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Synthesis, Characterization and Antimicrobial Investigation of Transition Metal Complexes by using Schiff Base Containing N-, O- Donor Atoms

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Abstract

Schiff base was prepared by the condensation of 5-aminosalicylic acid and 2-hydroxy-3-methoxybenzaldehyde in methanol. A series of transition metal complexes of Cu (II), Cd (II), Co (II), Zn (II) and Ni (II) were synthesized by using Schiff base as ligand. Schiff base and metal complexes were characterized on the bases of melting point, solubility, colour of product and FTIR analysis. The ligand and metal complexes were screened for antibacterial analysis against two of which Gram positive bacteria (*B. subtillus, S. aureus*) and two Gram negative bacteria (*E. coli, P. multocida*) and antifungal analysis against *Ganoderma lucidum, Alterharia lterhata, Penicillium notatum* and *Aspergillus flavus* by using disc diffusion method.

Key Words: *Schiff base; Metal Complexes; Transition Metals; Antibacterial; Antifungal activities*

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Introduction

The Schiff bases and their metal complexes have more importance recently because of their application as biological, biochemical, analytical, antimicrobial, anticancer, antibacterial, and antifungal and anti-tumor activity [1]. They have been studied as a class of ligands and are known to coordinate with metal ions through the azomethine nitrogen atom. The synthesis of transition metal complexes with Schiff base ligands are studied due to sensitivity, selectivity and synthetic flexibility towards metal atoms. They used as catalyst, in medicine like antibiotics and anti-inflammatory agents and in the industry as anticorrosion. In this paper we describe the behavior of the bidentate aromatic Schiff base ligand with various transition metal (II) ions [2]. Now a days, the use of Schiff bases as ligand is very common. They are used as intermediates for preparation of amino acid and for synthesis of complex with transition metals. Coordination of Schiff base to the metal takes place through the nitrogen atom of azomethine group and oxygen atom of the phenolic group. Schiff bases are widely used for metal complexation because of formation of stable complexes [3].

Many metallic elements are very important in living organism because they play a very important role in proper functioning of living organism. These metals are also called metals of life [4]. Four metals are very important these are sodium, magnesium, calcium and potassium. But transition elements i.e. chromium, manganese, iron, cobalt, nickel, copper and zinc are also very important in living organisms to perform their proper functioning [5]. In living organism, transition metals are present in very minute quantities usually at trace levels at molecular level these transition metals are also very important in the formation of Schiff base complexes [6]. From the nineteenth century, Schiff base metal complexes are well known in different field of life synthesis of Schiff base metal complexes are very important rather than Schiff bases in the development of coordination chemistry [7].

The aim of the present study was to prepare the Schiff bases by the condensation of 5-aminosalicylic acid and 2-hydroxy-3-methoxy-benzaldehyde in methanol. These Schiff bases were used to synthesize the different transition metal complexes of Cu (II), Cd (II), Co (II), Zn (II) and Ni (II) as ligand. The characterization of these Schiff base and metal complexes were carried out on the bases of melting point, solubility, colour of product and FTIR analysis. The ligand and metal complexes were screened for antibacterial analysis against two of which Gram positive bacteria (*B. subtillus, S. aureus*) and two Gram negative bacteria (*E. coli, P. multocida*) and antifungal analysis against *Ganoderma lucidum, Alterharia lterhata, Penicillium notatum* and *Aspergillus flavus* by using disc diffusion method.

