

## **Synthesis Spectral, Thermal Stability and Bacterial Activity of Schiff Bases Derived From Selective Amino Acid and Their Complexes**

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### **ABSTRACT**

*Different Schiff bases (L) ligands derived from the condensation reaction of 4-chlorobenzaldehyde and some amino acid (DL- Alanine, DL-Phenylalanine and DL-valine) have been synthesized by using microwave irradiation. Their Ni (II), Zn (II), Cd (II), Cu (II) and Co(III) complexes have been prepared. The ligands are characterized based on elemental analysis, FT-IR, mass spectrometry and <sup>1</sup>HNMR. Metal complexes are characterized by elemental analysis, FTIR, <sup>1</sup>HNMR, molar conductance and thermogravimetric (TG/DG) analysis. Ligands acts as bidentate coordinating with, 1:2 [M:L]. These complexes are [M [L]<sub>2</sub>]. H<sub>2</sub>O for [M=Cd (II),Ni(II),Zn(II) Cu II], [Cd[L]<sub>2</sub>].2H<sub>2</sub>O, [Zn[L]<sub>2</sub>].3H<sub>2</sub>O and [Ni[L]<sub>2</sub>[H<sub>2</sub>O]<sub>2</sub>].H<sub>2</sub>O using their carboxylate oxygen's. Another complexes are prepared from these ligands and they act as bidentate when they coordinating with Co (III) by using their carboxylate oxygen's and the azomethane nitrogen. The ratio are 1:3 [M:L]. The complexes are prepared with formula Co (L)<sub>3</sub>.2H<sub>2</sub>O and [Co (L)<sub>3</sub>].H<sub>2</sub>O. The thermogravimetric (TG / DG) analysis for one complex was studied and also the antibacterial activity of these ligands and there complexes were tested against some bacteria.*

**Keywords:** Amino acid; Ligand; Schiff base complexes

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### **INTRODUCTION**

Amino acids are molecules that contain both a carboxyl, and amino group with a side chain that varies between different amino acids. Although, many types of amino acids are known as  $\alpha$ -amino acids. The monomers from which proteins are constructed [1]. Since amino acid contains these groups therefore they contain potential donor sites such as (COOH) and (NH<sub>2</sub>) which they could coordinate with metals ions [2-4].The reaction of aldehyde and ketones with primary amines compounds of the type RNH<sub>2</sub> and ArNH<sub>2</sub> afforded an imine.

N-Substituted imines are sometimes called Schiff's bases, after Hugo Schiff who describes their formation in 1864. Schiff base ligands have significant importance, especially in the development of Schiff base complexes, because Schiff base ligands are potentially capable of forming stable complexes with metal ions [5,6].

Also Schiff bases have been studied extensively due to their applications in biological, clinical, analytical and pharmacological study [7-9].

Solvent free synthesis by microwave irradiation technique is observed here and this technique is rapid and efficient and very economical. Another wise the yield of products also high and purification is done by simple recrystallization [10]. The present study deals with the synthesis, characterization and biological studies of Schiff bases derived from *p*-chlorobenzaldehyde and DL-alanine, DL-valine and L- phenylalanine with its Ni (II), Zn (II), Cd (II), Cu (II) and Co (III) complexes.

### **EXPERIMENTAL PART**

#### **Reagents**

DL-Alanine, DL-valine, DL-phenylalanine and *p*-chlorobenzaldehyde from Fluka. Metal chloride salts, Sodium hydroxide were obtained from Sigma – Aldrich. All solvents were of reagent grade and use without further purification.

### Analytical methods

Carbon, hydrogen and nitrogen (CHN) analyses were performed by using CHNS-932 LECO Apparatus. Infrared absorption spectrum was recorded on a FTIR spectrometer in the range of 4000-400  $\text{cm}^{-1}$  and recorded by using Shimadzu FTIR as KBr pellet.

The HNMR spectra were recorded in DMSO – d<sub>6</sub>. On Bruker 400 MHz spectrometer and use TMS as internal standard. Mass spectra scanned by EI. Technique at 70 eV on Agilent technologies model 5975 spectrometer. Molar conductance were obtained using conduct meter – corning model 441 at room temperature. TGA analysis was recorded by using thermogravimetric Analyzer. TGAQ50 instruments in dynamic nitrogen atmosphere (50 mL.min<sup>-1</sup>) in the 20-800°C temperature range with heating rate of 10°C/min.

