*Egypt. J. Chem.* **Vol. 64**, No. 7 pp. 3235 - 3243 (2021)

**Egyptian Journal of Chemistry**

**http://ejchem.journals.ekb.eg/**

# Synthesis, structural, thermal and biological studies for new Schiff base derived from Isoniazid and it's complexes with metal ions.

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**Abstract**

A new ligand of Schiff base (L") [methyl (E)-(6-((2-isonicotinoylhydrazineylidene) (phenyl) methyl) -1H -benzo[d]imidazol-2'-yl) carbamate] (3) was prepared from the reaction of isoniazid (1) and mebendazol (2) in presence of HBr (48%) in ethanol. The complexes were prepared from the reaction metal ions M(II)=V, Mn, Co, Ni, Cu, Cd, Zn and Hg with (L") in ethanol and acetone. All compounds were characterized by FT-IR, 1H NMR,13C NMR, UV.Vis, Mass spectroscopy, magnetic susceptibility measurements, atomic absorption, thermal analyses, elemental microanalysis, molar conductance and chloride content. The results obtained when evaluating the antibacterial and antifungal activities of the compounds prepared against two types of bacteria and one type of fungi showed that the complexes are more effective than free ligand, while based on a date for all techniques It has been suggested that all prepared complexes have octahedral geometry.

Keywords: Isoniazid, Schiff Base, TGA, Complexes Of Metals, Biological Activities .

## 1. Introduction

Schiff bases form an essential class of organic compounds, containing azomethine (imine) group (RC= N), are usually prepared from primary amine condensation with an active carbonyl compound. [1-4]. Schiff bases have been synthesized as bi-dentate as well as multi-dentate ligands [5, 6]. Isoniazid (Is nicotinic acid hydrazide, INH) is the most commonly used drug for active infection and prophylaxis since its introduction for treatment of TB in 1952 [7]. Isonicotinic acid hydrazide (isoniazid, INH), a well-known ant tuberculosis drug [8,9] Schiff base metal complexes have been widely studied like industrial, biological applications, pharmaceutical [10], Treatments, Anti tuberculosis [11], anticancer, antifungal, antiviral agents and as herbicides [12].

In our study, we report new metal complexes that were obtained from the reaction of new ligand [L"] with metal ions M(II)= V, Mn, Co, Ni, Cu, Cd, Zn and Hg. These complexes were characterized by FT-IR, UV-Vis, microanalysis, TGA, magnetic susceptibility measurements, chloride content and molar conductance, and two separate groups of bacteria and one fungal type have also been investigated into antibacterial activity for prepared complexes.

## 2. Experimental

### 2.1 Materials and Methods of Characterization

Isoniazid (INH), vanadyl (II) sulfate mono hydrate, Manganese (II) Chloride tetrahydrate, Nickel (II) Chloride hexahydrate, Cobalt (II) Chloride hexahydrate, Cupper (II) Chloride dehydrate, Zinc (II) Chloride, Cadmium (II) Chloride and Mercury (II) Chloride, DMSO, Acetone, Mebendazol (MBZ), and Ethanol all compound from Aldrich company. Melting points for compounds measured by using (Stuart melting point apparatus) electro thermal, while Shimadzu (FT–IR)–8400S used to measured Infrared spectra. An electronic spectrum of all compounds measured by [(U.V-Vis) spectrophotometer type U.V 160A (Shimadzu)], Euro Vector, model EA 3000

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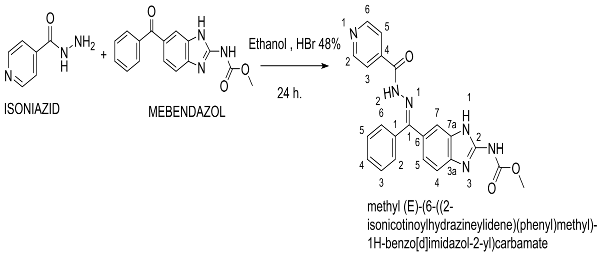
Received date 12 September 2020; revised date 09 March 2021; accepted date 18 March 2021

DOI: 10.21608/EJCHEM.2021.42643.2861

single V.3.O single in used to measured Elemental microanalysis, while the Chloride contents measured by using (686-Titro processor-665. Dosimat Matron Swiss). A conductivity meter, model 4070 used to measure the electrical conductivity of complexes at (25 °C) of a solution ($10^{-3}$ mo. Liter $^{-1}$) of complexes in DMSO. Magnetic Susceptibility of the mineral complexes measured by using Magnetic Susceptibility Balance (MSB _MKI). $^{1}$HNMR and$^{13}$C-NMR measured by using a Brucker-400 MHz and 200 MHz respectively, while thermos gravimetric analysis (TGA) has been performed by STA PT-1000 Linseys company /Germany.

