

## Synthesis, Identification and biological study of Some mixed ligand complexes of tridentate heterocyclic azo ligands

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### الخلاصة

تم تحضير ليكاند أزو ايمدازول من خلال أزوتة 2-أمينو حامض البنزويك وأزواج ملح الديازونيوم الناتج مع قاعدة الأزواج 4,5-ثنائي فنيل ايمدازول بعدها تم تحضير معقدات لكل من النيكل(II) و النحاس(II) والخراسين(II) و الزنق(II) وذلك بخلط كميات محسوبة من محاليل الايونات الفلزية سالفة الذكر مع محلول مزيج لكل من ليكاندي الأزو ايمدازول المحضر وصبغة الـ (PAN) وبنسبة مولية (1:1:1). استعملت عدة تقنيات تحليلية لتشخيص المركبات المحضرة حيث شملت مطيافية الأشعة تحت الحمراء و الأشعة فوق البنفسجية- المرئية والحساسية المغناطيسية والتحليل الدقيق للعناصر والامتصاص الذري والتوصيلية المولارية, إذ بينت النتائج المستحصل عليها سلوك كل من الليكاندين هو ثلاثي المخلب نوع (N,N,O) واتخاذ جميع المعقدات الشكل ثنائي السطوح. تم دراسة الفعالية الحيوية لليكاندين ومعقدات مزيجهما تجاه خمسة انواع من البكتريا المرضية حيث شملت (Enterococcus fecalius , Staphylococcus haemolyticus , Staphylococcus warneri, Escherichia coli, Klebsilla Pneumonia) بالإضافة الى دراسة الفعالية الحيوية المضادة للفطريات تجاه نوعين من الفطريات هما (Aspergillus flavus and Aspergillus terreus).

### Abstract

azo imidazole ligand has been prepared by diazolation of 2-amino benzoic acid and coupling the diazonium salt that formed with 4,5diphenyl imidazole as coupling base, the complexes of Ni(II), Cu(II), Zn(II), Hg(II) synthesized by mixing a calculated amount of these ion salt solutions with a mixture of azo imidazole ligand that prepared and azo pyridyl dye (PAN) solution with molar ratio (1: 1: 1). Several spectral techniques used to characterization these compounds include: FT-IR , UV-VIS the magnetic susceptibility ,C.H.N analysis ,atomic absorption and molar conductivity, The results confirmed both ligands behave as tridentate ligands type (N,N,O) and all complexes of mixed tridentate azo ligands that prepared have octahedral geometry. The antimicrobial activity of the ligands and their mixed ligand complexes was studied against five types of pathogenic bacteria (Enterococcus fecalius , Staphylococcus haemolyticus , Staphylococcus warneri, Escherichia coli, Klebsilla Pneumonia). Furthermore , the antifungal activity against two fungi (Aspergillus flavus and Aspergillus terreus) was studied .

**Keywords:** azo imidazole , mixed ligands complexes, azo compounds, tridentate lignds

## Introduction

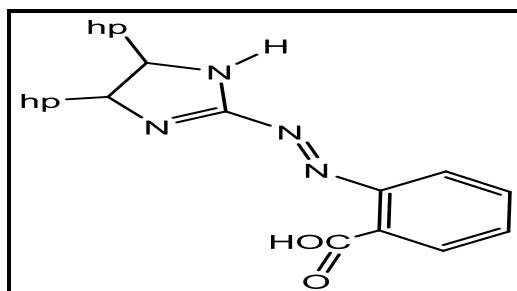
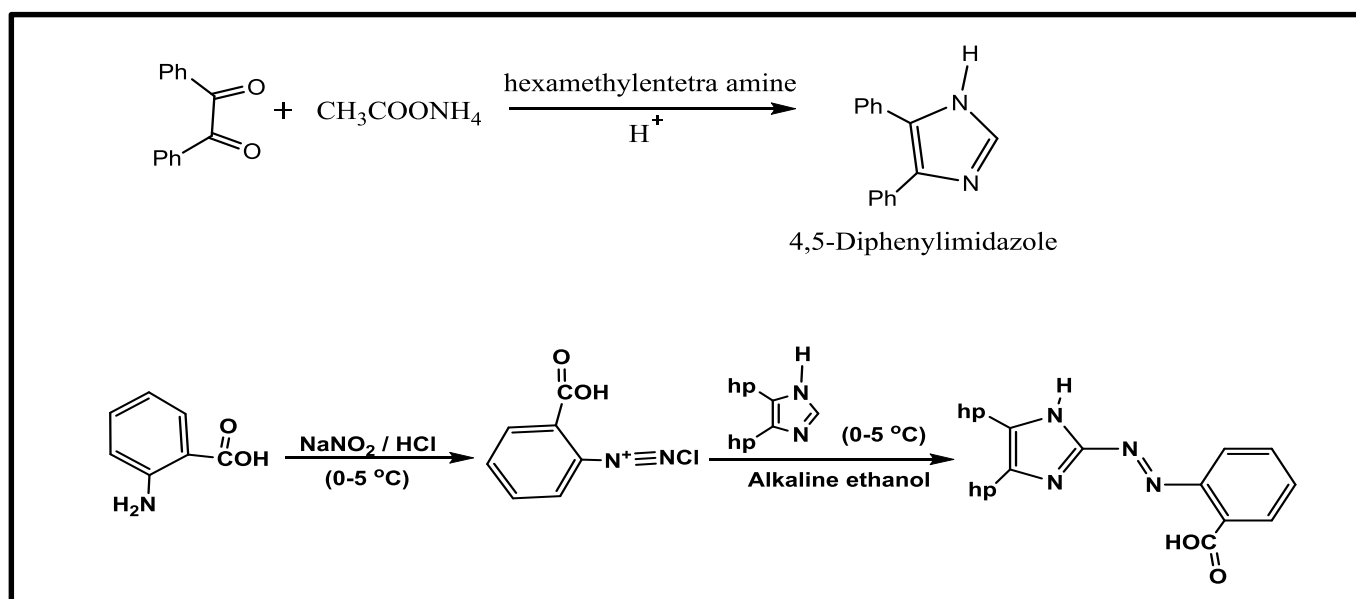
Metal complexes are compounds that result when a metal ion (electron acceptor) reacts with specific molecules called ligands (electron donor) [1], these ligands may be organic or inorganic molecules [2], a metal may react with the same species of ligands or with various ligands in this case the complex is called mixed ligands complex [3], The last type of complexes have wide applications in analytical chemistry especially spectral determination for different metal ions [4]. Heterocyclic cyclic azo compounds have been used widely in inorganic chemistry field as ligands because of their abilities as chelating ligands, they coordinate with a metal ion by one of nitrogen atoms of the bridge azo group and hetero donor atom on the ring [5], also this type of ligands could behave as tridentate ligands when one of the azo groups is attached to a substituted group that is able to coordinate with a metal ion in addition to the azo group and hetero donor atom on the ring [6].

