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Synthesis, Characterization and *In Vitro* Biological Activities of Schiff Base Cu (ii), Mn (ii) and Ni(ii) Complexes Derived From Ferrocenecarboxaldehyde and Aminoacids

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Abstract

Organometallic compounds derived from Schiff base metal complexes occupies a major area of coordination research in recent years. Schiff base metal complexes prepared from ferrocenecarboxaldehyde were found to exhibit good biological activities. In the present work, a Schiff base Cu (II), Mn (II) and Ni(II) complexes of the type $[ML_2(H_2O)_2]$ were synthesized from ferrocenecarboxaldehyde with L-tryptophan and L-valine. All the complexes were characterized by using physicochemical and spectral studies such as UV-Visible, FTIR, EPR, ESI-MS and TGA. *In vitro* antimicrobial studies were carried out against bacterial strains such as *S. aureus*, *E. coli* and *P. aeruginosa* and fungal strains such as *A. flavus, A. nigerA. fumigates* and *Rhizopus.* In addition, *in vitro* larvicidal activity against *Culex quiquefasciatus* and antioxidant studies were also performed. The conductivity measurements indicate the non-electrolytic of the complexes. The FTIR spectra confirmed that the coordination of metal ion to imine nitrogen, oxygen atom presents in the carboxylate group and coordinated water molecule. The synthesized complexes showed better antimicrobial, antioxidant and larvicidal activities.

Keywords

Schiff base, spectral studies, *in vitro* antibacterial, antifungal, larvicidal and antioxidant studies.

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INTRODUCTION:

The organometallic Schiff base exhibits various applications in the field of dye, drug industries and polymer industry. [1] These complexes also show biological properties such as anticancer and antitumor activities. [2] Schiff bases are considered as privileged ligand in the field of coordination chemistry [3] when it is synthesized from organometallic carbonyl compounds, it shows enhanced biological activities. [4] A compound in which >C=O is replaced by >C=N through the reaction between amines and carbonyl compounds are known as Schiff base as well as recognized as imine or azomethine. [5,6] Ferrocenecarboxaldehyde is one of the organometallic

carbonyl compounds which exhibits good biological activities. Schiff base ligand derived from ferrocene carboxaldehyde is a significant component in enlightening the geometries and possess attractive biological activities. [7] These complexes also show various properties such as biochemical, antifungal, antimicrobial activities [8] and also used as catalysts.

In this study, we aim to gather insight on the synthesis, spectral studies, antimicrobial, antioxidant and larvicidal activities of the Schiff base metal complexes derived from ferrocene carboxaldehyde.

MATERIALS AND METHODS:

The commercially available analytical grade chemicals were used as such without purification. The electronic absorption spectra of the complexes were recorded on a SYSTRONICS 2201 spectrometer at room temperature with the sample concentration of 10^{-3} M in DMSO. The FITR spectra were recorded on SHIMADZU spectrometer between 4000-400 cm^{-1} range, using KBr pellet. The EPR spectra were recorded in solid state at room temperature using Bruker EMX - 10/2.7 spectrometer. Thermal stability of the two complexes were carried out on the instrument SDT Q600 V20.9 Build 20 model from 33˚C to 800 ˚C under a nitrogen atmosphere at the heating rate of 20°C/min. The mass spectra of the complex $[CuL^2_2 (H_2O)_2]$ was recorded in powdered state using acquisition SW Version 6200 series TOF/6500 series Q-TOF B.08.00 (B8058.0).

Synthesis of organometallic Schiff base metal complexes:

The common method was adopted for the synthesis of Schiff base ligand and its metal complexes. [9] **An** ethanolic solution of ferrocenecarboxaldehyde (0.428 g, 2 mmol) was taken. To this an aqueous solution of L-tryptophan (0.408 g, 2 mmol) and KOH (0.112 g, 2 mmol) were added. The reaction mixture was stirred for 2 h at 60˚C. The solution turned yellowish orange. To this an ethanolic solution of appropriate metal salts [Cu (II) (0.319 g, 2 mmol), Mn(II) (0.229 g, 2 mmol) and Ni(II) (0.259 g, 2 mmol)] were added. The mixture was stirred for another 2 h at the same temperature. The resultant brown coloured product was filtered, washed several times with ethanol and dried.

