

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL SCREENING OF SCHIFF BASE METAL COMPLEXES DERIVED FROM ISONIAZID

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Abstract

New Schiff base was synthesized by reacting isonicotinoylhydrazones with anisaldehyde to get a new Schiff base ligand *N*-(4-methoxybenzylidene) pyridine-4-carbohydrazide. Newly synthesized ligand was successfully complexed with Cu(II), Co(II) and Zn(II) metal ions in alcoholic medium. The ligand and complexes obtained were characterized quantitatively and qualitatively by using micro elemental analysis, IR spectroscopy and UV–Vis spectroscopy. Spectral study reveals that, metal centre moieties are four-coordinated with square planar geometry for Cu(II) and Co(II) complexes and tetrahedral geometry for and Zn(II) complex. Synthesized compounds were tested for antimicrobial and antifungal activity. The preliminary *in vitro* antibacterial screening activity revealed that complexes showed moderate activity against tested bacterial strains and slightly higher compared to the ligand. The synthesized Schiff base ligand and the complexes were also screened for the *in vitro* antifungal studies and studies show that the metal complexes were found to be more active than the ligand.

Key words

Isonicotinoylhydrazones, anisaldehyde, antimicrobial activity, antifungal activity

1. Introduction

Schiff bases ligands and their metal complexes have a variety of applications in biological, clinical, analytical and industrial fields [1], which makes them extensively studied class of compounds in chemistry [2]. Schiff bases represent an important class of ligands in coordination chemistry, producing very stable complexes with several transition metals generally in (II) oxidation states [3]. Among these, heterocyclic Schiff base ligands and their metal complexes do have significant interest because of their pharmacological properties [4]. Present investigation deals with the synthesis of Schiff base derived from an amino and carbonyl compound which is an important class of ligands that coordinate to metal ions via azomethine nitrogen [5-8]. In literature, several azomethine derivatives have been reported to possess remarkable anticancer, antibacterial, antifungal and antimalarial activities [9] due to the presence of C, N linkage.

Due to the easy synthetic approachability and structural variability, schiff base complexes are considered to be important stereo chemical simulations in transition metal - coordination complex chemistry [10, 11]. Study of the interaction between drugs and transition metals is an important and active research area in chemistry [12]. Metal ions used to play a vital role during the biological process of drug utilization in the body. The high affinity of the Schiff bases toward the transition metal ions to form complexes upon chelation is utilized in preparing their solid complexes [13]. Through the present work studies were conducted to synthesize some biologically active Schiff base ligand and their transition metal complexes.

2. Experimental

All the reagents were used namely Isonicotinoylhydrazones (INH), anisaldehyde, metal chlorides and ethanol is of synthetic grade.

Elemental microanalysis of the compounds, C, H and N were performed using Shimadzu elemental vario EL III model elemental analyser. Melting points of the ligand and its complexes were recorded on an OMEGA melting point apparatus. The pH measurement was done on the Elico-16 pH meter. The IR spectra were obtained in a KBr disk using a BIO-RAD FTS 135 spectrometer, the UV-VIS spectra were recorded in PC based double beam spectrophotometer 2202 in N,N'-dimethylformamide (DMF) solution.

2.1 Synthesis of Schiff base ligand

To an ethanoic solution of INH (0.01M), solution of anisaldehyde (0.01M) in ethanol was added dropwise. The above mixture was refluxed for 4-5 hours in a water bath. Then the reaction mixture was evaporated and cooled at room temperature. The separated white crystals of ligand was washed with alcohol, ether and recrystallized from ethanol (yield: 76%). The prepared compound was checked for purity by TLC using glass plates percolated with silica gel 60 GF254 and suitable solvent system as mobile phase.

2.2 Synthesis of metal complexes

Alcoholic solution of metal salt (Copper (II) chloride, Cobalt (II) chloride and Zinc (II) chloride of 0.01M) were added to alcoholic solution of newly synthesized Schiff base *N'*-(4-methoxybenzylidene) pyridine-4-carbohydrazide (0.02M) in 1:2 (metal: ligand) molar ratio. Then, the mixture was heated under reflux for half an hour. The resulting coloured precipitate was collected by filtration and washed with methanol and finally recrystallized from ethanol.

