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SYNTHESIS, SPECTROSCOPIC CHARACTERISATION AND *IN VITRO* BIOLOGICAL STUDIES OF SCHIFF BASE TRANSITION METAL (II) COMPLEXES

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Abstract:

Coordination chemistry is the one of the interesting branch in inorganic chemistry. The research in coordination chemistry plays very important role in many fields including medical, industrial, analytical and material science. Schiff base is one of the important ligands in the field of Coordination Chemistry research. Schiff base is a condensation product of primary amines with carbonyl compounds. Amino acids are biologically important organic compounds and are very essential for every metabolic process. The research area encircling the coordination compounds with azomethine ligands derived from amino acids as main characters is widely expansive especially due to their potential interest aroused in various interdisciplinary fields such as bioinorganic chemistry. In the present work, we report the synthesis and characterization of ternary Schiff base transition metal complexes with the general formula $[ML^1L^2]ClO_4$ (where, M = Mn(II), Co(II), Ni(II), Cu(II) and Zn(II)] ; L^1= Schiff base ligand derived from L-valine and vanillin; L^2 = N,N,N',N'-tetramethyl-1,3-diaminopropane). The synthesized metal complexes were characterized by physicochemical techniques, spectroscopic analyses such as UV-Visible, FTIR, EPR and mass spectra. To facilitate the biological activities of the Schiff base and the transition metal complexes *in vitro* antibacterial, antifungal, antioxidant and larvicidal studies were carried out. The molar conductivity values revealed 1:1 electrolytic nature of all the metal complexes. The FTIR spectra confirmed that the metal centre is coordinated to imine nitrogen, phenolic oxygen atom present in carboxylate group of Schiff base ligand. It also suggested the presence of uncoordinated perchlorate anion. *In vitro* biological studies revealed that the Schiff base transition metal complexes possess good biological activities.

Key Words: Schiff Base, Amino Acids, Spectroscopic Analyses & In Vitro Biological Studies

1. Introduction:

Today the research in coordination chemistry plays a vital role in the development of inorganic chemistry. Coordination chemistry in particular comprises a large body of coordination compounds. Any compounds containing a metal atom or ion with one or more ligands are called as coordination compound or complex. A ligand is a chemical moiety which bond with the metal ions either covalent or coordination modes. One of the most interesting aspects of transition metals is their ability to form coordination compounds. Schiff bases are considered as privileged ligand in Coordination chemistry[1] which was first reported by Scientist Hugo Schiff in 1864. Schiff base ligand is formed as a product of the reaction between any amine moiety and carbonyl compound by condensation[2,3]. The common structural feature of these compounds is the azomethine group with a general formula $R^1R^2C = N\text{-}R^1$ where, R^1 and R^2 can be alkyl, aryl, cyclo alkyl or heterocyclic groups which may be variously substituted. These compounds are also known as anils, imines or azomethines[4,5]. Literature review reveals that the azomethine group (>C=N-) in Schiff base complexes are responsible for various biological functions[6-10] like antimicrobial, antibacterial, antifungal, anti-inflammatory, anticonvulsant and antitumor activities[11-16]. Schiff bases also have immense applications as catalysts, stabilizers, pigments, dyes and drugs.

In recent years Schiff base complexes of amino acid have received considerable attention because of their physiological and pharmacological activities[17,18]. The emergence and increase of pathogens resistant to many available drugs is of great concern and as a result of the spread of resistance to the inexpensive drugs widely used for treatment of diseases like malaria and tuberculosis[19]. Schiff base metal complexes in particular offer great promise for novel activity. L-valine is one of the essential amino acids and exhibit numerous biological activities [20]. Based on the above facts the prime aim of the present work is to synthesize a series of Schiff base transition metal complexes derived from L-valine and to characterize the synthesized metal complexes using various analytical, spectral studies and to explore the biological activities like antimicrobial, antioxidant and larvicidal using *in vitro* method.