The similar procedure was followed to synthesize Schiff base metal [Cu(II), Mn(II) and Ni(II)] complexes using L-valine (0.2343 g, 2 mmol) instead of L-tryphtophan.

Antimicrobial studies (*in vitro*):

The bioefficacy of the synthesised organometallic Schiff base Cu(II), Mn(II) and Ni(II) complexes were tested as per standard procedure. [10] In order to investigate the antimicrobial activities of the synthesized complexes the bacterial strains *Staphylococcus aureus, Pseudomonas aeruginosa*, *E.coli* and fungal strains *Aspergillus flavus, Aspergillus fumigates*, *Rhizopus* were taken. The synthesized complexes with the concentration 10^{-3} M in DMSO was used as test a solution for the study. The standard drug Ampicillin and Gentamicin were taken as reference for antibacterial and antifungal activities respectively.

Larvicidal activity:

Zonal Entomological Unit, Vellore, has provided the eggs and egg rafts of *Culex quinquefasciatus.* The standard procedure was followed to maintain the procured larvae. [11] The procedure of WHO guidelines with some modification has been used to study the larvicidal activity [12] and number of dead larvae, percentage of mortality in each batch were counted for every 24 h exposure period by using various concentration (6 mg, 4 mg, 2 mg and 1mg) of synthesized organometallic Schiff base Cu(II), Mn(II) and Ni(II) complexes. The treated larvae was mounted on a slide and examined under a microscope for image capture.

Antioxidant activity:

Hydrogen peroxide scavenging activity:

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4). The concentration of hydrogen peroxide is determined by adsorption at 230 nm using a UV-Vis. spectrophotometer. Synthesized complexes with the concentration of 2 mg /mL, 4 mg /mL, 6 mg /mL in DMF are added to hydrogen peroxide and the absorption at 230 nm was observed after 10 min against blank solution containing phosphate buffer without hydrogen peroxide.[13] The radical scavenging activity was calculated in percentage using following equations. [14]

% of radical scavenging activity (H_2O_2)

$$= \frac{A_c - A_s}{A_c} \times 100$$

where,

A_c is the absorbance of the control solution; As is the absorbance of the sample solution.

RESULTS: The analytical data such as molecular formula, molar conductance and colour of the synthesized Mn (II), Ni(II) and Cu(II) complexes are shown in **Table 1.**

Table 1. Analytical and physical data of the Schiff base complexes

Complex	Molecular formula	Molecular weight	Melting Point	Colour	Molar conductance $Ohm^{-1}cm^2\ mol^{-1}$
$[CuL^1_2(H_2O)_2]$	$C_{44}H_{46}Fe_2N_4O_6Cu$	899.5	>300	Dark brown	8.05
$[MnL^1_2(H_2O)_2]$	$C_{44}H_{46}Fe_2N_4O_6Mn$	890.9	>300	Dark brown	6.8
$[NiL^1_2(H_2O)_2]$	$C_{44}H_{46}Fe_2N_4O_6Ni$	894.6	>300	Dark brown	1.2
$[CuL^2_2(H_2O)_2]$	$C_{32}H_{44}Fe_2N_2O_6Cu$	725.5	>300	Dark brown	7.05
$[MnL^2_2(H_2O)_2]$	$C_{32}H_{44}Fe_2N_2O_6Mn$	716.7	>300	Dark brown	5.08
$[NiL^2_2(H_2O)_2]$	$C_{44}H_{46}Fe_2N_4O_6Ni$	894.6	>300	Dark brown	1.28

Where, L^1 - Schiff base ligand prepared with L-tryptophan. L^2 - Schiff base ligand prepared with L-valine.

UV-Vis. spectra:

The UV-Visible spectral data are represented in the **Table 2**.

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

Complex	Absorption (λ_{max} nm)		
	π→π*	n→π*	d→d
$[CuL^1_2(H_2O)_2]$	241	377	649
$[MnL^1_2(H_2O)_2]$	236	371	572
$[NiL^1_2(H_2O)_2]$	273	387	592
$[CuL^2_2(H_2O)_2]$	302	395	618
$[MnL^2_2(H_2O)_2]$	342	376	598
$[NiL^2_2(H_2O)_2]$	272	358	584

FTIR spectra:

The assignments of important infrared spectral data of the Schiff base metal complexes are listed in **Table 3**.

