and its co-ordination compounds against a variety of pathogenic strains of bacteria and fungi [17]. In DMSO, stock solutions of the Schiff base ligand and its co-ordination compounds CoL2 and CuL2 were prepared. The concentration of each was adjusted to 100 µg mL-1. The Disc Diffusion method uses Whatman No. 1 filter paper discs with a diameter of 6mm. A sterile apparatus was utilized to position the discs in the Petri plates on the media. Then, the discs were treated with Schiff base CoL2 and CuL2, which were present at concentrations of 100 and 200 µg per 1 and 2 mL, respectively. The positive controls for gram-positive bacteria were tetracycline at a concentration of 50 µg mL-1, while cephradine at a concentration of 30 µg mL-1 was used for gram-negative bacteria. Fluconazole (50 µg mL-1) was used as a positive control for fungal isolates. The plates were analyzed next day, and inhibition zones were observed in millimeters around the discs. The diameter of inhibition zones was expressed as the mean ±SD for each concentration of the Schiff base, complexes, and standard, and each activity was performed in triplicate.

2.6. Antioxidant Activity

The antioxidant activity of the Schiff base ligand and its co-ordination compounds was examined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. Schiff base and its active metal complexes were prepared as stock solutions (1.0 mg/mL) and subsequently diluted to 500, 400, 300, 200, and 100 µg mL-1 in methanol. The methanolic solution of DPPH (1.0 mL of 0.4 mM) was thoroughly stirring after the test solutions were added. Ascorbic acid was selected as the standard. The scavenging capacity of the standard Schiff base ligand and its co-ordination compounds was determined after a 30-minute period at room temperature. The decrease in the absorbance of DPPH at 517nm was used to determine the antioxidant properties of the standard Schiff base ligand and its co-ordination compounds. Additionally, the absorbance of neutral methanol was calculated. The DPPH radical scavenging activity of standard, ligand, and metal complexes was reported as the mean ± SD for each concentration (g/mL) in a triplicate assay. The %inhibition was determined using the equation provided below[17].

$$\text{DPPH Scavenged (\%)} = \frac{\text{Absorb. (c)} - \text{Absorb. (s)}}{\text{Absorb. (c)}} \, x \, 100$$

Where Absorb. (c) is the absorbance performance of the control Absorb. (s) is the absorbance performance of the sample.

3. RESULTS AND DISCUSSIONS

The Table 1 shows the physical characteristics of the synthesized Schiff base and its metal complexes. In air, the Schiff base and its transition complexes are resistant to decomposition. The melting points of all synthesized compounds are high, and they are non-hygroscopic. Schiff base is partially soluble in water, whereas complexes are insoluble in water. Schiff base and its metal complexes are moderately soluble in ethyl acetate and ethanol, but they are completely soluble in methanol, DMSO, and DMF.

Table 1. Physical properties of Schiff base ligand and its co-ordination compounds

Compounds	Yield (%)	Color	M.Pt ºC	Solubility					
				H20	n-Hexane	EtOH	MeOH	DMF	DMSO

Shiff base		Orange Yellow	184	PS	PS	PS	S	S	S
CoL_2		Bright yellow	267	IS	PS	PS	S	S	S
CuL_2		Vivid yellow	253	IS	PS	PS	S	S	S

S= soluble, PS= partially soluble, IS= insoluble

3.1. Infrared Spectroscopy

To know about the successful synthesis of the ligand and the two complexes, FTIR was performed which gives information about different types of functional groups and type of interactions. The type of functional group is given in Table 2 while the spectra of ligand and the two metal complexes are given in Figure 1-3. The band at 1630 cm^{-1}in ligandFTIR data correspond to the C=N linkage of azomethane that is present in the ligand [18]. A peak at 3340 cm^{-1}is due to hydroxyl group stretching[19]. The peak at 2948 cm^{-1}correspond to the presence of (C-H) group of aromatic alkanesin ligand and the peak at 2610 cm^{-1} is due to the (C-H) of alkene group. Two absorption bands at 1580 cm^{-1} and 1436 cm^{-1}correspond to the (C=C) ofaromatic and (C=C)of aliphatic groups respectively(Figure 1). The azomethine linkage bandthat appeared at 1630 cm^{-1} in ligand wasshifted to higher frequency in the complex which confirmsuccessful complexation of ligand to metal(Table 2). This shifting of azomethine band shows that nitrogen atom of azomethine group was successfullycoordinated to the metal ions [20]. The appearance of band in a range from 451-510cm^{-1}confirm the azomethane nitrogen(Figure 2, 3).[21].

