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Research Article

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Study of Antimicrobial Activity of 3-Methoxy-2-{[2-(2-Methoxy-Phenoxy)-Ethylimino]-Methyl}-Phenol and its Transition Metal Complexes on *E. coli* and *Staphylococcus aureus*

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ABSTRACT

Coordination complexes of transition metals with Schiff base ligands were synthesized. The characterization of these compounds were carried out by physical parameters and spectral analysis namely colour, melting point, IR, NMR, UV, Magnetic measurements, TGA and ESR studies. The spectral analyses are used for elucidating the structure of ligand and metal complexes. Biological activity of the compounds has been studied for E. coli and Staphylococcus aureus.

Keywords: Schiff bases; Metal complexes; Spectral analysis; Antimicrobial activity

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INTRODUCTION

The importance of metal complexes as drugs, their role in the biological systems and in the biological action of certain drugs has been realized. They are based upon the drug certain physical properties, e.g., low dissociation constants resulting in tightly metal ions, special oxidation-reduction potentials, solubility and electron distribution. The majority of the important metal complexes are chelates [1]. Studies on the relationship of metal complexes and biological response have been reported [2,3]. The Schiff bases and their metal complexes are of biological importance. The Schiff bases possess various activities such as antibacterial activity, antifugal activity, anticancer activity, antitumor activity and antitubercular activity [4]. Metal complexes of the Schiff bases also show these activities. These complexes are often more active than the ligands due to complexation with less side effects. In the present work the Schiff bases have been screened for their antibacterial and antifungal activity. Present work deals with synthesis of Schiff base ligand and its transition metal complexes by condensing with metal salts of Ni (II), Cu (II), Co (II), Mn (II) and Zn (II).

MATERIALS AND METHODS

Chemicals and Reagents

The chemicals used are 1-Naphthalen-1-yl-ethylamine (Merck, AR grade) and Salicyldehyde (Merck, AR grade), Ethyl alcohol (Merck, AR grade), Cobalt (II) chloride dihydrate (Sigma Aldrich), Nickel(II) chloride hexahydrate (Sigma Aldrich), Copper(II) chloride dihydrate (Sigma Aldrich), Zinc (II) chloride (Sigma Aldrich), Manganese (II) chloride tetrahydrate (Sigma Aldrich).

Synthesis of Ligand

The Schiff Base ligand 3-Methoxy-2-{[2-(2-methoxy-phenoxy)-ethylimino]-methyl}-phenol (Figure 1) was synthesized by condensing amine 2-(2-Methoxy-phenoxy)-ethylamine with o- Vanilline in equimolar proportions. To an ethanolic solution (10 ml) of the amine (0.01 mole) was added o- Vanilline (0.01 mole) in ethanol (10 mL) with stirring. The mixture was then refluxed for 30 mins. The reaction mixture was then cooled which immediately gave a precipitated product. The product then obtained was filtered, washed with ethanol and then dried. The crude product was then crystallized from aqueous ethanol to give a yield of 86%.

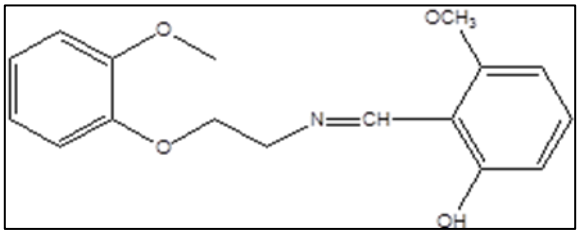

Figure 1: Structure of ligand: 3-methoxy-2-{[2-(2-methoxy-phenoxy)-ethylimino]-methyl}-phenol

Synthesis of Metal Complexes

The ligand and metal salt in the molar ratio of 2:1 was dissolved in a ethanol and the reaction mixture was heated on water bath for about one hour. It was then cooled when coloured solid separated out which was washed with ethanol and dried. This is the general method employed for the synthesis of metal complexes of ligand with metal chlorides viz Ni(II), Cu(II), Co(II), Mn(II) and Zn(II).

Antimicrobial Studies

The various screening studies carried out include the *in vitro* study against Gram-positive and Gram-negative bacteria viz. *Staphylococcus aureus* and *E. coli.*

RESULTS AND DISCUSSION

Formation of the complex was indicated by colour change and melting point. Physical characteristics of Schiff base and metal complexes are given in Table 1.