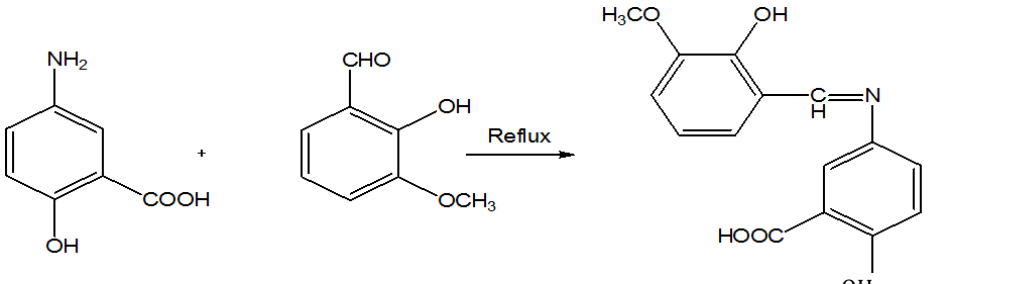
Experimental

Present work describes the synthesis of Schiff bases and their metal complexes. For the synthesis of these compounds, different glass wares are used which were oven and flame dried. All the commercially available reagents and chemicals were used as such without further purification. By using standard procedures solvents were dried, where necessary. Thin-layer chromatography was used to determine the progress and completion of reactions. In thin-layer chromatography (TLC) commercial aluminium backed Merck plates, coated with silica gel GF_{254} (0.25 mm thick), was used having a fluorescent indicator which was active at 254 nm wavelength. UV light at 254 nm was used to visualize the chromatograms.

Melting points were determined in open capillary tubes on an electro thermal (Griffin 1090) melting point. For the determination of IR, Perkin Elmer 1600-FT spectrometer and Matter son Satellite spectrometer fitted with a Spec arch Golden Gate ATR sampling platform were used and IR spectra were recorded on it in the range 4000-250 cm^{-1}. The results of IR were reported in wavenumbers (cm^{-1}). For IR analysis, samples were applied as solids or as neat liquids or in the form of KBr discs.

Synthesis of Schiff base 1

5-aminosalicylic acid (9.7 mmol) was dissolved in 15 ml methanol in beaker, (9.7 mmol) of 2-hydroxy-3-methoxybenzaldehyde was also dissolved in 15 ml methanol. Mix these two solutions in round bottom flask and 3-4 drops of acetic acid was also added. Reflux it for about two and half hours. Reddish orange colour precipitate appeared. Reaction mixture was cooled to room temperature and placed in refrigerator for about 24 hours. The precipitated solid was filtered and washed with methanol.



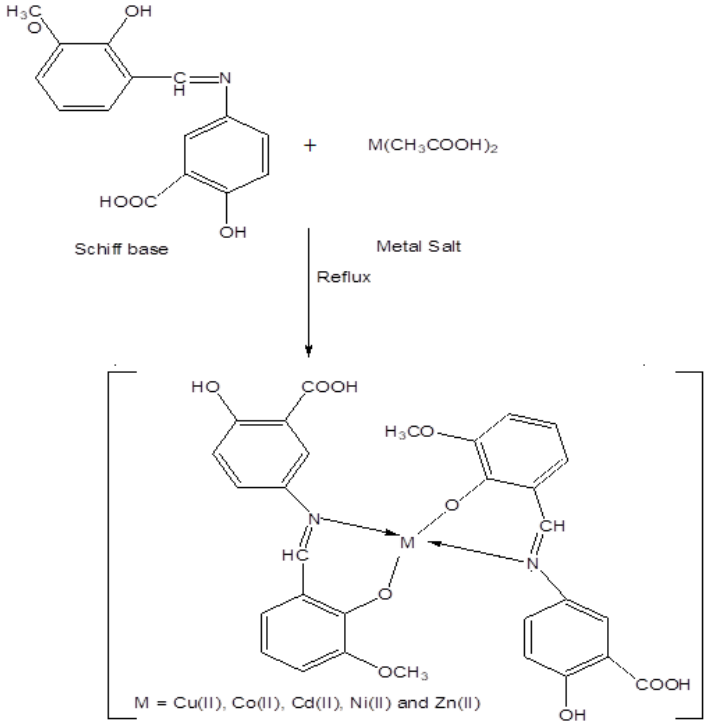
5-aminosalicylic acid

2-hydroxy-3-methoxy benzaldehyde

2-Hydroxy-5-[(2-hydroxy-3-methoxy benzlidene)-amino]-benzoic acid

Synthesis of Metal Complexes

Schiff base (3.5 mmol) was dissolved in 10 ml ethanol in a beaker and metal salt (1.75 mmol) was dissolved in 10 ml ethanol in a beaker. Mix these two solutions in round bottom flask. Reflux it for four hours. Metal to ligand ratio was 1:2 (w/w). Coloured precipitates appear. Filter these precipitate and washed with ethanol.