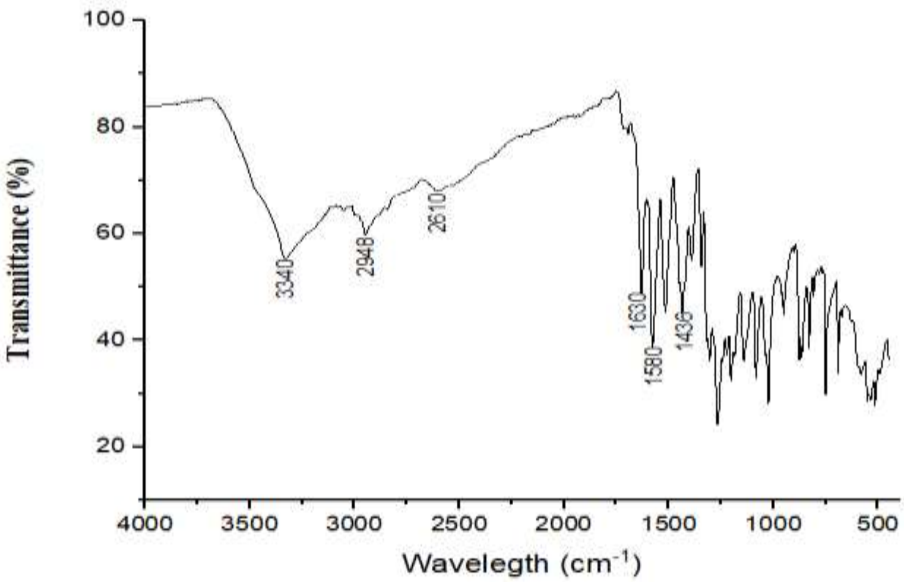


Fig. 1. IR spectral data of Ligand

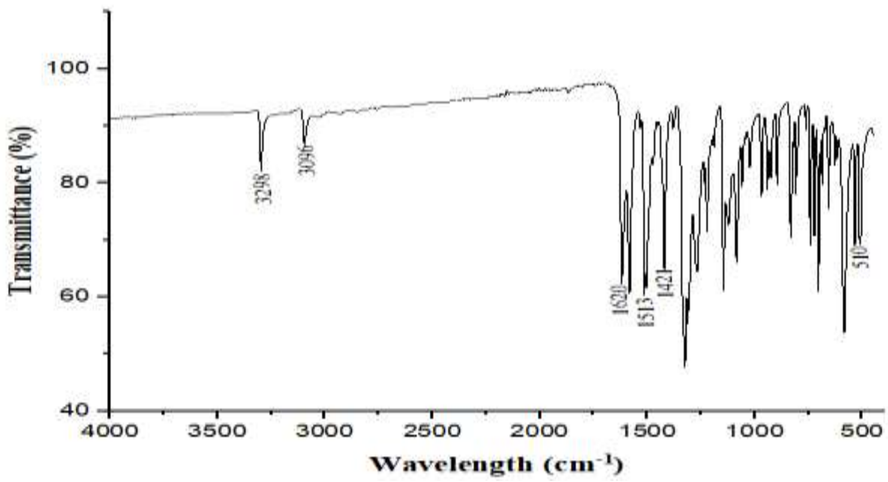


Fig. 2. IR spectral data of Cu (II) Metal Complex

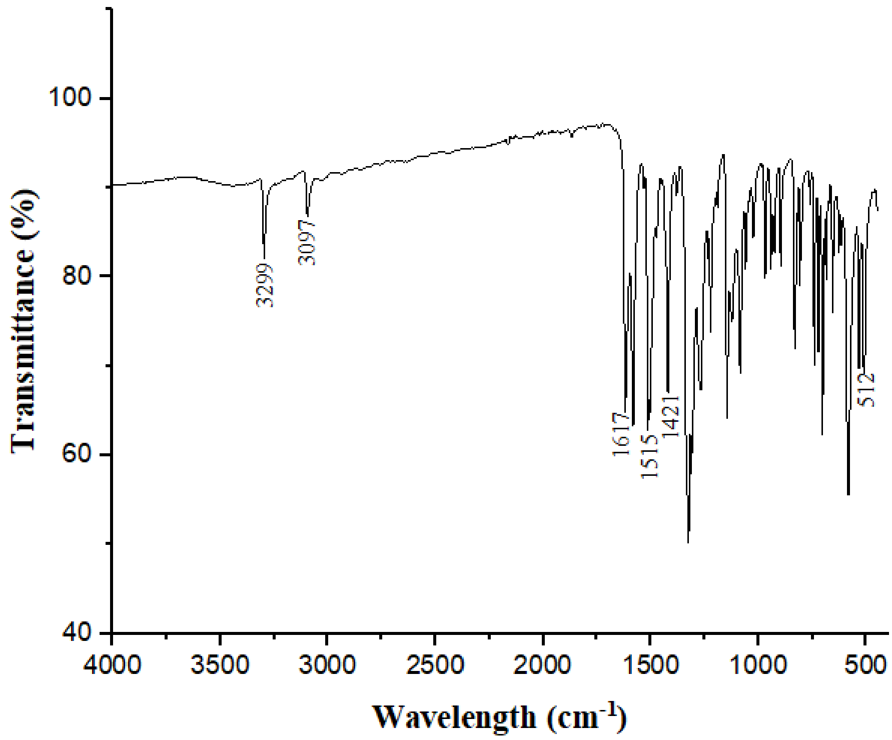


Fig. 3. IR spectral data of Co (II) Metal Complex

Table 2: IR (KBr υ_{max} cm^{-1}) of ligand and its metal complexes

Compound	O-H	C-H Aromatic alkene	C=H alkenes	C=N	C=N Aromatic	C=C Aliphatic	M-N
L	3340	2948	2610	1630	1580	1436	----
$[\{Cu(L)_2\}_2]$	3298	3096	----	1620	1513	1421	510
$[\{Co(L)_2\}_2]$	3299	3097	----	1617	1515	1421	512

3.2. ^{1}H NMR Spectra

The characteristic 1 H-NMR signals of the synthesized Schiff base and its Cu (II) and Co (II) complex are presented in Table 3. The spectrum exhibited a characteristic sharp singlet at 11.76 ppm corresponding to the hydrazone –NH proton, while the azomethine (–CH=N–) proton appeared as a singlet at 8.15–8.20 ppm. The aromatic region displayed well-resolved signals between 7.40 and 9.05 ppm integrating for eight protons, consistent with the pyridine and 2,4-dinitrophenyl rings. Notably, the absence of the aldehyde proton signal (expected at ~10.0 ppm) confirmed complete conversion of the starting material. [9]. The observed chemical shifts and integration values are in excellent agreement with the proposed structure. In both complexes, the hydrazone –NH signal at 11.76 ppm was either absent or significantly broadened, indicating deprotonation and involvement of the hydrazone moiety in coordination. The azomethine and aromatic protons exhibited moderate shifts and considerable broadening, particularly in the Cu(II) complex, consistent with the paramagnetic nature of Cu(II) (d^9) and high-spin Co(II) (d^7) ions. These spectral changes confirm the successful formation of the metal complexes through coordination via the imine nitrogen and/or pyridine nitrogen atoms.

The characteristic ^{13}C-NMR signals of the synthesized Schiff base are presented in Table 4. The azomethine carbon (CH=N) appears at 143.3 ppm, confirming the successful condensation between pyridine-3-carbaldehyde and 2,4-dinitrophenylhydrazine. The complete disappearance of the aldehydic carbon signal (expected around 190–195 ppm) further supports the formation of the hydrazone linkage. A distinctive downfield signal at 156.2 ppm is attributed to the quaternary aromatic carbons attached to the nitro groups. The signals appearing in the region 123.0–151.9 ppm[22]correspond to the remaining aromatic carbons of both the pyridine and dinitrophenyl rings. The aromatic CH carbons resonate at 110.0 ppm, while the methyl carbon (CH_3) of the dinitrophenyl moiety shows an absorption at 17.3 ppm. The ^{1}H-NMR, ^{13}C-NMR, and other spectral data are in complete agreement with the proposed structure of the Schiff base ligand and its metal complexes.