## Material and Experimental

All chemicals that were used to prepare the azo imidazole ligand and complexes were provided from Fluka, B.D.H and Riedel-deHaën. 4,5-diphenyl imidazole was prepared as described earlier [7]. Melting points were determined by using the capillary tube method and are uncorrected by using a Stuart melting point SMP10. Elemental analyses (C, H, N) were carried out using a C.H.N.S EA-99.01 instrument. The metal contents of the complexes were measured using atomic absorption technique by Shimadzu AA-6300. The IR spectra were recorded on a Shimadzu 8400S FTIR spectrophotometer in the (4000-400)  $\text{cm}^{-1}$  range using KBr discs. Electronic spectra were obtained on a Shimadzu 1700 UV spectrometer using ethanol as solvent in the (1100-200) nm range. Magnetic susceptibilities were determined by the Faraday method at room temperature using a balance magnetic (MSB-MKI) apparatus, and diamagnetic corrections for the ligand were calculated using Pascal's constant (Hatfield, 1976). Molar conductance of the transition metal complexes were determined in (DMF) using a digital conductivity series Ino. Lab- 720 at 25°C, The concentration of the solutions was ( $10^{-3}$  mol  $\text{L}^{-1}$ ). Antibacterial screening was done at the department of Biology, Faculty of Education for Girls, University of Kufa.

**Preparation of 2-[(Carboxy phenyl)azo]-4,5diphenyl imidazole**

The azo imidazole ligand has been prepared by classical method for azo dyes preparation that include dissolving (1.37g 0.01 mol) of anthranlic acid (*p*- amimo benzoic acid) in (10 mL) distilled water contain (4 mL) diluted hydrochloric acid. This solution was cooling to (0°C) then slowly and stirred solution of Sodium nitrite (0.75 g 0.012mol in 20 mL distilled water ) was adding to cooling solution to form diazonium salt of anthranlic acid, after that adding diazonium solution with gradually and continuous stirring to mixture that consist of (2.2 g 0.01 mol) of 4,5-diphenyl imidazole dissolving in (150 mL) ethyl alcohol and sodium hydroxide solution (10%). Red precipitate was appearing. After leaving in the refrigerator for 24 hour ,the pH of mixture was modifying to (pH = 6) . The precipitate filtered and twice recrystallized from hot ethanol, then dried in the oven at 70°C for several hours .

**Fig.1. Structure of the ligand (CPI)****Fig.2. Equations of preparation of azo imidazole ligand (CPI)**

### Preparation of metal complexes

The mixed ligand metal complexes (1 mmol) were prepared by dissolving 0.125 g of (PAN) in 25 mL hot ethanol and added to (CPI) solution which prepared by dissolving 0.184 g in 25 mL hot ethanol, Then the mixture of these ligands was added to a stoichiometry amounts of (1:1:1) (metal: mixed ligand) molar ratio of Ni(II), Cu(II), Hg(II) chloride salts and Zn(II) sulfate that, dissolved in 10 mL hot distilled water. The colored precipitates formed at room temperature were filtered off, washed with 5 mL hot (1:1) ethanol-water to remove any traces of the unreacted starting materials and dried at 80 °C for overnight.

