

Synthesis, Physico-Chemical and Antimicrobial Activities Co(II), Ni (II) ,Cu(II), Zn(II),Cd(II) and Hg(II) Mixed-Ligand Complexes of L- Alanine and Trimethoprim Antibiotic

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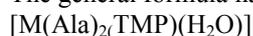
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Abstract

The Co (II), Ni (II) ,Cu(II), Zn(II) ,Cd(II) and Hg(II) complexes of mixed of amino acid (L-Alanine) and Trimethoprim antibiotic were synthesized.

The complexes were characterized using melting point, conductivity measurement and determination the percentage of the metal in the complexes by flame (AAS). Magnetic susceptibility, Spectroscopic Method [FT-IR and UV-Vis].

The general formula have been given for the prepared mixed ligand complexes



where L- alanine (abbreviated as (Ala)) = (C₅H₉NO₂)

deprotonated primary ligand, L- Alanine ion . = (C₅H₈NO₂⁻)

Trimethoprim (abbreviated as (TMP)) = C₁₀H₁₁N₃O₃S

M(II) = Co (II), Ni(II) ,Cu(II), Zn(II) ,Cd(II) and Hg(II).

The results showed that the deprotonated L- Alanine by KOH (Ala- K⁺) act as uni negative bi dentate ligand , was coordinated to the metal ions through the oxygen of the carboxylic group (–COO⁻), and the nitrogen of the amine group (–NH₂) while Trimethoprim antibiotic act as mono dentate ligand , was coordinated to the metal ions through the nitrogen of the pyrimidine group.

Keywords: Trimethoprim antibiotics , L- Alanine) complexes & Antibacterial activity

1. Introduction

α-Amino acids are very necessary in biological bodies. Such interaction of transition-metal ions with amino acids and peptides is of immense biological importance [1–2]. There are many reports on the metal- Alaninate complexes along with the structure of many of these compounds. [3-4] .The effects of chelating agents and drugs used clinically as antidotes to metal toxicity were reviewed in several articles [5].

The chemistry of metal-drug coordination compounds is more popular now than before in importance particularly in the design of more biologically active drugs. [6]

Trimethoprim (Systematic name)5-(3,4,5-Trimethoxybenzyl)pyrimidine-2,4-diamine is on the world health organization's list of essential medicines, the most important medications needed in a basic health system and is abroad-spectrum antimicrobial and also exhibits antiparasitic activities [7]. In the search for novel drugs against drug resistant diseases, the use of metal complexes has received tremendous attention [8–10]

In this paper we present the synthesis and study of Co (II), Ni(II) ,Cu(II), Zn(II) ,Cd(II) and Hg(II) complexes with L- Alanine amino acid as a primary ligand and Trimethoprim antibiotic as secondary ligand.

2. Experimental

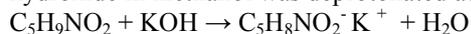
2.1-Reagents and instruments: Most of the chemicals used were of Analytical Grade. The drug (Trimethoprim) was obtained from (the state Enterprise for the drugs Industries and Medical Appliances) in Samarra- Iraq (SDI). L- Alanine, metals chloride and solvents from (B.D.H). The reagents were used without further purification .

2.2- Instruments: FT-I.R spectra were recorded as KBr discs using Fourier transform Infrared Spectrophotometer Shimadzu 24 FT-I.R 8400s. Electronic spectra of the prepared complexes were measured in the region (200-1100) nm for 10⁻³M solutions in DMSO at 25°C using shimadzu-U.V-160. A Ultra Violet Visible-Spectrophotometer with 1.000 ± 0.001 cm matched quartz cell. While metal contents of the complexes were determined by Atomic Absorption (A.A) Technique using Japan A.A-67G Shimadzu. Electrical conductivity measurements of the complexes were recorded at 25°C for 10⁻³ M solutions of the samples in DMSO using pw 9527 Digital conductivity meter (Philips). Melting points were recorded by using Stuart melting point apparatus. chloride ion content were also evolution by (Mohr method), Magnetic susceptibility measurements were measured using Bruker magnet BM6 instrument at 298°K following the Faraday's method. The proposed molecular structure of the complexes were drawing by using chem. office program, 3DX (2006)