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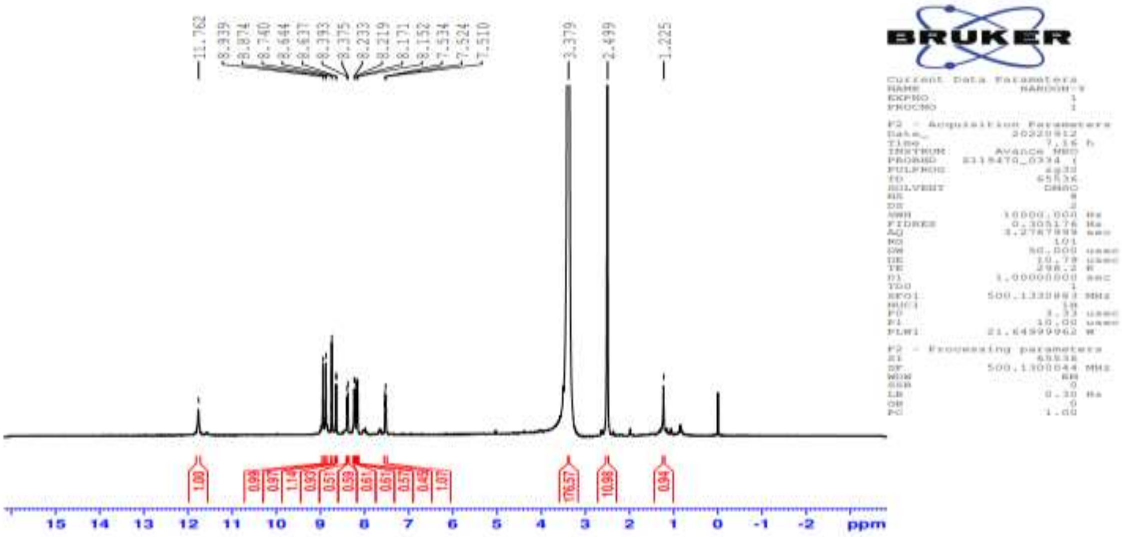

Fig. 4: ^{1}H NMR data of Schiff base

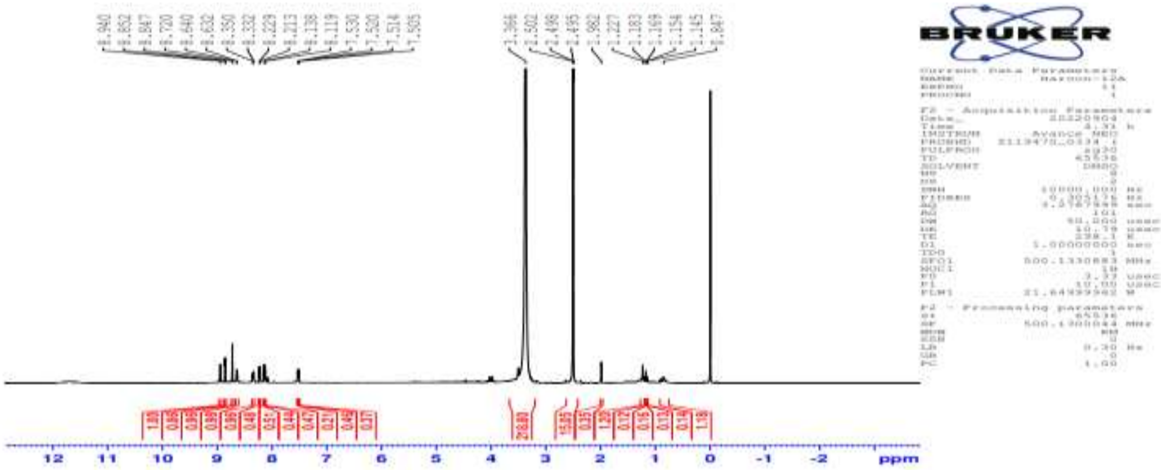

Fig. 5: ^{1}H NMR data of Cu (II) metal complex

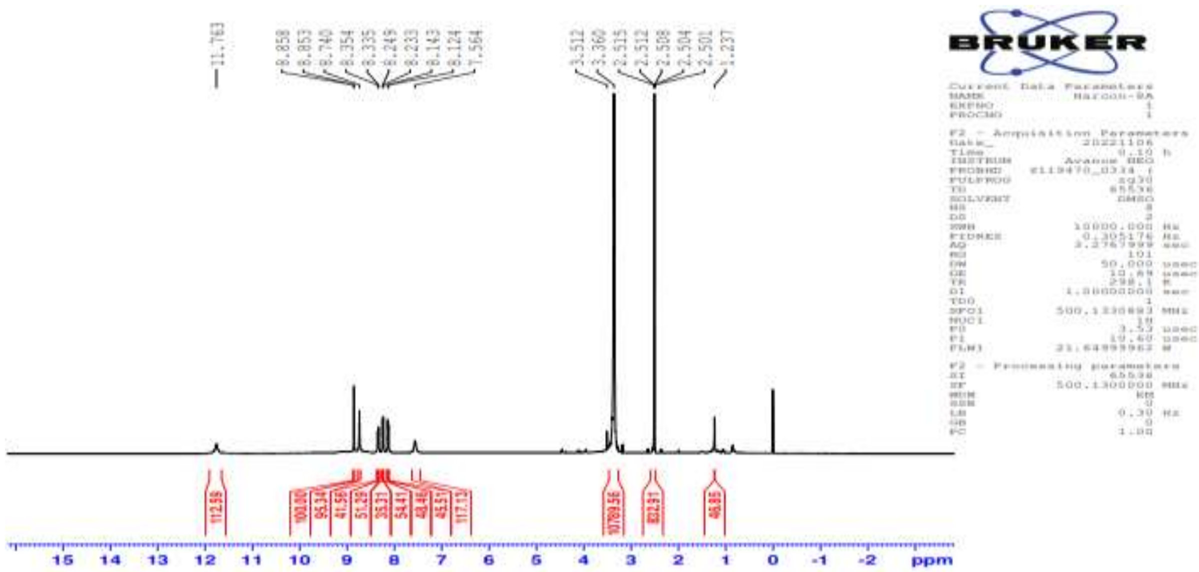

Fig. 6. ^{1}H NMR data of Co (II) metal Complex

Table 3. 1 H NMR spectral data of the Schiff base and its metal complexes.