### *2.2. Synthesis of ligand (L")*

A solution formed by dissolving [0.14g, 1mmole] of Isoniazid (1) in ethanol (10 ml) was added to a solution Mebndazole (2) [0.29g, 1 mmole] in Ethanol (10 ml) with 3 drops of 48% HBr. The mixture was refluxed for 24 h. with continued stirring. During relaxation, at room temperature, the yellow solid mass of 3 was formed, then washed with Ethanol and recrystallized the sample with Acetone to get a pure substance. Yield 86% , M.P.: 198 - 200 °C , M.wt : 414 .16 gm / mol ($C_{22}H_{18}N_6O_3$) . Scheme (1)



Scheme (1). The synthesis route of new Schiff base (L").

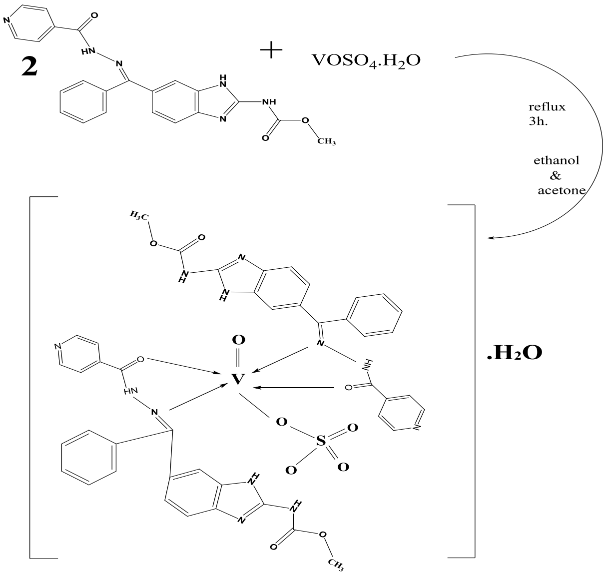
### *2.3. Synthesis of complexes*

#### *Synthesis of $[(VO)(L")_2SO_4]. H_2O$*

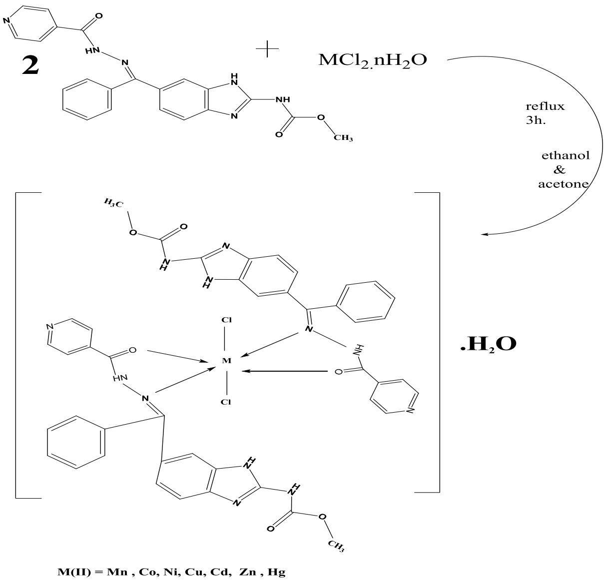
Mixed EtOH solution (10 ml) of VOSO4H2O (0.181 g, 1 mmol) with acetone solution (15 ml) of (L ") (0.828 g, 2 mmol) With continuous stirring at (72°C), for 3 h. A brown precipitate was formed, the precipitate was filtered and washed with cold acetone. Scheme (2).

#### *Synthesis of $[M (L")_2Cl_2] .H_2O$*

Same as the previous method (10 mL) EtOH solution of (0.197, 0.237 g, 0.237, 0.170, 0.136, 0.138,0.271) g) (1mmole) of M (II) = Mn, Co, Ni, Cu, Cd, Zn, and Hg respectively it was mixed with (15 mL) Acetone solution of (L") (0.828g, 2mmole), colored precipitates were formed, the precipitates were filtered and washed with cold acetone. Scheme (3). Table (1) appeared some physical properties of metal complexes.