Structure of Metal Complexes

Antibacterial Assay by Disc Diffusion Method

Disc diffusion method was used to determine the antibacterial activities against two gram positive bacteria (*B. subtillus, S. aureus*) and two Gram negative bacteria (*E. coli, P. multocida*). Nutrient agar media was prepared by suspending the 28 g nutrient agar in one liter distilled water. The media was autoclaved for 15 min at 121°C. Nutrient media (100 mL) was taken in beaker, to the media 100 µL of inocula was added and mixed well. Transfer the mixture (media and inocula) to the petri plate. Sample (100 µL) was applied on each flat paper discs. These discs were laid on the growth medium having bacterial strain in petri plates and incubated it for 37°C for 24 hours for the growth of bacteria. The active samples inhibit the growth of bacteria and clear zone were formed. Zone reader was used to measure the zone of inhibition [8].

Antifungal Assay by Disc Diffusion Method

Disc diffusion method was used to determine the antifungal activities against *Ganoderma lucidum, Alterharia lterhata, Penicillium notatum* and *Aspergillus flavus*. Potato dextrose agar media was prepared. The media was autoclaved for 15 min at 121°C. Potato dextrose agar media (100 mL) was taken in beaker, to the media 100 µL of inocula was added and mixed well. Transfer the mixture (media and inocula) to the petri plate. Sample (100 µL) was applied on each flat paper discs. These discs were laid on the growth medium having fungal strain in petri plates and incubated it for 28°C for 48 hours for the growth of fungus. The active samples inhibit the growth of fungus and clear zone were formed. Zone reader was used to measure the zone of inhibition [9].

Results and Discussion

The transition metal (II) complexes with bidentate aromatic Schiff base as ligand were prepared by stirring stoichiometric amounts of metal (II) salts and aromatic Schiff base derived from 5-aminosalicylic acid and 2-hydroxy-3-methoxybenzaldehyde. Analytical data and some Physical properties of the Schiff base as ligand and their metal complexes are listed in (Table 1).

Sample code	R_1	R_2	Physical state	Molecular formula	Molecular mass (g/mol)	Melting Point (°C)	Yield (%)
Schiff base 1	5-aminosalicylic acid	2-hydroxy-3-methoxybenz-aldehyde	Reddish orange solid	$C_{15}H_{13}O_5N$	287	207-211	71
1Cu	Schiff base 1	Copper acetate	Greenish solid	$C_{30}H_{24}O_{10}N_2Cu$	635.5	291-294	68
1Cd	Schiff base 1	Cadmium acetate	Light orange Solid	$C_{30}H_{24}O_{10}N_2Cd$	684.4	314-319	65
1Co	Schiff base 1	Cobalt acetate	Dark yellowish Solid	$C_{30}H_{24}O_{10}N_2Co$	631	301-304	71
1Zn	Schiff base 1	Zinc acetate	Light yellowish Solid	$C_{30}H_{24}O_{10}N_2Zn$	637.4	321-325	69
1Ni	Schiff base 1	Nickel Chloride	Yellowish solid	$C_{30}H_{24}O_{10}N_2Ni$	630	333-337	73

Table 1: *Physical characteristics data of Schiff base and metal complexes.*

The complexes were characterized by the usual methods melting point, colour of product, solubility and FTIR analysis. The complexes are stable in air and light and are soluble in organic solvents such as DMF and DMSO. (Table 2).

Sample Code	Dist. Water	Methanol	Ethanol	Ethyl acetate	n-Hexane	$CHCl_3$	DMF	DMSO
Schiff base 1	I	I	S	S	PS	S	S	S
1Cu	I	I	I	PS	I	PS	S	S
1Cd	I	PS	I	I	I	PS	S	S
1Co	I	I	I	PS	I	S	S	S
1Zn	I	I	PS	PS	I	PS	S	S
1Ni	I	PS	I	PS	I	PS	S	S

Table 2: *Solubility of Schiff bases and complexes.*

I = Insoluble

S = Soluble

PS = Partially soluble

Antibacterial Activity of Schiff base and Complexes

The Schiff bases and complexes were screened for their antibacterial activity against four bacterial strains by using disc diffusion method. Two of which, gram positive bacteria (*B. subtillus, S. aureus*) and two gram negative bacteria (*E. coli, P. multocida*). Results are summarized in (Table 3). In order to compare the results, Ampicillin is used as standard control drug. It was observed from the tabulated data of that the antibacterial activity of the Schiff bases are lower than the standard drug Ampicillin and the complexes showed higher activity than that of Schiff bases which are used as ligands. From the results, it is clear that complexes showed more activity towards Gram positive bacteria such as *B. subtillus* and *S. aureus* as compare to Gram negative strains such as *E. coli* and *P. multocida* due to the fact that structures of cell wall of Gram negative bacteria's are more complex as compare to Gram positive bacteria's.

