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Schiff Base Complexes of Copper (II) and Cobalt (II) and Their Biological Studies

Abstract

The Schiff base (BAP), benzilideneaminophenol was synthesized by refluxing ethanolic solutions of benzaldehyde and 2-aminophenol. Cu (II) and Co (II) complexes of the Schiff base with stoichiometry $[M(BAP)_2(H_2O)_2]$ have also been synthesized. The IR studies indicate that the Schiff base is acting as a bidentate ligand, and is co-ordinated to the metal through azomethine nitrogen and phenolic oxygen atom. IR spectra also indicate the presence of co-ordinated water. Octahedral geometry of these complexes could be confirmed from the electronic and NMR spectral data and magnetic moment values. The Schiff base acts as a promising candidate as an antimicrobial agent for E. coli and A. Niger.

Keywords: Schiff base, copper, cobalt, benzilideneaminophenol, benzaldehyde, 2-aminophenol, antimicrobial agent

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INTRODUCTION

The increase in the mortality rate associated with infectious diseases is directly related to bacteria that exhibit multiple resistances to antibiotics. The lack of effective treatments is the main cause of this problem. The development of new antibacterial agents with novel and more efficient mechanisms of action is definitely an urgent medical need [1-3]. Schiff bases of benzophenone derivatives are reported to show biological activities such as cytotoxic activities against human oral squamous carcinoma cells. Schiff base derived from furylglyoxal and para toluidine show antibacterial activity against Escherica coli. Complexes of thallium (II) with benzothiazolines show antibacterial activity against pathogenic bacteria. Isatin derived Schiff bases show anti HIV activity. Thiazole and benzothiazole Schiff bases possess effective antifungal activity [4]. Amino Schiff bases derived with aromatic and heterocyclic amines possess high activity against human tumor cell lines [5]. Synthetic flexibility of Schiff base permits the synthesis of multi dentate ligands of diverse structural type. Schiff bases are reported to have antimicrobial activities.

The aim of the present work is to synthesize a Schiff base from benzaldehyde and o-aminophenol. The objective of the work is to investigate the coordination character of the Schiff base formed with biologically important metals like Cu (II) and Co (II) and to investigate the antimicrobial activities of the ligand and complexes.

MATERIALS AND METHODS

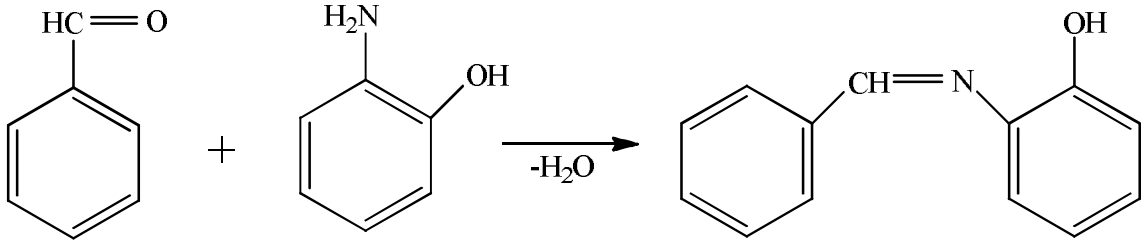
Reagents

All the chemicals used for synthetic purposes were of analar grade. A.R quality copper sulphate and cobalt nitrate were used. Ethanol was used as the solvent for the preparation of complexes. Commercial ethanol was purified by standard methods [6]. Other reagents employed are LR samples of 2-aminophenol and benzaldehyde.

Methods of Synthesis

Preparation of ligand (Schiff Base)

The Schiff base was prepared using **Scheme 1**. A mixture of 1.06g of benzaldehyde (1M) in 10mL ethanol and 1.09g of 2-aminophenol (1M) in ethanol was refluxed for 6-7 hours and left overnight at room temperature. The solid colored product formed was filtered, washed with ethanol. It was dried at room temperature and re-crystallized with ethanol to form the required Schiff base.