2.3- General Synthesis of (mixing ligands complexes with some metal ions

2.3-1 -Synthesis of Potassium alaninate ($C_5H_8NO_2^- K^+$):

A solution [0.178, gm (2mmol)] of L-alanine ($C_5H_9NO_2$) with [0.122 g m (2mmol)] solution of Potassium hydroxide in methanol was deprotonated according to the following reaction .

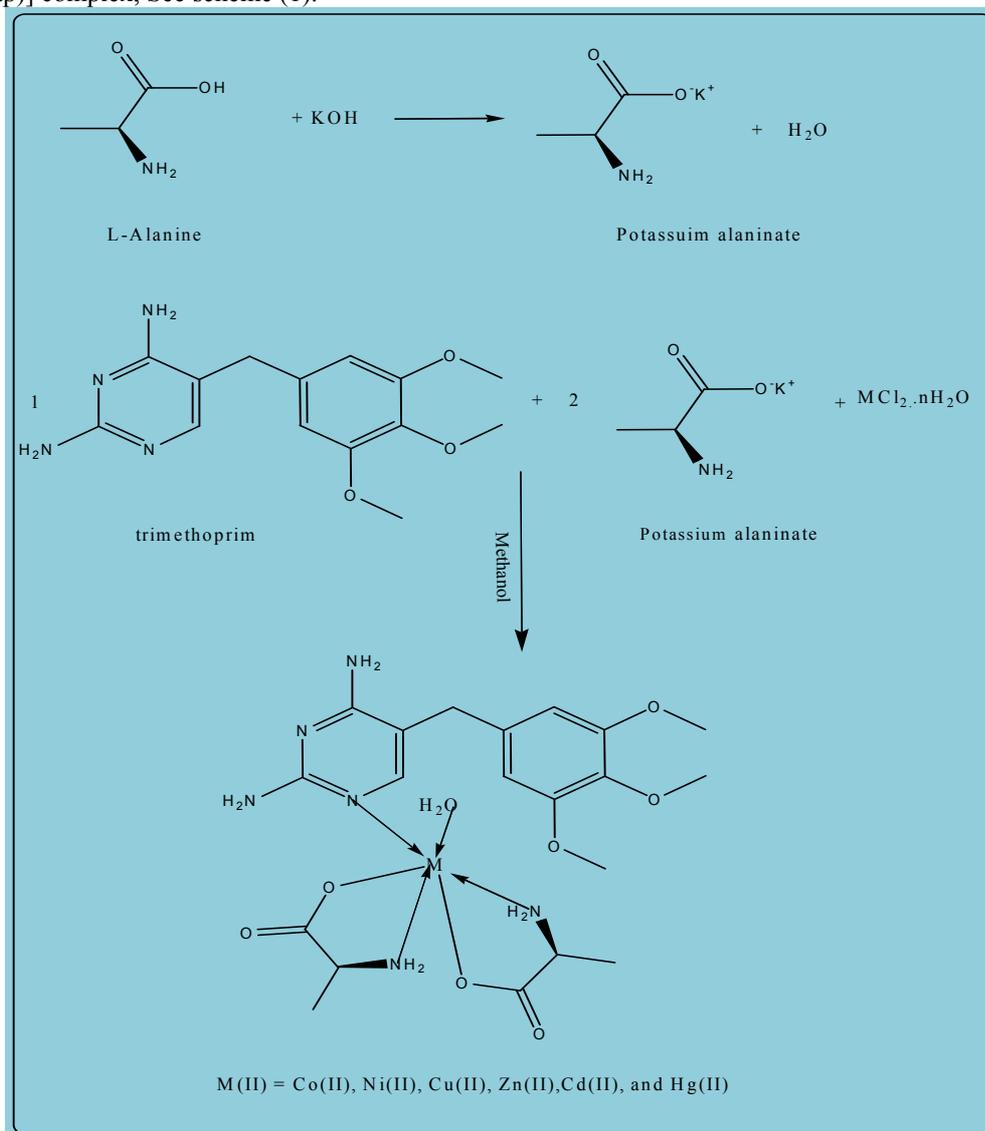


2.3-2 -Synthesis of $[Cu(Ala)_2(TMP)(H_2O)]$ complex:

A solution of (TMP) (0.290 gm, 1 mmole) in methanol (5 mL) and a solution of L-Potassium alaninate (0.178 gm,2 m mole) were added to stirred of Cu(II) Chloride dihydrate (0.17gm, 1 mmole) in methanol (5 mL).The resulting mixture was stirred for (1 hours). The PH of the solution was adjusted to 7.2 by using 1% KOH). Then the mixture was filtered and washed with an excess of ethanol and dried at room temperature during (24 hours). A green-blue solid was obtained, m. p (235° C), yield 85%.

2.3-3-Synthesis of $[Co(Ala)_2(TMP)(H_2O)]$, $[Ni(Ala)_2(TMP)(H_2O)]$, $[Zn(Ala)_2(TMP)(H_2O)]$, $[Cd(Ala)_2(TMP)(H_2O)]$ and $[Hg(Ala)_2(TMP)(H_2O)]$, complexes:

The method used to prepare these complexes was similar method to that mentioned in preparation of $[Cu(Ala)_2(TMP)]$ complex, See scheme (1).



Scheme (1): The Synthesis Route of Metal (II) Mixed Ligand Complexes

Results And Discussion:

Experimental section and synthetic pathway of M(I I) complexes of L- Alanine and trimethoprim were successfully synthesized from the reaction of the chloride salt of M(II) with the appropriate ligands in methanol explained in Scheme (1).

Table (1) shows the physical data for the prepared complexes which show different melting points. All the metal

complexes are coloured powders, and stable towards air and moisture at room temperature. The complexes are insoluble in common organic solvents but soluble in DMF and DMSO. See Table (2). The observed molar conductance values ($\Lambda_m = 3.3 - 8.3 \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$) are too low to account for any dissociation of the mixed ligand complexes in DMSO as solvent at room temperature, indicating non-electrolytic nature of the complexes [11]. This in turn suggests that the water molecules are coordinated with the metal ions and present inside the coordination sphere in all the complexes. [12] The atomic absorption measurements (Table 1) for all complexes gave approximated values for theoretical values. [11]. In conclusion, our investigation this suggest that the ligands L- Alanine and trimethoprim coordinate with M (II) forming octahedral geometry (Scheme 1).

Table (1) : Some Physical Characteristics of ligands and their complexes

Compounds Chemical Formula	M. wt Calc	Color	Yield %	M .p °c (de) °c	Λ_m $\Omega^{-1} \text{cm}^2 \text{mol}^{-1}$	(%) Metal content
						theoretical experimental)
TMP = $\text{C}_{10}\text{H}_{11}\text{N}_5\text{O}_3\text{S}$	290.32	white	—	283	1,3	
L-Alanine = $\text{C}_3\text{H}_9\text{NO}_2$	89.09	White	—	314	1.0	—
[Co(Ala) ₂ (TMP)(H ₂ O)]	543.44	Violet	70	202	4.6	10.84 (11.61)
[Ni(Ala) ₂ (TMP)(H ₂ O)]	543.20	blue	79	225	8.3	10.81 (10.75)
[Cu(Ala) ₂ (TMP)(H ₂ O)]	548.05	Green-blue	85	235	5.01	11.59 (10.67)
[Zn(Ala) ₂ (TMP)(H ₂ O)]	549.89	white	66	235	3.3	11.89 (11.05)
[Cd(Ala) ₂ (TMP)(H ₂ O)]	596.91	white	89	183	6.8	18.83 (19.90)
[Hg(Ala) ₂ (TMP)(H ₂ O)]	685.09	Yellow	58	208	3.7	29.28 (30.93)