Sample No.	Bacterial inhibition zone (mm)			
	E. coli	*S. aureus*	*B. subtilis*	*P. multocida*
Schiff base 1	14.5	15.5	22.5	16
1Cu	17.5	21	24.5	20
1Cd	22	30	29	24
1Co	16	26	15.5	17
1Zn	24.5	22	26	18
1Ni	23	25.5	23	22.5
Ampicillin	32	30	31	29

Table 3: *Antibacterial activity of Schiff base and Complexes.*

Values are mean of three individual replicates
Concentration = 10 mg/ml of DMSO - = No activity

Antifungal Activity of Schiff base and Complexes

In vitro tests for antifungal activity of Schiff bases and their complexes were screened against four fungal strains *Ganoderma lucidum, Alterharia lterhata, Penicillium notatum* and *Aspergillus* flavus by using disc diffusion method. Fluconazole was used as standard control drug for the determination of antifungal activity. The results of antifungal activity were shown in (Table 4). According to results, complexes (1Cd, 1Ni) show more activity against *Ganoderma lucidum, Alterharia lternaria* and *Penicillium notatum.* The increased activity of complexes may be due to coordination of Schiff base with transition metals [10].

Sample No.	Fungal inhibition zone (mm)			
	G. lucidum	*A. lternaria*	*P. notatum*	*A. flavus*
Schiff base 1	26	19.5	24	17.5
1Cu	31	23	27	23.5
1Cd	33	30	35.5	26
1Co	29	28.5	19	18
1Zn	26.5	22	27	21.5
1Ni	28	17.5	29	24
Fluconazole	37	36	35	31

Table 4: *Antifungal activity of Schiff base and Complexes.*

Values are mean of three individual replicates
Concentration = 10mg/ml of DMSO - = No activity

Infrared Spectroscopy

The infrared spectroscopy of Schiff base 1 and complexes (1Cu-1Ni) were recorded in the range 4000-250 cm^{-1} as KBr pellets. IR spectra of Schiff bases and their complexes showed remarkable broad bands as shown in (Table 5). The IR spectra provide valuable information regarding the nature of functional groups attached to ligand and metal atom in complexes. In order to study the bonding bond of Schiff base and metal complexes, IR spectrum of free ligand was compared with the spectra of complexes. A band in free Schiff base appeared at 1638.06 cm^{-1} was due to C=N vibrational frequency. The azomethine peak (1638.06 cm^{-1}) in Schiff base 1 was shifted

to lower value 1610.56-1623.64 cm^{-1} suggesting the coordination. The shifting of this group to lower frequency in the metal complexes when compared to free Schiff base, suggested the coordination of metal ion through nitrogen atom of azomethine group. It is expected that coordination of metal atom would reduce the electron density in the azomethine link and thus lower the –HC=N absorption. Peaks in the range 540.07-582.50 cm^{-1} in complexes were due to M-N bonding and peaks in the range 435.04-480.82 cm^{-1} in complexes were due to M-O bonding [11].