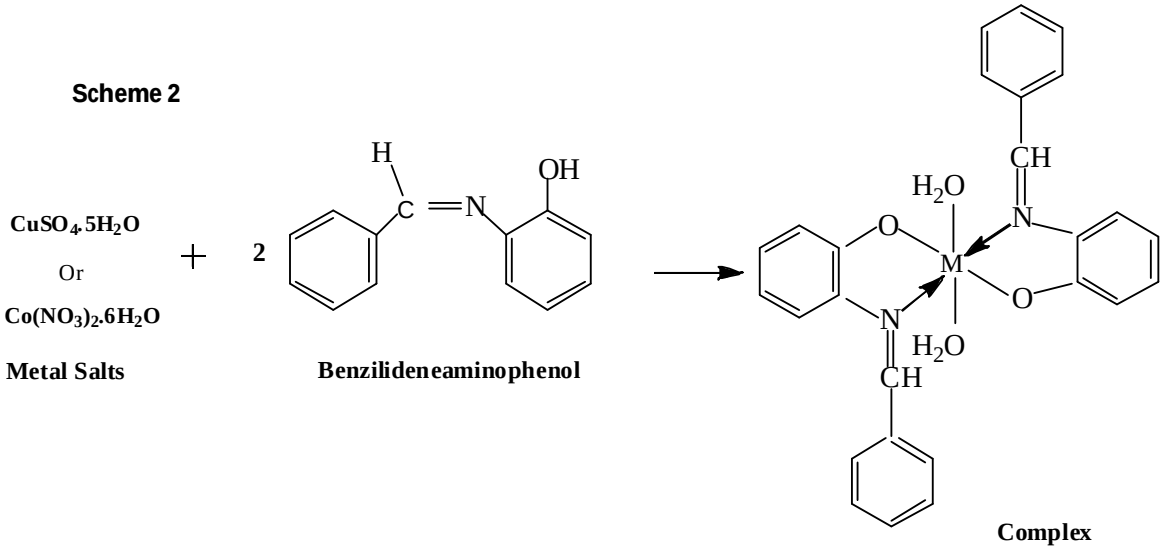
Benzaldehyde **2-aminophenol** **Benzilideneaminophenol**

Scheme 1

Preparation of complexes

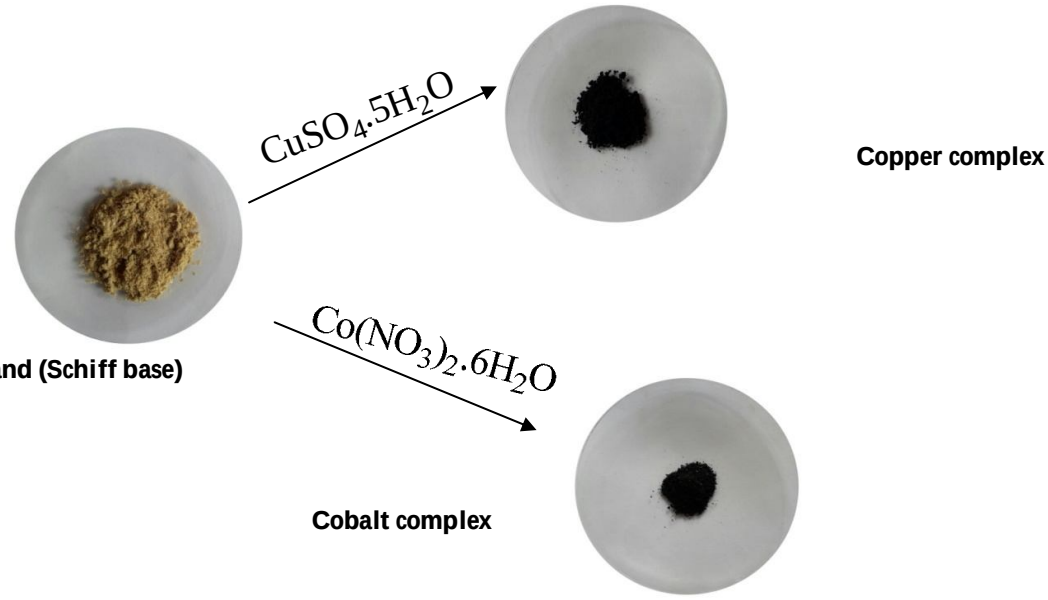
The complexes have been prepared using **Scheme 2**. To the metal salt solution, an ethanolic solution of the ligand was added gradually in small portions with good stirring, keeping the metal to ligand mole ratio at 1:2. Immediate color change indicates the formation of complexes. The contents were refluxed in a water bath for 6 hours. The solid complexes separated were filtered and washed several times with ethanol. The complexes were dried in a desiccator over anhydrous calcium chloride.