### Synthesis of Schiff bases

The Schiff bases ligands have been synthesis by condensation of *p*-chlorobenzaldehyde with different amino acid by using microwave-assisted.

In this method we carried in an oven, Sanyo, Em-G8586 V and is subjected to microwave for an optimized time (2-3 min) on the (MW, low 27W) setting [3].

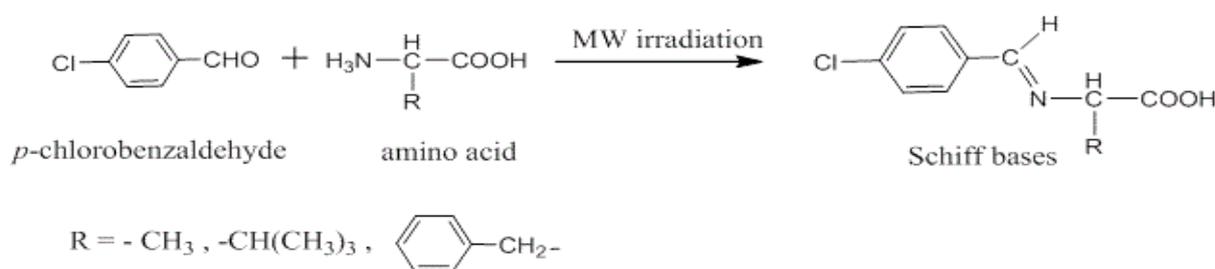
The reactions were executed worthily and we got good products in short time. The reaction can be illustrated in Scheme 1.

This reaction proceeds with no solvent. This method of technique has many frugalities like low risk of prohibition and has been done in short time, purified and carries out by simple recrystallization by using  $\text{CCl}_4$ . Table 1 show the analytical and physical data of Schiff bases ligands.

### Synthesis of metal complexes Ni (II), Zn (II), Cd (II), Cu (II) with Schiff base ligands

The Schiff base complexes were obtained as describe previously [2,3] and we could elucidation as below.

An ethanolic solution (10 mmol) of ligand were added drops wise to ehanolic solution of metal salts and the mixture were adjusted to pH 7-8 by adding NaOH solution. The above mixture was magnetically stirred for 30 min at room temperature. The metal complexes which separated were filtered off, wash with ethanol and dried in air. Table 2 describe the analytical and physical data of these complexes.



Scheme 1: Synthesis of Schiff bases

Table 1: Analytical and physical data of ligands and their complexes

Compound	Formula	Colour	M.P.°C	Elemental analysis found (calc.)			$\Omega$ Omb <sup>-1</sup> .cm <sup>2</sup> . mol <sup>-1</sup>	Yield %
				C%	H%	N%		
L <sub>2</sub>	C <sub>10</sub> H <sub>10</sub> NO <sub>2</sub> Cl	Orange	55.57	56.75 (56.91)	4.76 (4.36)	6.62 (7.82)	-	60
L <sub>3</sub>	C <sub>16</sub> H <sub>14</sub> NO <sub>2</sub> Cl	Orange	59.61	66.79 (66.25)	4.90 (4.97)	4.87 (3.55)	-	68
L <sub>4</sub>	C <sub>12</sub> H <sub>14</sub> NO <sub>2</sub> Cl	Yellow	52.53	60.13 (60.03)	5.89 (5.39)	5.83 (4.72)	-	64
Ni(L <sub>3</sub> ) <sub>2</sub>	Ni[(C <sub>16</sub> H <sub>14</sub> NO <sub>2</sub> Cl) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ].H <sub>2</sub> O	Green	220 (dec.)	56.01 (55.71)	4.70 (4.70)	4.08 (4.08)	1.34	60
Zn(L <sub>3</sub> ) <sub>2</sub>	Zn[(C <sub>16</sub> H <sub>14</sub> NO <sub>2</sub> Cl) <sub>2</sub> ].3H <sub>2</sub> O	White	175 (dec.)	55.47 (55.44)	4.65 (4.62)	4.04 (4.04)	3.11	85
Cd(L <sub>3</sub> ) <sub>2</sub>	Cd[(C <sub>16</sub> H <sub>14</sub> NO <sub>2</sub> Cl) <sub>2</sub> ].2H <sub>2</sub> O	White	270 (dec.)	53.24 (53.25)	4.19 (4.16)	3.88 (3.88)	1.45	64
Cd(L <sub>4</sub> ) <sub>2</sub>	Cd[(C <sub>12</sub> H <sub>14</sub> NO <sub>2</sub> Cl) <sub>2</sub> ].H <sub>2</sub> O	White	150 (dec.)	47.43 (47.52)	4.64 (4.45)	4.61 (5.62)	3.87	80
Cu(L <sub>3</sub> ) <sub>2</sub>	Cu[(C <sub>16</sub> H <sub>14</sub> NO <sub>2</sub> Cl) <sub>2</sub> ].H <sub>2</sub> O	Light Red	285 (dec.)	58.68 (58.43)	4.31 (4.56)	4.28 (4.39)	1.27	80
Co(L <sub>2</sub> ) <sub>3</sub>	Co[(C <sub>10</sub> H <sub>10</sub> NO <sub>2</sub> Cl) <sub>3</sub> ].H <sub>2</sub> O	light Brown	290 (dec.)	49.85 (53.31)	3.76 (5.34)	4.81 (5.18)	2.56	65
Co(L <sub>3</sub> ) <sub>3</sub>	Co[(C <sub>16</sub> H <sub>14</sub> NO <sub>2</sub> Cl) <sub>3</sub> ].2H <sub>2</sub> O	Brown	175 (dec.)	60.61 (60.36)	4.13 (4.54)	4.42 (5.40)	12.65	66