Scheme (2): Synthesis route of $[VO(L")_2 (SO_4)]. H_2O$



Scheme (3): Synthesis route of ligand [L"] complexes

Table 1: *Some Physical properties of metal complexes.*

| *Compounds* | *M. wt.* | *Colour* | *M.p.* *°c* | *Yield* *%* | *Metal analysis* *found / (Calc.) %* | *chloride content* *found / Calc.) %* | *Λm* *($S.cm^2.mol^{-1}$)* | *μeff* *(B.M)* |
|---|---|---|---|---|---|---|---|---|
| [VO(L")$_2$SO$_4$]. H$_2$O | 1010.2 | Green | 264 | 89 | 4.79 / (5.06) | - | 8.21 | 1.86 |
| [Mn(L")$_2$Cl$_2$]. H$_2$O | 972.18 | Light yellow | 220 | 90 | 5.62 / (5.66) | 7.02 / (7.13) | 9.52 | 5.60 |
| [Co(L")$_2$Cl$_2$]. H$_2$O | 975.94 | Blue | 200 | 77 | 5.86 / (6.05) | 7.06 / (7.15) | 17.01 | 4.86 |
| [Ni (L")$_2$Cl$_2$]. H$_2$O | 975.94 | Dark green | 214 | 85 | 5.72 / (6.07) | 6.88 / (7.91) | 19.20 | 2.83 |
| [Cu (L")$_2$Cl$_2$]. H$_2$O | 980.79 | Dark brown | 195 | 91 | 6.44 / (6. 53) | 6.89 / (7.06) | 23.41 | 1.72 |
| [Zn (L")2Cl2]. H$_2$O | 982.34 | Light yellow | 241 | 80 | 6.54 / (6. 61) | 6.29 / (6.38) | 5.57 | _ |
| [Cd (L")$_2$Cl$_2$]. H$_2$O | 1029.65 | Light yellow | 269 | 79 | 10.33 / (10. 88) | 5.75 / (6.12) | 5.19 | _ |
| [Hg (L")$_2$Cl$_2$]. H$_2$O | 1117.24 | Yellow | 210 | 74 | 17.82 / (17. 90) | 5.53 / (5.70) | 12.71 | _ |

## 3. Results and Discussion

### *3.1. Characterization of ligand*

The I.R. spectrum for (L")**:** Fig **(**1), appeared band at (3344) cm$^{-1}$, due to the frequency of NH. A strong band observed at (1689) cm$^{-1}$ that for to υ(C=O) group of INH [13]. A band of υ(C=N) appeared at (1643) cm$^{-1}$ [14], while the stretching frequency of υ(C = N) in MBZ appear at (1597) cm$^{-1}$. The bands at (1577) cm$^{-1}$ and (1365) allocated to υ(C=C) and υ(C-N) respectively. The iminic band appeared while the carbonyl(C=O) band of MBZ and amino group (NH$_2$) band were disappeared in new ligand spectrum, this confirms that the Schiff base ligand is formed. [15].

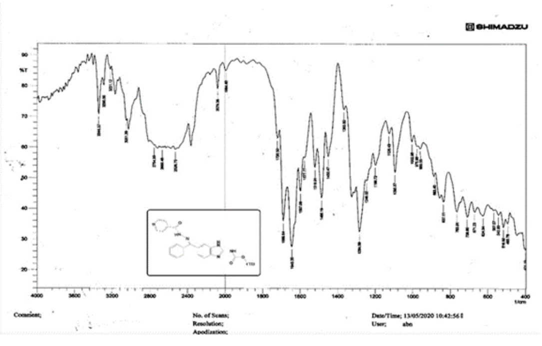


Fig. (1): FT-IR spectrum of the ligand [L"]

The (UV_Vis) spectrum of (L") Fig (2), appeared two absorption peaks, the first peak at (272) nm (36764) cm$^{-1}$ because of the electronic transmission of the type π→π*, while the second peak showed at (318) nm (31446) cm-1 because of the electronic transmission of the type π→π* [16].

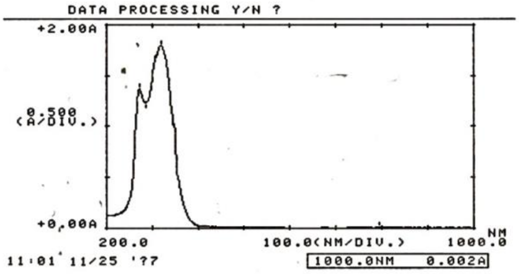


Fig. (2): Electronic spectrum of ligand [L"]

Ligand's (L") 1H-NMR spectrum: 1H NMR spectrum with regard to [L"], Fig. (3) in DMSO-d6 showed chemical shift at (δH =12.74 ppm, 11.34ppm) due to protons group (NH) of mebendazol [17]. The chemical shift at (δH =10.97 ppm) due to protons (NH) of isoniazid. The resonances at chemical shifts (δH = 7.57 - 8.75 ppm) (Ar–H) refers to protons of the aromatic ring, while at (δH = 3.84 ppm) pointing to (CO$_2$CH$_3$) [18]. In the end chemical shifts at. (δH = 3.39 ppm and 2.56 ppm) referred to water molecules are present in the solvent and (DMSO) solvent that respectively.

