

Structural, Characterization, Biological and Studying the Enzyme Activity of some transition metal complexes with 4- ((2- hydroxy phenyl imino) methyl) -2, 6-dimethoxy phenol

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Abstract

A new Schiff bases ligand 4- ((2-hydroxy phenylimino) methyl) -2, 6-dimethoxyphenol [L^2] derived from condensation of 2- amino phenol with 4-hydroxy -3, 5-dimethoxy benzaldehyde have been synthesized and characterized by $FT - IR, Uv - Vis$ spectroscopy, $^1H, ^{13}C - NMR$ spectra, Mass spectrum and elemental microanalysis (C.H.N). Metal Complexes with $Co(II), Ni(II), Cu(II)$ and $Pd(II)$ ions have been also synthesized and characterized spectroscopic methods ($FT - IR, Uv - Vis$) spectroscopy, flame atomic absorption, molar conductivity measurements and magnetic susceptibility. These studies indicate that the molar ratio ($L:M$), (2: 1) for the complexes. The complexes $Co(II)$ and $Ni(II)$ showed characteristics octahedral geometry with the (O,N) ligand coordinated in bidentate mode while with $Cu(II)$ and $Pd(II)$ showed square planer. The enzyme activity of the ligand and its metal complexes with esterase (AST) have also been studied . the study of enzyme activity indicates that the ligand its metal complexes revealed different inhibition behaviors. The synthesized ligands and their metal complexes have been tested against one type of fungi (candida albicans).

Keyword: 2-amino phenol, Schiff bases complexes, enzyme activity.

Introduction

Schiff bases have azomethine ($-C=N$) functional group and are the most promising target due to their wide range of biological activities which encompass antifungal, antibacteria^[1], anti malarial^[2], anticonvulsant^[3], antiprotiferative, antileishmanial, anti-inflammatory^[4], anti-viral and antipyretic properties. Coordination chemistry employs Schiff bases which have achieved prime importance in this era^[5-8]. The extensive studies have been conducted on complexation of Schiff bases with metals due to the attractive physicochemical properties of metal complexes and broad range of utilization in various areas of science^[9-12]. Such types of complexes have paved the way exploration and findings of variety of metal complexes in recent years^[13-15].

Transition metal complexes of 2-aminophenol based Schiff bases have been the subject of extensive investigation because of their wide use in various fields^[16-18]. These Schiff base complexes have wide applications in biological field, as anti-depressants, antimicrobial, antitumor, antiphlogistic, nematocide, and other medicinal agents have been reported based on these compound^[19,20] furthermore these complexes have good catalytic role in many reactions. In present work 4-((2-hydroxyphenylimino) methyl)-2,6- dimethoxy phenol with *Co (II)*, *Ni(II)*, *Cu(II)* and *Pd (II)* are synthesized

and their physical properties, enzyme activity were investigated.