**Table 2:** Important IR spectral Data (cm<sup>-1</sup>) of Schiff base and their complexes

Compound	$\nu$ Para Sub	$\nu$ COO- asym	COO- asym	$\nu$ HC=N	$\nu$ C=O	$\nu$ C-H aliph	$\nu$ C-H arom	$\nu$ OH COOH	$\nu$ OH H <sub>2</sub> O
L2	821	-	-	1645	1701	2927	3066	3373	-
Co(L2) <sub>3</sub>	825	1404	1490	1595	1650	2950	-	-	-
L3	821	-	-	1645	1670	2926	3086	3224	H <sub>2</sub> O
Ni(L3) <sub>2</sub>	831	1404	1492	1595	1598	2929	3030	-	3358
Cu(L3) <sub>2</sub>	833	1325	1390	1616	1622	2939	3030	-	3329
Co(L3) <sub>2</sub>	833	1330	1408	1593	1593	2931	3030	-	3356
Zn(L3) <sub>2</sub>	833	1320	1411	1597	1597	3028	3062	-	3429
Cd(L3) <sub>2</sub>	833	1340	1408	1571	1671	2929	3010	-	3429
L4	823			1597	1647	2870	2972		H <sub>2</sub> O
Cd(L4) <sub>2</sub>	856	-	1402	1550	1591	2964	-	-	3525

### Synthesis of cobalt complexes

An ethanolic solution of Schiff base ligands (15mmole) was added to ethanolic solution of cobalt (III) salt (5 mmol-10 mmol) and an aqueous solution of Sodium hydroxide was added. The mixture was magnetically stirred for 30 min at room temperature. The Schiff base complex was filtered, washes with ethanol and dried in air. Light brown precipitate was obtained as describe in Table 1.

### Antibacterial assay

All synthesized Schiff base ligands and their complexes were screened for their antibacterial activity against gram (+ve) *Staphylococcus aureus* (*S. aureus*) and gram (-ve) *Auromonas hydrophile* (*A. hydrophile*) [11-13]. The test carry out by agar well Diffusion method [14,15].

The bacterial and yeast cultures were incubated at 37°C for 18 h. The Schiff base ligands and their complexes were stored at room temperature and dissolved 500 g/ml in DMSO. Paper disc of 5 mm diameter 1 mm thickness were soaked in solution of test sample and inserted on nutrient agar plates. The plates were incubated at 37°C for 48 h. The diameter of inhibition zone around each disc was measured for each kind of the test organisms.