Compounds	1 H NMR (DMSO-d6 400 Hz) Frequencies (ppm)				
	NH	CH=N	Ar-H (Pyridine)	Ar-H (Dinitrophenyl)	Other Protons
L	11.76 (s,1H)	8.15-8.20 (s, 1H)	8.95-9.05 (s/d, 1H)	8.58-8.74 (m, 2H)	3.30-3.40

			7.40-7.64 (m,2H)	8.30-8.40 (m, 2H)	(s, Residual H20/DMSO)
CuL2	---	7.05-9.40 (br m, 16H)	---	---	3.38 (s, DMSO) 0.87-2.96 (m, minor)
CoL2	11.76 (Br s, 1H)	7.51-8.94 (br m, 16H)	---	---	3.38 (s, DMSO) 2.50 (s,DMSO- d5) 1.23 (S, Minor)

Table 4. 13C NMR data of the Schiff

Carbons	**^{13}C NMR (DMSO-d6 100 Hz) Frequencies (ppm)**
C (Aromatic)	156.2
CH=N	143.3
Ar C	123 - 151.9
Ar CH	110
CH_3	17.3

3.3. Molecular Geometry and Structural Analysis of the Complexes Using Computational Methods

An in-depth analysis of the analytical and spectroscopic data, combined with computational modeling, was conducted to propose the molecular geometries of the synthesized complexes. Computational software integrates established principles and a comprehensive database to assist in designing the molecular structures of these complexes. Key bonding parameters, such as bond lengths, and bond angle in Table5around the metal center, were explored by evaluating the proposed molecular structures.

In this study, ChemDraw and ArgusLab software packages were employed to model the molecular architecture of both the ligand and its metal complexes. These computational tools facilitated the visualization of possible molecular geometries and helped confirm experimental findings. The proposed structures of the complexes, based on both experimental data and computational simulations, are presented in Figure 7-9.

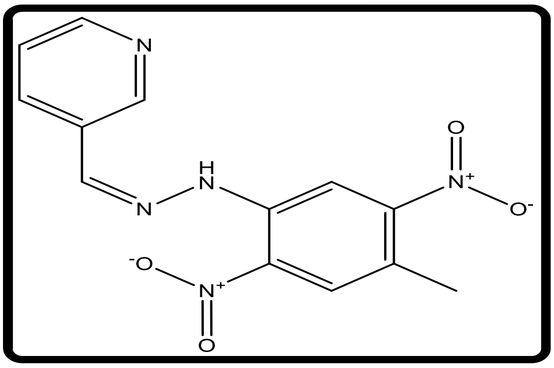

Fig. 7: Proposed Structure of Ligand [(Z)-3-((2-(4-methyl 2, 5-dinitrophenyl hydrazinylidene) methyl pyridine]