Sample code	υ (C = N) (cm^{-1})	υ (-OCH_3) (cm^{-1})	υ (M-N) (cm^{-1})	υ (M-O) (cm^{-1})
Schiff base 1	1638.06	1242.16	-	-
1Cu	1610.56	1238.30	540.07	480.82
1Cd	1623.64	1234.44	576.72	422.41
1Co	1614.42	1236.37	578.64	435.04
1Zn	1620.21	1244.09	572.86	439.77
1Ni	1613.78	1232.51	582.50	435.91

Table 5: *IR data of Schiff base and complexes.*

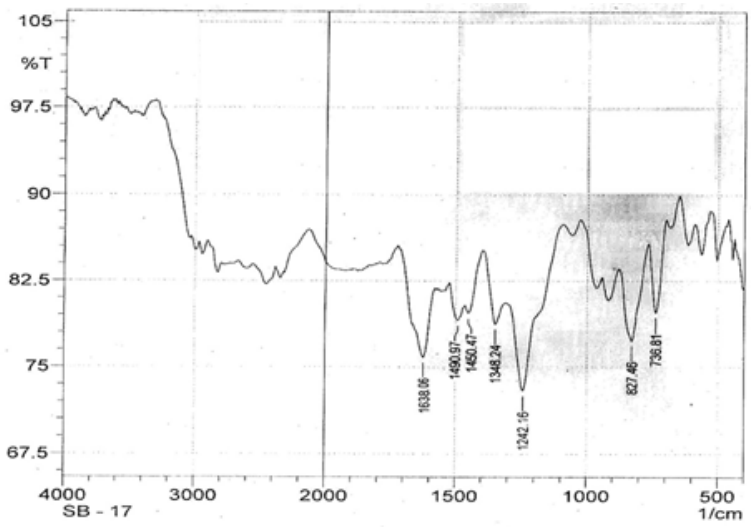


Figure 1: *IR Spectra of Schiff base 1.*

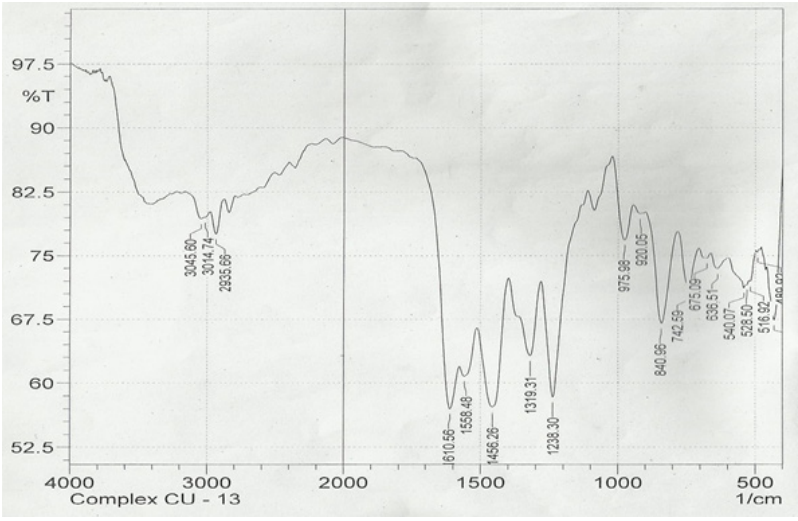


Figure 2: *IR Spectra of Complex 1Cu.*

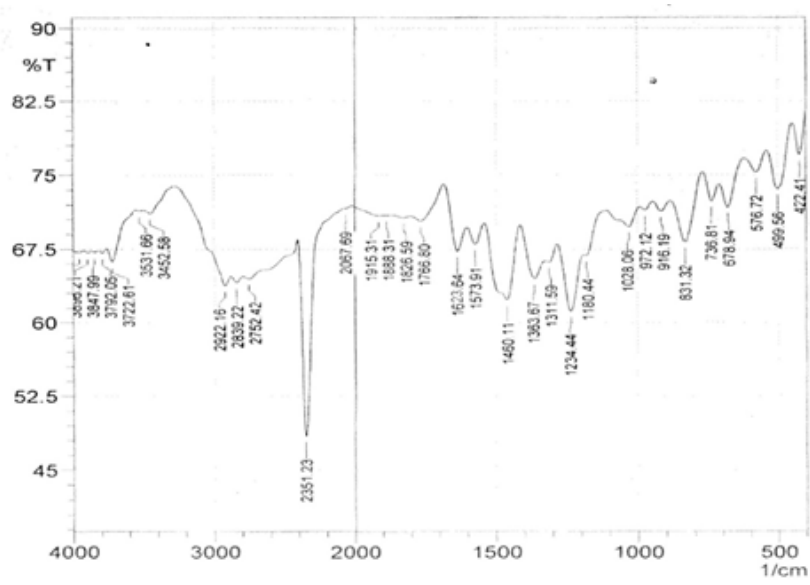


Figure 3: *IR Spectra of Complex 1Cd.*

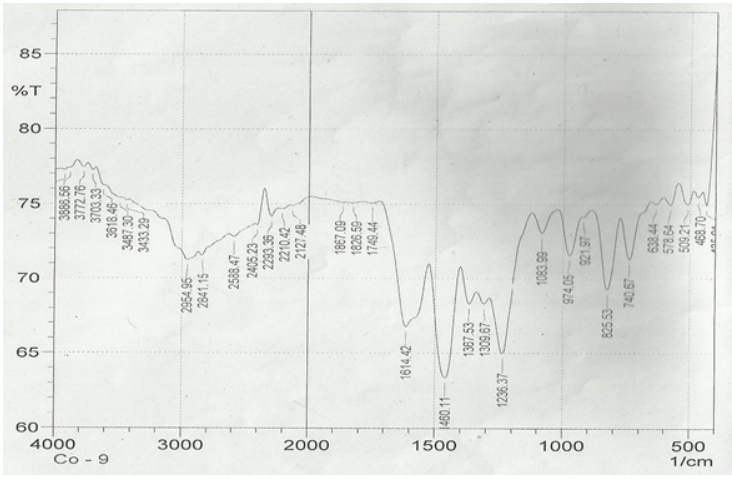


Figure 4: *IR Spectra of Complex 1Co.*

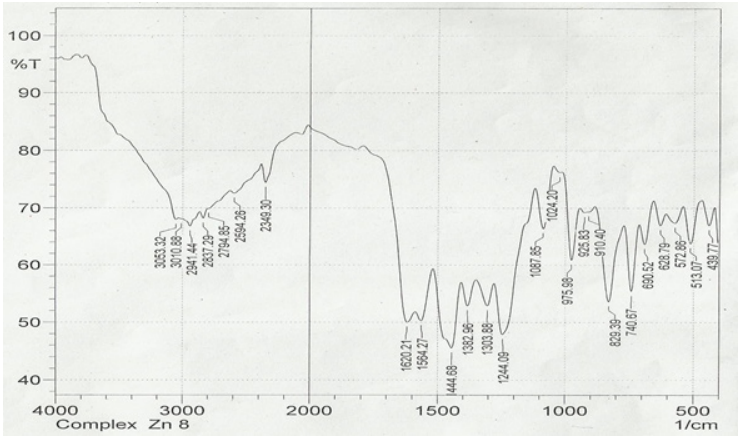