2. Materials and Methods:

2a. Physical Measurements: All chemicals and reagents used were of analytical grade. 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. The Ultraviolet-Visible spectra of the complexes in DMSO were recorded using Systronics 2201 spectrophotometer between 800 to 200

nm. The FTIR spectra of the complexes were recorded using Shimadzu spectrometer in the range of 4000-400 cm^{-1} using KBr pellet. The EPR spectra of the complexes were recorded in the polycrystalline state at room temperature using Bruker EMX -10/2.7 Spectrometer. The mass spectrum of Cu(II) complex was recorded mass spectrophotometer in powered state.

2b. Synthesis of Schiff Base Metal Complexes: To an aqueous solution of L-valine (0.58 g, 5 mmol) and KOH (0.56 g, 10 mmol) an ethanolic solution of vanillin (0.76 g, 5 mmol) was added in the ratio 1:2:1. The reaction mixture was stirred for about 1 h in a magnetic stirrer at 333 K. The solution turned yellow. To the above solution an appropriate metal salt [Copper(II) acetate monohydrate (0.99g, 5mmol), nickel (II) acetate(1.24g, 5mmol, cobalt (II) acetate (1.24g, 5mmol), zinc (II)acetate (1.09 g 5 mmol), manganese (II) sulphate (0.98 g, 5 mmol) was added and the reaction mixture was stirred for 1 h at 333 K followed by the addition of N,N,N',N' tetramethylpropane-1,3-diamine(0.83 mL, 5 mmol). The reaction mixture was stirred for another 2 h at the same temperature. At the end of the reaction aqueous solution of sodium perchlorate monohydrate (0.7 g, 5 mmol) was added. The resultant dark colored product washed with ethanol and filtered and dried.

2c. Antimicrobial Studies (in vitro): The Schiff base and the metal complexes were screened for their biological activities by *in vitro* method to test their bioefficacy [21]against Gram-positive bacteria such as *Bacillus subtilis*, Gram-negative bacteria such as *Escherichia coli, Pseudomonas aeruginosa* and fungi *Rhizopus nigricans, Aspergillus flavus and Penicillim notatum.* The agar well diffusion method was used to evaluate the antimicrobial activity of synthesized metal complexes [22]. The effect of various metal complexes on the several bacterial and fungal strains was assayed. The micro organisms were inoculated on Muller Hinton Agar and spread uniformly using sterile spreader in Petri plates. Three wells of 0.85cm 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 DMF solvent were poured into two wells and negative control DMF 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 °C for 4 h. Antimicrobial activity was determined by measuring the diameter of zones showing complete inhibition. Ampicillin and Polymyxin were used as positive controls for antibacterial and antifungal studies respectively[23].

Minimum Inhibitory Concentration (MIC): The minimum inhibitory concentration (MIC) is the lowest concentration at which the growth of a microorganism is inhibited [24]. 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 is serially diluted in to six test tubes using sterile pipette so that concentrations varied. One drop of the test organism was added in all test tubes. Finally the tubes were incubated at 37 °C for 24 h. The same procedure is followed for fungal strains using SDS nutrient broth and were incubated fro 48 h at room temperature.

2d. Antioxidant Activity: An antioxidant is a molecule that inhibits the oxidation of other molecules. Free radicals are responsible for causing a large number of diseases including cancer [25] cardiovascular disease [26], neural disorders, alzheimer's disease, mild cognitive impairment [27], Parkinson's disease, alcohol induced liver disease [28]. Antioxidants plays an essential role in the prevention of diseases. Hence the present work based on the study of the antioxidant property of the prepared Schiff base metal complexes.