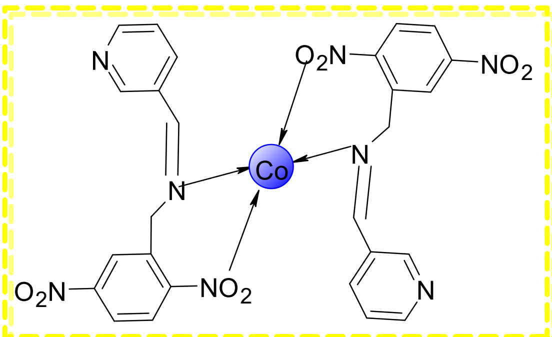

Fig. 8: Proposed Structure of Co Metal Complex

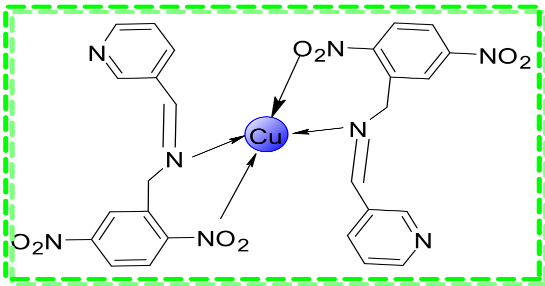

Fig. 9. Proposed Structure of Cu Metal Complex

Table 5: Bond Length and Bond Angle of L, CuL_2 and CoL_2

L		**CuL_2**		**CoL_2**	
Bond Length	**Bond Angle**	**Bond Length**	**Bond Angle**	**Bond Length**	**Bond Angle**
ATOMS Å	ATOMS Å	ATOMS Å	ATOMS Å	ATOMS Å	ATOMS Å
C(18)-H(33) 1.1130	O(22)-N(20)-O(21) 120.0000	N(21)-H(54) 1.0500	N(21)-Cu(40)-N(39) 109.5000	N(48)-O(49) 1.3100	O(49)-N(48)-O(31) 120.0000
C(18)-H(32) 1.1130	O(22)-N(20)-C(19) 120.0000	N(21)-Cu(40) 1.8460	H(65)-N(39)-Cu(40) 120.0000	N(48)-O(31) 1.3100	O(49)-N(48)-C(35) 120.0000
C(18)-H(31) 1.1130	O(21)-N(20)-C(19) 120.0000	C(15)-N(21) 1.2660	H(65)-N(39)-C(25) 120.0000	O(29)-O(30) 1.1008	O(31)-N(48)-C(35) 120.0000
C(14)-H(30) 1.1000	C(17)-C(19)-C(12) 120.0002	N(16)-O(43) 1.3100	Cu(40)-N(39)-C(25) 120.0000	N(18)-O(17) 1.3100	H(61)-N(47)-Co(50) 120.0000
C(12)-H(29) 1.1000	C(17)-C(19)-N(20) 119.9999	N(16)-O(42) 1.3100	O(20)-N(38)-O(19) 120.0000	N(18)-O(16) 1.3100	H(61)-N(47)-C(32) 120.0000
N(10)-H(28) 1.0500	C(12)-C(19)-N(20) 119.9999	O(22)-O(41) 1.1008	O(20)-N(38)-C(28) 120.0000	N(47)-Co(50) 1.8360	Co(50)-N(47)-C(32) 120.0000
C(7)-H(27) 1.1000	H(33)-C(18)-H(32) 109.5200	N(38)-O(20) 1.3100	O(19)-N(38)-C(28) 120.0000	N(47)-H(61) 1.0500	N(45)-C(46)-H(60) 118.0987
C(6)-H(26) 1.1000	H(33)-C(18)-H(31) 109.4618	N(38)-O(19) 1.3100	N(36)-C(37)-H(64) 118.0987	N(39)-C(40) 1.2600	N(45)-C(46)-C(41) 123.8025
C(4)-H(25) 1.1000	H(33)-C(18)-C(17) 109.4618	O(17)-O(18) 1.1008	N(36)-C(37)-C(32) 123.8025	C(38)-N(39) 1.4700	H(60)-C(46)-C(41) 118.0987
C(2)-H(24) 1.1000	H(32)-C(18)-H(31) 109.4418	N(39)-Cu(40) 1.8460	H(64)-C(37)-C(32) 118.0987	C(35)-N(48) 1.2480	C(46)-N(45)-C(44) 116.6193
C(1)-H(23) 1.1000	H(32)-C(18)-C(17) 109.4418	N(39)-H(65) 1.0500	C(37)-N(36)-C(35) 116.6193	C(44)-N(45) 1.3509	N(45)-C(44)-H(59) 118.1020
C(11)-C(13) 1.3948	H(31)-C(18)-C(17) 109.5000	C(25)-N(39) 1.2660	N(36)-C(35)-H(63) 118.1020	N(45)-C(46) 1.3509	N(45)-C(44)-C(43) 123.7959
C(14)-C(13) 1.3949	C(14)-C(17)-C(19) 120.0033	C(35)-N(36) 1.3509	N(36)-C(35)-C(34) 123.7959	C(43)-H(58) 1.1000	H(59)-C(44)-C(43) 118.1020
C(17)-C(14) 1.3948	C(14)-C(17)-C(18) 119.9984	N(36)-C(37) 1.3509	H(63)-C(35)-C(34) 118.1020	C(44)-H(59) 1.1000	H(58)-C(43)-C(44) 120.8827
C(19)-C(17) 1.3948	C(19)-C(17)-C(18) 119.9984	C(35)-H(63) 1.1000	H(62)-C(34)-C(35) 120.8827	C(41)-C(46) 1.3858	H(58)-C(43)-C(42) 120.8827
C(12)-C(19) 1.3949	O(16)-N(15)-O(9)	C(34)-H(62) 1.1000	H(62)-C(34)-C(33) 120.8827	C(46)-H(60) 1.1000	C(44)-C(43)-C(42) 118.2346

	120.0000				
C(11)-C(12) 1.3948	O(16)-N(15)-C(13) 120.0000	C(37)-H(64) 1.1000	C(35)-C(34)-C(33) 118.2346	C(40)-H(56) 1.1000	H(57)-C(42)-C(43) 120.3420
C(1)-C(6) 1.3858	O(9)-N(15)-C(13) 120.0000	C(31)-H(60) 1.1000	H(61)-C(33)-C(34) 120.3420	C(42)-C(43) 1.3910	H(57)-C(42)-C(41) 120.3420
N(5)-C(6) 1.3509	H(30)-C(14)-C(13) 120.0014	C(29)-H(59) 1.1130	H(61)-C(33)-C(32) 120.3420	C(41)-C(42) 1.3910	C(43)-C(42)-C(41) 119.3159
C(4)-N(5) 1.3509	H(30)-C(14)-C(17) 120.0014	C(34)-C(35) 1.3858	C(34)-C(33)-C(32) 119.3159	C(43)-C(44) 1.3858	C(46)-C(41)-C(42) 118.2317
C(3)-C(4) 1.3858	C(13)-C(14)-C(17) 119.9972	C(33)-C(34) 1.3910	C(37)-C(32)-C(33) 118.2317	C(38)-H(54) 1.1130	C(46)-C(41)-C(40) 120.8842
C(2)-C(3) 1.3910	C(11)-C(13)-C(14) 119.9996	C(32)-C(37) 1.3858	C(37)-C(32)-C(31) 120.8842	C(38)-H(55) 1.1130	C(42)-C(41)-C(40) 120.8842
C(1)-C(2) 1.3910	C(11)-C(13)-N(15) 120.0002	C(31)-C(32) 1.3370	C(33)-C(32)-C(31) 120.8842	C(33)-C(38) 1.4970	N(39)-C(40)-C(41) 120.0000
N(20)-O(22) 1.3100	C(14)-C(13)-N(15) 120.0002	C(32)-C(33) 1.3910	N(30)-C(31)-C(32) 120.0000	C(42)-H(57) 1.1000	N(39)-C(40)-H(56) 120.0000
N(20)-O(21) 1.3100	H(29)-C(12)-C(19) 120.0015	C(29)-H(58) 1.1130	N(30)-C(31)-H(60) 120.0000	C(32)-C(37) 1.3948	C(41)-C(40)-H(56) 120.0000
C(19)-N(20) 1.2480	H(29)-C(12)-C(11) 120.0015	C(33)-H(61) 1.1000	C(32)-C(31)-H(60) 120.0000	C(36)-C(37) 1.3949	C(40)-N(39)-C(38) 108.0000
C(17)-C(18) 1.4970	C(19)-C(12)-C(11) 119.9969	C(26)-C(29) 1.4970	C(31)-N(30)-C(29) 108.0000	C(35)-C(36) 1.3948	N(39)-C(38)-H(55) 109.4618
N(15)-O(16) 1.3100	C(13)-C(11)-C(12) 120.0029	C(23)-H(55) 1.1000	N(30)-C(29)-H(59) 109.4618	C(34)-C(35) 1.3948	N(39)-C(38)-H(54) 109.4418
N(15)-O(9) 1.3100	C(13)-C(11)-N(10) 119.9986	C(23)-C(28) 1.3948	N(30)-C(29)-H(58) 109.4418	C(33)-C(34) 1.3949	N(39)-C(38)-C(33) 109.5000
C(13)-N(15) 1.2480	C(12)-C(11)-N(10) 119.9986	C(27)-C(28) 1.3949	N(30)-C(29)-C(26) 109.5000	C(32)-C(33) 1.3948	H(55)-C(38)-H(54) 109.5200
N(10)-C(11) 1.2660	H(28)-N(10)-C(11) 120.0000	C(26)-C(27) 1.3948	H(59)-C(29)-H(58) 109.5200	C(34)-H(51) 1.1000	H(55)-C(38)-C(33) 109.4618
N(8)-N(10) 1.2300	H(28)-N(10)-N(8) 120.0000	C(25)-C(26) 1.3948	H(59)-C(29)-C(26) 109.4618	C(40)-C(41) 1.3370	H(54)-C(38)-C(33) 109.4418
C(7)-N(8) 1.2600	C(11)-N(10)-N(8) 120.0000	C(24)-C(25) 1.3949	H(58)-C(29)-C(26) 109.4418	C(36)-H(52) 1.1000	H(53)-C(37)-C(32) 120.0002
C(3)-C(7) 1.3370	N(10)-N(8)-C(7) 115.0000	C(23)-C(24) 1.3948	N(38)-C(28)-C(23) 120.0002	C(37)-H(53) 1.1000	H(53)-C(37)-C(36) 120.0002
C(18)-H(33) 1.1130	H(27)-C(7)-N(8) 120.0000	N(30)-C(31) 1.2600	N(38)-C(28)-C(27) 120.0002	C(32)-N(47) 1.2660	C(32)-C(37)-C(36) 119.9996
C(18)-H(32) 1.1130	H(27)-C(7)-C(3) 120.0000	C(24)-H(56) 1.1000	C(23)-C(28)-C(27) 119.9996	C(12)-N(18) 1.2480	H(52)-C(36)-C(37) 120.0014