## RESULTS AND DISCUSSION

### Analytical methods and spectral measurement

#### IR spectrum

Schiff base ligands are soluble in polar solvents such as methanol, ethanol. Spectral data of Schiff base ligands and their complexes were given in Table 2. In this table the IR of the free ligands were compared with their complexes to describe the coordination sites of the metals that may be involved in chelating. The infra-red spectral analysis of the Schiff base ligands showed abroad absorption bond appeared in the region – 3733-3224 cm<sup>-1</sup> which is assigned to hydrogen bonded  $\nu$ (OH). This band disappears on complexation suggesting chelation of the oxygen of the Schiff base ligand and with different metals [3,16]. The IR spectra of Schiff base ligands showed the absence of NH<sub>2</sub> bands which marked by amino acids and  $\nu_{C=O}$  of *P*-chlorobenzaldehyde. Instead of that a new band at the range 1597-1645 cm<sup>-1</sup> which attributed to azomethane  $\nu$  (CH=N). This band appeared in each Schiff base ligands [17,18] which indicating the condensation between aldehyde moiety of *P*-chlorobenzaldehyde and that of amino group of the studies amino acids. When the ligands coordinating with metals, the band of azomethane group was shifted to lower or higher frequency indicating the coordinated through nitrogen of azomethane group. Also the spectra of the complexes can observed in the region (1320-1406) cm<sup>-1</sup> and 1390-1492 cm<sup>-1</sup>, respectively band which assigned to symmetric and asymmetric of COO<sup>-</sup> group in the same time, the IR spectrum of the complexes shows a weak bands at 500-520 cm<sup>-1</sup> attributed to M–N and M–O stretching vibration which described the chelation between the studies metals and the nitrogen atom of the azomethane group and the coordination of metals with oxygen atom of the carboxylate anions group of the studies amino acids [19,20]. Also a medium intensity band in the higher frequency region is assigned to  $\nu$ (OH) vibration of water in the complexes [21].

### The <sup>1</sup>HNMR spectra

The spectra of Schiff base ligand and two of their complexes have been recorded [3,14] as shown in Table 3. All the proton of Schiff base ligands were found as to be in their expected regions. The <sup>1</sup>HNMR spectral data of the Schiff base ligands in accord with the proposed structures and confirms the nature of bonding which discussed in their IR spectra. In these <sup>1</sup>HNMR spectra of the Schiff base ligands in DMSO exhibits single signals at 8.3-8.6 ppm attributed to CH=N, while the signals at 13.2-13.5 ppm, respectively assigned to the proton of COOH group. In <sup>1</sup>HNMR spectra of the complexes the signals of the proton of COOH was absent, further support to the mode of bonding was established by the downfield shifting of these signals in the complexes, that make sure the coordination have been done via nitrogen atom of azomethane and carboxylate oxygen [2,3,15].

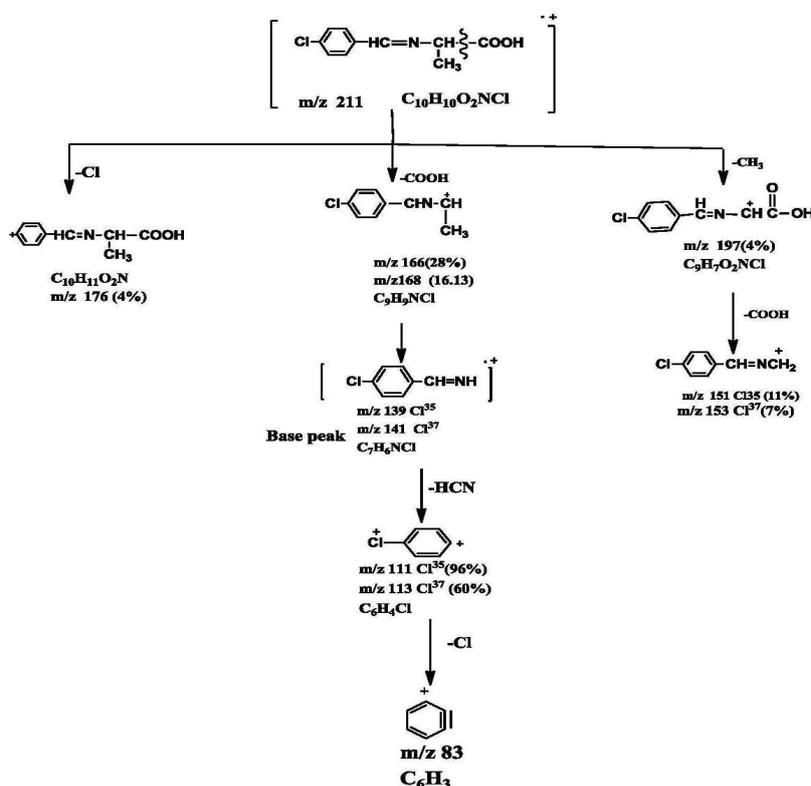
The Schiff base ligands give multiplet signal in the region 6.5-7.3 ppm which can be assigned to the aromatic protons of the ring and these signals keep almost at the same location of spectra in the metal complexes. The protons of aliphatic groups of alanine, valine and phenylalanine amino acid residues of the Schiff base ligands and their complexes were appeared in these spectra [21].

