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Synthesis and Spectral Investigation (^{1}H and ^{13}C) of Tetradentate Schiff Base Ligands for the Preparation of Post Transition Bimetallic Complexes of Antimicrobial Importance

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A tetradentate Schiff base ligand 2-(*bis*-2-hydroxylphenylidene)-1,2-diiminoethanne (L) was synthesized and then its structure was investigated by ^{1}H NMR and ^{13}C NMR techniques. The ligand and its bimetallic complexes were screened *in vitro* for antibacterial and antifungal activities by using disc diffusion method. The results showed that in case of antibacterial activity the Co_2L_2 exhibited better efficiency (9 ± 0.816) against *E. coli* while binuclear complex of Mn_2L_2 showed better potential against all the three strains (*E. coli*, *S. aureus* and *A. alternate*) as compared with other attempted bimetallic (Cu, Mn and Zn) complexes when compared with standard one. Similarly the antifungal potential of binuclear (Co_2L_2) complex was satisfactory (12.25 ± 0.062) for *A. alternate* while the binuclear Cu_2L_2 complex exhibited good effect (7.75 ± 0.957) against *A. niger*. Similarly binuclear Mn_2L_2 complex showed better efficiency (10.25 ± 0.5) against *A. flavous*. The biological activity data justified that binuclear post transition metal complexes with Schiff base can be successfully used against fungal as well as bacterial infections.

Keywords: Tetradentate Schiff base, Binuclear complexes, Bacterial strains, Fungal strains.

INTRODUCTION

The Schiff bases and their metal complexes are considered to be very important due to their interest in a variety of biological mechanisms[1]. Many transition metal complexes were found to exhibit greater stability and are very active against different diseases. Depending upon the stability, the transition metals and their complexes were used against tumor and cancer treatments[2]. The stability of complexes mainly depends on the chelating and donating powers of the ligand which ultimately facilitates oxidizing and electrophilic reactions. The less stability of metal complexes restricts their applications in some biological processes[3]. Ligands made from salicylaldehyde exhibit higher values of antibacterial activities against different species including *S. aureus*, *P. mirabilis*, *H. influenza* and *Salmonella spp*[4]. Apart from ligands and metal complexes, salicylaldehyde is more frequently used as larvicide and fungicides.

A number of biological components such as glucose, vitamins, drugs, nitrogenous bases and some heterocyclic compounds containing nitrogen atom act as bioligands. These bioligands exhibit average antimicrobial activities which can be enhanced by coordination with transition metals. Complex formation promotes dissolving power of different drugs in lipid containing organisms and ultimately enhances the drugs effect against different diseases. The activities of anticancer, antitumor, antianalgesic and antibiotic drugs can be increased by complexing them with transition metals[5]. Metallo-elements specially zinc and copper play significant role in the viral growth and restrict virus to enter into the host cell, for example sulfur and zinc occur in viruses and bacteria respectively. Zinc quickly coordinates with sulfur present at the outer layer of virus and can transfer virus into the host cell, so to break this coordination, transition metals were considered to be very important to arrest the sulfur. So viral growth depends on these metals and can be controlled by varying concentrations of metallo-elements[6]. Literature justifies that much more interest is being focused on the effect of ligands and their mono-transition metal complexes for their biological potential. Keeping in mind the importance of the transition metal complexes, the present study was carried out to see the effect of binuclear post transition metal complexes for antimicrobial activities.

EXPERIMENTAL

All solvents and reagents including ethylenediamine, cobalt acetate, copper acetate, manganese acetate, zinc acetate, ethanol, salicylaldehyde, acetic acid, toluene and ethyl acetate of AR grade were purchased from Sigma-Aldrich (Germany).

Synthesis of ligand: This ligand (tetradentate Schiff base) was synthesized by condensation reaction of equimolar quantities of salicylaldehyde (23.4 g, 0.1 mol) and ethylenediammine (6 g, 0.1 mol). The reactants along with glacial acetic acid (1 mL) were refluxed in 40 mL of ethanol for 7 h[7]. After refluxing, the progress of the reaction was checked by TLC test in a pet ether-ethyl acetate (1:1) solvent system. The crude product was then subjected to column chromatography by using silica gel and pet ether-ethyl acetate (1:1) solvent system. The eluent of the column chromatography was then concentrated up to dryness by rotary evaporator. After drying well the productivity was calculated to be 72 %. The ligand was subjected to spectral investigation (^1H, ^{13}C) after dissolving the sample in $CDCl_3$ and calibration was achieved with TMS.

