



Research Article

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***In vitro* antioxidant, antimicrobial and larvicidal studies of schiff base transition metal complexes**

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ABSTRACT

A series of mononuclear Schiff base transition metal complexes of the type $[ML^1L^2]$ (where, $M = \text{Mn(II)}, \text{Co(II)}, \text{Ni(II)}, \text{Cu(II)}$ and Zn(II) ; $L^1 =$ Schiff base ligand derived from *o*-hydroxyacetophenone and L-cysteine; $L^2 =$ ethylenediamine) have been synthesized and characterized on the basis of elemental analysis, molar conductance and spectroscopic studies such as UV-Visible, FTIR and EPR. A square pyramidal geometry has been assigned based on the analyses. All the synthesized complexes and the Schiff base ligand were screened for their *in vitro* antibacterial against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli* and antifungal activities against *Aspergillus niger*, *Aspergillus flavus* and *Penicillium notatum* by well diffusion method. In addition, the antioxidant and larvicidal activities were also done for all the metal complexes. The percentage of antioxidant scavenging activity and mortality of the larvicidal activity were also determined.

Keywords: Schiff base transition metal complexes, spectroscopic studies, antibacterial, antifungal, antioxidant and larvicidal activities.

INTRODUCTION

Coordination chemistry, comprise a large body of current inorganic research. Schiff bases are widely used as ligands in coordination chemistry [1]. Schiff bases are prepared by the condensation of aldehydes or ketones with primary amines. Schiff bases are used as ligands in the preparation of transition metal complexes because of their preparative accessibility, solubility in common solvents, stereo chemical and electronic properties. Further, Schiff base transition metal complexes exhibits promising applications like biological activity and biological modeling [2,3]. Since interdisciplinary research is found to be a hallmark of coordination chemistry, many researchers focus on the areas like catalysis, photochemistry, molecular modeling and biological studies [4]. The physiological and pharmacological activities of the complexes derived from amino acid Schiff bases have gained importance from research point of view [5-7]. L-cysteine is one of the non-essential amino acids. The thiol group of L-cysteine exhibit numerous biological activities [8], biological functions and has antioxidant properties [9].

Based on the above facts the prime aim of our present work is to synthesis a series of Schiff base transition metal complexes derived from L-cysteine and to characterize the synthesized metal complexes using various analytical and spectral studies. Also, the synthesized metal complexes were screened for larvicidal and *in vitro* antioxidant and antimicrobial studies.

EXPERIMENTAL SECTION

Materials

All chemicals and reagents used were of analytical grade and used as such.

Molar conductance

The molar conductance of the freshly prepared Schiff base transition metal complexes (10^{-3} M) were recorded in DMF at room temperature using Digital conductivity meter, Global electronics, Model: DCM 900.

UV-Vis. Spectroscopy

The Ultraviolet-Visible spectra of the complexes in DMSO were recorded using Systronics 2201 spectrophotometer, between 200 to 800 nm.

FTIR Spectroscopy

The FTIR spectra of the complexes were recorded using Shimadzu spectrometer in the range of $4000-400\text{ cm}^{-1}$ using KBr pellet.

EPR Spectroscopy

The EPR spectra of the complexes were recorded in the polycrystalline state at room temperature using Bruker EMX -10/2.7 Spectrometer.

Synthesis of Schiff base metal complexes

To an aqueous solution of L-cysteine (0.606 g, 5 mmol) and KOH (0.562 g, 10 mmol) an ethanolic solution of *o*-hydroxyacetophenone (0.68 g, 5 mmol) was added in drops. The reaction mixture was stirred for about 1 h in a magnetic stirrer at 333 K. The solution turned yellow. To this, an ethanolic solution of an appropriate metal salt [copper(II) acetate (1.1 g, 5 mmol), cobalt chloride (1.185 g, 5 mmol), nickel sulphate (0.77 g, 5 mmol) and zinc sulphate (1.435 g, 5 mmol)] was added and the reaction mixture was stirred for another 1 h. Then ethylenediamine (0.3 g, 5 mmol) was added in drops and the mixture was stirred for additional 2 h at the same temperature. The resultant product was filtered, washed with ethanol and dried.

Antimicrobial studies (in vitro)

All the synthesized complexes and the Schiff base ligand were tested for their antimicrobial studies by *in vitro* method against bacterial strains such as *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and fungal strains such as *Aspergillus flavus*, *Aspergillus niger* and *Penicillium notatum*.