Experimental

Reagents and physical Measurements

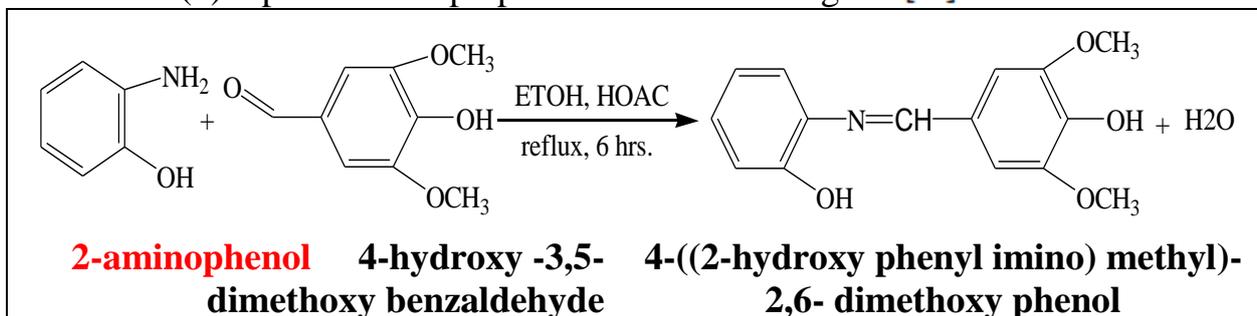
All reagents and solvent were obtained from commercial sources and used as received with further purification. Melting point were recorded by astuart melting point digital SMP30 apparatus. *FT – IR* spectra were recorded by a shimadzu (*FT – IR*) model 4800S spectrophotometer in the range $(4000 - 400) \text{ cm}^{-1}$. as KBr discs. UV-Visible spectra were recorded by shimadzu. Uv-vis 160 Ultraviolet spectrophotometer at 25C° using 1cm quartz cell and examined at the range at (200-1100) nm at $10^{-3}M$ in DMSO. Atomic absorption (A.A) technique has been measured using ashimadzu AA 680 G atomic absorption spectro photometer at the laboratories of Ibn- Sinaa company. Elemental analysis (C,H,N) for the new ligand [L^2] and complexes were determined by calibration type: linear Regression Euro EA elemental analysis were made intran Mass analysis was performed for ligand on Gc-MS (DIRECT Probe). 1H , $^{13}C - NMR$ spectra of ligand was recorded at a Bruker DMx-500 spectro photometer (300 MHz), by using $DMSO - d_6$ and $(CD_3)_2CO$.

Magnetic susceptibility of prepared complexes were determined at $(R.T) C^{\circ}$ by Auto magnetic susceptibility balance. Conductivity measurements were recorded at $(R.T) C^{\circ}$ for solutions of samples in DMSO solvent using an Inolab multi 740, WTW 82362-Germany. These measurements have been done at Al-Mustansiriyah university, college of science, chemistry Department.

Preparation of ligand $[L^2]$

The new Schiff base ligand $[L^2]$ was synthesized by condensation method of a solution of 2-aminophenol (0.109 gm, 0.001 mol) in absolute (10 ml) was added gradually to acidified solution 4-hydroxy-3,5-dimethoxy Benzaldehyde (0.182 gm, 0.001 mol) in (10 ml) from same solvent. The final reaction mixture refluxed for (6 hrs.) the formed brown precipitate filtered off, washed with ethanol, dried at room temperature and finally recrystallized from hot absolute ethanol. The synthesized ligand dissolved in the following solvent ethanol, methanol, chloroform tetrachlorocarbon, DMF and, DMSO. Purity of ligand $[L^2]$ was detected by (TLC) using silica gel as stationary phase and (Hexane/ Ethyl acetate) as eluent in ratio 71% melting point $(122 - 124) C^{\circ}$.

Scheme (1) represents the preparation reaction of ligand $[L^2]$.



Scheme (1): synthesis of 4-((2-hydroxy phenyl imino) methyl)-2,6-dimethoxy phenol

Preparation of metal complexes

One mole of ethanoic solution of metal salts was added to two moles of the ligand $[L^2]$. where the salts of

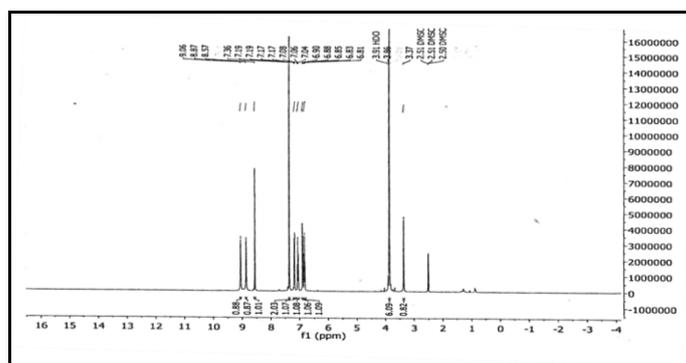
$[CoCl_2 \cdot 6H_2O (0.24gm, 1.00m mol)]$, $[NiCl_2 \cdot 6H_2O (0.24gm, 1.00m mol)]$
 $[CuCl_2 \cdot 2H_2O (0.169 gm, 1.00 mmol)]$ and $[PdCl_2 (0.170gm, 1.00 mmol)]$ was added to $(0.273 gm, 2.00 mmol)$ of the ligand $[L^2]$. color change has been noticed after mixing both solution. The reaction mixture then heated under reflux for 2 hrs. the product was filtered and washed with ethanol, then dried at room temperature. The color, melting point, yield, metal analysis and solubility of the ligand and its complexes are given in table (1):