Table 3. The FTIR spectral data of the Schiff base metal complexes

Complex	H_2O	C=N	COO^-		$\Delta\upsilon = [\upsilon_{as} - \upsilon_s]$	M-N	M-O
			υ_{as}	υ_s			
$[CuL^1_2(H_2O)_2]$	3387	1613	1585	1350	235	609	478
$[MnL^1_2(H_2O)_2]$	3302	1629	1577	1355	222	598	474
$[NiL^1_2(H_2O)_2]$	3236	1658	1585	1354	211	658	498
$[CuL^2_2(H_2O)_2]$	3490	1618	1595	1367	229	523	487
$[MnL^2_2(H_2O)_2]$	3410	1581	1502	1327	175	540	478
$[NiL^2_2(H_2O)_2]$	3434	1633	1499	1350	149	567	473

EPR spectra:

The ESR spectra was reordered at room temperature and the g-value of $[CuL^1_2(H_2O)_2]$ was and found to be 2.0757.

TGA analysis:

The thermal stability of the complexes can be studied using TGA. The TGA curve of the complexes $[CuL^1_2(H_2O)_2]$ and $[CuL^2_2(H_2O)_2]$ were represented in Fig1 & 2 respectively.

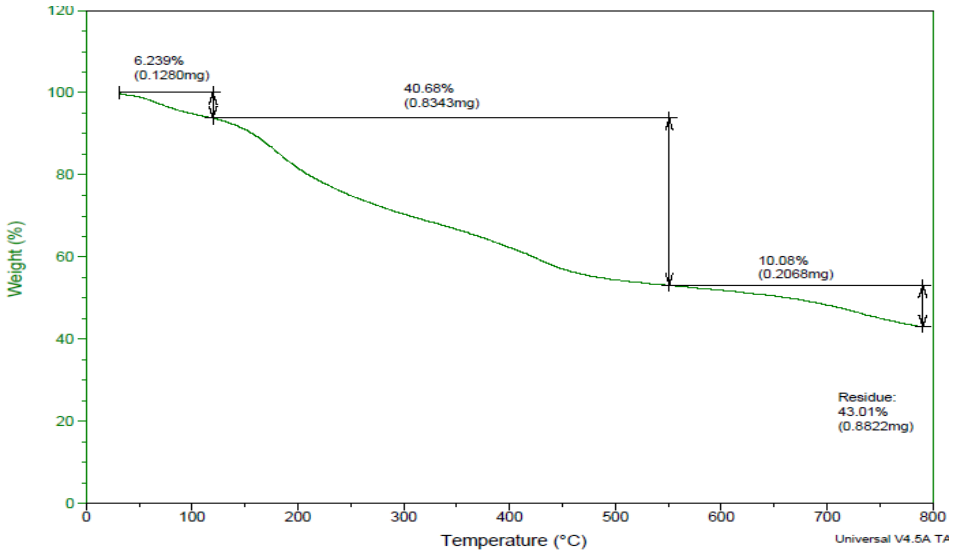

Figure 1. TGA curve of $[CuL^1{}_2(H_2O)_2]$

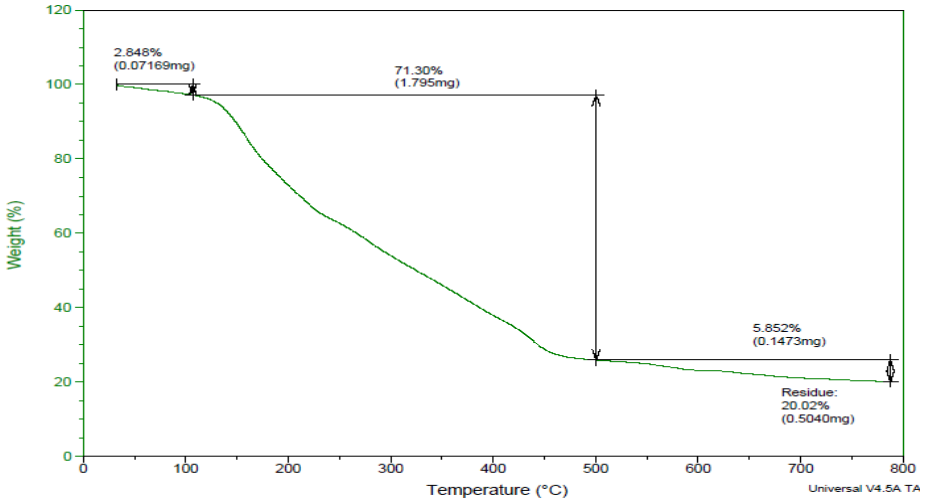

Figure 2. TGA curve of $[CuL^2{}_2(H_2O)_2]$

ESI-MS Spectra:

ESI mass spectra provide a vital clue for elucidating the structure of complex. The mass spectra of $[CuL^2{}_2(H_2O)_2]$ was recorded at room temperature and shown in the Fig 3.

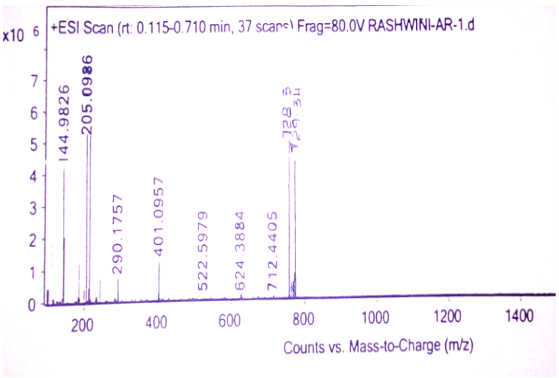

Figure 3. Mass spectra of $[CuL^2{}_2(H_2O)_2]$

Antibacterial and antifungal activity:

The zone of inhibition values of the synthesized metal complexes against the growth of the bacteria and fungi under investigation were measured in mm. The corresponding data summarized in **Table 4 & 5**.