	N(8)-C(7)-C(3) 120.0000	C(27)-H(57) 1.1000	H(57)-C(27)-C(28) 120.0014	N(8)-C(9) 1.4700	H(52)-C(36)-C(35) 120.0014
	H(26)-C(6)-C(1) 118.0987	C(29)-N(30) 1.4700	H(57)-C(27)-C(26) 120.0014	C(7)-N(8) 1.2600	C(37)-C(36)-C(35) 119.9972
	H(26)-C(6)-N(5) 118.0987	C(1)-N(8) 1.2600	C(28)-C(27)-C(26) 119.9972	N(3)-C(4) 1.3509	N(48)-C(35)-C(36) 119.9984
	C(1)-C(6)-N(5) 123.8025	C(28)-N(38) 1.2480	C(29)-C(26)-C(27) 119.9984	C(2)-N(3) 1.3509	N(48)-C(35)-C(34) 119.9984
	C(6)-N(5)-C(4) 116.6193	C(12)-N(16) 1.2480	C(29)-C(26)-C(25) 119.9984	C(9)-H(25) 1.1130	C(36)-C(35)-C(34) 120.0033
	H(25)-C(4)-N(5) 118.1020	N(8)-C(10) 1.4700	C(27)-C(26)-C(25) 120.0033	C(9)-C(10) 1.4970	H(51)-C(34)-C(35) 119.9999
	H(25)-C(4)-C(3) 118.1020	C(4)-N(2) 1.3509	N(39)-C(25)-C(26) 119.9999	C(10)-C(15) 1.3948	H(51)-C(34)-C(33) 119.9999
	N(5)-C(4)-C(3) 123.7959	N(2)-C(5) 1.3509	N(39)-C(25)-C(24) 119.9999	C(11)-H(26) 1.1000	C(35)-C(34)-C(33) 120.0002
	C(4)-C(3)-C(2) 118.2346	C(1)-H(44) 1.1000	C(26)-C(25)-C(24) 120.0002	C(9)-H(24) 1.1130	C(38)-C(33)-C(34) 120.0015
	C(4)-C(3)-C(7) 120.8827	C(5)-C(6) 1.3858	H(56)-C(24)-C(25) 120.0015	C(14)-C(15) 1.3949	C(38)-C(33)-C(32) 120.0015
	C(2)-C(3)-C(7) 120.8827	C(3)-C(7) 1.3910	H(56)-C(24)-C(23) 120.0015	C(10)-C(11) 1.3948	C(34)-C(33)-C(32) 119.9969
	H(24)-C(2)-C(3) 120.3420	C(10)-H(50) 1.1130	C(25)-C(24)-C(23) 119.9969	C(13)-H(27) 1.1000	N(47)-C(32)-C(37) 119.9986
	H(24)-C(2)-C(1) 120.3420	C(10)-H(49) 1.1130	H(55)-C(23)-C(28) 119.9986	C(11)-C(12) 1.3949	N(47)-C(32)-C(33) 119.9986
	C(3)-C(2)-C(1) 119.3159	C(3)-C(4) 1.3858	H(55)-C(23)-C(24) 119.9986	C(12)-C(13) 1.3948	C(37)-C(32)-C(33) 120.0029
	H(23)-C(1)-C(6) 120.8842	C(13)-C(14) 1.3948	C(28)-C(23)-C(24) 120.0029	C(13)-C(14) 1.3948	O(17)-N(18)-O(16) 120.0000
	H(23)-C(1)-C(2) 120.8842	C(9)-C(11) 1.3948	H(54)-N(21)-Cu(40) 120.0000	C(6)-H(22) 1.1000	O(17)-N(18)-C(12) 120.0000
	C(6)-C(1)-C(2) 118.2317	C(10)-C(9) 1.4970	H(54)-N(21)-C(15) 120.0000	C(4)-H(21) 1.1000	O(16)-N(18)-C(12) 120.0000
		C(6)-C(1) 1.3370	Cu(40)-N(21)-C(15) 120.0000	C(5)-C(7) 1.3370	C(10)-C(15)-C(14) 119.9996
		C(14)-H(53) 1.1000	O(43)-N(16)-O(42) 120.0000	C(2)-H(20) 1.1000	H(28)-C(14)-C(15) 120.0014
		C(6)-C(7) 1.3910	O(43)-N(16)-C(12) 120.0000	C(1)-H(19) 1.1000	H(28)-C(14)-C(13) 120.0014
		C(5)-H(47) 1.1000	O(42)-N(16)-C(12) 120.0000	C(1)-C(6) 1.3910	C(15)-C(14)-C(13) 119.9972
		C(7)-H(48) 1.1000	N(21)-C(15)-C(9) 120.0002	C(5)-C(6) 1.3910	H(27)-C(13)-C(14) 119.9984