Scheme 2



Metal Salts **Benzilideneaminophenol** **Complex**

M= Cu (II)
Co (II)



Instruments Used

The IR Spectra were recorded by KBr pellet method in the range 400-4000 cm^{-1} on a Schimadzu DR-43S FT-IR Spectrometer at Govt. College for Women, Thiruvananthapuram. The thermal stability of the complexes was investigated using TG-DTA technique, in which the mass of the sample is continuously recorded as a function of time or temperature. The TGs were recorded on a Perkin Elmer thermal analysis system with a heating rate of $10^{o}C$/min in nitrogen atmosphere in the temperature range 35-950°C. The electronic spectra of the ligand and the complexes were taken in ethanol in the range 200 nm to 700 nm on a Perkin Elmer λ25- UV Visible Spectrophotometer. Magnetic susceptibility of the complexes was measured at room temperature (28±2^{0}C) on a Sherwood Scientific Magnetic Susceptibility Balance. The gram susceptibility was determined from the equation,

$\chi_g = I(R-R_0)/W\times10^9$, R = reading with sample, R_0 = reading with empty tube, I = length of sample column, W = weight of sample

$\chi_m = \chi_g \times M$, M = Molecular mass of the complex,

$\chi_m^{corre} = \chi_m$ + diamagnetic correction

The effective magnetic moment, μ_{eff} was calculated using the relation,

$\mu_{eff} = 2.84\sqrt{\chi_m^{corre}\times T}$, where μ_{eff} is the observed magnetic moment and T is the temperature in Kelvin.

The ^{1}H NMR and ^{13}C NMR spectra of the ligand and complexes were recorded in JEOL GSX 400 NBFT NMR Spectrometer at CSIR-NIIST, Thiruvananthapuram. Elemental analyses of the complexes were determined by micro analytical method using Elementar Variomicrocube CHNS analyser at CSIR-NIIST, Pappanamcode, Thiruvananthapuram. Molar conductance of the complex in methanol was determined at room temperature (28°C) using direct reading type Systronic Conductivity Meter Model No. 306. The cell constant of the conductivity meter was 1.02 cm^{-1}. Approximately 10^{-3} M solutions were used.

In vitro antimicrobial screening was performed by disc diffusion method. The compounds $C_{13}H_{11}NO$, $[Cu(C_{13}H_{11}NO)_2(H_2O)_2]$ and $[Co(C_{13}H_{11}NO)_2(H_2O)_2]$ were tested against the bacteria E. Coli and the fungus A. Niger. The activity was determined after 24 hours of incubation at room temperature (37°C) and was expressed in terms of mm by measuring the diameter of zone of inhibition [7].

Procedure: Disc Diffusion Method

The antibacterial activity of ligand and complexes were determined by the disc diffusion method. The investigated isolates of Escherichia coli bacteria were seeded in tubes with nutrient broth (NB). The seeded NB (1cm^3) was homogenized in the tubes with 9cm^3 of melted (45°C) nutrient agar (NA). The homogeneous suspensions were poured into Petri dishes. The discs of filter paper (diameter 5mm) were ranged on the cool medium. After cooling on the formed solid medium, 2×10^{-5} dm^3 of the investigated compounds were applied using a micropipette. After incubation for 24 h in a thermostat at 25-27°C, the inhibition (sterile) zone diameters (including disc) were measured and expressed in mm. The concentration of each solution was 1.0×10^{-3} mol dm^3. Commercial DMF was employed to dissolve the tested samples and was also used as control. Erythromycin was used as positive standard drug. An inhibition zone diameter over 8mm indicates that the tested compounds are active against the bacteria under investigation.