Table 4. Antibacterial activity data of Schiff base metal complexes

	Zone of Inhibition (mm)						
Bacteria	**$[CuL^1_2(H_2O)_2]$**	**$[MnL^1_2(H_2O)_2]$**	**$[NiL^1_2(H_2O)_2]$**	**$[CuL^2_2(H_2O)_2]$**	**$[MnL^2_2(H_2O)_2]$**	**$[NiL^2_2(H_2O)_2]$**	**Ampicillin**
S. aureus	15	9	15	18	14	10	20
Pseudomonas	17	13	9	17	10	13	20
E.coli	15	10	13	10	11	9	20

Table 5. Antifungal activity data of Schiff base metal complexes

Fungi	**$[CuL^1_2(H_2O)_2]$**	**Zone of Inhibition (mm)**					
		$[MnL^1_2(H_2O)_2]$	**$[NiL^1_2(H_2O)_2]$**	**$[CuL^2_2(H_2O)_2]$**	**$[MnL^2_2(H_2O)_2]$**	**$[NiL^2_2(H_2O)_2]$**	**Gentamicin**
A. niger	20	16	15	14	13	18	18
A.fumigatus	22	16	12	17	14	14	18
Rhizophus	20	14	16	18	12	18	18
A.flavus	24	15	14	20	15	17	18

Antioxidant activity:

The antioxidant scavenging activity of the synthesized Schiff base metal complexes in hydrogen peroxide (H_2O_2) was monitored and the values are given in the **Table 6.**

Table 6. Scavenging activity data of the Schiff base metal complexes

	% of antioxidant scavenging activity		
COMPLEX	**6 mg**	**4 mg**	**2 mg**
$[CuL^1_2(H_2O)_2]$	80.58	70.87	67.96
$[MnL^1_2(H_2O)_2]$	66.01	59.22	52.42
$[NiL^1_2(H_2O)_2]$	66.99	61.16	56.31
$[CuL^2_2(H_2O)_2]$	68.00	50.48	41.74
$[MnL^2_2(H_2O)_2]$	75.72	67.96	59.22
$[NiL^2_2(H_2O)_2]$	73.78	68.93	63.10

Larvicidal activity:

The larvicidal activity of synthesized Cu (II), Mn (II) and Ni (II) complexes was studied against *C. quinquefasciatus* and the values are depicted in **Table 7 & 8**.