2.3 Biological assay: preparation of nutrient broth

Nutrient broth gel was prepared to study antimicrobial activity of the synthesised compounds. Different concentrations of metal complex in a test tube (0.05, 0.1, 0.15, 0.2 and

0.25 M) were taken for Gram positive and Gram negative bacteria respectively. Nutrient broth gel was added to each test tube and covered with cotton plug. The solution is then kept in an autoclave about an hour and after autoclaving it is cooled at room temperature. By using Broth Dilution Method, it was tested for gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Escherichia coli*. Added two drops of Schiff base solution and complex solution to each test tube using pipette and stirred well before keeping it in BOD incubator. After 24 hours microbial growth was examined by comparing it with the control solution. Both showed positive results.

The newly synthesized compounds were also screened for their antifungal activity against *Aspergillus flavus*, *Chrysosporium keratinophilum* and *Candida albicans* (MTCC 227). The compounds were dissolved in DMSO and antifungal activity was determined by well plate method at concentration of 1 and 0.5 mg/mL. The required amounts of each fungal strain were removed from the stock and suspended in 5ml of distilled water with 2 drops of Tween 80. This suspension was uniformly spread on Petri plates containing Potato dextrose agar media using sterile swabs. After applying the samples into the wells formed by using the same technique for tests on bacteria, the plates were incubated at 25°C for 3 days. The plates were then examined for the presence of zone of inhibition and the results were recorded. Flucanazole was used as a positive control at a concentration of 0.5 and 1 mg/ml.

3 Result And Discussion

3.1 Physical measurements

Schiff base complexes are obtained by heating under reflux of respective metal salt with ligand in 1:2 molar ratios. The purity of the ligand and complexes were checked by TLC using glass plates percolated with silica gel 60 GF254 and suitable solvent system as mobile phase. The ligand and metal complexes are of different colours, the ligand is white while the complex of Cu(II), Co(II) and Zn(II) are red, orange and yellow in colour respectively. The molar conductance of all the complex was measured in DMF using 10^{-3} M solutions at room

temperature. The low molar conductivity values suggest the non-electrolytic nature of the metal complex [14]. The Schiff base ligand is soluble in common organic solvents. The resultant Schiff base complexes are soluble in DMF and insoluble in other common organic solvents. Complexes gave a sharp melting point indicating the isolation of fairly pure complex. The micro-elemental analysis for C, H and N of the complex obtained was in agreement with the predicted formula for complex. Physical properties of the ligand and its complexes were listed in Table 1.

Table 1: Analysis physical data of Schiff base and its complex

Compound	Formula	Colour	M.P(°C)	Yield %	Cal (found) %			UV (λ_{max})
					C	H	N	
Ligand	C ₁₄ H ₁₃ N ₃ O ₂	White	118-120	76	65.87 (65.72)	5.13 (5.24)	16.46 (16.24)	440 nm
Copper complex	[Cu(C ₅₆ H ₅₂ N ₁₂ O) Cl ₂ .H ₂ O]	Red	240-248	84	65.46 (65.34)	5.18 (5.16)	16.65 (16.53)	650 nm
Cobalt complex	[Co(C ₅₆ H ₅₂ N ₁₂ O) Cl ₂ .H ₂ O]	Orange	280-285	86	65.46 (65.41)	5.18 (5.20)	16.65 (16.62)	640 nm
Zinc complex	[Zn(C ₅₆ H ₅₂ N ₁₂ O) Cl ₂ .H ₂ O]	Yellow	245-250	79	65.46 (65.39)	5.18 (5.22)	16.65 (16.60)	550 nm

3.2 Chemistry and electronic spectra

The condensation reaction of INH and anisaldehyde under reflux conditions results in the formation of the Schiff base ligand namely *N'*-(4-methoxybenzylidene) pyridine-4-carbohydrazide. The ligand is subsequently reacted with chloride salts of Cu(II), Co(II) and