Table (1) physical properties, yield, percentage and Elemental Analysis for ligand and its metal complexes

No	Compounds	Chemical formula (M.wt) $g. mol^{-1}$	Color	M.PC°	Yield%	Metal analysis found (calculated)				Solubility
						C%	H%	N%	M%	
1.	$C_{11}H_{11}NO_4 [L^2]$	273.0	Brown	122-124	71	65.90 (65.93)	5.50 (5.49)	5.10 (5.12)	-	EtOH, MeOH $CCl_4, CHCl_3$ DMF, DMSO
2.	$[Co (L^2)_2 (H_2O)_2]$	640.9	Light blue	219-221	78	56.19 (56.17)	5.31 (5.30)	4.32 (4.36)	9.22 (9.20)	DMF, DMSO
3.	$[Ni (L^2)_2 (H_2O)_2]$	640.7	Dark green	220-222	62	56.19 (56.18)	5.29 (5.30)	4.38 (4.37)	9.18 (9.20)	DMF, DMSO
4.	$[Cu (L^2)_2]$	609.5	Light brown	180-182	88	59.04 (59.06)	4.90 (4.92)	4.60 (4.59)	10.38 (10.41)	DMF, DMSO
5.	$[Pd (L^2)_2]$	652.4	Light brown	152-154	86	55.15 (55.18)	4.56 (4.59)	4.23 (4.29)	16.27 (16.3)	DMF, DMSO

1. 1H -NMR Spectrum for the ligand $[L^2]$

1H -NMR Spectrum for the ligand $[L^2]$ are summarized in the chemic shift at ($\delta=9.06$ ppm, $1H$) and (8.87 ppm, $1H$) assigned to phenolic (O-H) group ^[21]. While the singlet signal appeared at ($\delta=6.81$ ppm, $1H$) refers to azomet proton ^[22]. The spectrum signals related to protons of aromatic rings ($\delta=6.81$ - 7.36 PPM, s,m, 2H, benzo). signals at (3.37 ppm, 3.86 ppm, $S,3H$) belongs to the (OCH_3) group beside the signal of $DMSO - d_6$ water molecules ^[23]. The signal of methyl group appeared at ($\delta=0.86$ ppm and 1.28 ppm, 6H) and the signal for $DMSO - d_6$, at (2.51 ppm, 3H) fig. (1)



were assigned to carbon atoms of aromatic rings. A signal at (56.55ppm) due to methoxy group^[25]. and a signal at (δ 40.16 ppm) due to the solvent. fig (2).