### Mass spectra (EI)

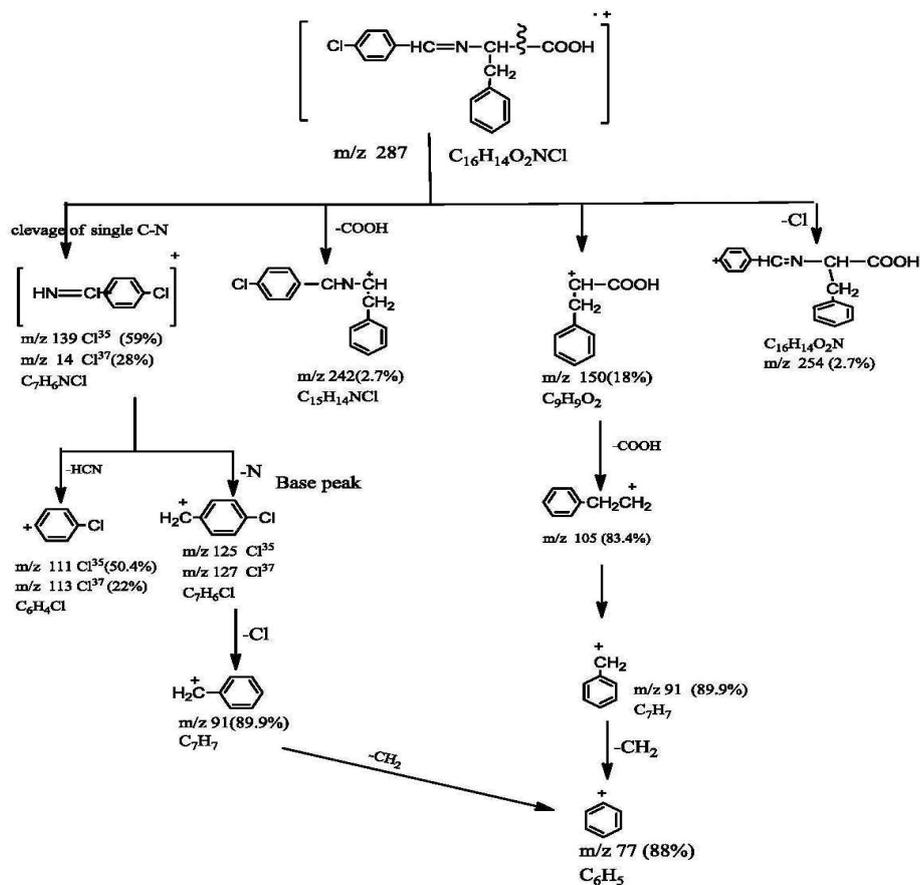
Mass spectra of all ligands show the molecular ions in agreements with the suggested structures of each ligand As shown in figures we recognized the exact molecular ion at m/z=211 for ligand L<sub>2</sub>, m/z=287 for ligand L<sub>3</sub> and m/z=239 for L<sub>4</sub>, respectively [14-16]. Schemes 2-4 suggested mechanism of the formation of the importments fragments.