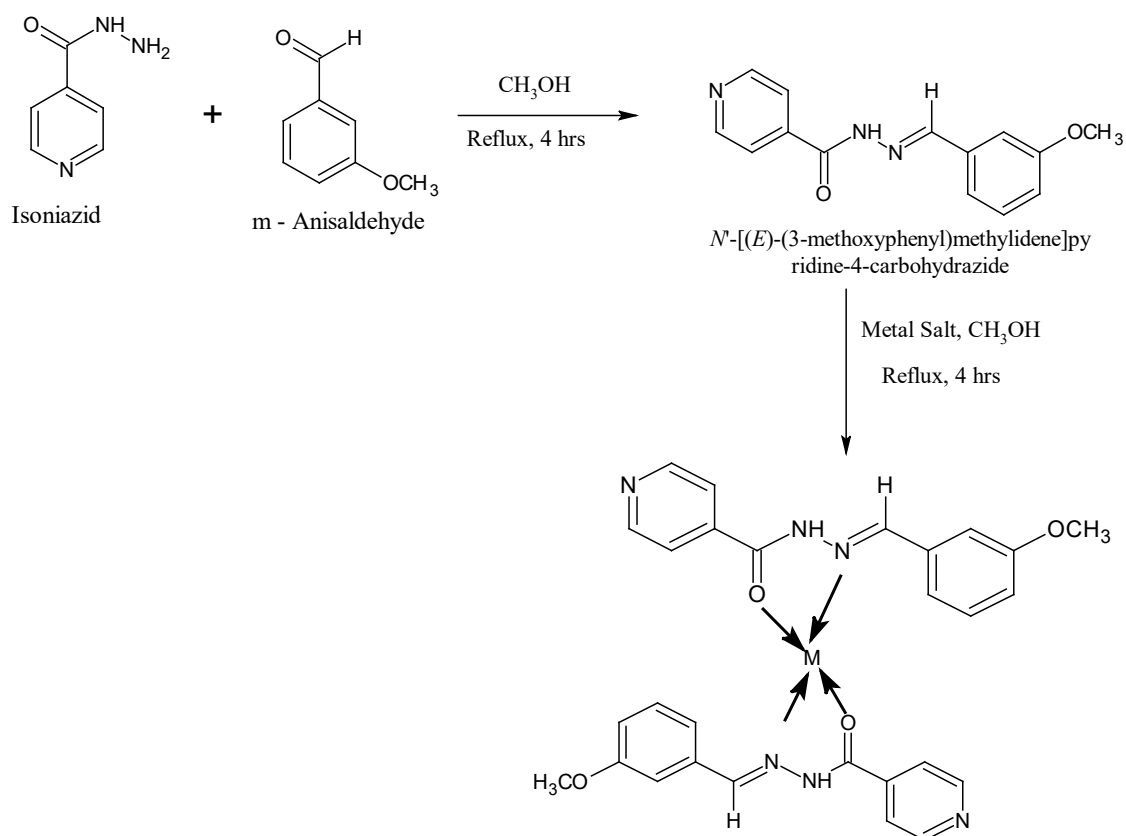
Zn(II) to give corresponding metal complexes. The proposed synthetic route of the ligand and the complexes are given in Scheme 1.

The electronic spectra of ligands and complexes are recorded in the 200–800 nm range. Transitions for the Schiff base ligands which corresponded to the imine ($-C=N$) attributed to $\pi-\pi^*$ and $n-\pi^*$ transitions around 335 nm and 433 nm, respectively [15]. In complex the transitions $n-\pi^*$ appeared in Schiff base may shift either to the lower or higher wavelength region [16]. The shift indicated metal ion coordination via nitrogen atoms of imine groups of the Schiff bases [17]. Due to diamagnetic nature of zinc (II) metal complexes shows $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ transitions attributed to imine groups. The electronic spectra of copper and nickel complex shows one broad band around 650 and 640 nm due to ${}^2B_{1g} \rightarrow {}^2A_{1g}$ transition suggesting a square planar geometry [18].

3.3 Stoichiometry of the reaction product

Complex obtained are studied in DMF solution to determine the M/L ratio in the complex by Job's Method [19]. A series of solutions are prepared with a constant concentration (10^{-3} M) of the metal ion and ligand. The M/L ratio was determined from the relationship between the absorption of the absorbed light and the mole ratio of M/L. The study shows that the metal to ligand, ratio is 1:2 stoichiometric in nature. In view of this result, a reaction mechanism is proposed and metal to ligand ratio is to be considered as 1:2.