*$^{13}$CNMR spectrum of (L"),*

Fig. (4) in DMSO-d6 solvent showed a chemical shift in the range (δ= 115.65-149.39 ppm) refer to aromatic carbon atoms, while carbon atom in the carbonyl group (C17) appeared at (δ=185.65 ppm) and carbon atom in the imine group (C10) was appeared at (δ=174.57 ppm). A carbon atom in the carbonyl group (C2) appeared at (δ = 154.40 ppm), while a shift at (δ=147.32ppm) due to (C3) in the end, a shift at (δ= 52.62ppm) due to the methoxy group's carbon (C1) [19].

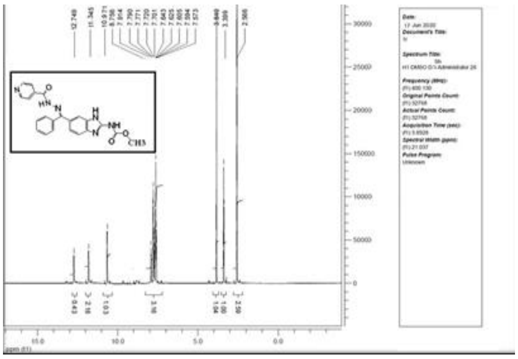


Fig. (3): 1HNMR spectrum for [L"] in DMSO-d$^6$

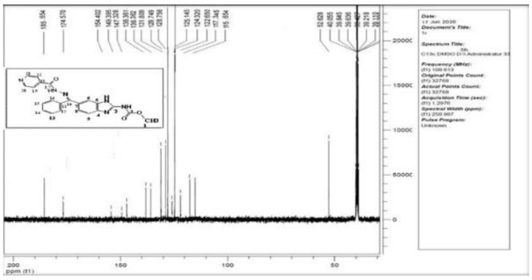


Fig. (4): $^{13}$CNMR spectrum for [L"] in DMSO-d6.

Mass spectrum of (L") Figure (5). The ligand molecular ion peak appears at m/z = 414.16 $(M)^+$ for C22H18N6O$_3$. The other peaks were located in m/z = 355.4 -77.3 get along with $[C_{20}H_{15}N_6O]^+$- $[C_6H_5]^+$. A fragmentation pattern of [L"] is tabled in table (2). Scheme [4] showed the suggested mass fragmentation (L").

Table (2): The pattern of fragmentation of [L"]

| Fragment | Mass/charge (m/z) |
|---|---|
| M]$^+$ = [ C22H18N6O$_3$]$^+$ | 414.2 |
| $[C_{20}H_{15}N_6O]^+$ | 355.4 |
| $[C_{18}H_{15}N_4O]^+$ | 303.1 |
| $[C_{17}H_{15}N_2O]^+$ | 263.1 |
| $[C_{14}H_{13}N_2]^+$ | 209.2 |
| $[C_{13}H_9N]^+$ | 180.2 |
| $[C_9H_9N]^+$ | 131.1 |
| $[C_7H_6N]^+$ | 104.2 |
| $[C_6H_5]^+$ | 77.3 |

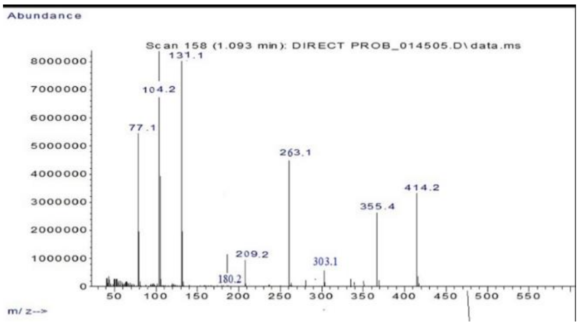
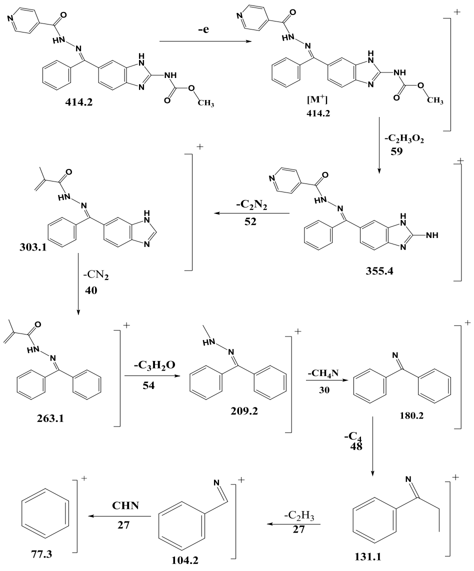