### Antimicrobial activity studies

#### Antibacterial activity

The prepared compounds were evaluated for their antibacterial activity against bacterial strain *Enterococcus fecalius* , *Staphylococcus haemolyticus* , *Staphylococcus warnneri* (Gram +ve) and *Escherichia coli* and *Klebsiella pneumonia* (Gram – ve) by well diffusion method using muller Hinton agar[8] as medium. The tested compounds were dissolved in DMF to obtain a solution 1000 ppm concentration. After 24h of incubation at 37°C. Zones of inhibition is given as average of three independent determinations, the diameter of the inhibition zones measured in mm.

#### Antifungal activity

The compounds were assessed for their antifungal activity against pathogenic fungus species namely *Aspergillus flevus* and *Aspergillus terreus* using potato dextrose agar as the medium by well diffusion method[9] at 10,100 and 1000 ppm concentrations with triplicate determinations in each case . The average percentage inhibition was calculated by using the following formula[10]

$$\text{Inhibition (\%)} = (C - T) 100/C .$$

Were C is diameter of colony fungs in center plates and T is diameter of the colony fungus in the test plates .

## Results and Discussions

### Characterization of prepared compounds

The azo imidazole ligand was red color semi-crystal, while the chelates complexes of this ligand were varying in color because it depending on metal ions. The preparing compounds were stable at room temperature and insoluble in water, but It's have good solubility in hot ethanol and some common organic solvents such as Dimethyl Formamide (DMF) and dimethyl sulfoxide(DMSO). The elemental analyses results showed a good agreement with theoretical expectations to the prepared ligand and their metal chelates, and these results indicate that both ligands behave as tridentate ligands (N,N,O), which, supported the metal-ligand ratios (1:1:1). Table 1 illustrated some physical and analytical data of prepared compounds.

**Table 1. Some physical and analytical data of the ligand and its complexe**

### Infrared Spectral Studies

No.	Chemical formula of compounds	m.p.°c	Yield %	Color	C % (cal.)	H % (cal.)	N % (cal.)	M % (cal.)
1	$C_{15}H_{11}N_3O = HL_1$ (PAN)	140	----	orang	---	---	----	----
2	$C_{22}H_{16}N_4O_2 = HL_2$ (CPI)	235-233	87	Deep red	71.54 (71.73)	3.26 (3.34)	15.06 (15.21)	----
3	$C_{37}H_{25}N_7O_3Ni$	>300	85	Brown	62.31 (62.56)	3.38 (3.52)	13.51 (13.80)	7.92 (8.27)
4	$C_{37}H_{25}N_7O_3Cu$	>300	78	red	61.89 (62.14)	3.27 (3.49)	13.44 (13.71)	8.6 (8.88)
5	$C_{37}H_{25}N_7O_3Zn$	>300	81	orang	61.88 (61.97)	3.28 (3.48)	13.39 (13.67)	8.89 (9.12)
7	$C_{37}H_{25}N_7O_3Hg$	164-162	81	red	51.99 (52.11)	2.72 (2.93)	11.23 (11.50)	----

Infrared spectrum technology have been used widely in coordination chemistry field it can be recognize the coordinate sites between ligands and metal ions by observing the changes in density, shape and locations of these band sites when ligands spectrum compared with spectrum of metal complex in addition to appear new band of ligand- metal bond in spectrum of metal complex. In present study we note the spectrum of azo imidazole ligand(CPI) showed a medium band in at ( $1410\text{ cm}^{-1}$ ) to (N=N) bridge group and ( $1589\text{ cm}^{-1}$ ) [11] due to  $\nu(\text{C}=\text{N})$  of the imidazole ring, and weak bands of OH to carboxylic group and ( N-H ) of the imidazole ring can be observed at ( $3448\text{ cm}^{-1}$ ) and ( $3144\text{ cm}^{-1}$ ) respectively, The reason of less density and shape of these bands belong to hydrogen bond formation[12]. On the other hand the spectrum of (PAN) ligand showed several stretching vibration bands include medium band at ( $1404\text{ cm}^{-1}$ ) belong to (N=N) bridge group ,Also appear band of ( $\text{C}=\text{N}$ ) of pyridine ring at ( $1650\text{ cm}^{-1}$ ) position and at ( $3500\text{ cm}^{-1}$ ) to phenolic hydroxyl group , also appear band at ( $3059\text{ cm}^{-1}$ ) belong to aromatic (C-H)[13]. Infrared spectra of metal complexes of mixed ligands can observed clearly change in some of these bands ,whereas stretching vibration bands of ( N=N) groups, OH carboxylic group of (CPI) ligand, ( $\text{C}=\text{N}$ ) of the imidazole ring, ( $\text{C}=\text{N}$ ) of pyridine ring and phenolic hydroxyl group of naphthol in (PAN) ligand were shifting to lower frequencies that indicate for both ligand coordinated with metal ions . Furthermore we observed that vibration frequency of carbonyl group and N-H of the imidazole ring in (CPI) ligand were affected as result of coordination process and new band of M-O and M-N was appear[14]. Finely, the Infrared spectra of metal complexes showed appearance of stretching vibration band between ( $3452\text{-}3537\text{ cm}^{-1}$ ) region belong to crystallization water molecules. Table 2 showed a values of vibration frequency of ligands and its chelate metal complexes while fig.3,4 ,5 showed the IR spectrum of the free ligands (PAN) ,(CPI) and Ni(II) complex respectively.