Scheme: Reaction of INH with anisaldehyde followed by the complex formation (M=Cu, Co, Zn)



3.4 Infrared spectroscopy

The IR spectra of the free ligand is compared with those of the complex in order to determine the coordination sites that may be involved in chelation. There are some characteristic peaks in the spectra of the ligands, which are helpful in predicting the bonding mode of ligand and metal. The main bands in the IR spectra of ligand and its complexes are presented in (Table 2). The IR spectrum of the ligand exhibits a strong band at 1661 cm^{-1} due to $\nu(\text{C}=\text{O})$ of the amide group (Figure 1). This has strongly displaced in complex indicating coordination through the carbonyl oxygen. A band at 1598 cm^{-1} due to $\nu(\text{C}=\text{N})$ azomethine group has shifted to lower frequency, 1514 , 1550 , 1512 cm^{-1} in complexes [30-22]. This suggests the involvement of the azomethine nitrogen in chelation. In the lower frequency region, the weak bands observed at 500 to 400 cm^{-1} (Figures 2a, 2b, 2c) have been assigned

respectively to the M-N and M-O vibrations in metal complexes [23]. Accordingly, spectral study reveals the involvement of azomethine nitrogen and the carboxylate oxygen in chelation.

Fig 1. IR spectra of the Schiff base

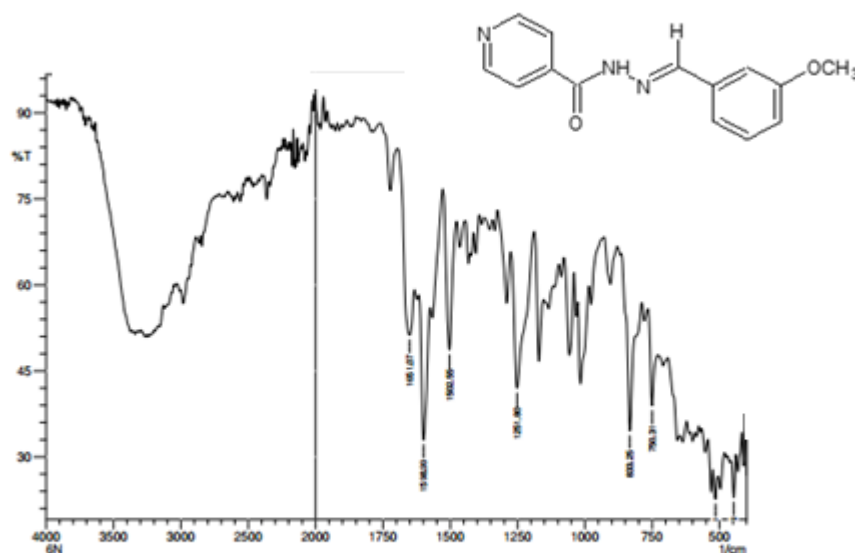


Fig 2a. IR Spectrum of the metal complex (M=Cu)

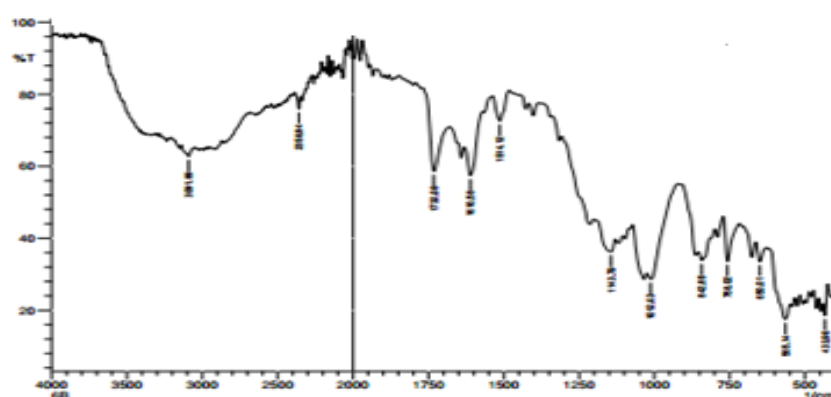


Fig 2b. IR Spectrum of the metal complex (M=Co)