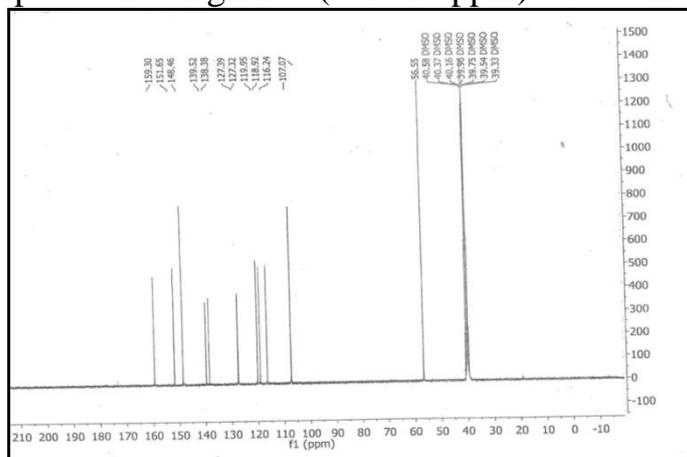
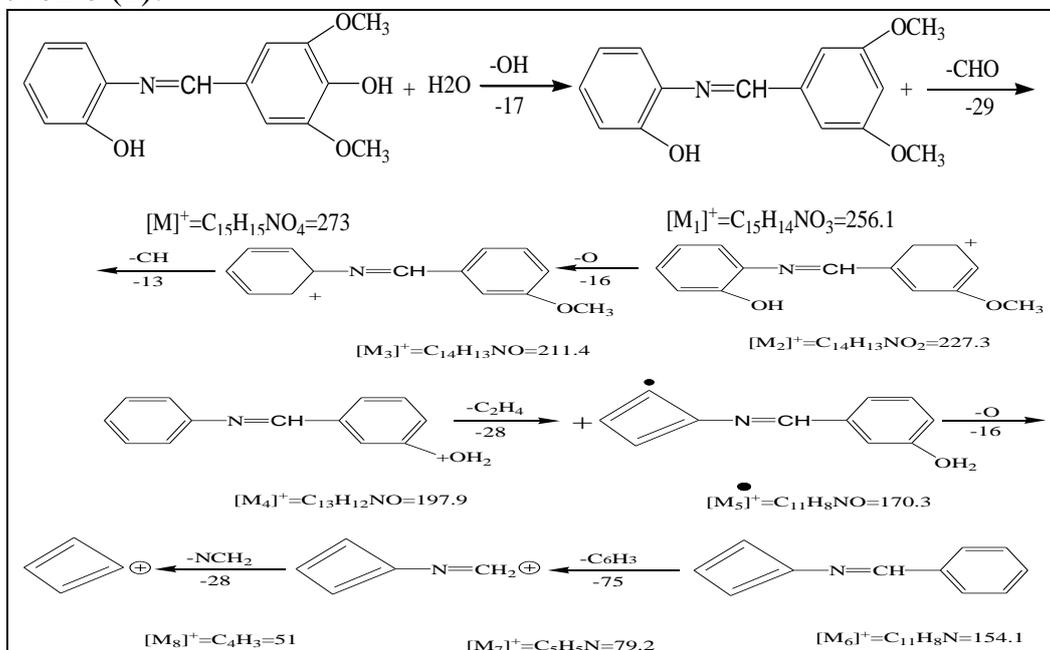


Fig (2): The ^{13}C -NMR Spectrum for the ligand $[L^2]$

3. Mass spectrum for the ligand $[L^2]$

The mass spectrum of ligand fig (3) showed the mother ion peak at ($m/z = 273$), as a base peak, which corresponds to (M^+) suggested fragmentation pathways and structural assignments if fragments are described in scheme (2):



Scheme (2): proposed fragmentation path ways of ligand $[L^2]$

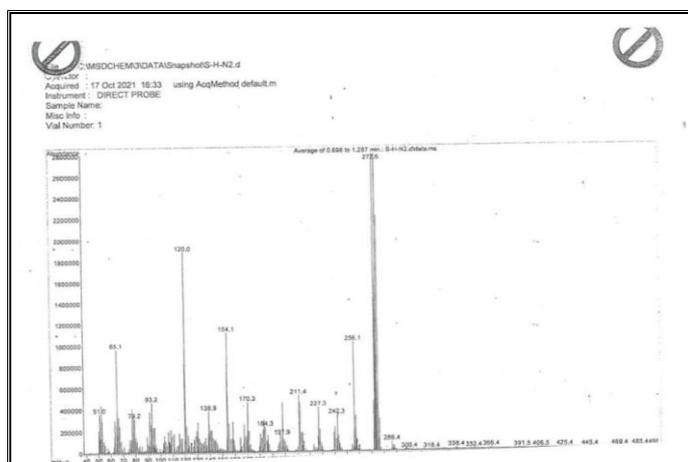


Fig (3): Mass Spectrum for the ligand [L^2]