Antibacterial screening

The in vitro antibacterial activities were investigated using Disc diffusion method. The activity of tested compounds was studied against the human pathogenic bacteria species, Escherichia coli (MTCC-433) (as gram negative bacteria). Centrifuged pellets of bacteria from a 24h old culture containing approximately 10^4-10^6 CFU (colony forming unit) per ml were spread on the surface of Nutrient agar (tryptone1%, yeast extract 0.5%, NaCl 0.5%, agar 1%, 1000 ml of distilled water, pH 7.0) which was autoclaved under 121°C for at least 20 min. Wells were created in medium with the help of a sterile metallic bores and then cooled down to 45°C. The activity was determined by measuring the diameter of the inhibition zone (in mm). 100ml of the tested samples (10 mg mL^{-1}) were loaded into the wells of the plates. All compounds were prepared in dimethyl formamide (DMF). DMF was loaded as control. The plates were kept for incubation at 37°C for 24h and then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each bacterium culture. Streptomycin was used as antibacterial standard drugs.

Antifungal screening

The prepared compounds were screened separately in vitro for their antifungal activity against fungal species, namely, Aspergillus Niger on Sabouraud dextrose agar plates. The culture of fungi was purified by single spore isolation technique. The antifungal activity was determined by disc diffusion method by the following procedure: Sabouraud dextrose agar plates: A homogeneous mixture of glucose-peptone-agar (40:10:15) was sterilized by autoclaving at 121°C for 20 min. The sterilized solution (25 mL) was poured in each sterilized petri dish in laminar flow and left for 20 min to form the solidified sabouraud dextrose agar plate. These plates were inverted and kept at 30°C in incubator to remove the moisture and to check for any contamination. Dimethyl formamide (DMF) was loaded as control. The plates were kept for incubation at 30°C for 3-4 days and then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each fungus. Clotrimazole was used as references to evaluate the antifungal activity.

RESULTS AND DISCUSSION

The complexes reported here are stable, coloured and non hygroscopic. They are soluble in ethanol and methanol.

Elemental Analysis

The data of elemental analysis are shown in the Table 1. The experimental values are in good agreement with the calculated values.

Table 1: Percentage composition of the ligand and complexes

Compound	% C Calcd.(found)	% H Calcd.(found)	% N Calcd.(found)	% M Calcd.(found)
$C_{13}H_{11}NO$	79.16 (79.33)	5.63 (5.25)	7.11 (6.96)	----
$[Cu(C_{13}H_{11}NO)_2(H_2O)_2]$	63.46 (63.04)	4.93 (4.55)	5.69 (5.23)	12.92 (12.69)
$[Co(C_{13}H_{11}NO)_2(H_2O)_2]$	68.56 (68.25)	5.32 (5.54)	6.15 (5.97)	12.94 (12.76)

Magnetic moment

Generally the magnetic moments of Cu (II) complexes range from 1.85-2.20 BM. However stereochemistry of the molecule influences the magnetic moment. For octahedral complexes, the magnetic moments are in the range 1.9-2.0 BM. The magnetic moment measured at room temperature for the present Cu (II) complex is 1.95BM. This suggests an octahedral structure for the synthesized copper (II) complex. Usually at room temperature, the magnetic moment of octahedral Co (II) complex ranges from 4.7 to 5.2 BM. The present Co (II) complex has a magnetic moment of 4.75 BM. This value suggests an octahedral structure for the cobalt (II) complex.

Molar conductance

The molar conductance measurements of complexes were carried out using 10^{-3} molar concentration of the complexes in methanol. The values indicate that copper and cobalt complexes are non electrolytes. The values are presented in Table 2.

Table 2: Molar conductance of Cu(II) and Co(II) complexes

Complex	Molar conductance in methanol ($ohm^{-1}cm^2mol^{-1}$)	Assignment
$[Cu(C_{13}H_{11}NO)_2(H_2O)_2]$	10.5	Non electrolyte
$[Co(C_{13}H_{11}NO)_2(H_2O)_2]$	15.6	Non electrolyte