Modified antibacterial test was performed using the agar well diffusion method. The microorganisms were inoculated on Muller Hinton Agar and spread uniformly using sterile spreader in Petri plates. Three wells of 6 mm in diameter were made on Muller Hinton Agar using a sterile well puncher. The cut agar blocks were carefully removed by the use of forceps sterilized by flaming. 50 μL and 100 μL of the freshly prepared solution of metal complex (1 mg/mL) in DMSO were poured into two wells and negative control DMSO is poured in one well and the plates were allowed to stand for 1 h at room temperature for the diffusion of the substances and before the growth of organism commenced, the plates were incubated at 37 $^{\circ}\text{C}$ for 4 h. Antimicrobial activity was determined by measuring the diameter of zones showing complete inhibition.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) is the lowest concentration at which the growth of a microorganism is inhibited [10]. A set of sterile test tubes were arranged in a rack. 1 mL of sterile Muller-Hinton broth was taken in all the test tubes. Stock solutions of synthesized metal complexes were prepared by dissolving the complexes in DMSO (1mg/1 mL). To the first test tube 1ml of stock solution was added then it was serially diluted in six test tubes using sterile pipette so that concentrations varied. One drop of the test organism was added in all the test tubes. The same procedure was followed for fungal strains using SDS nutrient broth. Finally the bacterial strains in the test tubes were incubated at 37 $^{\circ}\text{C}$ for 24 h, whereas the fungal strains were incubated at room temperature for 48 h.

Antioxidant activity**DPPH scavenging activity**

DPPH radical scavenging activity is a standard assay to study the antioxidant activities. It is a rapid technique for screening the radical scavenging activity of specific compounds [11-16]. The free radical scavenging effects of all the complexes and the ligand with DPPH radical were evaluated using concentrations 2 mg/mL of the synthesized complexes in DMF with 2 mL of 0.05 M methanolic solution of DPPH. The reaction mixture was incubated in the dark for 30 min at room temperature. The control contained all reagents without the sample while methanol was used as blank. The antiradical scavenging ability of synthesized metal complexes was determined by measuring the decrease in the absorbance of DPPH at 517 nm. The absorbance decreased when the DPPH is scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule. This lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percent of inhibition (I %) of free radical production from DPPH was calculated by using the following equation.

$$\% \text{ of inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

where, A_c - absorbance of the control; A_s - absorbance in the presence of sample solution

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity is one of the best method to study the antioxidant property [12-16]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Synthesized complexes with the concentration of 2 mg/mL in DMF were added to hydrogen peroxide and absorbance at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging is calculated as given in DPPH method.

Larvicidal bioassay

The eggs and egg rafts of *Culex quinquefasciatus* were procured from Zonal Entomological Unit, Vellore, Tamil Nadu. The eggs and egg rafts of *C. quinquefasciatus* were dipped into a plastic bottle containing 500 mL of dechlorinated water for 30-40 min to hatch out larvae. They were maintained in the laboratory as per literature [17]. Mosquito larvae were fed with powdered nutrient broth once a day. After 4 days the hatched larvae turned into larvae in early fourth stage and were subjected for further experiment.

The larvicidal activity was assessed by the procedure of WHO guide lines with some modification [18]. For the preliminary screening bioassay test a total of 20 reared mosquito larvae of *C. quinquefasciatus* was placed in 200 mL of double distilled sterilized water containing 1.0 mg of synthesized metal complexes. The negative control was set up with sterile distilled water without metal complex while the positive control was the commercial larvicide with test solution. Percentage of mortality was assessed after 24 h of incubation. A number of dead larvae in each batch were counted every hour for 24 h exposure period. The treated larvae was mounted on a slide and examined under a microscope for image capture.

Dose-response bioassay

Based on the preliminary screening results, all the synthesized complexes were subjected to dose-response bioassay for larvicidal activity against the larvae of *C. quinquefasciatus*. A total of 20 reared mosquito larvae were placed in 200 mL of double distilled sterilized water containing various concentrations (4 mg, 2 mg, 1 mg, 0.5 mg) of synthesized metal complexes. Percentage of mortality was assessed after 24 h of incubation. A number of dead larvae in each batch were counted every hour for 24 h exposure period and the percentage of mortality was reported from the average of triplicates.

RESULTS AND DISCUSSION

All the synthesized transition metal complexes are found to be freely soluble in DMSO, DMF and ethanol. The analytical data of the synthesized metal complexes are shown in Table 1. The lower molar conductivity value of the complexes (10^{-3} M) in DMSO at 25 °C indicates their non-electrolytic nature [19, 20].