Table 2. Infrared spectral data (cm<sup>-1</sup>) of the ligands and its mixed complexes

Comp.	v(O-H) hydr-	v(O-H)	v(C=O)	v(N=N) (CPI)	v(C=N) imi.	v(N-H) imi.	v(N=N) (PAN)	v(C=N) <sub>y</sub> .	M-O	M-N
HL <sub>1</sub> (PAN)	---	3500	---	---	---	---	1404	1650	---	---
HL <sub>2</sub> (CPI)	---	3448	1689	1410	1589	3144	---	---	---	---
C <sub>37</sub> H <sub>25</sub> N <sub>7</sub> O <sub>3</sub> Ni	3452	---	1610	1371	1564	---	1350	1593	510	470
C <sub>37</sub> H <sub>25</sub> N <sub>7</sub> O <sub>3</sub> Cu	3440	---	1608	1373	1417	---	1334	1597	515	470
C <sub>37</sub> H <sub>25</sub> N <sub>7</sub> O <sub>3</sub> Zn	3417	---	1610	1379	1548	---	1350	1597	510	460
C <sub>37</sub> H <sub>25</sub> N <sub>7</sub> O <sub>3</sub> Hg	3537	---	1668	1400	1570	---	1317	1618	510	460

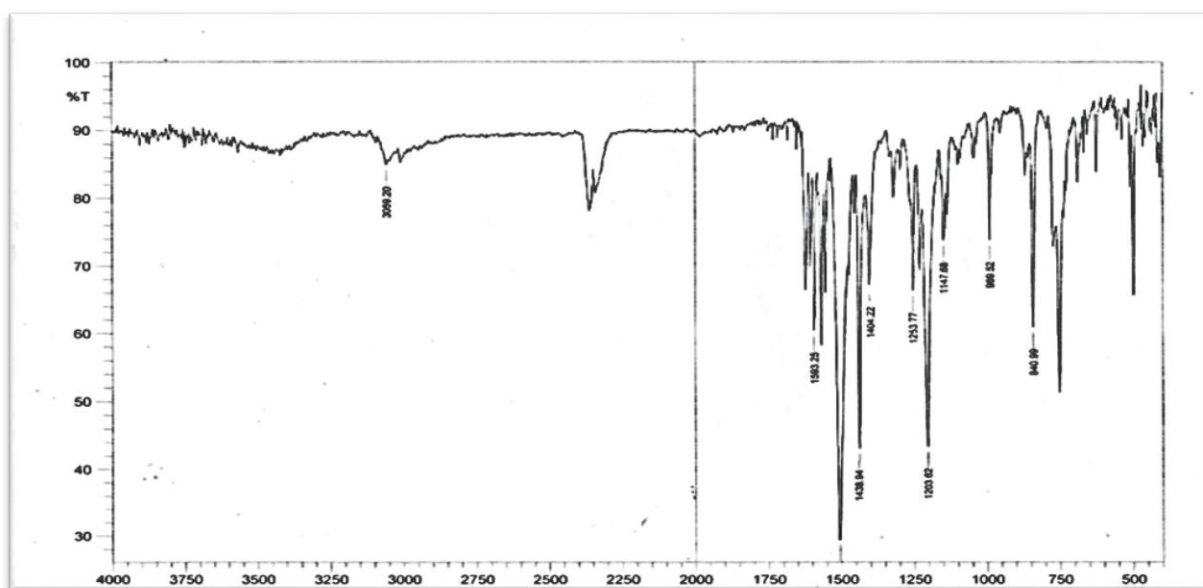


Fig.3. The IR spectrum of the ligand (PAN)