DPPH Scavenging Activity: The antioxidant activity of the complexes was evaluated by DPPH radical scavenging assay DPPH radicals scavenging activity evaluation is a standard assay in antioxidant activity studies. It is a rapid technique for screening the radical scavenging activity of the specific compounds [29]. The free radicals scavenging effects of the all the compounds and ligand with the DPPH radical were evaluated using concentration 2 mg / mL of the test complex in DMF with 2 mL of 0.05 M methanolic solution of DPPH. After 30 min incubation period at room temperature, the antiradical scavenging ability of synthesized metal complexes was determined by measuring the decrease in the absorbance of DPPH at 517 nm. Resulting from a color change, the absorbance decreased when the DPPH is scavenging by an antioxidant, though donation of hydrogen atom to from a stable DPPH molecule. DPPH solution without sample is considered as control. 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 antioxidant property [30]. A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (50 mM, pH 7.4). The concentration of hydrogen peroxide is determined by adsorption at 230 nm using a spectrophotometer. Synthesized complexes with the concentration of 2 mg/ 1mL in DMF are added to hydrogen peroxide and absorption at 230 nm is determined after 10 min against blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging activity was calculated as DPPH method.

2e. Larvicidal Activity: The eggs and egg rafts of *Culex quinquefasciatus* were procured from Zonal Entomological Unit, Vellore, Tamilnadu. 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[31]. 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 [32]. A total of 20 reared mosquito larvae of *C. quinquefasciatus* was placed in 200 mL of double distilled sterilized water containing various concentration (4 mg, 2 mg, 1 mg, 0.5 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.

3. Results and Discussion:

All the synthesized transition metal complexes are found to be freely soluble in DMSO, DMF, methanol, ethanol and partially soluble in water at room temperature. The analytical data of the synthesized metal complexes are shown in Table 1. The molar conductivity values were in the range of 67-81 ohm $^{-1}$ cm^2 mol^{-1} and hence confirmed 1:1 electrolytic nature [33]of the complexes.

Table 1: Analytical data of the Schiff base metal complexes

Complex	Molecular Formula	Molecular weight	Melting/ decomposition point	Colour	Molar conductance ohm^{-1} cm^2 mol^{-1}	Elemental analysis found % (calculated)		
						C	H	N
[MnL^{1}L^2] ClO$_4$	$C_{20}H_{33}O_8N_3$ ClMn	533.95	360	Light Brown	80	44. 67 (44.98)	6.51 (6.24)	7.81 (7.87)
[Co L^{1}L^2]ClO$_4$	$C_{20}H_{33}O_8N_3$ ClCo	537.94	270	Dark Brown	67	44. 55 (44.67)	5.72 (5.80)	7.85 (7.81)
[Ni L^{1}L^2]ClO$_4$	$C_{20}H_{33}O_8N_3$ ClNi	537.7	270	Pale Green	79	44. 01 (44.65)	6.45 (6.19)	7.55 (7.81)
[Cu L^{1}L^2]ClO$_4$	$C_{20}H_{33}O_8N_3$ ClCu	542.56	270	Dark Green	80	44. 09 (44.27)	5.97 (6.10)	7.28 (7.70)
[Zn L^{1}L^2]ClO$_4$	$C_{20}H_{33}O_8N_3$ ClZn	548.4	355	Light White	81	43. 75 (43.80)	6.21 (6.07)	7.55 (7.66)

Where L^1=Schiff base; L^2=N,N,N',N'-tetramethyl-1,3-propanediamine.

3.1 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 spectrum of the free Schiff base (L1) displays bands at 238 nm and 312 nm corresponds to π-π* transition of the phenyl ring and n-π*transition of the imine moiety [34] respectively. In the case of metal complexes, these bands are shifted to longer wavelength with increasing the intensity. Such a shift may be attributed to the donation of lone pair of electron present in the nitrogen atom of the Schiff base to the metal ions. The absorption band observed around 266 nm for all the metal complexes corresponding π-π* transition of aromatic chromospheres. The absorption bands observed in between in the the range of 305 to 309 nm for metal complexes corresponds to n-π* transition of imine moiety[35]. Due to d^{10} configuration d-d transition is not appeared in Zn (II) complex while all other complexes showed d-d transition in the range of 692-696 nm [35].