Λ_m = Molar Conductivity, Dec. =decomposition

Table (2): The solubility of the mixed ligands complexes in different solvents

Compounds	H ₂ O	DMF	DMSO	CH ₃ OH	C ₂ H ₅ OH	Acetone	C ₆ H ₆	CCl ₄	CHCl ₃	Petroleum ether
[Co(Ala) ₂ (TMP)(H ₂ O)]	-	+	+	÷	÷	÷	-	-	-	-
[Ni(Ala) ₂ (TMP)(H ₂ O)]	-	+	+	÷	÷	÷	-	-	-	-
[Cu(Ala) ₂ (TMP)(H ₂ O)]	-	+	+	÷	÷	÷	-	-	-	-
[Zn(Ala) ₂ (TMP)(H ₂ O)]	-	+	+	÷	÷	÷	-	-	-	-
[Cd(Ala) ₂ (TMP)(H ₂ O)]	-	+	+	÷	÷	÷	-	-	-	-
[Hg(Ala) ₂ (TMP)(H ₂ O)]	-	+	+	÷	÷	÷	-	-	-	-

(+) Soluble , (-) Insoluble , (÷) Sparingly

The ultra violet visible spectra and Magnetic measurements for the mixed- ligand :

Magnetic moments μ_{eff} (μ B) of each of the complexes are given in Table (3). These values suggest octahedral geometry which is in good agreement with data of electronic transition [12-13].

The magnetic moments of Ni(II) complex is χ_{g} , ($\text{cm}^3 \text{g}^{-1}$) = 5.48 X10⁻⁶, χ_{M} , ($\text{cm}^3 \text{mol}^{-1}$) = 3.80X10⁻³ and calculate magnetic moments = 2.989 BM . These value is typical of d⁸ systems with two unpaired electrons and is in agreement with octahedral structure. The observed value of magnetic moment for Ni(II) complex was higher than the spin only value, which indicates the presence of spin-orbit coupling contributions. The three mixed ligand Zn(II),Cd(II) and Hg(II) complexes are diamagnetic as expected for d¹⁰ electronic configuration.

The μ_{eff} of 4.28 BM for the Cobalt(II) complex suggests octahedral arrangement.

The Cu (II) complex shows a normal magnetic moment of 1.73 BM observed. [12-14].

UV/Visible spectra of the ligands

Table 3 . Shows the electronic spectra data of free ligands TMP and L-Alanine , M(II) complexes and their assignment. The Uv- Vis spectrum of TMP in DMSO solvent appeared a high intense absorption band at (257nm) (38910cm⁻¹) (ϵ_{max} =2431 molar¹.cm⁻¹) is attributed to [$\pi \rightarrow \pi^*/n \rightarrow \pi^*$ (overlap of the two peaks) (C= C) and (C=N) of the pyrimidine group] transitions [10].The electronic absorption spectrum of the L- Alanine showed three bands at 240, 284 and 349 nm. The first one may be assigned to intraligand $\pi \rightarrow \pi^*$ transition, whereas the second and third band may be assigned to the $n \rightarrow \pi^*$ transition of the (-COO⁻) group. [10, 15]

V/Visible spectra of the mixed ligand complexes:

The d-d transition bands observed for Co(II) mixed ligand complex are found to have ν_{max} in the range 11173-11173 cm⁻¹ could be attributed to 4T1g(F) \rightarrow 4A2g(v2) and 4T1g(F) \rightarrow 4T1g(P)(v3) transitions, suggesting distorted octahedral environment around Co(II) ions. [13, 14].