Table 1. Analytical data of the metal complexes

Complex	Molecular Formula	Molecular weight	Decomposition point	Color	Molar conductance $\text{Ohm}^{-1}\text{cm}^2\text{mol}^{-1}$	Elemental analyses found (calculated) %		
						C	H	N
[Mn L ¹ L ²]	C ₁₂ H ₁₇ O ₃ N ₃ SMn	338	>350 °C	Brown	3.8	42.68 (42.60)	5.12 (5.07)	12.50 (12.42)
[Co L ¹ L ²]	C ₁₂ H ₁₇ O ₃ N ₃ SCo	342	>350 °C	Brown	3.3	42.16 (42.10)	5.09 (5.02)	12.30 (12.28)
[Ni L ¹ L ²]	C ₁₂ H ₁₇ O ₃ N ₃ SNi	342	>360 °C	Green	2.9	42.18 (42.13)	5.08 (5.02)	12.35 (12.29)
[Cu L ¹ L ²]	C ₁₂ H ₁₇ O ₃ N ₃ SCu	346	>360 °C	Green	3.3	41.59 (41.54)	5.00 (4.95)	12.18 (12.11)
[Zn L ¹ L ²]	C ₁₂ H ₁₇ O ₃ N ₃ SZn	348	>360 °C	Yellow	3.8	41.39 (41.32)	5.14 (4.92)	12.09 (12.05)

UV-Vis. Spectra

The electronic absorption spectra of Schiff base metal complexes (10^{-3} M) were recorded in DMSO at room temperature and the data are represented in the Table 2. The band appeared around 276 nm and 340 nm corresponds

to $\pi \rightarrow \pi^*$ transition of aromatic chromophore and to $n \rightarrow \pi^*$ transitions of imine moiety respectively [21]. Except zinc complex all the other complexes exhibited a broad band between 526 to 660 nm due to d-d transition [22, 23].

Table 2. The UV-Vis. spectral data of Schiff base metal complexes

Complex	Absorption (λ_{\max} nm)		
	$\pi \rightarrow \pi^*$	$n \rightarrow \pi^*$	d-d
[Mn L ¹ L ²]	276	325	662
[Co L ¹ L ²]	348	389	526
[Ni L ¹ L ²]	273	339	585
[Cu L ¹ L ²]	353	411	565
[Zn L ¹ L ²]	273	359	-

FTIR spectra

The characteristic FTIR bands of the synthesized complexes are given in Table 3. All the complexes exhibited an intense band around 1604 cm^{-1} due to the coordinated imine group with the metal ions [24-26]. FTIR spectra is an important tool in investigating the coordination mode of the carboxylate anion with the metal ions. In the present case the difference between asymmetric and symmetric stretching frequencies of the carboxylate group lies above the free carboxylate anion which is 192 cm^{-1} . This confirms the monodentate coordination nature of the carboxylate ion with the metal ions [21, 23]. The bands observed around 590 cm^{-1} and 450 cm^{-1} confirms the M-N and M-O linkages in the coordination complexes [26].

Table 3. FTIR absorption bands of the Schiff base metal complexes in cm^{-1}

Complex	C=N	COO ⁻		$\Delta\nu = [\nu_{\text{as}} - \nu_{\text{s}}]$	M-O	M-N
		(ν_{as})	(ν_{s})			
[Mn L ¹ L ²]	1604	1598	1390	208	497	599
[Co L ¹ L ²]	1604	1600	1400	200	470	580
[Ni L ¹ L ²]	1598	1597	1394	203	457	578
[Cu L ¹ L ²]	1602	1598	1398	200	435	590
[Zn L ¹ L ²]	1606	1595	1390	205	418	599

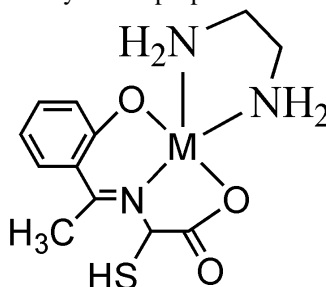
EPR Spectra

The paramagnetic nature of the complexes was investigated with the help of EPR spectroscopy. EPR spectral data of the polycrystalline metal complexes at room temperature are given in Table 4. Zn(II) complex is found to be EPR inactive and hence confirms the diamagnetic as expected for the d^{10} configuration. Whereas all the other complexes were found to be paramagnetic in nature. The g_{iso} values of the complexes shows axial symmetry i.e., $g_{xx} = g_{yy} = g_{zz}$, where all the axis are aligned parallel to the principal axial. Such a spectra is expected for complexes with symmetrical environment like octahedral, square planar and square pyramidal geometry [24].