Table 2: The UV-Vis. spectral data of Schiff base ligand and its metal complexes

Complex	Absorption (λ_{max} nm)		
	π-π*	n-π*	d- d
Schiff Base Ligand	238	312	-
[MnL^{1}L^2]ClO$_4$	266	308	692
[Co L^{1}L^2]ClO$_4$	266	309	696
[Ni L^{1}L^2]ClO$_4$	266	309	696
[Cu L^{1}L^2]ClO$_4$	266	304	696
[Zn L^{1}L^2]ClO$_4$	266	305	-

3.2 FTIR Spectra: The FITR spectrum provides valuable information regarding the nature of the functional group attached with the metal ion in the synthesized Schiff base metal complexes. The synthesized complexes were characterized mainly using the C=N (azomethine), COO-, M-O, and M-N bands. The assignments of important infrared spectral data are listed in table 3. The strong bands appeared around 1600 cm^{-1} are related to the C=N linkage of the Schiff base moiety in metal complexes. The formation of M-N and M-O linkage was confirmed due to appearance of bands around 540 and 480 cm^{-1} respectively[36].

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

Complex	C=N	COO^-		$\Delta\upsilon=[\upsilon_{as}-\upsilon_s]$	M-O	M-N
		(υ_{as})	(υ_s)			
$[MnL^1L^2]ClO_4$	1666	1585	1330	172	570	484
$[Co\ L^1L^2]ClO_4$	1645	1577	1311	206	597	466
$[Ni\ L^1L^2]ClO_4$	1660	1577	1346	231	559	484
$[Cu\ L^1L^2]ClO_4$	1649	1577	1392	185	564	468
$[Zn\ L^1L^2]ClO_4$	1643	1575	1313	262	570	484

The bands appeared around 1020 cm^{-1} indicated the presence of uncoordinated perchlorate anion [36]. The characteristic band was obtained for asymmetric and symmetric carboxylate group. The difference between asymmetric and symmetric stretching values of the carboxylate group ($\Delta\upsilon = \upsilon_{as} - \upsilon_s$) were found to be greater than that of the free carboxylate ion. This confirms the monodenate coordination of the carboxylate anion [36] with the metal ions. The FTIR spectra revealed bidentate nature of the Schiff base ligand with the metal ions.

3.3 EPR Spectra: The EPR spectrum is mainly used to analyse the paramagnetic nature of the complexes. The EPR spectra of the synthesized complexes were recorded in solid state at room temperature The EPR spectra of the complexes were isotropic and the corresponding g-values are given in table 4.

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

Complex	g value
$[MnL^1L^2]ClO_4$	3.06
$[Co\ L^1L^2]ClO_4$	2.05
$[Ni\ L^1L^2]ClO_4$	2.35
$[Cu\ L^1L^2]ClO_4$	2.40
$[Zn\ L^1L^2]ClO_4$	-

The g-values for Mn(II), Co(II), Ni(II), Cu(II) confirmed the paramagnetic nature of those complexes. Zn (II) complex is found to be EPR inactive and hence confirmed the diamagnetic nature as expected for the d^{10} configuration[37]. The g_{iso} values of the complexes shows axial symmetry i.e, $g_{xx}=g_{yy}=g_{zz}$, where the entire axis aligned parallel to the principal axis. Such the spectra are expected for the complexes with symmetrical environment like octahedral, square planar and square pyramidal [38].

3.4 Mass Spectra: The positive ionization spectrum of the Cu(II) complex showed two prominent peaks which were characterized as follows. The theoretical exact mass of the copper complex is 443.1845 Da. The time-of-flight electron spray ionisation (TOF-ESI) spectrum showed the m/z value of 461.9435 Da., which was characteristic peak of as [M + Na]+. The theoretical exact mass of sodium adduct was calculated as 462.1799 Da. The calculated mass defect is around 51 ppm. Hence mass spectra confirmed the molecular weight of proposed formula of Cu(II) complex. Based on the physicochemical and spectral analyses the proposed structure of the complexes is given in Figure 1.