Fig. (5): Mass spectrum for [L"]



Scheme (4): Suggested mass fragmentation of Schiff base (L")

### 3.2. *Characterization of complexes*

Thermal gravimetric analysis (TGA) of [Cu $(L")_2Cl_2$]. $H_2O$ :

TGA peak Fig. (6) observed at 279.29 ºC indicated the loss of [$H_2O$, 2Cl, ($C_8H_9N_2O_2$)] fragment, (det. = 5.455 mg, 25.9787 %; calc. = 5.439 mg). The second step of the decomposition of compound at 597.09 ºC is related to the loss of ($C_5H_8N_3O_3$) segment, (obs. = 3.397 mg, 16.1782 %; calc. = 3.385 mg). The molecular formula of [Cu $(L")_2Cl_2$]. $H_2O$ complex agreement with the TGA data [20,21].

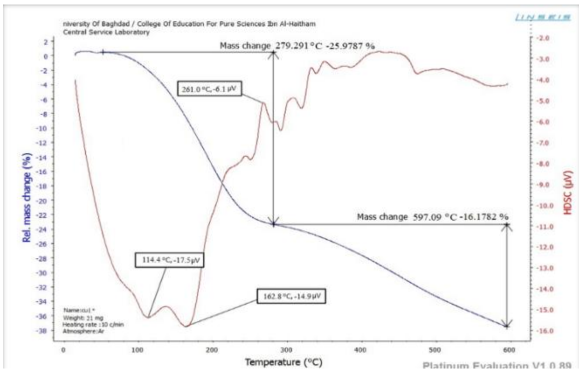


Fig. (6): (TGA and DSC) of $[Cu (L'')_2Cl_2]. H_2O$

*The IR spectra*

The IR spectra of synthesized complexes Fig. (7) showed the band at (1643) $cm^{-1}$ which indicates the azomethine group stretching frequency (C=N) in free ligand, this band appeared at range (1630-1639 $cm^{-1}$), this lower frequency happened due to the azomethine group nitrogen atom was coordinated with metal ions in spectra of all prepared complexes.

In another way, a stretching vibration band (υC=O) for INH in free ligand appeared at (1689) $cm^{-1}$, this band was shifted to lower frequency at range (1664-1679) $cm^{-1}$ in all complexes' spectra, shows that coordination has occurred between the group's oxygen atom (C= O) and metal ions.

A new band appeared in VO(II) complex spectrum at (955) $cm^{-1}$, it was referred to (υV=O) group, in the same time new bands appeared at (479, 646) $cm^{-1}$ and (988, 1039) $cm^{-1}$ that refers to $\delta$ ($SO_4^{-2}$) and υ ($SO_4^{-2}$) respectively, that indicates the coordination happened between $SO_4^{-2}$ and VO(II) ion in the form a monodentate ligand [22]. The IR spectrum for all prepared complexes in the low-frequency region shows new bands that do not appear in the free ligand spectrum, these bands are shown in (470– 605) $cm^{-1}$ and (451–547) $cm^{-1}$ these bands refer to υ(M-N) and υ(M-O) respectively [23].

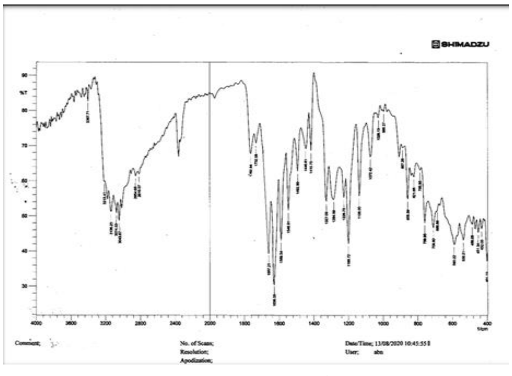


Fig. (7): FT-IR spectrum of $[Cu(L'')_2Cl_2]. H_2O$

*Electronic spectra*

In the (UV_Vis) transitions spectrum all complexes appeared octahedral geometry about the metal ion, while the complexes of Zn (II), Cd (II), Hg (II) appeared no any type of electronic transition (d-d) because these complexes contain the full d subshell [20].