Figure 5: *IR Spectra of Complex 1Zn.*

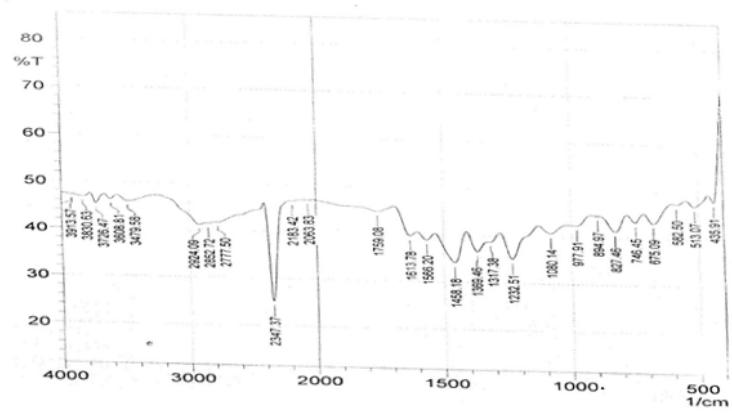


Figure 6: *IR Spectra of Complex 1Ni.*

Conclusion

Schiff base of 5-aminosalicylic acid, 2-hydroxy-3-methoxybenzaldehyde and its metal complexes of Cu (II), Cd (II), Co (II), Zn (II) and Ni (II) were synthesized and characterized by analytical and spectral techniques. These compounds were tested against different of bacterial and fungal strains and exhibited promising activity against all the tested microorganisms.

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Conflict of Interest

Authors declare that they have no conflict of interest.

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