Table 4. EPR spectral g value for Schiff base metal complexes

Complex	g value
[Co L ¹ L ²]	2.41
[Ni L ¹ L ²]	2.62
[Cu L ¹ L ²]	2.83
[Zn L ¹ L ²]	-

Based on the physico-chemical and spectral analyses the proposed structure of the complexes are given in Figure 1.



where $M = \text{Cu(II)}, \text{Ni(II)}, \text{Mn(II)}, \text{Co(II)}$ and Zn(II) .

Figure 1. Proposed structure of the metal complexes

Antimicrobial activity

All the synthesized Schiff base ligand and its metal complexes were screened *in vitro* for their antibacterial activity against bacteria such as *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *E. coli* by well diffusion method. Similarly antifungal activity against fungi such as *A. niger*, *A. flavus* and *P. notatum*.

The zone of inhibition values of the synthesized metal complexes against the growth of the selected bacteria and fungi are measured in mm. The zone of inhibition value less than 10 mm is considered as resistant towards the corresponding microorganism. The standard Ampicillin was used as positive control for antibacterial activity and standard drug Polymyxin B sulphate was taken as positive control for antifungal activity and DMSO was used as negative control.

The antimicrobial activity results are depicted in Table 5-8. When compared to the Schiff base ligand all the complexes exhibited higher zone of inhibition against the bacteria under investigation. *E. coli* is found to be resistant towards the Schiff base ligand and the metal complexes. Cu(II), Ni(II) and Co(II) complexes exhibited higher zone of inhibition (28 mm) against *S. aureus*.

Against *P. notatum*, except Co(II) complex, all the other metal complexes showed higher zone of inhibition than the standard drug Polymyxin B sulphate and the Schiff base ligand against the fungi under study. Among the complexes Ni(II) and Cu(II) complexes showed 32 mm zone of inhibition against *A. niger* and *P. notatum* respectively. Co(II) and Cu(II) complexes exhibited 29 mm zone of inhibition against *A. niger*.

The MIC value of the Schiff base ligand, Co(II) and Ni(II) complexes against *A. niger*, Schiff base ligand and Ni(II) complex against *A. flavus* as well as Schiff base ligand and Cu(II) complex against *P. notatum* showed least MIC value of 1.25 µg/mL.

Table 5. Antibacterial activity of the Schiff base ligand and its metal complexes

Bacteria	Zone of inhibition(mm)						Ampicillin (Standard)
	[Mn L ¹ L ²]	[Co L ¹ L ²]	[Ni L ¹ L ²]	[Cu L ¹ L ²]	[Zn L ¹ L ²]	L ¹	
<i>S. aureus</i>	27	28	28	28	24	18	30
<i>P. aeruginosa</i>	22	18	23	18	22	14	25
<i>K. pneumoniae</i>	21	9	20	10	10	12	33
<i>E. coli</i>	9	10	10	9	13	10	16

Table 6. Antifungal activity of the Schiff base ligand and its metal complexes

Fungi	Zone of inhibition(mm)						Polymyxin B sulphate (Standard)
	[Mn L ¹ L ²]	[Co L ¹ L ²]	[Ni L ¹ L ²]	[Cu L ¹ L ²]	[Zn L ¹ L ²]	L ¹	
<i>A. niger</i>	28	29	32	29	26	18	11
<i>A. flavus</i>	20	17	20	18	19	14	11
<i>P. notatum</i>	27	10	26	32	25	14	11

Table 7. Antibacterial MIC values of Schiff base ligand and the metal complexes in µg /mL

Compound	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>E. coli</i>
[Mn L ¹ L ²]	2.5	5	2.5	5
[Co L ¹ L ²]	1.25	5	5	5
[Ni L ¹ L ²]	2.5	1.25	2.5	5
[Cu L ¹ L ²]	2.5	5	5	5
[Zn L ¹ L ²]	5	2.5	5	2.5
L ¹	2.5	5	5	5

Table 8. Antifungal MIC values Schiff base ligand and the metal complexes in µg /mL

Compound	<i>A. niger</i>	<i>A. flavus</i>	<i>P. notatum</i>
[Mn L ¹ L ²]	2.5	2.5	2.5
[Co L ¹ L ²]	1.25	10	10
[Ni L ¹ L ²]	1.25	1.25	2.5
[Cu L ¹ L ²]	2.5	2.5	1.25
[Zn L ¹ L ²]	2.5	2.5	2.5
L ¹	1.25	1.25	1.25

Antioxidant activity

An antioxidant can be defined as any substance that when present at low concentrations, compared with those of the oxidizable substrate, significantly delays or inhibits oxidation of that substrate. To study the *in vitro* antioxidant

activity of the metal complexes two methods were adopted viz., DPPH and H₂O₂ method. The results revealed that Zn(II) complex showed highest scavenging potential of 58.96% and 57.29% in DPPH and H₂O₂ method respectively, whereas all the other synthesized complexes showed moderate to mild antioxidant activity ranging from 38-56%. The DPPH and H₂O₂ radical scavenging activity of standard antioxidant α -tocopherol was also assayed for comparison [27] and the values are listed in Table 9.