Table 3: The <sup>1</sup>HNMR spectra data for Schiff bases and the complex

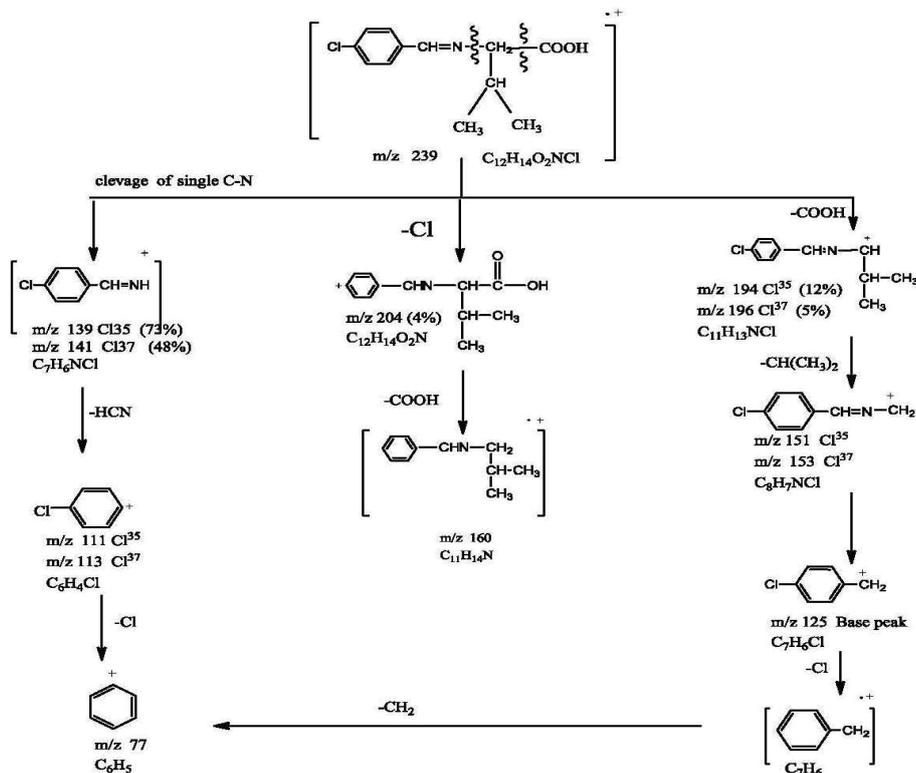
Compound	Chemical Shift (ppm)
L2	1,3 (s, 3H, -CH <sub>3</sub> ), 4.7 (q, 1H, -C=N-CH-, 8.3 (s, 1H, CH=N), 6.8-7.7 (m, 4H, Ar-H), 13.2 (s, 1H, -COOH)
L3	3.6 (d, 2H, -CH-CH <sub>2</sub> -), 4.3 (t, 1H, -C=N-CH-), 8.5 (s, 1H, -CH=N-), 6.5-7.5 (m, 4H, Ar-H) 13.5 (s, 1H, -COOH)
L4	1.9 (d, 6H, -CH <sub>3</sub> ), 1.1 (m, 1H, -CH-(CH <sub>3</sub> ) <sub>2</sub> ), 6.5-7.5 (m, 4H, Ar-H), 8.6 (s, 1H, -CH=N), 13.8 (s, COOH)
Zn(L3)2	3.6 (d, 2H, -CH-CH <sub>2</sub> ), 4.9 (t, 1H, -C=N-CH), 8.4 (1H, -CH=N, 6.5-7.5 (m, 4H, Ar-H)