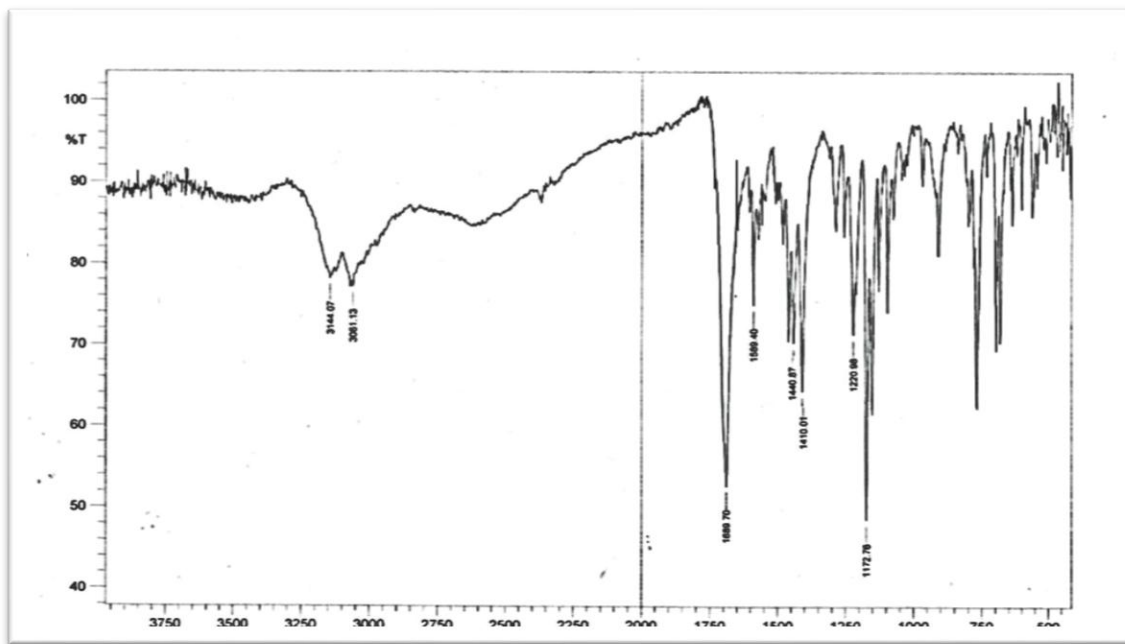


Fig.4. The IR spectrum of the ligand (CPI)

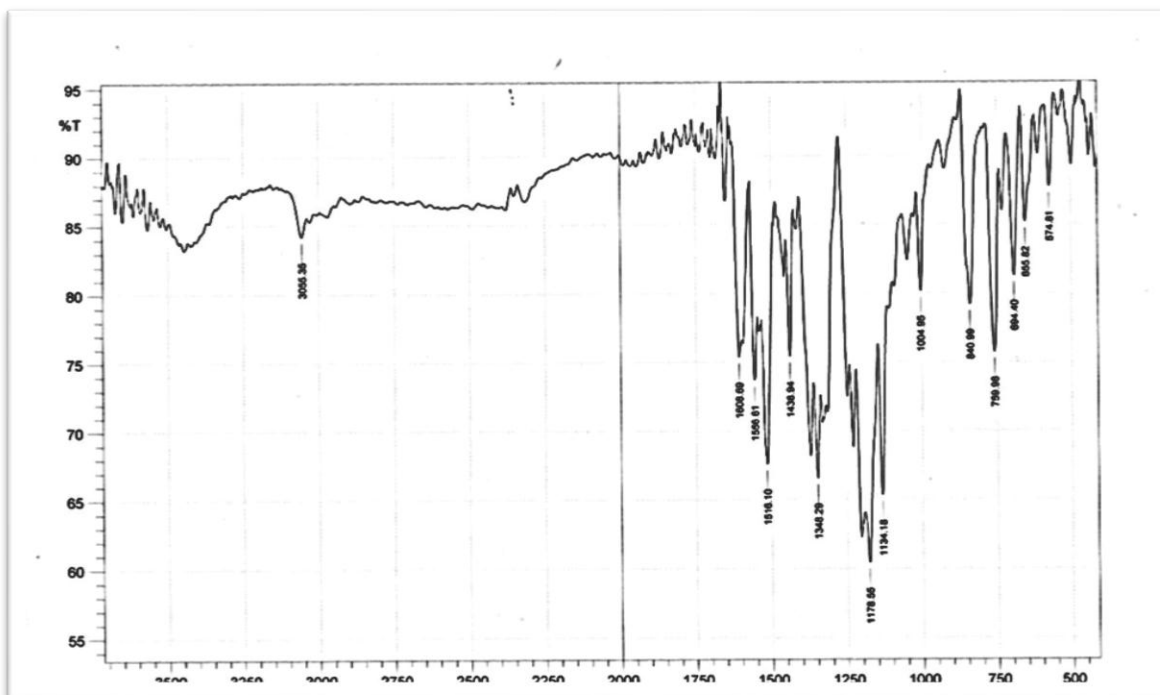


Fig. 5. The IR spectrum of the Ni(II) Chelate complex



### Electronic Spectral Studies

Electronic spectra of the complexes were recorded in di methyl formamide (DMF) solution in the UV-Vis region of (200-1100 nm). The spectral data and the magnetic moment of prepared complexes are listed in Table.3. UV-Vis spectral studies of the complexes exhibit transition at more than 400 nm [14] corresponding to intramolecular  $n \rightarrow \pi^*$  and  $\pi \rightarrow \pi^*$  charge transfer transitions. Intense absorption bands ( $\epsilon \sim 10^4$ ) appear in the range of 568-508 nm for the complexes which may belong to  $d \rightarrow \pi^*$  (ligand) charge transfer transitions[15-17].

### Magnetic measurements

Magnetic measurements have been used to characterize the coordination complexes, These measurements provide information about electrons configuration in d orbital and oxidation state of metal ion, Thus , Its helps the researcher to suggest a geometrical shape of metal complexes. In present study the values of magnetic moment listed in table 3 which showed that the complexes of Zn(II) and Hg(II) had diamagnetic characteristic, while the complex of Ni(II) exhibited a magnetic moment value of 3.01 BM that indicate to presence of tow unpaired electron in d orbital which suggests an octahedral geometry[18]. Cu(II) complex had a magnetic moment value of 1.38 BM, this value was corresponding to the range (2.20-1.70) that stated in the literature and showed one unpaired electron, as where as to led suggested the distorted octahedral geometrical shape[19].