IR Spectral Studies

In the IR spectrum of the ligand (Fig.1), there is a strong band at 3329cm^{-1}. This band is assignable to H bonded OH group in the ligand. Also there is an intense band at 1622cm^{-1} which can be assigned to the stretching of the azomethine C=N group [8]. In the IR spectra of complexes, the band around 3329cm^{-1} corresponding to H bonded OH is found to be absent which suggests that the ligand is co-ordinated to the metal after deprotonation at the phenolic OH. The IR spectrum shows a broad absorption in the region 3000-3500 cm^{-1} indicating the presence of coordinated water molecules in both the complexes [9,10]. The two weaker bands at 842 cm^{-1} and 748 cm^{-1} in copper complex (Fig.2) and those at 844 cm^{-1} and 752 cm^{-1} in the cobalt complex (Fig.3) can be assigned to the rocking and wagging vibrations of water in these complexes. Hence the IR spectrum suggests the presence of co-ordinated water molecules in these complexes. The band observed at 1622cm^{-1} in the spectrum of ligand shows an upward shift to 1695cm^{-1} in these complexes indicating that the co-ordination is through azomethine nitrogen. The bands at 582 cm^{-1} and 499 cm^{-1} in copper complex and at 582cm^{-1} and 455cm^{-1} in cobalt complex, which are not present in spectrum of ligand can be assigned to υ_{M-N} and υ_{M-O} vibrations respectively [11]. The spectral data are shown in Table 3.