Scheme 2: The proposed fragmentation key pathway for L2



Scheme 3: The proposed fragmentation key pathway for L3

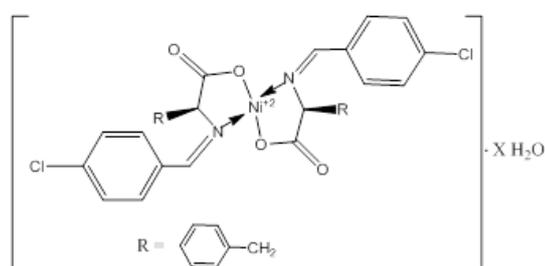


Scheme 4: The proposed fragmentation key pathway for L3

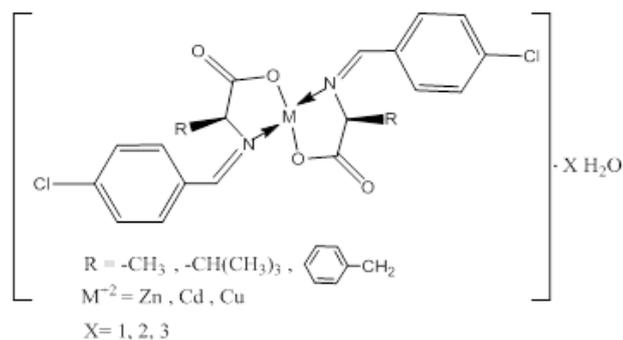
### Thermal analysis

To approve and conform the structure of  $[\text{Co}(\text{L}_2)_3]\cdot\text{H}_2\text{O}$  complex we established on the basis of differential thermal analysis (DTA) and thermogravimetric (TG) and its structures under a nitrogen flow and carried out from room temperature to  $800^\circ\text{C}$  with heating rate of  $10^\circ\text{C}/\text{min}$ . The thermal analysis of other complexes were the same studies in previous research [3]. In this decomposition curve of the complex shows three steps. The first step of decomposition within at  $(50-150)^\circ\text{C}$  temperature range with 5% weight (theoretical 4.9%) corresponds to the loss of one lattice water molecule [3,22]. The second step of degradation occurs at  $(150 \rightarrow 390)^\circ\text{C}$  ( $\text{DTG}_{\text{max}} 302^\circ\text{C}$ ) accompanied by a weight loss of 28% (theoretical 28.2%) which is consistent with the loss of ligand molecules. In the theoretical step of decomposition will continue and located at  $700^\circ\text{C}$  temperature corresponding to the loss of  $(\text{CaO}+2\text{C})$  and then give the final mass 13% (theoretical 10%) which corresponding to  $\text{CoO}$  as reduce.

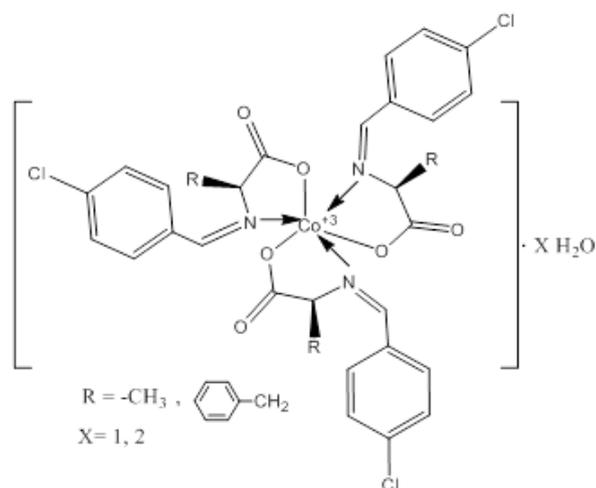
The above spectral data IR,  $^1\text{H}$ NMR, mass and thermal analysis, the proposed structures for the studies complexes are as follows:



Octahedral geometries for the complex  $[\text{Ni}(\text{L}_3)_2(\text{H}_2\text{O})_2]\cdot\text{H}_2\text{O}$



Tetrahedral geometries have been proposed to another studies complex



While octahedral geometries for the complexes of cobalt

### BIOLOGICAL ACTIVITY STUDIES

The synthesized Schiff base ligand and corresponding complexes was tested against Gram positive (*S. aureus*) and Gram negative (*A. hydrophila*) bacteria. In vitro antibacterial activity of these compounds, DMSO is used as negative control. The zone of inhibition based upon size around the disc was measured in mm. The result of the antibacterial inspected of these compounds at concentration 100 g/ml) against these bacteria are shown in Table 4. The results of antimicrobial screening (Figure 1) indicate the amino acid Schiff base (L4) show activity against *S. aureus* while another ligands (L2, L3) gave negative test against Gram positive (*S. aureus*). On the other hand the three Schiff base ligands show good activity against the gram negative (*A. hydrophila*) which are described may be to carboxyl group of each amino acid [23]. Also from the results, it has been observed that the metal complexes illustrate that they have more and better activity than the free Schiff base ligand. The promoted activity of the complexes can be explained in terms of the nature of the cell membrane which contain deferent lipid, and we can explained in terms of greater lipid solubility and cellular penetration of the complexes. It indicates that chelating increases the antibacterial activity. These complexes distribute the respiration process of the cell and thus block the synthesis of the protein that restricts further growth of the organism and as result the bacteria die [24].