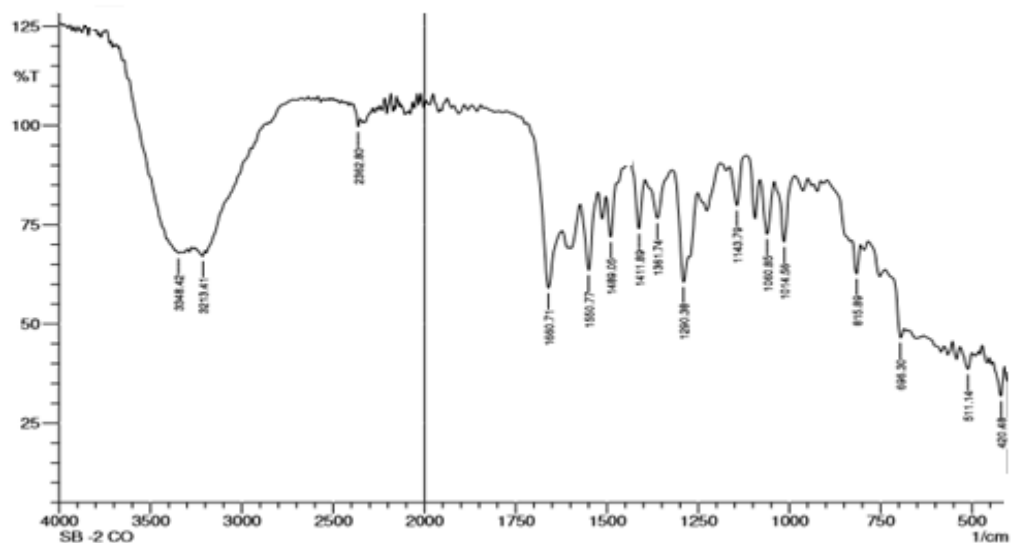


Fig 2c. IR Spectrum of the metal complex (M=Zn)

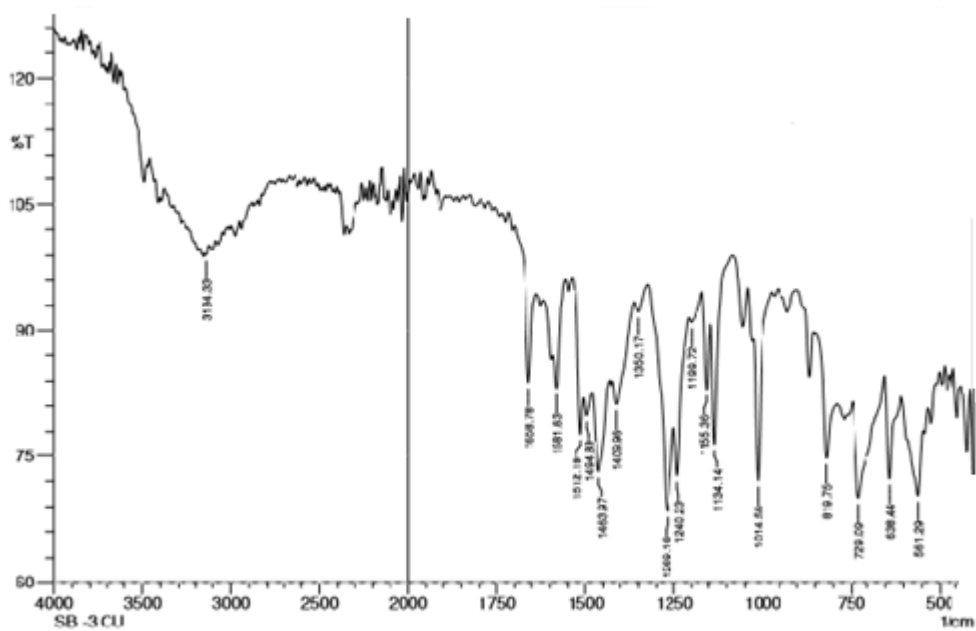


Table 2. IR frequencies in (cm^{-1}) of the ligand and its metal complex

Ligand/complex	ν (C=O)	ν (C=N)	ν (M-N)	ν (M-O)
L=(Schiff base)	1661	1598	-	-
[Cu(L) Cl ₂ H ₂ O]	-	1514	565	433
[Co(L) Cl ₂ H ₂ O]	-	1550	511	420
[Zn(L) Cl ₂ H ₂ O]	-	1512	541	440

3.4 Antimicrobial activity

The antibacterial activity of synthesized ligand and their metal complexes were screened against two bacteria, *Staphylococcus aureus* (Gram positive) and *Escherichia coli* (Gram negative) by using Broth Dilution Method. Results reveal that the metal complexes shows better inhibitory activity than Schiff base ligand (Table 3). Chelation of organic species with metal atom results in the enhancement of biological activity [25]. According to Tweedy's chelation theory [26], chelation reduces the polarity of the metal atom by the partial sharing of its positive charge with donor groups which leads to greater π -electron delocalization in the entire metal complex ring [27]. Thus, enhances the lipophilicity of the complex. This increased lipophilic character of the complex favours the complex permeation through the lipid layers of the cell membrane of the bacteria.