Table 7. Larvicidal activity data of Schiff base metal complexes

Complex	**Concentration /Mortality**			
	6mg/200mL	**4mg/200mL**	**2mg/200mL**	**1mg/200mL**
$[CuL^1_2(H_2O)_2]$	16	14	8	4
$[MnL^1_2(H_2O)_2]$	14	10	4	6
$[NiL^1_2(H_2O)_2]$	13	7	3	5
$[CuL^2_2(H_2O)_2]$	15	11	7	10
$[MnL^2_2(H_2O)_2]$	12	6	4	4
$[CuL^1_2(H_2O)_2]$	10	6	3	4

Table 8. Statistical analysis of larvicidal activity of Schiff base metal complexes

Complex	**Concentration /Mortality ± SD**					
	6mg/200mL	**4mg/200mL**	**2mg/200mL**	**1mg/200mL**	**χ2**	**df**
$[CuL^1_2(H_2O)_2]$	80±0.25	40±1.50	10±4.00	50±3.26	21.30	
$[MnL^1_2(H_2O)_2]$	70±4.00	50±2.80	20±9.00	30±4.20	10.20	
$[NiL^1_2(H_2O)_2]$	65±3.38	35±2.28	15±4.2	40±3.87	14.37	3
$[CuL^2_2(H_2O)_2]$	75±1.60	55±5.45	35±7.42	45±2.84	06.46	
$[MnL^2_2(H_2O)_2]$	60±3.00	30±6.00	20±11	30±6.51	07.46	

Complex	Concentration /Mortality ± SD					
	6mg/200mL	4mg/200mL	2mg/200mL	1mg/200mL	χ2	df
$[CuL^1_2(H_2O)_2]$	50±2.40	30±2.66	15±8.00	20±4.56	05.69	

Mean value of triplicates; Control-Nil mortality; df- significant at $p < 7.81$

DISCUSSION:

The synthesized organometallic Schiff base metal complexes are freely soluble in DMSO, DMF, ethanol at room temperature. The lower molar conductivity value of the complexes denotes the non-electrolytic behaviour of the complexes.[15] The absorption band observed around 270 nm and 300 nm corresponds to π→π* and n→π* transitions respectively of synthesized metal complexes. [16] The broad absorption band appeared around 600 nm corresponds to d→d transitions which confirmed the colour of the complexes.

In the FTIR spectra, an intense band appeared around 1620 cm^{-1} for the complexes corresponds to the coordination of imine nitrogen with metal ions. [17] The difference between asymmetric and symmetric stretching frequencies ($\Delta\upsilon = [\upsilon_{as} COO^- - \upsilon_s COO^-]$) were found to be higher than the corresponding free carboxylate anion. This confirmed the monodentate coordination of the carboxylate anion present in the Schiff base ligand with the Cu (II), Mn (II) and Ni (II) ions. [18] The bands appeared around 480 cm^{-1} and 580 cm^{-1} are assigned to the formation of M-O and M-N coordination respectively. [19] The FT-IR suggests that the metal ions are coordinated to imine nitrogen and oxygen atom present in carboxylate group of the Schiff base ligand and coordinated water molecule.

The g-value obtained from the ESR spectra of $[CuL^1_2(H_2O)_2]$ was 2.07 and the data confirmed the paramagnetic nature of the complex. 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 axis. Such spectra are expected for complexes with symmetrical environment like octahedral geometry. [20]

The TGA curve of the complex $[CuL^1_2(H_2O)_2]$ exhibits no mass loss up to 40 °C. The 1st stage of decomposition from the range of 50 °C -130 °C with a weight loss of 6.2 % corresponding to the elimination of two water molecules from coordinated complex. The 2nd stage of decomposition occurs rapidly in the range of 140 ºC - 550 ºC, indicating the loss of smaller and less stable groups of the complex with the mass loss of 40.68 %. The 3rd stage of decomposition takes place in the range 550 °C - 750 °C is due to loss of larger groups about 10.08 % of weight loss in the synthesized complex. [21] The mass of the final residue corresponds to 43.01% which confirmed the stability of the metal complex. [22]