Assignment of infrared frequencies in $cm^{-1}$ and Electronic spectral Data of the complexes is seen in table (3) Seen [Fig. (8), Fig. (9)].

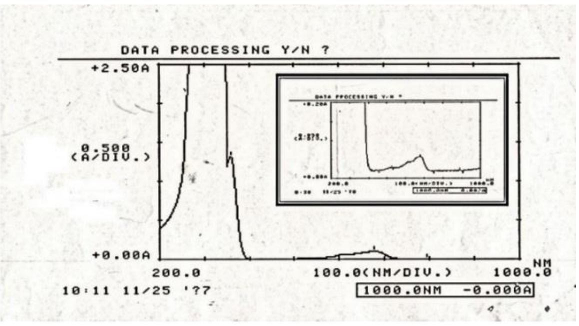


Fig. (8): Electronic spectrum of $[Cu(L'')_2Cl_2]$ in $10^{-3}$M

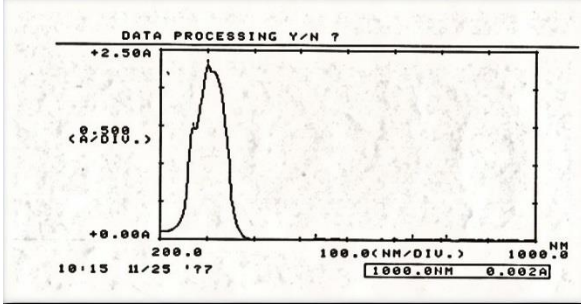


Fig. (9): Electronic spectrum of $[Cu(L'')_2Cl_2]$ in $5.8x10^{-4}$M.

*Elemental analysis*

The findings also match well with the values measured. Finally, where through the study of molar conductivity it became clear that all the complexes are non-electrolytic seen in table (1).

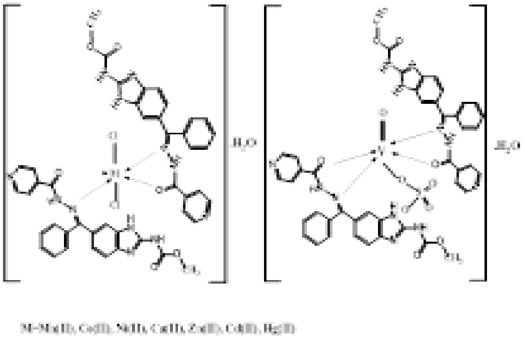


Fig. (10): Proposed structures of complex

Table 3: Assignment of infrared frequencies in $cm^{-1}$ and electronic spectral data of the complexes