4. FT-IR. Spectrum of ligand [L^2] and it's metallic complexes

The main stretching frequencies of characteristic bands related to the free ligand and its metal complexes and their assignments are presented in table (2). The $\nu(O-H)$ vibration of phenolic hydroxyl group which appeared at (3417 Cm^{-1}) in free ligand spectrum disappeared^[26] at all spectrum of metal complexes which indicate clearly. The IR spectrum of ligand exhibited two bands at (1253 Cm^{-1} and 794 Cm^{-1}) which can be assigned to $\nu(c-o)$ and $\delta(c-o)$ for phenolic group respectively. The observed band at (1585 Cm^{-1}) and at (3028 Cm^{-1}) can be assigned to aromatic ($C=C$) and ($C-H$) stretching frequencies respectively^[27]. The $\nu(C=N)$ vibration frequency of the Schiff base which appeared at (1624 Cm^{-1}) shifted to higher or lower frequencies in all complexes. The two bands at ($1261-1280\text{ Cm}^{-1}$) and at range ($748-759\text{ Cm}^{-1}$) in spectra of ligand complexes were attributed to $\nu(c-o)$ and $\delta(c-o)$ of phenolic group. Which were shifted to lower or higher frequency where its comparison with that of free ligand [L^2]. This shifted refers to involved oxygen atom of phenolic group in chelation with metal ions. In the spectrum of the complexes. Showed to the two bands at (3414 Cm^{-1}) and ($840\text{ Cm}^{-1}-844\text{ Cm}^{-1}$) can be attributed to coordinated H_2O (aqua)^[28] IR spectrum of all complexes showed new bands which are not present in spectrum of Free ligand, these bands were noted at range ($520-547\text{ Cm}^{-1}$) and ($420-439\text{ Cm}^{-1}$) were attributed to $\nu(M-N)$ and $\nu(M-O)$ respectively^[29].

Table (2) : FT-IR spectra Data (Cm^{-1}) for ligand [L^2] and its metal complexes

No	Compounds	$\nu(O-H)$ Aqua H_2O	$\nu(C=N)$ imine	$\nu(O-H)$ $\delta(O-H)$	$\nu(C=C)$ Aro.	$\nu(C-H)$ Aro.	$\nu(C-H)$ alph.	$\nu(M-N)$	$\nu(M-O)$
1.	$C_{12}H_{12}NO_4$ [L^2]	3417 S	1624 S	1235 S 744 S	1585 m	3028 S	2943 S 2839 S	-	-
2.	[Co (L^2) ₂ (H_2O) ₂]	3414m 3330 m 840 S	1639 S	1250 S 752 S	1589 S	3020 m	2939 m 2839 m	547 S	482 S
3.	[Ni (L^2) ₂ (H_2O) ₂]	3414 S 3321 S 840 S	1674 S	1249 S 729 S	1585 S	3055 m	2943 m 2839 S	551 S	450 S
4.	[Cu (L^2) ₂]	3371 m	1639 S	1240 S 752 S	1593 S	3040 S	2970 m 2843 m	551 S	459 S
5.	[Pd (L^2) ₂]	3410 m 3302 m	1634 Sh	1261 S 748 S	1558 m	3059 S	2930 S 2877 S	536 S	462 S