		C(13)-H(52) 1.1000	N(21)-C(15)-C(14) 120.0002	C(4)-C(5) 1.3858	H(27)-C(13)-C(12) 119.9984
		C(4)-H(46) 1.1000	C(9)-C(15)-C(14) 119.9996	C(7)-H(23) 1.1000	C(14)-C(13)-C(12) 120.0033
		C(9)-C(15) 1.3948	H(53)-C(14)-C(15) 120.0014	C(14)-H(28) 1.1000	N(18)-C(12)-C(13) 119.9999
		C(14)-C(15) 1.3949	H(53)-C(14)-C(13) 120.0014	C(1)-C(2) 1.3858	N(18)-C(12)-C(11) 119.9999
		C(12)-C(13) 1.3948	C(15)-C(14)-C(13) 119.9972		C(13)-C(12)-C(11) 120.0002
		C(11)-H(51) 1.1000	H(52)-C(13)-C(14) 119.9984		H(26)-C(11)-C(12) 120.0015
		C(11)-C(12) 1.3949	H(52)-C(13)-C(12) 119.9984		H(26)-C(11)-C(10) 120.0015
		C(3)-H(45) 1.1000	C(14)-C(13)-C(12) 120.0033		C(12)-C(11)-C(10) 119.9969
			N(16)-C(12)-C(13) 119.9999		C(15)-C(10)-C(11) 120.0029
			N(16)-C(12)-C(11) 119.9999		C(15)-C(10)-C(9) 119.9986
			C(13)-C(12)-C(11) 120.0002		C(11)-C(10)-C(9) 119.9986
			H(51)-C(11)-C(12) 120.0015		N(8)-C(9)-H(24) 109.4618
			H(51)-C(11)-C(9) 120.0015		N(8)-C(9)-C(10) 109.5000
			C(12)-C(11)-C(9) 119.9969		N(8)-C(9)-H(25) 109.4418
			N(8)-C(10)-C(9) 109.5000		H(24)-C(9)-C(10) 109.4618
			N(8)-C(10)-H(49) 109.4618		H(24)-C(9)-H(25) 109.5200
			N(8)-C(10)-H(50) 109.4418		C(10)-C(9)-H(25) 109.4418
			C(9)-C(10)-H(49) 109.4618		C(9)-N(8)-C(7) 108.0000
			C(9)-C(10)-H(50) 109.4418		N(8)-C(7)-H(23) 120.0000
			H(49)-C(10)-H(50) 109.5200		N(8)-C(7)-C(5) 120.0000
			C(15)-C(9)-C(11) 120.0029		H(23)-C(7)-C(5) 120.0000
			C(15)-C(9)-C(10) 119.9986		H(22)-C(6)-C(1) 120.3420
			C(11)-C(9)-C(10) 119.9986		H(22)-C(6)-C(5) 120.3420
			C(10)-N(8)-C(1) 108.0000		C(1)-C(6)-C(5) 119.3159
			C(3)-C(7)-C(6) 119.3159		C(7)-C(5)-C(6) 120.8842
			C(3)-C(7)-H(48) 120.3420		C(7)-C(5)-C(4) 120.8842
			C(6)-C(7)-H(48) 120.3420		C(6)-C(5)-C(4) 118.2317
			C(7)-C(6)-C(5) 118.2317		N(3)-C(4)-H(21) 118.0987
			C(7)-C(6)-C(1) 120.8842		N(3)-C(4)-C(5) 123.8025
			C(5)-C(6)-C(1) 120.8842		H(21)-C(4)-C(5) 118.0987
			N(2)-C(5)-C(6) 123.8025		C(4)-N(3)-C(2) 116.6193

			N(2)-C(5)-H(47) 118.0987		N(3)-C(2)-H(20) 118.1020
			C(6)-C(5)-H(47) 118.0987		N(3)-C(2)-C(1) 123.7959
			N(2)-C(4)-H(46) 118.1020		H(20)-C(2)-C(1) 118.1020
			N(2)-C(4)-C(3) 123.7959		H(19)-C(1)-C(6) 120.8827
			H(46)-C(4)-C(3) 118.1020		H(19)-C(1)-C(2) 120.8827
			H(45)-C(3)-C(7) 120.8827		C(6)-C(1)-C(2) 118.2346
			H(45)-C(3)-C(4) 120.8827		
			C(7)-C(3)-C(4) 118.2346		
			C(5)-N(2)-C(4) 116.6193		
			N(8)-C(1)-C(6) 120.0000		
			C(6)-C(1)-H(44) 120.0000		

3.4. Anti-bacterial Activity

The antibacterial potential of the synthesized Schiff base ligandand its corresponding metal complexes is summarized in Table 3. The results showed that the Schiff base exhibited notable activity against most of the tested bacterial strains.

The free Schiff base ligand was active against all the pathogenic bacteria. It showed the highest inhibition against *E. coli* (14.0 ± 0.5 mm) followed by *A. baumannii* (13.0 ± 0.6 mm), *E. faecalis* (12.0 ± 0.5 mm), *C. freundii* (11.0 ± 0.6 mm), *S. typhi* (11.5 ± 0.5 mm), and *B. subtilis* (10.0 ± 0.7 mm) at 200 µg/mL.

The metal complexes displayed varied activity. Among them, the CuL_2 complex showed the best overall performance in comparison to the other compounds. It exhibited good inhibition against *B. subtilis* (12.4 ± 0.6 mm), *C. freundii* (13.0 ± 0.7 mm), and *A. baumannii* (12.0 ± 0.8 mm). The CoL_2 complex demonstrated moderate to good activity particularly against *E. faecalis* (13.0 ± 0.7 mm) and *E. coli* (10.0 ± 0.5 mm). Both complexes were inactive against *S. typhi*.

Although the standard antibiotics (tetracycline and cephradine) produced larger inhibition zones, the synthesized Schiff base and its metal complexes demonstrated promising antibacterial potential. These findings suggest that the ligand and its complexes could serve as useful leads for the development of new antimicrobial agents.[23].