Synthesis of bimetallic complexes: In order to prepare four binuclear (Cu, Co, Mn & Zn) complexes with Schiff base (L), the equi-molar quantities of metal acetates (Co, Cu, Mn, Zn) were dissolved seperately in ethanol/ toluene and were reacted with ligand dissolved in toluene. Reaction mixture in 250 mL flask was refluxed along with stirring for 4-5 h to complete the reaction. The success of each reaction was checked by TLC test. After the reaction the solvent was removed by the rotary evaporator, washed with pet ether-ethyl acetate (1:1) solvent. All the complexes were recrystallized from toluene/pet-ether system. After filtration each pure product was dried well under vacuum and productivity was calculated. These newly synthesized binuclear complexes along with the ligand were tested for their antibacterial as well as antifungal activities.

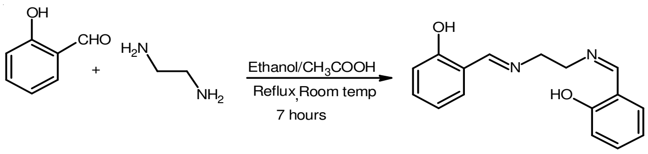
Antimicrobial activity: Disc diffusion method was used to test the antibacterial and antifungal activity. Different strains (bacterial and fungal strains) were engaged to check the antibacterial and antifungal activities.

Antibacterial activity: Different strains were used to test the antibacterial activity. The selected strains were *Escherichia coli*, *Staphylococcus aureus* and *Bacillus Subtilis*[8]. The ligand and bimetallic complexes were employed for antibacterial activity under Disc diffusion method. Nutrient agar was mixed in distilled water and dispersed homogenously. Sterilization of the medium was carried out by means of autoclave at 121 °C for 20 min. Medium was treated with inoculums before it was transferred to petri plates. The filter paper discs were placed parallel on growth medium (100 µL) containing bimetallic complexes and ligand separately. The incubation of petri plates was taken for 24 h at 37 °C for bacterial growth. The complexes as well as ligand successfully inhibited the growth of bacteria and formed clear zones. Zone reader was employed to measure the inhibition zones in mm. The results of the complexes and ligand were compared with drug rifamipicin[9].

Antifungal activity: Fungal strains were used to test the antifungal activity. The selected strains were *A. flavus*, *A. alternate* and *A. niger*[8]. The growth medium was synthesized, sterilized and then transferred to the petri plates. Filter paper discs were cited on growth medium. Appropriate fungal strain was transferred on the filter paper disc present in the growth medium containing Petri plate. The bimetallic complexes and ligand were applied (100 µL) separately on each disc and then the Petri plates were incubated for 48 h at 28 °C for fungus growth. The binuclear complexes as well as ligand itself successfully inhibited the fungal growth. The results of the complexes were compared antifungal drug fluconazol[10].

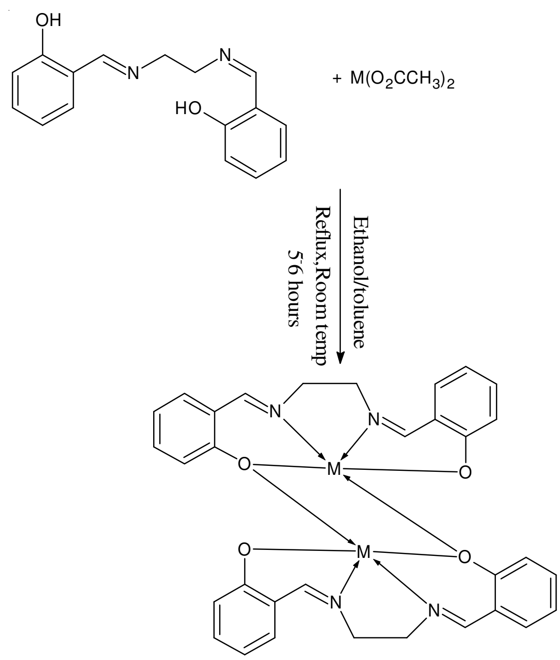
RESULTS AND DISCUSSION

The tetradentate Schiff base, 2-(*bis*-2-hydroxylphenylidene)-1,2-diiminoethanne (L) and its the binuclear complexes were prepared **Schemes I** and **II**.



L= $C_{16}H_{16}N_2O_2$

Scheme-I: Synthesis of tetradentate Schiff base, 2-(*bis*-2-hydroxylphenylidene)-1,2-diiminoethane (L)



(M= Co, Cu, Mn, Zn)

Scheme-II: Synthesis of teradentate Schiff base derivatives of binuclear complexes

The Ligand (L) and it binuclear complexes showed sharp melting points and were soluble in most of the organic solvents (Tables 1 and 2).