Sh. =Shoulder S. =strong , br. =Broad , m. =medium

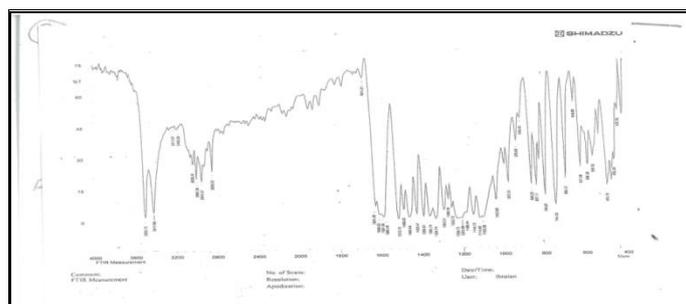


Fig (4): FT-IR for the ligand

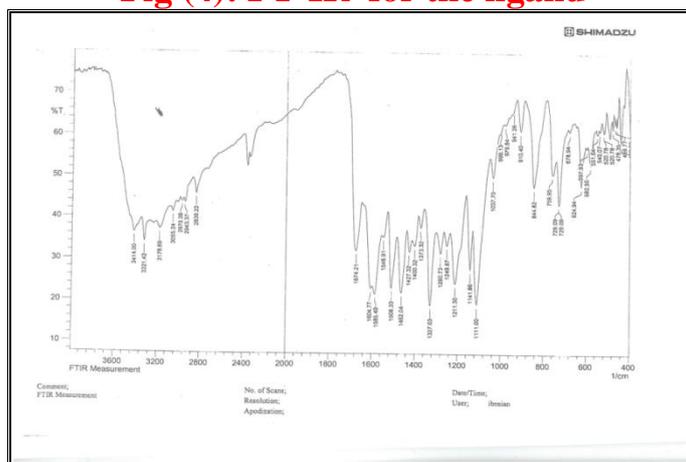


Fig (5): FT-IR for the $[Ni(L^2)_2(H_2O)_2]$

Electronic spectrum and magnetic properties of the ligand $[L^2]$ and its complexes.

The magnetic susceptibility measurements were used in combination with electronic spectral data to established the structure of complexes. The effective magnetic moment (μ_{eff}) values were observed at room temperature (300 K) for the complexes have been listed in table (3) with electronic spectrum of ligand and its complexes were recorded in DMSO solution at wave length range (200-1100)nm. The Uv-vis spectrum of brown solution of the prepared ligand reveals two peaks at (263 nm, 28022 Cm^{-1}) and (354 nm, 28248 Cm^{-1}). This may attributed to the ($\pi \rightarrow \pi^*$) and ($n \rightarrow \pi^*$)^[30] fig (6). Those electronic transition have been shifted toward higher or lower frequencies in the electronic spectrum of every prepared complexes, verify the ligand coordination with ions of the metal. The absorption peaks observed in spectrum of Co(II) at (265 nm, 37735 Cm^{-1}) and (353 nm, 28328 Cm^{-1}) were assigned to ligand field transition. The absorption peak at (452 nm, 22123 Cm^{-1}) due to ${}^4T_{1g(F)} \rightarrow {}^4T_{1g(P)}$,

(609 nm, 16420 Cm^{-1}) due to ${}^4T_{1g(F)} \rightarrow {}^4A_{2g(F)}$, and (682 nm, 14662 Cm^{-1}) due to

${}^4T_{1g(F)} \rightarrow {}^4T_{2g(F)}$.^[31] The calculated value of effective magnetic moment was seen at (4.83) B.M within the expect range of octahedral geometry^[32]. The spectrum of Ni(II) complexes exhibited three peaks in visible region at (480 nm, 20833 Cm^{-1}) (502 nm, 19920 Cm^{-1}) and (603 nm, 16583 Cm^{-1}) were assigned to (${}^3A_{2g(F)} \rightarrow {}^3T_{1g(P)}$), (${}^3A_{2g(F)} \rightarrow {}^3T_{1g(F)}$) and (${}^3A_{2g(F)} \rightarrow {}^3T_{1g(F)}$). Fig (7). The magnetic moment value was (2.84) B.M and the ligand field parameters confirmed an octahedral configuration around Ni(II)^[33]. The spectrum of Cu(II) complexes showed peak at (439 nm, 22779 Cm^{-1}) assigned to (${}^2A_{2g(F)} \rightarrow {}^2T_{2g(P)}$) transition. The position of this peak is a good agreement with square planer geometry the magnetic moment value (1.73) B.M^[34]. The Uv-vis spectrum of Pd(II) complexe exhibited a new absorption peak at (452 nm, 22123 Cm^{-1}) due to (${}^1A_{1g} \rightarrow {}^1T_{2g}$).

The Pd(II) complexe were square planer geometry in nature because of $4d^8$ – system. The magnetic mement of the Pd(II) complexe were found to be diamagnetic^[35]. Table (3) Electronic spectral data, magnetic moments, molar conductance and proposed geometry of ligand $[L^2]$ and its complexes.