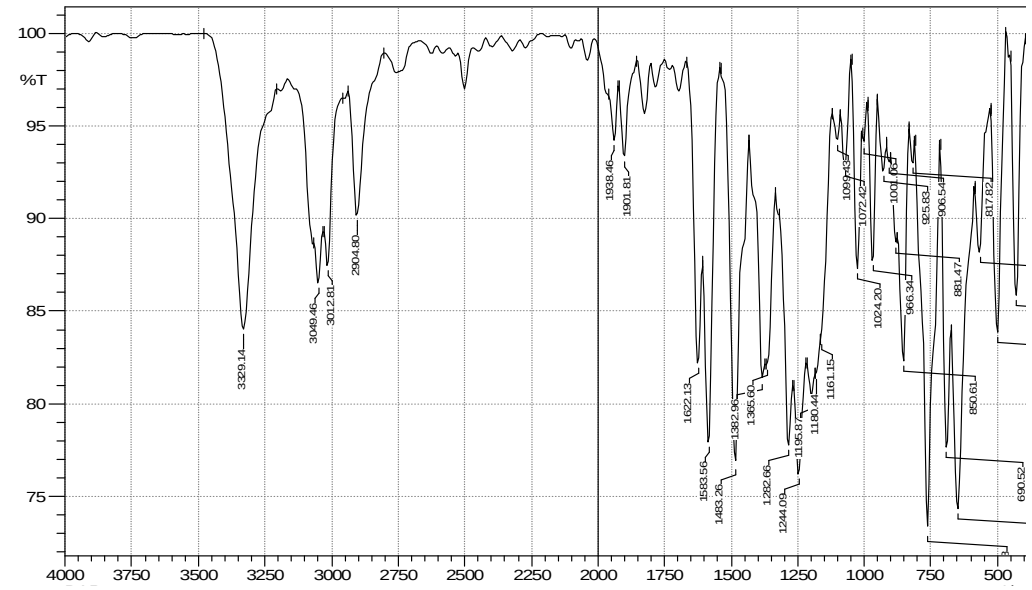


Figure 1: IR Spectrum of Ligand benzilidineaminophenol

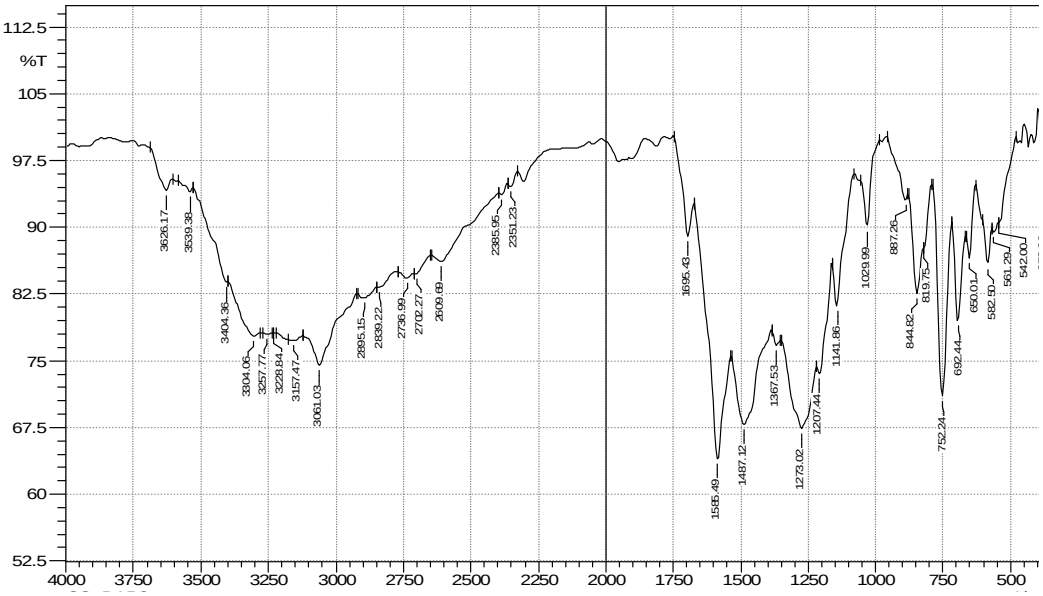


Figure 2: IR spectrum of cobalt complex

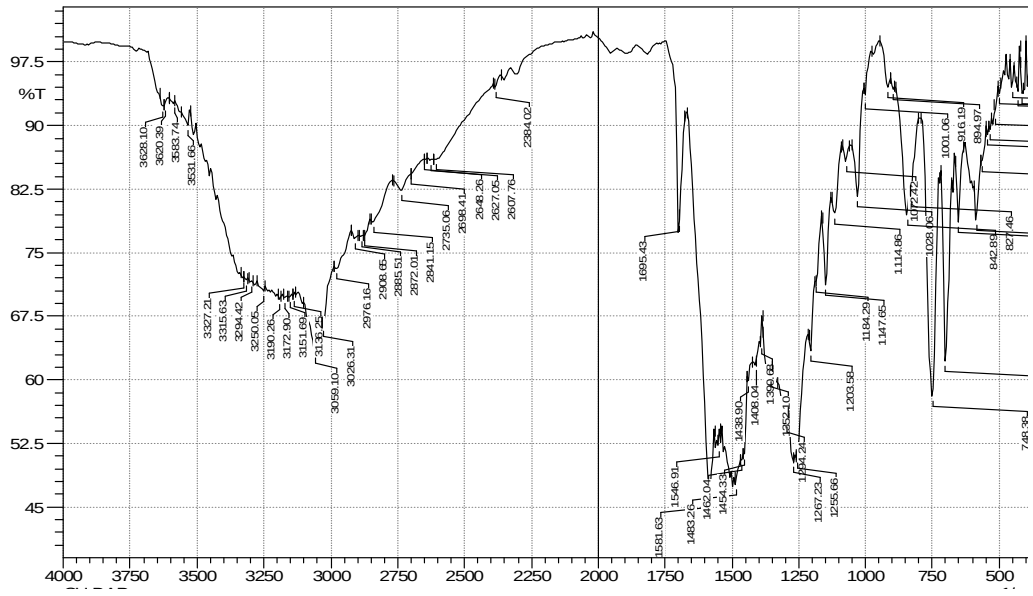


Figure 3: IR spectrum of copper complex

Table 3: IR bands of Schiff base and its Cu (II) and Co (II) complexes

Ligand (cm^{-1})	Copper complex (cm^{-1})	Cobalt complex (cm^{-1})	Assignment
3329	----	----	υH-bonded OH
1622	1695	1695	$\upsilon_{C=N}$
1483-1583	1483-1581	1487-1585	$\upsilon_{ring\ skeletal}$
3012-3049	3026-3059	3061	$\upsilon_{aromatic\ C-H}$
1244	1255	1273	$\upsilon_{phenolic\ C-O}$
----	3000-3500	3000-3500	$\upsilon_{coordinated\ water}$
----	582	582	υ_{M-N}
----	499	455	υ_{M-O}

UV-Vis Spectra

In the electronic spectra of the ligand in ethanol, there are two bands at 225nm and 317 nm. These bands are assignable to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions respectively. The absorption bands of the complexes are shifted to shorter wavelengths compared to that of the ligand (Table 4).