### Conductivity measurement

Molar conductance data of the complexes were measured in the solvent DMF and the complexes were found to be nonelectrolytic in nature. Conductivity value of the chelate complexes are lie in the range (13.08-6.4) S.  $\text{cm}^2.\text{mol}^{-1}$ .

Table 3. Spectral data, magnetic moment, conductivities, and proposed Structure of prepared complexes

Complex	Assignment	Absorption band(nm)	$\mu_{\text{eff}}$ (B.M)	$\Lambda_M$ (S.cm <sup>2</sup> .mol <sup>-1</sup> )	Proposed Structure
HL <sub>1</sub> = (PAN)	$n \rightarrow \pi^*$	458	-----	---	-----
	$\pi \rightarrow \pi^*$	273			
HL <sub>2</sub> = (CPI)	$n \rightarrow \pi^*$	472	-----	----	-----
	$\pi \rightarrow \pi^*$	292			
[Ni(L <sub>1</sub> )( L <sub>2</sub> ). 2H <sub>2</sub> O	C. T.	568	3.01	13.08	Oh
[Cu(L <sub>1</sub> )( L <sub>2</sub> )]. 2H <sub>2</sub> O	C. T.	561	1.38	6.4	Oh
[Zn(L <sub>1</sub> )( L <sub>2</sub> )]. 2H <sub>2</sub> O	C. T.	549	Dia	10.18	Oh
[Hg(L <sub>1</sub> )( L <sub>2</sub> )]. 2H <sub>2</sub> O	C. T.	508	Dia	11.30	Oh