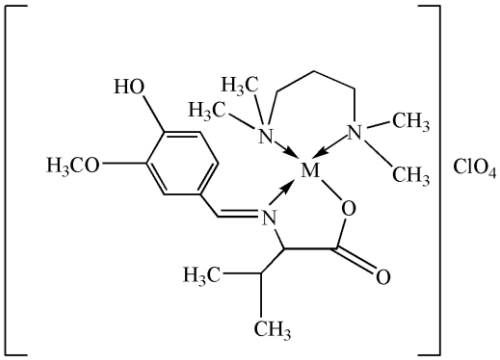

Figure 1: Proposed structure of the Schiff base metal(II) complexes

Where, M = Cu(II), Mn(II), Ni(II), Co(II) and Zn(II)

3.5 Antimicrobial Activity: The increase in prevalence of multiple drug resistance has slowed down the development of new synthetic antimicrobial drug and has necessitated the search for new antimicrobial from alternative sources. Hence we made another attempt to study the biological activities of the synthesized complexes. The Schiff base ligand and all the transition metal complexes were screened *in vitro* for their antibacterial activity against Gram-positive bacteria such as *Bacillus subtilis* and Gram-negative bacteria such as *Pseudomonas aeruginosa* and *E. coli* by well diffusion method as well as antifungal activity against *Aspergillus flavus, Rhizopus nigericans* and *Penicillim notatum.* The zone of inhibition values of the synthesized metal complexes against the growth of the selective bacteria and fungi are measured in mm. The zone of inhibition values less than 10 mm is considered to be a resistant towards the corresponding the microorganism. The standard *Ampicillin* and *Polymyxin* was used as positive control for antibacterial and antifungal activity

respectively and DMF was used as negative control. The corresponding data are summarized in table 5 and 6. The MIC values of the Schiff base ad its metal complexes are depicted in tables 7 and 8.

Table 5: Antibacterial activity of the Schiff base transition metal complexes

Bacteria	**Zone of inhibition(mm)**						**Ampicillin**
	$[MnL^1L^2]ClO_4$	$[Co\ L^1L^2]ClO_4$	$[Ni\ L^1L^2]ClO_4$	$[CuL^1L^2]ClO_4$	$[Zn\ L^1L^2]ClO_4$	L^1	
B. subtilis	24	15	16	27	19	18	12
P. aeruginosa	20	15	14	30	16	13	17
E. coli	12	10	13	29	11	12	20

Table 6: Antifungal activity of the Schiff base transition metal complexes

Fungi	**Zone of inhibition(mm)**						**Polymyxin B sulphate**
	$[MnL^1L^2]ClO_4$	$[Co\ L^1L^2]ClO_4$	$[Ni\ L^1L^2]ClO_4$	$[CuL^1L^2]ClO_4$	$[Zn\ L^1L^2]ClO_4$	L^1	
A. flavus	12	20	19	20	22	16	18
R. nigricans	16	18	15	26	18	16	16
P. notatum	18	20	18	20	22	17	17

All the synthesized metal complexes showed very good antibacterial activity against *Bacillus subtilis* than the corresponding standard *ampicillin*. The antibacterial activity of Mn(II), Cu(II) and Zn(II) complexes were found to the greater than the corresponding Schiff base ligand. Except Ni(II) complex all the synthesized metal complexes showed very good antibacterial activity against *Pseudomonas aeruginosa*. Cu(II) exhibited higher antibacterial activity against *E. coli* with the zone of inhibition 29 mm. All the synthesized metal complexes showed good antifungal activity against *Aspergillus flavus.* Among the series Zn(II) complex showed higher zone of inhibition value of 22 mm against *Aspergillus flavus*. Co(II) and Cu(II) complexes showed 20 mm zone of inhibition against *Aspergillus flavus*. All the synthesized complexes exhibited moderate to stronger antifungal activity against *Rhizopus nigricans.* Among the series of Zn(II), Co(II) and Cu(II) complexes showed higher zone of inhibition against *Penicillim notatum*.