Table 6: Susceptibility results of theAntibacterial against the Schiff base and its metal complexes. The diameter of inhibition zones is expressed as mean +SD for triplicate assay for each concentration

Microbial Species	Strain type	Mean inhibition zone (mm)							
		100 µg per 1 mL			200 µg per 2 mL				
		L	CuL_2	CoL_2	L	CuL_2	CoL_2	TCN	CEF
E.coli	Gram +ve	13 ±0.6	8 ±0.8	10 ±0.7	14 ±0.5	8.7 ±0.6	10. 5±0.5	17 ±0.5	
E.bacelisa	Gram +ve	11 ±0.5	11 ±0.7	12.5 ±0.6	12 ±0.6	11 ±0.5	13 ±0.7	18 ±0.7	
B.Subtilis	Gram +ve	10 ±0.8	12.6 ±0.5	10.6 ±0.5	10 ±0.7	12.4 ±0.6	11 ±0.6	16.5± 0.6	
A.bactor	Gram -ve	12 ±0.7	9 ±1.0	7 ±0.8	13 ±0.6	12 ±0.8	7.6 ±0.7		16 ±0.5
C.bactor	Gram -ve	10 ±0.5	8.7 ±0.6	10 ±0.6	11 ±0.6	13 ±0.7	11 ±0.5		17 ±0.6
S.typhi	Gram -ve	10.3± 0.4	---	---	11.5 ±0.5	---	---		16 ±0.6

3.5. Antifungal Activity

The antifungal potential of the synthesized Schiff base ligand and its corresponding metal complexes is summarized in Table 4. The results showed that all the tested compounds exhibited moderate antifungal activity against the selected fungal strains.

The Schiff base ligand was active against all the pathogenic fungi. It showed the highest inhibition against *A. niger* (3.13 ± 0.48 mm) followed by *C. albicans* (2.73 ± 0.52 mm), *T. rubrum* (2.22 ± 0.46 mm), and *A. flavus* (2.04 ± 0.50 mm) at 200 µg/2 mL.

The copper(II) complex (CuL_2) showed the best overall inhibition performance in comparison to the other compounds. It exhibited the highest antifungal activity against *C. albicans* (3.42 ± 0.61 mm) followed by *A. flavus* (3.03 ± 0.49 mm), *A. niger* (3.07 ± 0.52 mm), and *T. rubrum* (2.30 ± 0.50 mm). The cobalt(II) complex (CoL_2) performed moderate activity against most of the tested fungi.

Although the standard drug fluconazole produced significantly larger inhibition zones, the synthesized Schiff base ligand and its metal complexes demonstrated promising moderate antifungal potential. These findings suggest that the ligand and its complexes could serve as useful leads for the development of new antifungal agents[24].

Table 7: Susceptibility results of the anti-fungal against the Schiff base and its metal complexes. The diameter of inhibition zones is expressed as mean +SD for triplicate assay for each concentration

Microbial Species	**Strain type**	**Mean inhibition zone (mm)**						
		100 µg per 1 mL			**200 µg per 2 mL**			
		L	CuL_2	CoL_2	L	CuL_2	CoL_2	Fluconazole
C.albicans	Fungi	2.27±0.45	2.37±0.51	2.36±0.48	2.73±0.52	2.42±0.61	2.53±0.55	5.80±0.70
A.niger	Fungi	2.90±0.4	3.03±0.55	2.99±0.50	3.13±0.48	3.18±0.52	3.07±0.45	6.20±0.65
A.flavous	Fungi	1.99±0.38	2.99±0.47	2.47±0.42	2.04±0.50	3.03±0.49	2.43±0.51	5.05±0.60
T.viride	Fungi	2.18±0.42	2.27±0.45	2.47±0.48	2.22±0.46	2.30±0.44	2.27±0.44	5.50±0.55

3.6. Anti-Oxidant Activity

All the synthesized complexes showed better antioxidant activity in comparison to the uncomplexed free Schiff base. Schiff base has the lowest antioxidant potential (36.74 ± 0.18%) at the concentration of 500 µg/mL[25]. Among the complexes, CuL_2 showed the best scavenging activity (73.40 ± 0.23% inhibition) at the concentration of 500 µg/mL. The CoL_2 complex has also shown good antioxidant performance (62.40 ± 0.26%) at the same concentration[26].

Table 8: Antioxidant Activity of Schiff base at different concentrations

DPPH Antioxidant activity Schiff base				
S.No	**Concentration**	**Absorbance of sample**	**% Radical Scavenging**	**Standard %Inhibit**
1	1000 mcg/ml	0.742	2.62±0.03	18.12±0.23
2	500 mcg/ml	0.634	16.7±0.09	32.45±0.15
3	250 mcg/ml	0.482	36.7±0.18	48.18±0.19
4	125 mcg/ml	0.397	47.9±0.20	70.43±0.15
5	62.5 mcg/ml	0.364	52.2±0.23	80.63±0.05

mcg= microgram Absorbance of Control= 0.376

Table 9: Antioxidant Activity of copper (II)complex at different concentrations

DPPH Antioxidant activity Cu				
S.No	**Concentration**	**Absorbance of sample**	**% Radical Scavenging**	**Standard %Inhibit**
1	1000 mcg/ml	1.048	42.19±0.03	18.12±0.23
2	500 mcg/ml	0.63	52.07±0.09	32.45±0.15
3	250 mcg/ml	1.007	64.09±0.18	48.18±0.19
4	125 mcg/ml	1.036	73.40±0.20	70.43±0.15
5	62.5 mcg/ml	0.592	52.2±0.23	80.63±0.05

mcg= microgram Absorbance of Control= 0.376

Table 10: Antioxidant Activity of cobalt (II)complex at different concentrations

DPPH Antioxidant activity Co				
S.No	**Concentration**	**Absorbance of sample**	**% Radical Scavenging**	**Standard %Inhibit**
1	1000 mcg/ml	0.13	82.9±0.25	18.12±0.23
2	500 mcg/ml	0.287	62.3±0.22	32.45±0.15

3	250 mcg/ml	0.121	84.1±0.25	48.18±0.19
4	125 mcg/ml	0.135	82.2±0.24	70.43±0.15
5	62.5 mcg/ml	0.132	82.6±0.26	80.63±0.05

mcg= microgram Absorbance of Control= 0.376

CONCLUSION

By the condensation method afresh ligand has beensynthesized of 2, 4 Dinitrophenyl Hydrazine (DNP) and Pyridine-3-al. The synchronizationcapability of ligand has been described by physical characteristics, micro-analytical data, ^{1}H Nuclear magnetic resonance (NMR). According to FTIR data, the preferred orientation of the ligand is bidentate and coupled to oxygen through an azomethine group and phenol, generating a stable chelate. The coordinated octahedral form of the metal complexes was discovered through structural characterization of the ligand's metal chelates. According to a biological experiment, metal complexes are much more active than ligands.

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