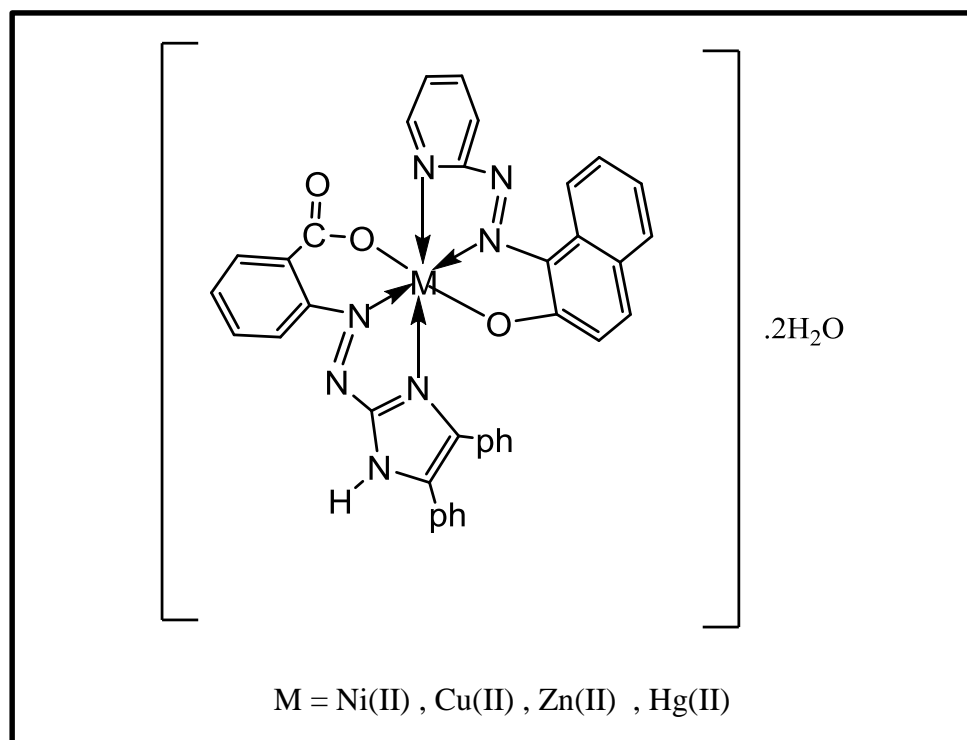


Fig.6 The suggested geometry of Chelate complexe

### Antimicrobial activity studies

In vitro antimicrobial activity of the free ligands HL<sub>1</sub>, HL<sub>2</sub> and their mixed ligands complexes were studied against five bacterial strains namely *Ent.fecalius*, *S. haemolyticus*, *S.warneri*, *E.coli*, *K.Pneumonia*, and two fungal species namely *A.flevus* and *A. terreus* by well diffusion method. Their zones of inhibition against different bacteria are summarized in table 4 and shown in fig.7. The results exhibited that the complexes show more activity and the ligands have less activity against same pathogenic bacterial strains under the identical experimental conditions. It is found that ligands HL<sub>1</sub> and HL<sub>2</sub> were shown moderately effect against gram positive and gram negative bacteria except the ligand HL<sub>1</sub> was shown slightly effect against *S.warneri*. The complex Hg(II) was exhibited highly activity the all bacteria. The complexes Cu(II) and Ni(II) was shown highly effect as antibacterial for most bacteria except *Ent.fecalius* moderately effect. The Zn(II) complex was indicated highly effect toward most bacteria with exception of *Ent.fecalius* and *K.Pneumonia* respectively moderately effect. The ligands and their complexes was shown highly to moderately activity against the same bacteria strain except the ligand HL<sub>1</sub> was indicated slightly activity against *S.warneri*. *S. haemolyticus* and *E.coli* was found to be more susceptible than rest of the other strains of bacteria. Also, it was observed that the compounds are more active against gram negative than gram positive bacteria. The results of antifungal activity of the prepared compounds are listed in table 5, which reveals that chelate complexes indicated highly antifungal potency in comparison to their parent ligand (HL<sub>1</sub> and HL<sub>2</sub>). Substantial activity is achieved in case of Hg(II), complex against *A. Terreus* and the remaining chelate complexes are significantly against the species. A comparative study of the compounds was shown significantly toxicity at 1000 ppm conc., against all species of fungi. However, all metal complexes are more active than the free ligands and the fungicidal activity decreases on dilution. Also, it was observed that compounds exhibit more significant effect *A. terreus* than *A.flevus*. When the comparison for the compounds was made between bacteria and fungi, it was observed that the compounds found to be more active against fungi than bacteria. This would suggest that the chelation of the complex easily cross a cell membrane[20]. The change in effectiveness of different biocidal species against microorganisms depend on the impermeability of the cell of the microbes or on differences in ribosome of microbial cells[21]. The higher activity of the complexes may be attribute to the different properties of metal ions upon chelation, in which the metal ion adsorbed on the cell wall of the microorganisms. There for metal ions are essential for the growth inhibitory

influence [22,23]. Such enhanced activity of meal chelates is due to lipophilic nature of the metal ions in complexes. The increase in activity with concentration is due to the effect metal ions on the normal process, and the action of compounds may involve the formation of the hydrogen bond with active center of cell constituent. resulting in interference with normal cell process[24]. Furthermore, other factor such as, solubility, conductivity and dipole moment influenced by the presence of metal ions may also be possible reasons for this activity [25].

**Table 4: antibacterial activity data (zone on inhibition in mm) in the ligands and their mixed complexes .**

Compound Bacteria	G(+ve)			G(-ve)	
	Ent.fecalius	S. haemolyticus	S.warneri	E.coli	K.Pneumonia
HL <sub>1</sub>	9	11	8	10	10
HL <sub>2</sub>	10	10	9	11	9
[Ni(L <sub>1</sub> )( L <sub>2</sub> )].2H <sub>2</sub> O	12	14	15	18	14
[Cu(L <sub>1</sub> )( L <sub>2</sub> )] .2H <sub>2</sub> O	11	18	14	19	13
[Zn(L <sub>1</sub> )( L <sub>2</sub> )] .2H <sub>2</sub> O	12	16	14	14	12
[Hg(L <sub>1</sub> )( L <sub>2</sub> )] .2H <sub>2</sub> O	16	22	19	20	17

**Note: Highly active = Inhibition zone > 12 mm , Moderately = Inhibition zone = 9-12 mm**

**Slightly = Inhibition zone = 7-8 .**

**Table 5:Antifungal activity of the ligands and their mixed complexes**

Compound Fungi	Average percentage inhibition (%) after 72 h					
	A.flevus			A. terreus		
	10	100	1000	10	100	1000
HL <sub>1</sub>	56.7	61.1	70	64.4	68.9	73.3
HL <sub>2</sub>	60	65.6	72.2	63.3	65.6	74.4
[Ni(L <sub>1</sub> )( L <sub>2</sub> )]. 2H <sub>2</sub> O	77.8	85.6	91.1	73.3	80	86.7
[Cu(L <sub>1</sub> )( L <sub>2</sub> )] . 2H <sub>2</sub> O	65.6	74.4	86.7	68.9	77.8	88.9

$[\text{Zn}(\text{L}_1)(\text{L}_2)] \cdot 2\text{H}_2\text{O}$	70	76.7	84.4	65.6	75.5	80
$[\text{Hg}(\text{L}_1)(\text{L}_2)] \cdot 2\text{H}_2\text{O}$	74.4	83.3	87.8	87.8	93.3	95.5