Table 7: Antibacterial MIC values in µg /mL

Compound	***B. subtilis***	***E. coli***	***P. aeruginosa***
$[MnL^1L^2]ClO_4$	1.25	5	2.5
$[Co\ L^1L^2]ClO_4$	2.5	2.5	1.25
$[Ni\ L^1L^2]ClO_4$	1.25	0.625	1.25
$[Cu\ L^1L^2]ClO_4$	2.5	1.25	2.5
$[Zn\ L^1L^2]ClO_4$	2.5	2. 5	0.625
L1	2.5	5	1.25

Table 8: Antifungal MIC values in µg /mL

Compound	***A. flavus***	***R. nigerians***	***P. notatum***
$[MnL^1L^2]ClO_4$	1.25	2.5	1.25
$[Co\ L^1L^2]ClO_4$	1.25	1.25	0.625
$[Ni\ L^1L^2]ClO_4$	1.25	1.25	1.25
$[Cu\ L^1L^2]ClO_4$	2.5	2.5	1.25
$[Zn\ L^1L^2]ClO_4$	2.5	1.25	2.5
L1	1.25	2.5	2.5

Among the Schiff base ligand and its metal complexes Mn(II) and Ni(II) complexes showed least MIC value of 1.25 µg/mL against *Bacillus subtilis.* Similarly, Ni(II) and Zn(II) complexes showed least value of 0.625 µg/mL against *E. coil* and *Pseudomonas aeruginosa.* the MIC value of Mn(II), Co(II), Ni(II) and Schiff base ligand complexes Shows 1.25 µg /mL against *Aspergillums flavus.* Cobalt (II) complex exhibit MIC value of 0.625 µg /mL against *Penicillim notatum.* Zn(II), Co(II), and Mn(II) complexes shows lowest inhibition concentration value 1.25 µg /mL against *Rhizopus nigricans.* It was observed that metal chelate have higher antibacterial activity because of an increase in cell permeability. Such as an increase in the activity of the complexes can be explained on the basis of chelation theory [39, 40]. Schiff base ligand may be due to the change in structure due to coordination and chelating tends to make metal complexes act as more powerful and potent bacteriostatic agents, thus inhibiting the growth of the bacteria also destroying them more forcefully [41]. In the present study, low activity of the some metal complexes may be due to the lower penetrating tendency of them through lipid membrane. Hence this could neither block nor inhibit the growth of the microorganism [42-43].

3.6 Antioxidant Activity: To study the *in vitro* antioxidant activity of the metal complexes two methods were adopted viz., DPPH and H_2O_2 method. α- tocopherol with 89.45 % of scavenging activity [44] is used as positive control and DMF is used as negative control.The results revealed that Mn(II) complex shows highest scavenging

potential of 91.02% and 89 % in H_2O_2 and DPPH method respectively, whereas all the other synthesized complexes showed moderate to mild antioxidant activity ranging from 69-88%. The values are listed in Table 9.

Table 9: Antioxidant scavenging activity of metal complexes

Complex	% of Antioxidant Scavenging Activity	
	DPPH	H_2O_2
$[MnL^1L^2]ClO_4$	91.02	89
$[Co\ L^1L^2]ClO_4$	88.89	85
$[Ni\ L^1L^2]ClO_4$	84.46	80
$[Cu\ L^1L^2]ClO_4$	76.28	73
$[Zn\ L^1L^2]ClO_4$	69.74	72

3.7 Larvicidal Activity: The larvicidal activity of synthesized metal complexes was studied against *C. quinquefasciatus* and the values are depicted in Tables 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, LC_{50} and LC_{90} 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. Though all the metal complexes are having larvicidal activityCu(II) and Ni(II) complexes were found to have higher mortality values among the synthesized metal complexes.