Table 4: Electronic spectral data of ligand and complexes

Compound	λ_{max} (nm)	Assignment
$C_{13}H_{11}NO$	225 317	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$
$[Cu(C_{13}H_{11}NO)_2(H_2O)_2]$	218 232	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$
$[Co(C_{13}H_{11}NO)_2(H_2O)_2]$	219 230	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$

NMR Spectral Studies

^{1}H NMR Studies

The ^{1}H NMR spectrum of ligand shows multiplet at δ6.9-7.5 ppm, a singlet at δ8.7 ppm and a singlet at δ1.6ppm corresponding to aromatic proton, OH proton and methine proton respectively. The complexes showed all expected signals for BAP protons. The signals of phenyl protons (δ6.9-7.5ppm) have been shifted to 6.3-8.3 ppm in both copper and cobalt complexes upon co-ordination to the metal ion. The signal corresponding to OH proton (δ8.7ppm) is absent in the ^{1}H NMR spectra of both the complexes indicating the co-ordination through phenolic oxygen atom to the metal. The signal corresponding to methine proton has also been shifted in both the complexes [12]. The spectral data are shown in Table 5.

Table 5: ^{1}H NMR spectral data of ligand and complexes

Compounds	Aromatic proton	-OH proton	-CH= proton
Ligand	6.9-7.5	8.7	1.6
Copper complex	6.3-8.3	----	1.29
Cobalt complex	6.4-8.3	----	1.29

^{13}C NMR Studies

^{13}C NMR of complexes show chemical shift values corresponding to all the carbon atoms present in the ligand, with shift in the δ values of each carbon indicating complexation [13]. The chemical shift values obtained are given in Table 6.

Table 6: ^{13}C NMR spectral data of ligand and complexes

Compounds	C-N or C-O	Aromatic	>C=N
Ligand	76.9-77.4	115-135	157
Copper complex	48.49-49.51	101-130	161
Cobalt complex	47.08-48.1	100-128	138

Thermogravimetric Studies

The thermograms of copper and cobalt complexes were recorded in an atmosphere of nitrogen at heating rate of 10°C/min up to a temperature 950°C. The TGA of $[Cu(C_{13}H_{11}NO)_2(H_2O)_2]$ shows a mass loss of 7% (7.3%, calculated) from 100-150°C and that of $[Co(C_{13}H_{11}NO)_2(H_2O)_2]$ indicates a mass loss of 7.4% (7.9%, calculated) from 100-175°C, corresponding to the removal of two water molecules co-ordinated to these metal ions. Further the decomposition takes place between 200-600°C. There is no step characteristic of "free" ligand, indicating that the ligands are co-ordinated to the metal centers.

Antimicrobial Screening

The in vitro biological screening effects of the investigated compounds were tested against the bacteria Escherichia Coli and Aspergillus Niger by using disc diffusion method. The inhibition zone of antibacterial and antifungal activities of ligand and its complexes are shown in Table 7. Photographs showing antibacterial and antifungal activities are given in figures 4-5.

Table 7: Inhibition zone of Antimicrobial Screening Studies

Compound	Zone of Inhibition (mm)	
	E. coli	A. Niger
$C_{13}H_{11}NO$ (Ligand)	19	9
$[Cu(C_{13}H_{11}NO)_2(H_2O)_2]$	NZ	NZ
$[Co(C_{13}H_{11}NO)_2(H_2O)_2]$	7	NZ