In the Nickel(II) complex the bands at [236 nm (42372 cm⁻¹) (ϵ_{max} =2469 molar⁻¹ .cm⁻¹),350 nm (28571cm⁻¹) and 375 nm (26666 cm⁻¹) are due to $\pi \rightarrow \pi^*$ (C=C), $\pi \rightarrow \pi^*$ (C=N) and $n \rightarrow \pi^*$ (C=N)]charge transfer transitions (CT) respectively. This indicates a shift to shorter wave lengths due to complexation metal with Trimethoprim . The bands at (681nm) (14684cm⁻¹) (ϵ_{max} =16molar⁻¹ .cm⁻¹) is due to 3A2g \rightarrow 3 T1g(P) and (1014nm) (9861cm⁻¹) (ϵ_{max} =28molar⁻¹ .cm⁻¹) is due to 3A2g \rightarrow 3T1 g(F).

The electronic spectrum of the Cu(II) complex exhibited two bands, the band at 235nm)(42553cm⁻¹) (ϵ_{max} =2426molar⁻¹ .cm⁻¹) assigned to the charge transfer transitions (CT) and only one broad d-d transition at (676nm) (14792cm⁻¹) (ϵ_{max} =27molar⁻¹ .cm⁻¹) assigned to the 2Eg \rightarrow 2T2g transition, which is in conformity with the octahedral configuration around the copper ion.

The Zn(II),Cd(II) and Hg(II)complexes do not show any d-d transitions but display charge transfer transition at range (36363- 40485) cm⁻¹ , attributed to the M \rightarrow L (charge transfer) transition, which is compatible with this complexes having a octahedral structure [16-17].

Table (3) :Electronic spectral data of the ligands and their metal complexes in DMSO (10⁻³) M.

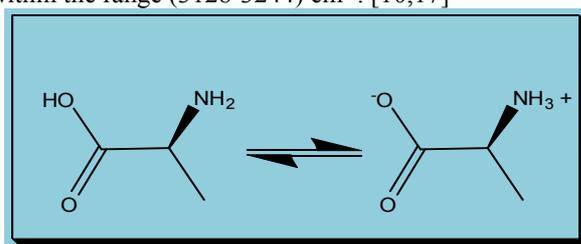
Compound	λ nm	ν cm ⁻¹	ϵ_{\max} molar ⁻¹ .cm ⁻¹	Assignments	μ_{eff} (BM)
TMP	257	38910	2431	($\pi \rightarrow \pi^*$)	-
Ala	236	42372	2469	($\pi \rightarrow \pi^*$)	-
[Co(Ala) ₂ (TMP)(H ₂ O)]	236	42372	2469	C.T	4.28
	537	18621	33	4T1g(F) → 4T1g(P)(v3)	
	895	11173	4	4T1g(F) → 4A2g(v2)	
[Ni(Ala) ₂ (TMP)(H ₂ O)]	236	42372	2467	(C.T) M → L	2.989
	263	38022	2414	(C.T) M → L	
	373	26809	33	(C.T) M → L	
	681	14684	16	3A2g → 3T1g(P)	
	1014	9861	28	3A2g → 3T1g(F)	
[Cu(Ala) ₂ (TMP)(H ₂ O)]	235	42553	2426	C.T	1.73
	676	14792	27	Eg(D) → 2T2g(D)	
[Zn(Ala) ₂ (TMP)(H ₂ O)]	234	42735	2378	C.T	Diamagnetic
	264	37878	2365	C.T	
[Cd(Ala) ₂ (TMP)(H ₂ O)]	225	44444	2192	C.T	Diamagnetic
	278	35971	2017	C.T	
[Hg(Ala) ₂ (TMP)(H ₂ O)]	207	48309	1664	C.T	Diamagnetic

Fourier-transform infrared spectra:

The relevant vibration bands of the free ligands and the complexes are in the region 400–4000cm⁻¹ [15-20]. Comparative study of IR spectra of the mixed ligand complexes with ligand reveals that several peaks are shifted, vanished or have newly appeared. The FT-IR spectra assignment off free ligand (L-Alanine), was summarized in (Table 4) , and (Trimethoprim),was summarized in Table (5)and the important IR peaks of the complexes are given in Table (6) .