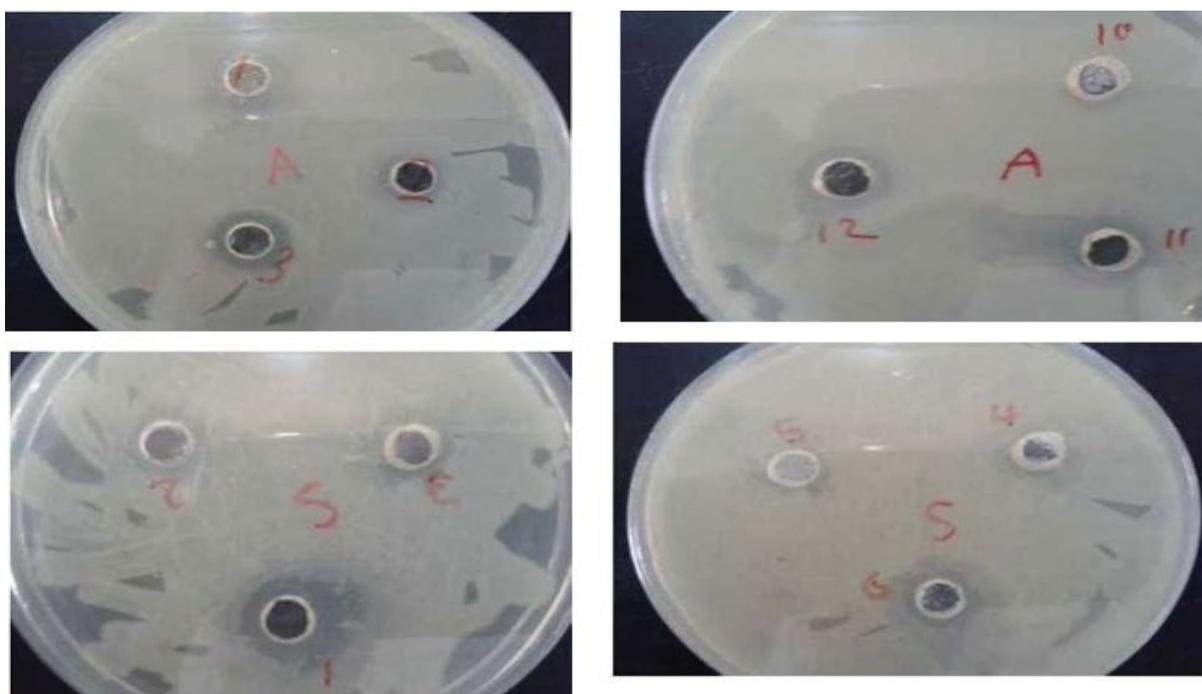


Figure 1: Results of antimicrobial screening

Table 4: The biological activity data for Schiff bases and their complexes

Bacterial Strains	Inhibition Zone (mm)						
	L4 (10)	L2 (11)	L3 (12)	Cu(L2) <sub>2</sub>	Co(L2) <sub>3</sub>	Cd(L3) <sub>2</sub>	Cu(L4) <sub>2</sub>
<i>S. aureus</i> gram positive	6	0	0	12	10	10	0
<i>A. hydrophila</i> gram negative	16	13	10	0	13	10	0

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## CONCLUSION

This article is concerned with the synthesis of some Schiff bases derived from condensation of selected  $\alpha$ -amino acid with *P*-chlorobenzaldehyde by using microwave irradiation. The synthesized Schiff bases act as bidentate ligands coordinated with some transition metal Co (III), Cu (II), Zn (II), Cd (II), Ni (II), via the carboxylate oxygen and the nitrogen of azomethane group. Two types of geometric have been proposed for these complexes. The first kinds of geometry suggested that the complexes of Zn (II), Cd (II) and Cu (II) were tetrahedral geometry. Octahedral geometry have been proposed for the Ni (II) complexes and for Co (III) complexes with the help of deferent spectral studies IR, <sup>1</sup>HNMR, CHN analysis and thermal gravimetry analysis. The low molar conductance of the complexes indicates that they are non-electrolyte and neutral. The antimicrobial activity of Schiff bases ligands and their complexes were tested against two types of bacteria.

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