Table 1: Physical characteristics

Compound	Color	Yield %	M.P (°C)
Ligand (SB4)	Bright yellow	86	96°C
SB4-Ni complex	Pale green	70	257°C
SB4-Cu complex	Blackish green	61	237°C
SB4-Co complex	Orange	54	227°C
SB4-Mn complex	Brownish green	71	246°C
SB4-Zn complex	Yellow	75	240°C

NMR and IR Spectra

In NMR spectra formation of ligand was confirmed by presence of CH=N peak at 8.4 δ and OH at 5.6 δ. In the present investigation the Infra-red values for major peaks are assigned. The IR spectrum of ligand gave a strong band at 1642.09 cm^{-1} and 2901.27 cm^{-1} which are attributed to the stretching frequencies of C=N (azomethine) and OH respectively. Complexes showed a lower shift of wave numbers for C=N. Also IR bands were observed for M-O and M-N. All complexes showed bands 3300 cm^{-1} to 3400 cm^{-1} indicating co-ordinated H_2O moiety in the complexes. Complex of SB1-Ni showed IR bands at 1617.98 cm^{-1} and 3331.31 cm^{-1} corresponding to C=N and H_2O, IR values of 469.582 cm^{-1} and 546.72 cm^{-1} were assigned to M-O and M-N respectively. Similarly complex of SB1-Zn complex showed bands at υ(C=N) 1622.8 cm^{-1}, υ(H_2O) 3452.54 cm^{-1}, υ(M-O) 479.224 cm^{-1} and υ(M-N) 673.035 cm^{-1}. Similarly bands were observed for Cu complex at υ(C=N) 1622.8cm^{-1}, υ(H_2O) 3409.86 cm^{-1} ,υ(M-O) 470.546 cm^{-1} and υ(M-N) 673.035 cm^{-1}. Co complex υ(C=N) 1613.16 cm^{-1}, υ(H_2O) 3375.81 cm^{-1}, υ (M-O) 461.868 cm^{-1} and υ(M-N) 563.112 cm^{-1}. Mn complex υ(C=N) 1539.88 cm^{-1}, υ(H_2O) 3446.17 cm^{-1}, υ(M-O) 454.154 cm^{-1} and υ(M-N) 496.58 cm^{-1}.

Electronic Absorption Spectra

In the electronic spectra the ligand exhibited energy peaks at 30211 cm^{-1} and 23640 cm^{-1}. The Co(II) complexes exhibited two energy peak at 18181, 22935 and 29325 cm^{-1}, which can be assigned [5] to the transitions 4T1g(F) → 4T2g(F), 4T1g(F) → 4A2g(F) and 4T1g (F) → 4T2g (P) for a high spin octahedral geometry respectively. The electronic spectra of the Ni(II) complexes showed d–d transition at 28985, 24390 cm^{-1} and 22883 cm^{-1} [5] while Mn complexes showed peaks at 30487 cm^{-1} and 24509 cm^{-1}. These are assigned to 3A2g(F) → 3T2g(F), 3A2g(F) → 3T1g(F) and 3A2g (F) → 3T2g(P) transitions, respectively. These are consistent with a well- -defined octahedral geometry. The Zn(II) complexes exhibited only a high intensity band at 26385 cm^{-1} and 29850 cm^{-1}, which is assigned to ligand–metal charge transfer. In case of the Cu(II) complexes, a broad band at 26809, 28571 cm^{-1} and 27027 cm^{-1} [5] was observed that is assigned to the 2Eg → 2T2g transition, which confirms its octahedral geometry.

Thermo Gravimetric Analysis

TGA analysis is carried out to explain the thermal stability of complexes. TGA study of complex showed weight loss in the temperature range of 110°C-200°C is due to elimination of coordinated water molecule. Also gradual decrease in mass is seen up to 300°C due to loss of volatile matter. And a plateau observed above 350°C respectively which corresponds to the formation of stable metal oxide.

ESR

The gll and g⊥ value for Copper complex is reported in the following Table 2. The spectrum showed asymmetric bands with two g values. The trend gll> g⊥> 2.00277, indicating that the unpaired electron lay predominately in the dx^2-y^2 orbital with possibly mixing of dz^2 orbital because of the low symmetry. The axial symmetry parameter 'G' is determined as $G = \frac{(gll - 2.00277)}{(g\perp - 2.00277)}$.

G values found to be more than 4 suggesting very weak or no interaction in the solid state.