TABLE-1
PHYSICAL PROPERTIES OF LIGANDS AND ITS METAL COMPLEXES

S. No.	Compound nature	m.w.	m.f.	m.p. (°C)
1	L	268	$C_{16}H_{16}O_2N_2$	116
2	Cu_2L_2	659	$Cu_2(C_{16}H_{14}O_2N_2)_2$	> 350
3	Co_2L_2	650	$Co_2(C_{16}H_{14}O_2N_2)_2$	> 350
4	Mn_2L_2	642	$Mn_2(C_{16}H_{14}O_2N_2)_2$	> 350
5	Zn_2L_2	662	$Zn_2(C_{16}H_{14}O_2N_2)_2$	> 350

TABLE-2
SOLUBILITY OF LIGAND AND ITS BINUCLEAR COMPLEXES

S. No.	Compound	Solubility			
		DMSO	Chlorofom	Ethyl alcohol	Mixed solvent system (Ethanol. Toluene. Ethyl acetate)
1	$C_{16}H_{16}N_2O_2$	+	+	+	+
2	$Cu_2(C_{16}H_{14}N_2O_2)_2$	Slightly soluble	Slightly soluble	Slightly soluble	+
3	$Co_2(C_{16}H_{14}N_2O_2)_2$	Slightly soluble	Slightly soluble	Slightly soluble	+
4	$Mn_2(C_{16}H_{14}N_2O_2)_2$	Slightly soluble	Slightly soluble	Slightly soluble	+
5	$Zn_2(C_{16}H_{14}N_2O_2)_2$	Slightly soluble	Slightly soluble	Slightly soluble	+

^{1}H NMR analysis of ligand: The singlet peak at 3.95 ppm is exhibited by protons of ethylene. Each aryl group has four protons in the molecule. Different environment of these protons is found (Table-3) with labeling from C_1-C_4. C_1, C_2, C_3 and C_4 protons showed doublet, triplet, triplet and doublet at 7.02, 7.52, 7.08 and 7.66 ppm correspondingly. Imines protons showed singlet at 8.54 ppm. Imine group have π electrons which are deshielded and showed high value. A singlet at 11.26 ppm is exhibited by phenolic a proton which is probably due to the strong effect of deshielded oxygen atom. The results can be visualized from the spectrum (Fig. 1).

TABLE-3
^{1}HNMR DATA OF LIGAND (L)

Position of Protons	Type of Peak	Value in ppm
C_1	Doublet	7.02
C_2	Triplet	7.08
C_3	Triplet	7.52
C_4	Doublet	7.66

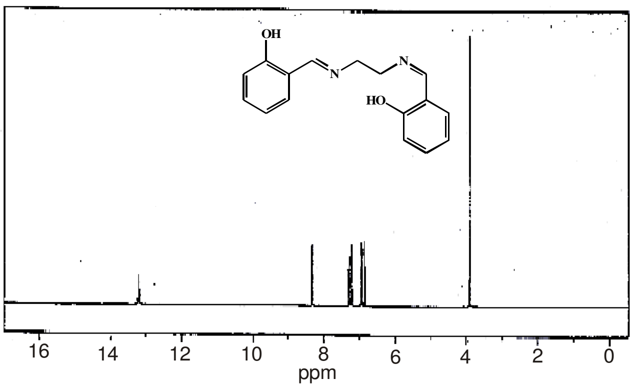


Fig. 1. ^{1}H NMR spectrum of Ligand

^{13}C NMR analysis of ligand: The environment of the ligand molecule containing different carbon atoms is supported easily by ^{13}C NMR spectrum. Two carbon atoms correspond to each peak in the molecule. First peak at 61.9 ppm is due to the CH_2-CH_2 group. The signals of sp^3 hybridized carbon atom present in the middle of the molecule mostly appears at about 30 ppm but due to the presence of electronegative atom (N) and the presence of π bonds adjacent to CH_2-CH_2 group resulted the signals at downfield (61.9 ppm). The CH carbon exhibits sp^2 hybridization which is not terminal and is found at 140 ppm. The presence of electronegative nitrogen (N) is correlated with the observed value of carbon which showed some deviation. Also the aryl group contains carbon atoms which are observed from 120-130 ppm whereas the phenolic carbons of the molecule showed peak at 161.1 ppm. This is perhaps due to the presence of more electronegative oxygen atom (O) which is bonded to carbon and described the downfield shift. On the contrary oxygen atoms also contain lone pair which is correlated with the *ortho* and *para* carbons of the phenols which exhibit up field shift by mesomeric effect. The meta carbon of phenol remained unchanged (Table-4). The results can be visualized from the respective spectrum (Fig. 2).