$[Cu\ L^1L^2]\ ClO_4 > [Ni\ L^1L^2]\ ClO_4 > [Co\ L^1L^2]\ ClO_4 > [Zn\ L^1L^2]\ ClO_4 = [Mn\ L^1L^2]ClO_4$

Table 10: Larvicidal activity of metal complexes

Complex	Concentration / mortality			
	4 mg/200 mL	2mg/200 mL	1mg/200 mL	0.5mg/200 mL
$[MnL^1L^2]ClO_4$	12	10	6	2
$[Co\ L^1L^2]ClO_4$	14	12	12	6
$[Ni\ L^1L^2]ClO_4$	16	16	10	6
$[Cu\ L^1L^2]ClO_4$	18	18	16	14
$[Zn\ L^1L^2]ClO_4$	12	8	8	4

Table 11: Statistical analysis of Larvicidal activity of metal complexes

Complex	Concentration / %Mortality±SD				LC50	LC90	χ^2	df
	4mg/200mL	2mg/200mL	1mg/200mL	0.5mg/200mL				
$[MnL^1L^2]ClO_4$	60 ± 4.49	50 ± 3.59	30 ± 3.26	10 ± 4.56	2	4	21.72	3
$[Co\ L^1L^2]ClO_4$	70 ± 7.16	60 ± 6.11	40 ± 3.89	30 ± 3.87	1.6	3.3	13.76	
$[Ni\ L^1L^2]ClO_4$	80 ± 7.36	80 ± 6.78	50 ± 5.65	30 ± 2.84	1.1	2.3	23.75	
$[Cu\ L^1L^2]ClO_4$	90 ± 6.26	90 ± 8.12	80 ± 7.28	70 ± 6.51	1.1	2.0	18.17	
$[Zn\ L^1L^2]ClO_4$	60 ± 7.64	40 ± 6.32	40 ± 3.89	20 ± 1.65	3.0	6.5	21.66	

Mean value of four replicates, Control-Nil mortality, df- significant at $P< 7.81$

4. Conclusion:

In the present study, Schiff base transition metal complexes were synthesized and characterized by various physicochemical and spectral analyses. The molar conductance of all the complexes suggested their electrolytic nature with 1:1 ratio. The electronic absorption spectra suggested the square planar geometry for the synthesized metal complexes. FTIR suggested that the metal centre is coordinated to imine nitrogen, phenolic oxygen atom present in carboxylate group of schiff base ligand. Hence confirms that the schiff base acted as a bidentated ligand and also confirmed that the presence of uncoordinated perchlorate anion. EPR spectra substantiate the paramagnetic nature of all the complexes except Zn(II) complex. The isotropic spectra of the complexes confirmed the presence of symmetrical environment around metal ions. The biological activity of the synthesized metal complexes were studied. The results revealed that Cu(II) complexes exhibits very good anti bacterial activity against *Bacillus subtilis, Pseudomonas aeruginosa* and *E. coli.* Zn(II), Cu(II) and Co(II) complexes showed good antifungal activity against *Aspergillus flavus, Rhizopus nigericans* and *Penicillim notatum.* Mn(II) complexes exhibited well scavenging activity with 91.02 % and 89 % by H_2O_2 and DPPH method respectively. Ni(II) and Cu(II) complexes (4mg/200mL) showed highest mortality of % and 80 % against *C. quinquefasciatus* respectively. Since all the complexes showed significant biological activities, the current results provide a lead for the *in vivo* studies to establish the possibility of these complexes as antimicrobial, antioxidant and larvicidal agents.

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