Table 3. Antimicrobial study of Schiff base and metal complexes

Compound name	<i>Escherichia coli</i> (Gram negative)					<i>Staphylococcus aureus</i> (Gram positive)				
	0.05	0.1	0.15	0.2	0.25	0.05	0.1	0.15	0.2	0.25
Schiff base (mm)	14.1 ±0.2	13.6± 0.2	16.0 ±0.2	14.6 ±0.2	14.6± 0.2	09.1± 0.2	12.1± 0.2	11.1 ±0.2	10.1± 0.2	09.1± 0.2
Copper complex (mm)	15.2 ±0.2	15.6± 0.2	16.6 ±0.2	17.6 ±0.2	17.6± 0.2	09.6± 0.2	11.6± 0.2	13.6 ±0.2	11.6± 0.2	12.6± 0.2
Cobalt Complex (mm)	16.9 ±0.2	15.3± 0.2	17.3 ±0.2	18.5 ±0.2	15.3± 0.2	09.2± 0.2	13.9± 0.2	15.6 ±0.2	11.6± 0.2	13.6± 0.2
Zinc Complex (mm)	18.7 ±0.2	16.8± 0.2	18.3 ±0.2	20.3 ±0.2	18.6± 0.2	09.9± 0.2	15.6± 0.2	16.6 ±0.2	11.6± 0.2	14.6± 0.2
Std. Streptomycin (mm)	17.2 ±0.1	18.3± 0.1	20.3 ±0.1	21.3 ±0.1	21.3± 0.1	10.6± 0.2	17.6± 0.2	19.6 ±0.2	19.6± 0.2	19.6± 0.2

3.5 Antifungal activity

The *in vitro* antifungal activities of the compounds were tested against *Aspergillus niger*, *Chrysosporium Keratinophilum* and *Candida Albicans* by well plate method [28, 29] at concentration of 0.5 and 1 mg/mL. Synthesized metal complexes showed stronger antifungal activities than the free ligand. Flucanazole was used as the standard. Although there is a sufficient increase in the fungicidal activity of the ligand complex as compared to the free ligand (Schiff base), they cannot attain the effectiveness of the conventional standard fungicides. The results of antifungal studies are summarized in Table 4.

Table 4. Antifungal study of Schiff base and complex

Microbs	<i>Aspergillus Flavus</i>		<i>Chrysosporium Keratinophilum</i>		<i>Candida Albicans</i>	
	1	0.5	1	0.5	1	0.5
Conc. in mg/ml	1	0.5	1	0.5	1	0.5
Control	00		00		00	
Schiff base (mm)	12±0.6	5±0.3	8±0.4	5±0.5	15±0.7	14±0.3
Copper complex	14±0.5	7±0.8	11±0.8	7±0.3	20±0.9	16±0.5
Cobalt complex	15±0.9	8±0.5	12±0.3	9±0.8	21±0.5	17±0.3
Zinc complex	16±0.3	9±0.8	13±0.5	11±0.3	22±0.9	19±0.3
Std. Flucanazole	17±0.2	10±0.1	16±0.2	14±0.2	23±0.2	20±0.2

4 Conclusion

In the present work we have synthesized Schiff base by condensation of INH with anisaldehyde and their metal complexes. Physico-chemical and various spectral studies revealed that ligand having oxygen and nitrogen donor atom were formed effectively. Synthesized Schiff base was complexed with Cu(II), Co(II) and Zn(II) metal with 1:2 (metal:ligand) stoichiometry. Compounds have shown to exhibit promising antimicrobial and antifungal activity. The increase in the anti-bacterial and antifungal activity of the ligand complexes may be attributed due to the effect of the metal ion on the normal cell processes. Further studies are required to explore these complexes as effective drug. To examine the possible antifungal effect of metal complexes on the fungal cell membrane, ergo sterol, sorbitol assay can be evaluated which gives further insights about the synthesised complexes as effective antifungal drug. This work can be further applied for the development of novel ligand – transition metal complexes as effective biological agents.

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