The FTIR spectrum of pure L-Alanine amino acid and pure Trimethoprim antibiotic are shown in table .4 and 5 respectively. The infrared spectra of the metal complexes [M (Ala)₂(TMP)(H₂O)]; M= Co (II), Ni (II) ,Cu(II), Zn(II) ,Cd(II) and Hg(II) , table .6 are compared with that of the free ligands to determine the changes that might have taken place during the complexation..

The FT- IR spectrum of the new complex shows a broad an intense peaks at range 3402-3444 cm⁻¹ due to $\nu(\text{OH})$ vibrational modes which are consistent with the presence of coordinated water. [10, 17].Most of the absorption modes appear in the 1454- 1609 cm⁻¹ range, which corresponds to stretching absorption of aromatic rings. [10]. In all free L-amino acids (act as the zwitterions) see [Scheme (3) zwitterion of L-Alanine) the stretching vibration of $\nu(\text{NH}_3^+)$ appears at (3030-3130) cm-1 region [10], in the spectrum of L-Alanine it appears at (3084) cm⁻¹. In complexation this band was vanished in all complexes with appearance bands of the coordinated amino group (NH₂) within the range (3128-3244) cm⁻¹. [10,17]



Scheme (3): zwitterions of L-Alanine

The bands appears at (1622) cm^{-1} and (1413) cm^{-1} were assigned to the $\nu(-\text{COO})_{\text{asy}}$ and $\nu(-\text{COO})_{\text{sym}}$ respectively. $\Delta\nu(-\text{COO})_{\text{asy-sym}}=209\text{cm}^{-1}$ [10].

The value of $\Delta\nu$ reveals to difference between the ν [wave number of the asymmetric (ν_{asym}) and symmetric (ν_{sym})] stretching vibration of carboxylate group =this value was appeared in the prepared complexes within the range (218-251) cm^{-1} table (5) indicating of participate the (COO^-) group as a mono dentate donor in the coordination process with the metal ion The energy difference between both vibrations [$\nu_{\text{asym}}(\text{COO}^-) - \nu_{\text{sym}}(\text{COO}^-)$], $> 200 \text{ cm}^{-1}$, from FT-IR data that's supports the collaboration of this group in monodentate binding [13 ,14]. Metal- oxygen (M-O) and metal – nitrogen) (M-N) bonds were further confirmed by the presence of the bands around (509-570) cm^{-1} and (432-493) cm^{-1} respectively [15-16].

IR spectra demonstrate that the L-alanine act as uninate bi dentate ligand in coordination with metal ion (M^{+2}) through the oxygen atom in the carboxyl group ($-\text{COO}^-$) and the nitrogen atom of the ($-\text{NH}_2$) and a sharp very strong frequency band at 1633 cm^{-1} in trimethoprim assigned to the pyrimidine nitrogen $\nu(\text{C}=\text{N})$ shifted to [1643,1666,1620,1636, and 1647] cm^{-1} in[Co(Ala)₂(TMP)(H₂O)], Ni(Ala)₂(TMP)(H₂O)],Cu(Ala)₂(TMP)(H₂O)], [Zn(Ala)₂(TMP)(H₂O)], [Cd(Ala)₂(TMP)(H₂O)]

and [Hg(Ala)₂(TMP)(H₂O)]respectively. The significant shifts to a higher frequency band for all complexes suggest the coordination of the metal ion to the ligand through the pyrimidine nitrogen of the ligands. Interestingly, the C-N in the imine aromatic which confirms that the nitrogen has interacted with the carboxylic group of alanine acid . [20]

Table (4): Assignment of infrared frequencies in Cm^{-1} of L-Alanine

compound	νNH_2 asym	$\nu(\text{NH}_2)$ sym	$\nu(\text{C-H})$ aliphatic	$\nu(-\text{COO}^-)_{\text{asy}}$	$\nu(-\text{COO}^-)_{\text{sy}}$	Δ (COO^- asy - COO^- sy)
Alanine	3379	3084	2939	1622	1413	209