Table 9. Antioxidant scavenging activity of the Schiff base metal complexes

Complex	% of antioxidant scavenging activity	
	DPPH	H ₂ O ₂
[Mn L ¹ L ²]	46.71 %	48.46 %
[Co L ¹ L ²]	56.20 %	45.26 %
[Ni L ¹ L ²]	43.85 %	38.42 %
[Cu L ¹ L ²]	56.49 %	50.26 %
[Zn L ¹ L ²]	58.96 %	57.29 %
α -tocopherol	89.45%	68.72%

Larvicidal activity

The larvicidal activity of synthesized metal complexes was studied against *C. quinquefasciatus* and the values are depicted in Table 10 and 11. The highest mortality was obtained for Cu(II) complex with 95%. The average larval mortality data were subjected to statistical analysis for calculating standard deviation, chi-square values, LC₅₀ and LC₉₀ for synthesized metal complexes. Minimum lethal concentration of the complexes indicates the more toxicity of the complex towards larvae. The calculated values are lesser than table value hence the results with $p < 7.81$ were considered to be statistically significant. The order % of mortality of the synthesized complexes is given below:

[Cu L¹L²] > [Zn L¹L²] > [Ni L¹L²] > [Mn L¹L²] = [Co L¹L²]

Table 10. Larvicidal activity of Schiff base metal complexes

Complex	Concentration / mortality			
	4 mg/200 mL	2mg/200 mL	1mg/200 mL	0.5mg/200 mL
[Mn L ¹ L ²]	11	7	3	2
[Co L ¹ L ²]	11	8	4	1
[Ni L ¹ L ²]	18	13	13	5
[Cu L ¹ L ²]	19	11	10	6
[Zn L ¹ L ²]	17	12	8	6

Table 11. Statistical analysis of Larvicidal activity of the Schiff base metal complexes

Complex	Concentration /Mortality \pm SD				LC50 (mg/200 mL)	LC90 (mg/200 mL)	χ^2	df
	4mg/200mL	2mg/200mL	1mg/200mL	0.5mg/200mL				
[Mn L ¹ L ²]	55 \pm 6.15	35 \pm 6.90	15 \pm 1.88	10 \pm 2.00	3.65	6.4	12.35	3
[Co L ¹ L ²]	55 \pm 6.29	40 \pm 3.66	20 \pm 1.5	05 \pm 0.00	3.6	6.75	13.76	
[Ni L ¹ L ²]	90 \pm 5.87	65 \pm 5.27	65 \pm 4.60	25 \pm 5.45	1.8	4	18.24	
[Cu L ¹ L ²]	95 \pm 6.56	55 \pm 6.19	50 \pm 6.28	30 \pm 4.75	1.6	3.75	18.17	
[Zn L ¹ L ²]	85 \pm 5.19	60 \pm 5.88	40 \pm 5.32	30 \pm 5.21	1.65	4.3	14.18	

Mean value of four replicates, Control-Nil mortality, df- significant at $P < 7.81$ LC₅₀ lethal concentration that kills 50% of the exposed larvae, LC₉₀ lethal concentration that kills 90% of the exposed larvae.

CONCLUSION

In the present study ternary Schiff base transition metal complexes has been synthesized and characterized by various physico-chemical and spectral analyses. The molar conductance of all the complexes suggested their non-electrolytic nature. The coordinating mode of the donor atoms of the ligand was confirmed by FTIR spectra. EPR spectra substantiate the paramagnetic nature of all the complexes except Zn(II) complex. Based on the studies square pyramidal geometry has been proposed for the complexes. Larvicidal and *in vitro* antimicrobial and antioxidant studies were carried out. The results revealed that all the complexes were found to exhibit very good antifungal activity against the fungi under study. Co(II), Ni(II) and Cu(II) complexes exhibited very good anti bacterial activity against *S. aureus*. Ni(II), Cu(II) and Zn(II) complexes showed highest mortality against *C. quinquefasciatus*. Among the synthesized complexes Zn(II) complex was found to have good antioxidant activity.

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