TABLE-4
^{13}C NMR DATA OF LIGAND (L)

Position of Carbon	Observed Value in ppm	Peak area equivalent to No of carbon
1 and 1'	61.9	Two
2 and 2'	157.5	Two
3 and 3'	124.6	Two
4 and 4'	161.1	Two
5 and 5'	117.8	Two
6 and 6	132.4	Two
7 and 7'	121.4	Two
8 and 8'	132.1	Two

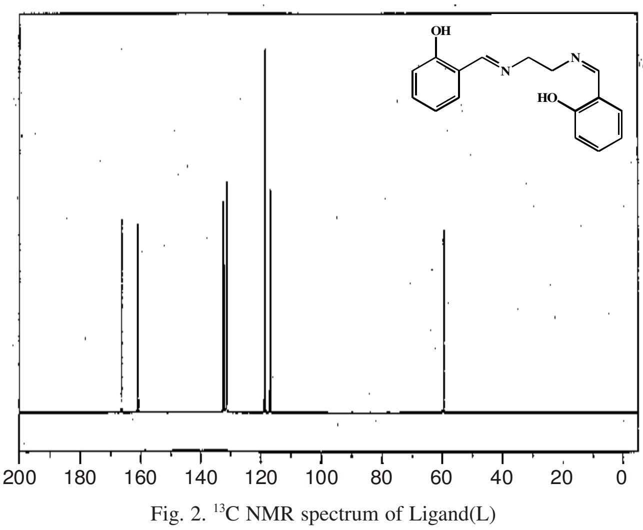


Fig. 2. ^{13}C NMR spectrum of Ligand(L)

Antimicrobial activity: The antimicrobial activity was determined by using disc diffusion method against different species of fungus and bacteria and the results were compared with standard drugs. The results confirmed that the activity of bimetallic complexes is considerable high against bacteria and fungus as compared to ligand.

Antibacterial activity: The tetradentate Schiff base (2-(*bis*-2-hydroxylphenylidene)-1,2-diiminoethane (L) ligand and its binuclear complexes were tested for their antibacterial activity against *B. subtilis, S.aureus* and *E. coli* along with rifamipicin as a standard one[8]. The results are explained as mean ± SD (Table-5). The ligand showed less activity as compared to bimetallic complexes. The Mn_2L_2 exhibited best activity (10.5 ± 0.577) against *S. aureus*. while Mn_2L_2 and Co_2L_2 expressed better antibacterial potential (9.75 ± 0.5* and 9 ± 0.816) against *B. subtilus* and *E. coli*, respectively. The results of the Mn_2L_2 against *S. aureus* and *B. subtilus* are statistically significant ($p < 0.05$) with zone 10.5 ± 0.577 mm and 9.75 ± 0.5 respectively. Similarly the activity Co_2L_2 against *E. coli* were also found statistically significant ($p < 0.05$) with zones 9.75 ± 0.5 mm. All results are given in comparison with standard drug (rifamipicin) which gave the zone 24.5 ± 0.577 mm. The anti bacterial potential of the titled complexes along with ligand can be verified (Fig. 3).

TABLE-5
ANTIBACTERIAL ACTIVITY DATA OF LIGAND (L) AND ITS BINUCLEAR COMPLEXES

Sr.No.	Comps.	Tested microorganism diameter of inhibition zone		
		E. coli	*S. aureus*	*B. subtilus*
1	(L)	4 ± 0.816	6 ± 0.816	4.25 ± 0.957
2	Co_2L_2	9 ± 0.816*	5.25 ± 0.957	8 ± 0.816
3	Cu_2L_2	5.25 ± 0.957	6.25 ± 0.957	6 ± 1.154
4	Mn_2L_2	8.75 ± 0.5*	10.5 ± 0.577*	9.75 ± 0.5*
5	Zn_2L_2	6.5 ± 0.577	5.75 ± 0.5	6.5 ± 0.577
6	Standard	23.25 ± 2.061	24.5 ± 0.577	23 ± 0.816

Values shown are mean ± SD.
*represents the significant difference at $p < 0.05$.