Table 2: ESR values for copper complex

Complex	gll value	g⊥ value	g_{avg}	G
SB4 Cu complex	2.297	2.047	2.13033	6.65227

Magnetic Susceptibility Measurements

The effective magnetic moment values for the complexes were determined. The magnetic moment value 4.24 B.M for Co(II) complex suggests an octahedral environment [6,7]. The magnetic moment value of the Cu (II) complexes of 1.63 B.M suggests distorted octahedral geometry [8,9]. The magnetic moment value of the Ni(II) complexes 3.13 B.M suggests an octahedral geometry. Mn (II) complexes with the value of 5.64 B.M indicate octahedral geometry [10]. The Zn(II) complexes were found to be diamagnetic, as expected for d^{10} configuration. From the discussion of the results of various physico-chemical studies presented above, it may be concluded that the most probable geometry for the transition metal complexes with general formula $ML_2.2xH_2O$ is octahedral and the bonding in the complexes can be represented in Figure 2.

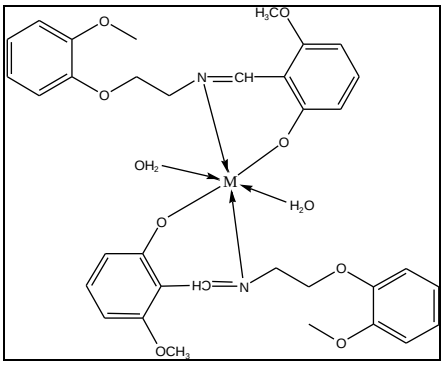

Figure 2: Structure of complex (M= Ni, Cu, Co, Mn, Zn)

Antimicrobial Activity

In the present work the Schiff bases have been screened for their antibacterial and antifungal activity. The test compounds have been subjected to *in vitro* screening against Gram-positive and Gram-negative bacteria viz.

Staphylococcus aureus and *E. coli* using Nutrient broth as the culture medium by agar cup diffusion method. The results of the studies for Schiff base and its complexes are summarized in Tables 3 and 4 below.

- Antibacterial activity for Organism-*Staphylococcus aureus*
- Antibacterial activity for Organism- *E. coli*

Table 3: Activity of compounds for organism-*Staphylococcus aureus*

Sample	Concentration	Zone of Inhibition in mm
Ligand- SB4	20	13
	40	10
	60	-
	80	1.1
	100	10
	Control	0
SB4-Ni complex	20	-
	40	-
	60	-
	80	-
	100	09
	Control	0
SB4-Cu complex	20	-
	40	-
	60	-
	80	09
	100	10
	Control	0
SB4-Co complex	20	-
	40	-
	60	-
	80	-
	100	09
	Control	0
SB4-Mn complex	20	-
	40	-
	60	-
	80	-
	100	-
	Control	0
SB4-Zn complex	20	-
	40	-
	60	-
	80	-
	100	11
	Control	0

RESULTS AND DISCUSSIONS

The evaluation of the antimicrobial activity was carried out after the incubation period by the measurement of the diameter of the inhibition zones. The different concentrations of ligand and metal complexes were found to inhibit the *E. coli* and *S. aureus* which can be seen from zone of inhibition in the above tables. However the activity of complexes was found to be less than that of the ligand. All the above results were compared with two standard antibiotics Erythromycin and Tetracyline. Erythromycin showed a zone of inhibition of 19 mm (intermediate range) and 16 mm (intermediate range) for *E. coli* and *S. aureus* respectively. Similarly Tetracyline showed a zone of inhibition of 15 mm (intermediate range) and 18 mm (intermediate range) for *E. coli* and *S. aureus* respectively. Hence it was concluded that the synthesized compounds exhibited weak antimicrobial activity on the microbes under study. The order of the activity can be summarized as follows: Standard > Ligands > Complexes.

Table 4: Activity of compounds for organism-*E. coli*

Sample	Concentration	Zone of Inhibition in mm
Ligand- SB4	20	-
	40	14
	60	11
	80	11
	100	12
	Control	0
SB4-Ni complex	20	-
	40	-
	60	07
	80	09
	100	11
	Control	0
SB4-Cu complex	20	-
	40	-
	60	08
	80	08
	100	09
	Control	0
SB4-Co complex	20	-
	40	-
	60	10
	80	11
	100	09
	Control	0
SB4-Mn complex	20	-
	40	10
	60	11
	80	11
	100	-
	Control	0
SB4-Zn complex	20	-
	40	-
	60	09
	80	08
	100	11
	Control	0

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