The TGA curve of the complex $[CuL^2_2(H_2O)_2]$ exhibits no mass loss up to 30 ºC. The 1st stage of decomposition from the range of 30 °C -120 °C with a weight loss of 2.84 % corresponding to the elimination of two water molecules from coordinated complex. The 2nd stage of decomposition occurs rapidly in the range of 120 ºC - 500 ºC, indicating the loss of smaller, and less stable groups of the complex with the mass loss of 71.30 %. The 3rd stage of decomposition takes place in the range 500 ºC - 780 ºC is due to loss of larger groups about 5.85 % of weight loss in the synthesized complex. The mass of the final residue corresponds to 20 % which confirms the stability of the metal complex.

The ESI mass spectra of the Schiff base Cu (II) complex $[CuL^2_2(H_2O)_2]$ was recorded at room temperature. It shows a molecular ion peak (M^+) at m / z =728.3 which is equivalent to its molecular weight and the isotopic peak ($M^+ + 1$) at m / z =729.30 due to C^{13} and N^{15} isotopes. The different molecular ion peaks appeared in the mass spectra of complexes are attributed to the fragmentation of the metal complex molecule obtained from the rupture off different bonds inside the molecule by successive degradation leading to many more important peaks due to formation of various radicals.

The base peak at m / z = 205.09 was obtained with highest intensity may be due to $(C_{11}H_{13}N_2O_2)^+$ ion. The peak obtained at m/z = 712 may be due to elimination of CH_3· from the parent molecule. The different competitive fragmentation pathways of complex gave the peak at different mass numbers at 401, 290 and 144 dues to $(C_{25}H_{23}NO_4)^+$, $(C_{18}H_{28}NO_2)^+$, $(C_9H_{22}N)^+$ ions respectively. The spectra of the complex show characteristic molecular ion peak at their expected m/z values confirming the empirical formula [23].

The structure of organometallic based Schiff base Cu (II), Mn (II) and Ni (II) complexes enhances the chelating ability, which was act as powerful

bacteriostatic agents, thus inhibiting the growth of the bacteria also destroying them effectively. [24] In the present study, the lower activity of 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.

All the complexes show higher antibacterial activity. Cu (II) complexes such as $[CuL^1_2(H_2O)_2]$ and $[CuL^2_2(H_2O)_2]$ exhibited potent activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and produce good zone of inhibition. Complexes of Cu (II), Mn (II) and Ni (II) showed effective antifungal activity against *Aspergillus flavus, Rhizopus nigericans, Aspergillus fumigates, Aspergilus niger* with good zone of inhibition values.

Among the synthesized complexes $[CuL^1_2(H_2O)_2]$ exhibits good scavenging activity with 80 % by H_2O_2 method. $[NiL^1_2(H_2O)_2]$ and $[MnL^2_2(H_2O)_2]$ complexes showed moderate antioxidant scavenging activity in the range between 50 % - 65 % when compared to Cu (II) complex.

The larvicidal activity of the synthesized metal complexes was performed against *Culex quiquefasciatus* and the percentage of mortality was determined. Among the synthesized complexes $[CuL^1_2(H_2O)_2]$ and $[CuL^2_2(H_2O)_2]$ (6 mg/200 ml) showed higher mortality against *Culex quiquefasciatus* when compared to other complexes. The calculated values were subjected to statistical analysis and it was found to be good agreement with theoretical value.

CONCLUSION:

Here in, we have reported the synthesis and characterization of two new Schiff base ligands obtained from ferrocenecarboxaldehyde, L-valine and L-tryptophan and their Cu (II), Mn (II) and Ni (II) complexes. The spectral data revealed that all the complexes were six coordinated and proposed to be octahedral around the metal ions with the general formula $[ML_2(H_2O)_2]$. Thermal property measurements showed that the synthesized Cu (II) complexes have good thermal stability. Mass spectra confirmed the empirical formula of the synthesized Cu (II) complex. *In vitro* antibacterial, antifungal, antioxidant and larvicidal activities of the complexes were found to be good and hence lead a path to *in vivo* studies.

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