| Complexes | N-H group | C=O amid | C=N imin | (M – N) | (M – O) | UV/visible in (DMSO, nm) | Assignments |
|---|---|---|---|---|---|---|---|
| [L"] | 3344 | 1689 | 1643 | - | - | 272 | $\pi\rightarrow\pi^*$ |
| | | | | | | 318 | $\pi\rightarrow\pi^*$ |
| $[VO(L")_2SO_4]. H_2O$ | 3402 | 1678 | 1639 | 551 | 509 | 270 | Intra-ligand |
| | | | | | | 314 | (In.L.) |
| | | | | | | 368 | (In.L.) |
| | | | | | | 479 | (LMC.T) |
| | | | | | | 682 | $({}^{2}B_{2}g\rightarrow{}^{2}A_{1}g)$ |
| | | | | | | 813 | $( {}^{2}B_{2}g\rightarrow{}^{2}B_{1}g$<br>${}^{2}B_{2}g\rightarrow {}^{2}Eg)$ |
| $[Mn(L")_2Cl_2]. H_2O$ | 3402 | 1679 | 1636 | 582 | 475 | 269 | (In.L.) |
| | | | | | | 318 | (In.L.) |
| | | | | | | 366 | (LMC.T ) |
| | | | | | | 420 | $( {}^{6}A_{1}g\rightarrow{}^{4}A_{1}g,{}^{4}Eg(G))$ |
| | | | | | | 484 | $( {}^{6}A_{1}g\rightarrow{}^{4}T_{1}g\ (P) )$ |
| | | | | | | 534 | $( {}^{6}A_{1}g\rightarrow{}^{4}T_{2}g\ (G) )$ |
| | | | | | | 736 | $( {}^{6}A_{1}g\rightarrow{}^{4}T_{1}g\ (G) )$ |
| | | | | | | 811 | $( {}^{6}A_{1}g\rightarrow{}^{4}Eg\ (G) )$ |
| $[Co\ (L")_2Cl_2]. H_2O$ | 3367 | 1675 | 1630 | 539 | 520 | 295 | (In.L.) |
| | | | | | | 301 | (In.L.) |
| | | | | | | 359 | (LMC.T ) |
| | | | | | | 523 | $({}^{4}T_{1}g(F) \rightarrow{}^{4}T_{1}g\ (P))$ |
| | | | | | | 678 | $({}^{4}T_{1}g\ (F) \rightarrow {}^{4}A_{2}g\ (F))$ |
| | | | | | | 898 | $({}^{4}T_{1}g\ (F) \rightarrow {}^{4}T_{2}g\ (F))$ |
| $[Ni\ (L")_2Cl_2]. H_2O$ | 3406 | 1669 | 1635 | 567 | 547 | 268 | (In.L.) |
| | | | | | | 313 | (In.L.) |
| | | | | | | 359 | (LMC.T) |
| | | | | | | 428 | ${}^{3}A_{2}g\ (F) \rightarrow {}^{3}T_{1}g\ (P))$ |
| | | | | | | 570 | $({}^{3}A_{2}g\ (F) \rightarrow {}^{3}T_{1}g\ (F))$ |
| | | | | | | 811 | $({}^{3}A_{2}g\ (F) \rightarrow {}^{3}T_{2}g\ (F))$ |
| $[Cu(L")_2Cl_2]. H_2O$ | 3367 | 1667 | 1638 | 590 | 436 | 269 | (In.L.) |
| | | | | | | 314 | (In.L.) |
| | | | | | | 360 | (LMC.T ) |
| | | | | | | 862 | $({}^{2}Eg \rightarrow {}^{2}T_{2}g)$ |
| $[Zn\ (L")_2Cl_2]. H_2O$ | 3402 | 1676 | 1636 | 605 | 470 | 270 | (In.L.) |
| | | | | | | 314 | (In.L.) |
| | | | | | | 360 | (LMC.T ) |
| $[Cd\ (L")_2Cl_2]. H_2O$ | 3367 | 1664 | 1637 | 578 | 470 | 268 | (In.L.) |
| | | | | | | 317 | (In.L.) |
| | | | | | | 364 | (LMC.T ) |
| $[Hg\ (L")_2Cl_2]. H_2O$ | 3367 | 1670 | 1634 | 470 | 451 | 270 | (In.L.) |
| | | | | | | 315 | (In.L.) |
| | | | | | | 368 | (LMC.T ) |

## 4. Biological activity:

Microorganisms

A method of Bauer was used to measure the effectiveness of the Gram-positive bacteria (Staphylococcus aureus) and Gram-negative bacteria (Escherichia coli) for all prepared complexes and ligand by using the method diffusion of agar, the media prepared from nutrient agar (25g) in distilled water (1000 mL) put in an autoclave at 121°C for 30 min to disinfected and to prevent pollution, after the rigidity of the media, the bacteria was spilled into a petri dish, and each complex was dissolved in afterward in (DMSO) to have a concentration (0.01) mg / ml and make sure the outgrowth of the inhibition zone against fungus ( Candida albicans) after incubating the media for 24 h and at 37 °C. The zone of inhibitions of the ligand (L'') and its complexes against the growth of bacteria and fungi were given in table (4) and Fig. (11-13) [24, 25]

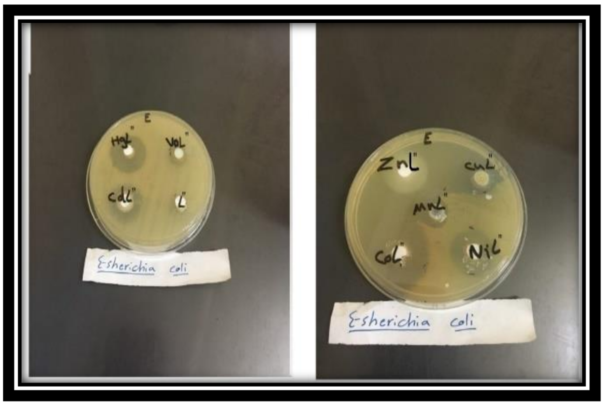


Fig. (11) Biological activity (Ecoli) for the(L'') and it's complexes.