Table (5):Assignment of infrared frequencies in Cm^{-1} of Trimethoprim

Compound	$\nu(\text{N-H})$ asym	$\nu(\text{N-H})$ sym	$\nu(\text{C}=\text{N})$ Pyrimidine nitrogen	C-O-C str. (asym.)	C-O-C str. (sym.)	$-\text{OCH}_3$
Trimethoprim	3471vs	3363	1633vs 1597vs	1263s	1236s	1128vs

Table(6):Assignment of infrared frequencies in Cm^{-1} of $[M(Ala)_2(TMP)(H_2O)]$ Complexes

Compound	ν OH(H_2O)	ν NH _{sym} NH _{asym} Str	ν (C=N) Pyrimidine nitrogen	ν O- CH ₃	ν (-COO) asym	ν (-COO) sym	Δ ν (COO) asym - sym	ν M-OH ₂	ν M-O	ν M-N
$[Co(Ala)_2(TMP)(H_2O)]$	3402vs	3321vs 3163vs	1643vs	1130vs	1589VS	1338vs	251	763	532w 509	478 443w
$[Ni(Ala)_2(TMP)(H_2O)]$	3448vs-br	3322vs 3244vs	1666vs	1122s	1570VS	1346s	224	779	567 543	451 432w
$[Cu(Ala)_2(TMP)(H_2O)]$	3405vs	3302vs	1620vs	1130vs	1583	1342m	241	763	570 513	486 451
$[Zn(Ala)_2(TMP)(H_2O)]$	3424vs	3335vs 3128	1635vs	-	1548VS	1330vs	218	783	536 513	474 459
$[Cd(Ala)_2(TMP)(H_2O)]$	3444vs-br	3332vs 3132vs	1647vs	1130vs	1548VS	1330vs	218	783	563 528	493 451

Biological Study [Antibacterial]

Table (7),figure (1) reveal that two ligand and the synthesized mixed ligands complexes have been tested against growth of *E.coli*, *Staphylococcus aureus*, *Pseudomonas* and *Acineto* bacteria. Applying the agar plate diffusion technique using agar as nutrient, all of newly synthesized mixed ligand complexes in DMSO medium were screened in-vitro for antimicrobial activities against four pathogenic bacteria. Measured zone of inhibition (in mm) of the compounds against the growth of bacteria were given in [Table 7].

Table (7) : antibacterial activity (IZ mm) data of compounds (ligands & complexes) with different microorganisms

Compound	<i>E-coli</i>	<i>Pseudomonas</i>	<i>Staphylococcus aureus</i>	<i>Bacillus</i>
Control	5	7	5	6
TMP	4	0	5	10
Ala	8	8	7	17
$[Co(Ala)_2(TMP)(H_2O)]$	0	0	11	36
$[Ni(Ala)_2(TMP)(H_2O)]$	13	0	17	37
$[Cu(Ala)_2(TMP)(H_2O)]$	0	0	13	26
$[Zn(Ala)_2(TMP)(H_2O)]$	0	0	13	41
$[Cd(Ala)_2(TMP)(H_2O)]$	0	0	16	40
$[Hg(Ala)_2(TMP)(H_2O)]$	23	25	35	37
Inhibition zone > 15mm ,		Highly active		
Inhibition zone >10mm		moderately active		
Inhibition zone > 5mm		Slightly active		
Inhibition zone \leq 5-0 mm		Inactive		

* The rate of inhibition diameter was varied according to the variation in the ligands type and bacteria type. [21].