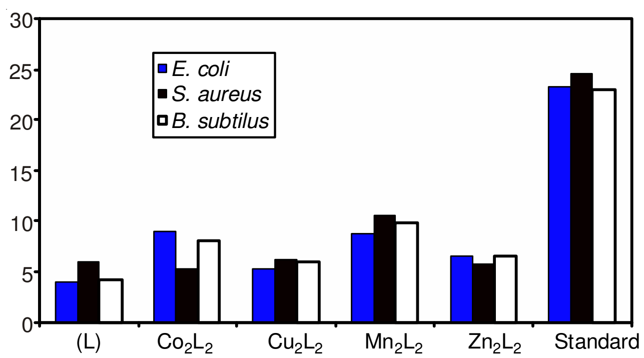


Fig. 3. Antibacterial activity data of Ligand (L) and its binuclear complexes

Antifungal activity: Similarly the antifungal potential of Schiff base and its bimetallic complexes were evaluated against various fungal strains (*A. alternata*, *A. niger*, *A. flavis*) along with fluconazol drug as a standard one by using disc diffusion method. (Table-6). Results are shown as mean ± SD. The results showed that Co_2L_2 exhibited highest activity against *A. alternate* (12.25 ± 0.062) while Cu_2L_2 and Mn_2L_2 expressed better antifungal potential against *A. niger* (7.75±0.957) and *A. flavous* (10.25 ± 0.5), respectively. The antifungal activity of Co_2L_2 against *A. alternata* and Cu_2L_2 against *A. niger* was found statistically significant with zone 12.25 ± 0.062 mm and 7.75 ± 0.957 mm respectively ($p < 0.05$). Similarly the activity of Mn_2L_2 against *A. flavus* with zones 10.25 ± 0.5 was observed statistically significant ($p < 0.05$). All above results are in comparison with standard drug (fluconazol) which gave the zone 24 ± 0.577 mm. The results can further be verified Fig. 4. The antibacterial as well as antifungal results of attempted complexes explained that antimicrobial potential of Mn_2L_2 is the best while the Zn_2L_2 exhibited the least one. This may be due to the least chelating ability of Zn (having greater number of outer *d* electrons and hence smaller vacancies are available for chelation) and good chelating capacity of Mn is due to less number of electrons and hence greater vacancies for available for for chelation. Earlier in a similar type of the study Schiff base and its post transition metal complexes were attempted for antimicrobial study. The results showed that metallation can increase the antibacterial activity rather than free ligand. Further it was observed that complexes exhibited variable antimicrobial potential due to different metals involved for metallatic[11]. Similarly the results of most studies showed that the antimicrobial activity of the complexes was observed higher than related ligand. The increased activity of the metal complexes was explained on the basis of chelating theory. Chelating reduces the polarity of the metal ion, because positive charges of the metal are partially shared with the donor atoms present in the ligands and there may be π-electron delocalization over the whole chelation. This phenomenon increases the lipophilic character of the metal chelate and favors its permeation more efficiently through the lipoid layer of the microorganism, thus destroying them more forcefully. The other factors like solubility, conductivity and bond length between the metal and ligand also increase the activity[12].

TABLE-6
ANTIFUNGAL ACTIVITY DATA OF LIGAND (L) AND ITS BINUCLEAR COMPLEXES

Sr. No.	Comps.	Tested microorganism diameter of inhibition zone		
		A. niger	*A. flavous*	*A. alternate*
1	(L)	Nil	4.25 ± 0.577	4 ± 1.825
2	Co_2L_2	4 ± 1.825	7.75 ± 0.957	12.25 ± 0.062*
3	Cu_2L_2	7.75 ± 0.957*	6.25 ± 0.957	6 ± 1.154
4	Mn_2L_2	6 ± 0.816	10.25 ± 0.5*	8.5 ± 0.577
5	Zn_2L_2	6.5 ± 0.577	5.75 ± 0.5	Nil
6	Standard	24.5 ± 0.577	23.25 ±2.061	23 ± 0.816

Values shown are mean ± SD.
*Represents the significant difference at $p < 0$.

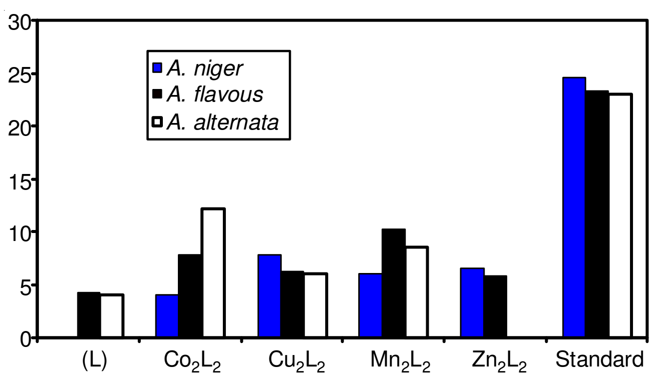


Fig. 4. Antifungal activity data of Ligand (L) and its binuclear complexes

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