NZ: no zone

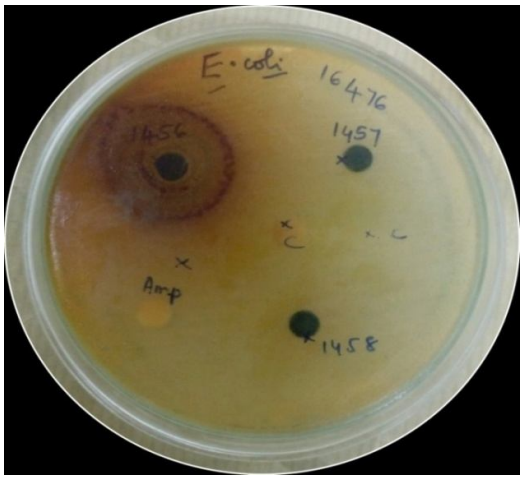


Figure 4: Antibacterial Activity

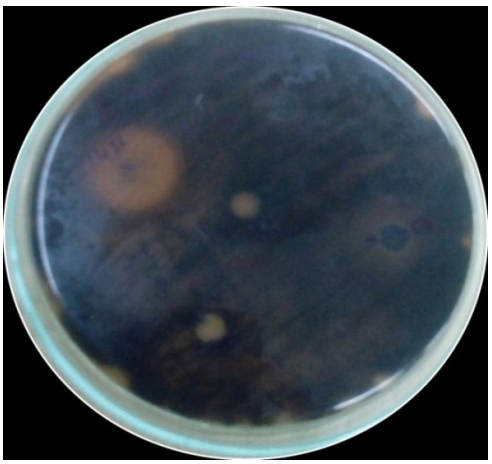


Figure 5: Antifungal Activity

1456- Ligand, **1457**- Cu complex and **1458**- Co complex

Result: The antimicrobial screening values indicate that the ligand shows the highest antibacterial and antifungal activity. Copper complex does not show any antimicrobial activity. Cobalt complex of Schiff base shows antibacterial activity to some extent.

Geometry of complexes

Elemental analyses and conductance measurements agree with the composition $[Cu(C_{13}H_{11}NO)_2(H_2O)_2]$ and $[Co(C_{13}H_{11}NO)_2(H_2O)_2]$ for copper and cobalt complex respectively. The IR spectral data indicate the co-ordination through the azomethine nitrogen and phenolic group in these complexes.

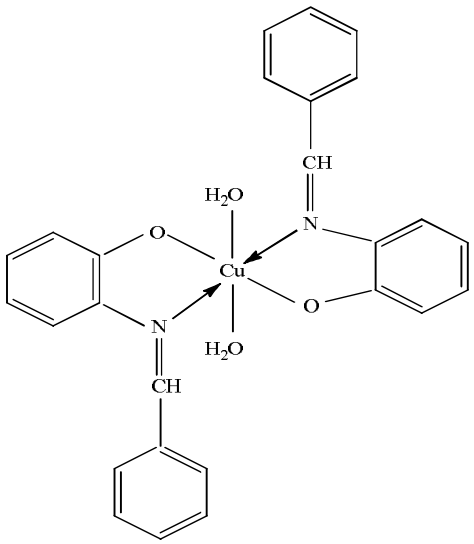


Figure 6: Proposed structure for octahedral Cu (II) complex

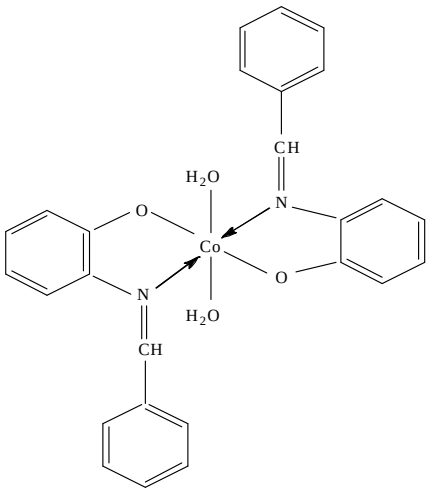


Figure 7: Proposed structure for octahedral Co (II) complex

CONCLUSIONS

Schiff base, benzilidineaminophenol (BAP) has been synthesized from benzaldehyde and 2-aminophenol. Cu (II) and Co (II) complexes of Schiff base have been synthesized and have been characterized by elemental analyses, molar conductance, magnetic moment, IR, TG analysis, UV-Vis and NMR spectral studies. The elemental analyses confirm the stoichiometry $[M(BAP)_2(H_2O)_2]$ for copper and cobalt complexes. IR studies show that the Schiff base is acting as a bidentate ligand, co-ordinated to the metal through azomethine nitrogen and phenolic oxygen atom. IR spectra also predict the presence of co-ordinated water. The conductance data indicates that these complexes behave as non electrolytes. Octahedral geometry of these complexes could be confirmed from the electronic and NMR spectral data and magnetic moment values. Antimicrobial studies indicate that the synthesized Schiff base shows better antibacterial and antifungal activities than the complexes. The Schiff base acts as a promising candidate as an antimicrobial agent for E. coli and A. Niger.

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