### Biological activity of compounds

The biological activity of compounds was evaluated with Staphylococcus aureus, E. Coli, and C. Albicans which showed in the table (4).

The ligand and all its complexes have increased inhibition activities in comparison to the control DMSO and starting agent, against two bacterial forms (Staphylococcus aureus and Escherichia Coli) and one type of fungus (Candida Albicans).

Hg complex with Staphylococcus aureus and Zn complex with E. Coli have antimicrobial activity more than other complexes while Cd complex with C. Albicans has antifungal activity more than other complexes.

This can depend on its electronic (d10) configuration and/or higher molecular weight than other metal ions. [26].

Any antimicrobial activity for different compounds against different organisms counts on [27]:

a- Their microbial cell impermeability.

b- The difference in the microbial cell ribosome.

## 5. Conclusions

1-The Schiff base (L'') acted in the form of bidentate ligand through a nitrogen atom in imine and oxygen atom in (C=O) of INH with the central metal ions M(II): V, Mn, Co, Ni, Cu, Zn, Cd and Hg with complexes of its general molecular formula: $[M(L'')_2Cl_2].H_2O$ and $[VO(L'')_2SO_4].H_2O$

2- The octahedral geometrical structure was suggested for all prepared complexes. See fig. (10).

3- To use these prepared complexes as drugs because these complexes have high inhibition activity against bacteria and fungus

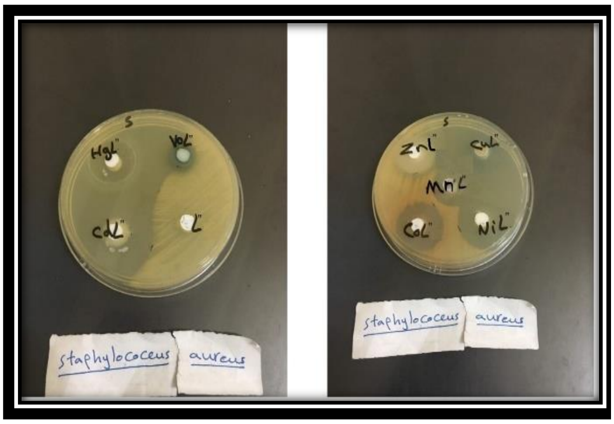


Fig.(12) Biological activity(Staph) for the( L'') and it's complexes

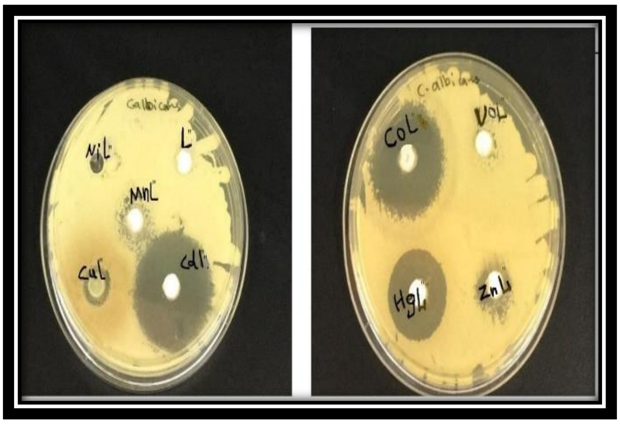


Fig.(13) Biological activity(C.albicans) for the( L'') and it's complexes

Table (4): The biological activities of prepared compounds

| Compound | *E. Coli* | *Staphylococcus aureus* | *C. Albicans* |
|---|---|---|---|
| DMSO | - | - | - |
| L" | 12 | - | - |
| [VO(L")$_2$SO$_4$]. H$_2$O | 15 | 14 | - |
| [Mn (L")$_2$Cl$_2$]. H$_2$O | 17 | 21 | 13 |
| [Co (L")$_2$Cl$_2$]. H$_2$O | 29 | 31 | 22 |
| [Ni (L")$_2$Cl$_2$]. H$_2$O | 21 | 26 | - |
| [Cu (L")$_2$Cl$_2$]. H$_2$O | 14 | 48 | 11 |
| [Zn (L")$_2$Cl$_2$]. H$_2$O | 42 | 25 | 13 |
| [Cd (L")$_2$Cl$_2$]. H$_2$O | 22 | 39 | 31 |
| [Hg (L")$_2$Cl$_2$] H$_2$O | 28 | 54 | 28 |

*St.aureus= Staphylococcus aureus* , *E. Coli = Escherichia coli*

*C. albicans = Candida albicans*

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