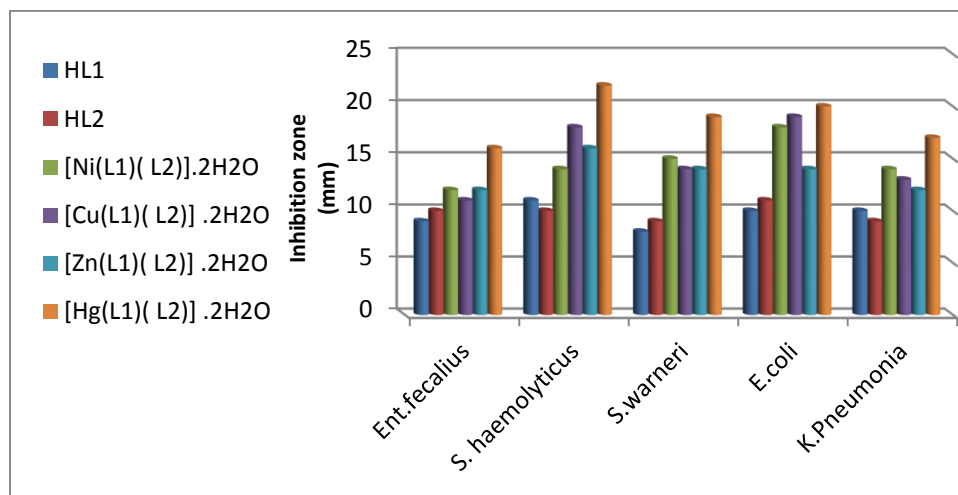


Fig.7:Statistical representation for antibacterial activity of the ligands and their mixed complexes .

## Conclusion

In the present study, the preparation and characterization of Ni(II) Cu(II), Zn(II) and Hg(II) complexes mixed ligands of (CPI) and (PAN) have been carried out by elemental analyses, IR, and UV-Vis spectral studies. Electronic spectral data and magnetic susceptibility. The invitro antimicrobial screening to the compounds that the mixed ligand complexes have the considerable therapeutic action and act as antimicroorganisms agents .

## References

1. Dhanaraj C J. and Johnson J. [2014] . *Res. J. Chem. Sci.* 4(11) p 80-102.
2. Housecroft C E. and Sharpe A G.[ 2005]. Inorganic chemistry. 2nd edition,.
3. Ali A.A.M and Abdul Zahra Z.A. [2014]. **International Journal of novel research in Interdisciplinary studies.** (2) p5-13.
4. Tanaka R., Viehmann P. and Hecht S. [2012] **organo metal** 31(4216).
5. Ali A.A.M, Taha D.K, Abdul Sahib A.A, Abd Alaly R, Abdul Kadhim H, Hassan Z, Salman A and Jawad A. [2013]. **J. of Babylon University Pure and Applied Science.** 12(2) p460-469.
6. Al-Adilee K.J, Al- Shamsi H.A.H and Dawood M.N.[ 2016]. **Research Journal of Pharmaceutical, Biological and Chemical Science.** 7(4) p2882-2905.
7. Hofmann K, (1953) : Imidazole and it's derivatives ,Interscience , New York.
8. Raman N., Raja JD. [2007]. **Indian J. of. Chem.** 46 , p.1611-1614.
9. Al-rammahi A S. Al- khafagy A H. and Al-rammahi F. [2015]. **World.J. of pharm. Res.** ,4(2), P.1668-1680.
10. Al- khafagy A H .[2016]. **J. of Chem. and pharm. Res .** , 8(8), P.296-302.
11. Abbas A.and Kadhim R S.[2016]. **J.of Applied Chem.** 9(8) P. 20-31.
12. Hasan M d R. , Hossain M A., Salam M d A. and Uddin M N.[ 2016]. **J. of Taibah University for Science** ,10, P.766–773.
13. Al-Saadi N M A. [2008].Chemistry department. College of Science-University of Baghdad M.Sc .**A Thesis.**
14. Al-hassani A A., Al-Khafagy A H. and Ali A A M. [2014]. **World J. of Pharm. Res.** 3(10) P. 218-231.
15. Chaulia S N. [2017]. **J.Bulgarian Chemical Communications** 49(1) P.46- 61.
16. Al-Adilee K. and Kyhoiesh H A K. [2017]. **Journal of Molecular Structure.** P.160-178.
17. Mehdi R. and Ali A M.[2005]. **National Journal of Chemistry.** 20 , P. 540-546.
18. نادية حسين عبيد و رحيم طاهر مهدي. [2011] **مجلة الكوفة لعلوم الكيمياء.** العدد 2, ص 21-32
19. Chaulia S N. [2016]. **Der Pharma Chemica.** 8 (3) p254-272.

20. Subramaniam S M V, Ramaswamy N. and Kartha B. [2015] **Chem . Sci. Rev. Lett.** 4(13) .P.121-128 .
21. Mary S S., Shobana S., Dharmaraja J., Narayaa S A.and Ralamurugan J.[2016].  
**J. of Chem. Pharm. Res.** 8(1S), P .12-18.
22. .Joseyphus R and Nair M. [2008]. **Mycoloiology** .36 . P. 93-98.
23. Raman N., Raja J D. and Sakthivel A. [2007]. **J. Chem. Sci.** 119(4), P.303-310.
24. Shelke V A., Jadhav S M.,Patharkar V R., Shankarwar S G., Munde A S. and Chordhekar T K., [2012]. **Arab. J. of Chem.** 5. P. 501-507.
25. Abd-Elzaher M M., Shakdofa M M E., Mousa H A. and Mostafa S A. [2014]. **Sop. Trans.on Appl.** 1(1). P. 42-52.