* The rate of inhibition diameter showed very good activity against *Bacillus* [22]

Complexation of antimicrobial active organic part with M (II) ions reduce the polarity of metal ions significantly because of partial sharing of its positive charge with donor groups and delocalization of π -electrons over the whole chelate ring resulting in high lipid solubility. - [21-24]

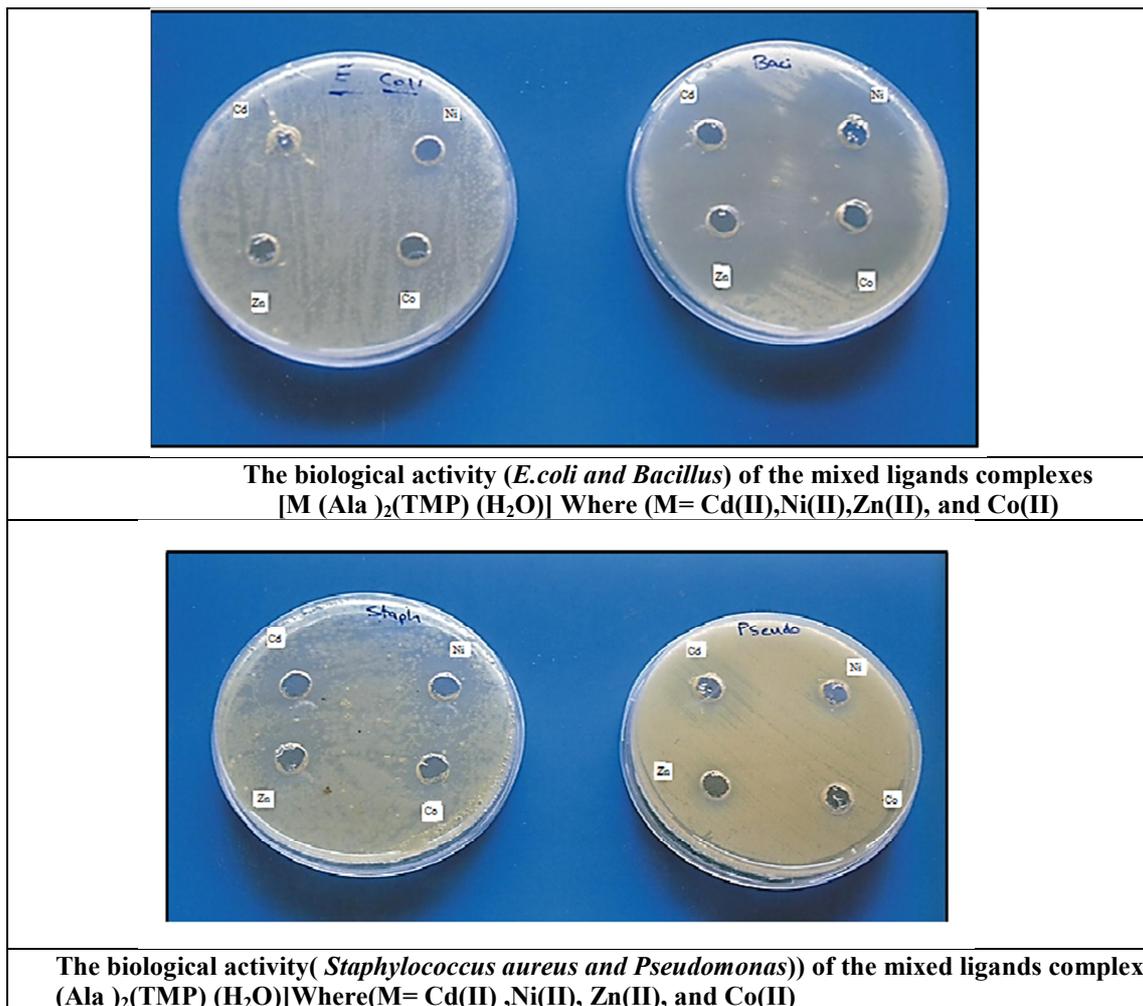


Figure (1) Photograph of Antimicrobial Activity of $[M(Ala)_2(TMP)(H_2O)]$ Complexes

CONCLUSION

The metal of Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) complexes of mixed ligands have been synthesized. The complexes undergo various characterization using solubility, electric conductivity, melting point, and infrared Spectroscopy. Electronic spectra and magnetic susceptibility measurement reveal octahedral geometry for all complexes.

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