Table (3) Electronic Spectral data, magnetic moment, Molar conductance and proposed geometries for ligand and its complexes

No	Compounds	λ nm	ν cm^{-1}	ϵ_{max} $Mol^{-1} \cdot L \cdot Cm^{-1}$	transition	Molar conductance $S \cdot Cm^2 \cdot Mol^{-1}$	(μ_{eff}) (B.M) suggested Geometry
1.	$C_{15}H_{15}NO_4 [L^2]$	363 354	28022 28248	1309 1934	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	-	-
2.	$[Co (L^2)_2 (H_2O)_2]$	265 316 353 452 609 682	37735 31645 28328 22123 16420 14662	1198 1915 2010 321 103 60	Intra - ligand Intra - ligand Intra - ligand ${}^4T_1g(F) \rightarrow {}^4T_1g(F)$ ${}^4T_1g(F) \rightarrow {}^4A_2g(F)$ ${}^4T_1g(F) \rightarrow {}^4T_2g(F)$	8.34	4.55 Octahedral
3.	$[Ni (L^2)_2 (H_2O)_2]$	268 309 414 480 502 603	37313 32362 24154 20833 19920 16583	1807 2409 1014 1020 660 120	Intra - ligand Intra - ligand MLC.T ${}^3A_2g(F) \rightarrow {}^3T_1g(F)$ ${}^3A_2g(F) \rightarrow {}^3T_1g(F)$ ${}^3A_2g(F) \rightarrow {}^3T_2g(F)$	5.63	2.83 Octahedral
4.	$[Cu (L^2)_2]$	264 313 354 439	37878 31948 28248 22779	1084 2325 2295 196	Intra - ligand Intra - ligand Intra - ligand ${}^2A_2g(F) \rightarrow {}^2T_2g(p)$	10.22	1.73 Square planer
5.	$[Pd (L^2)_2]$	266 294 356 452	37593 34013 28089 22123	1814 2435 1963 508	Intra - ligand Intra - ligand Intra - ligand ${}^1A_1g \rightarrow {}^1T_2g$	7.85	Diamagnetic Square planer

B.M= Bohr magneton

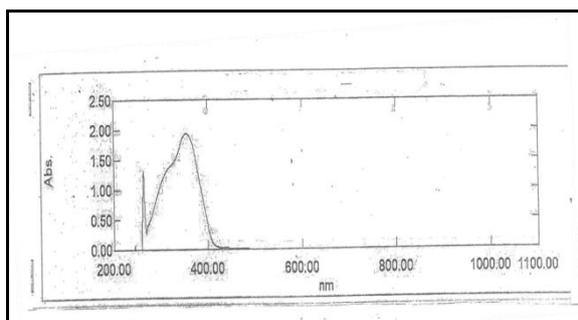
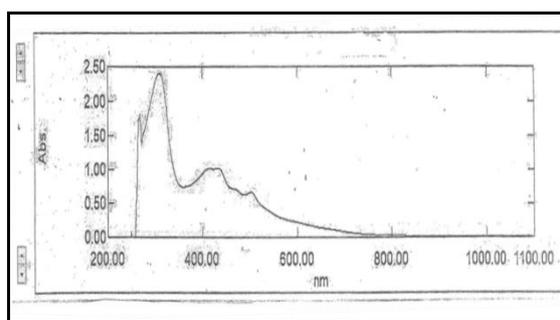


Fig (6): UV - Vis the ligand



**Fig (7): UV. Vis for the
 $[Ni (L^2)_2 (H_2O)_2]$**

Molar conductance for prepared complexes.

The values of molar conductance $Co(II)$, $Ni(II)$, $Cu(II)$ and $Pd(II)$ in DMSO were $(5.6 - 10.2) S.Cm^2 mol^{-1}$. This indicated non-electrolyte nature ^[36]. According to all previously mentioned analysis we proposed the following structure of prepared complexes as show in fig (8).

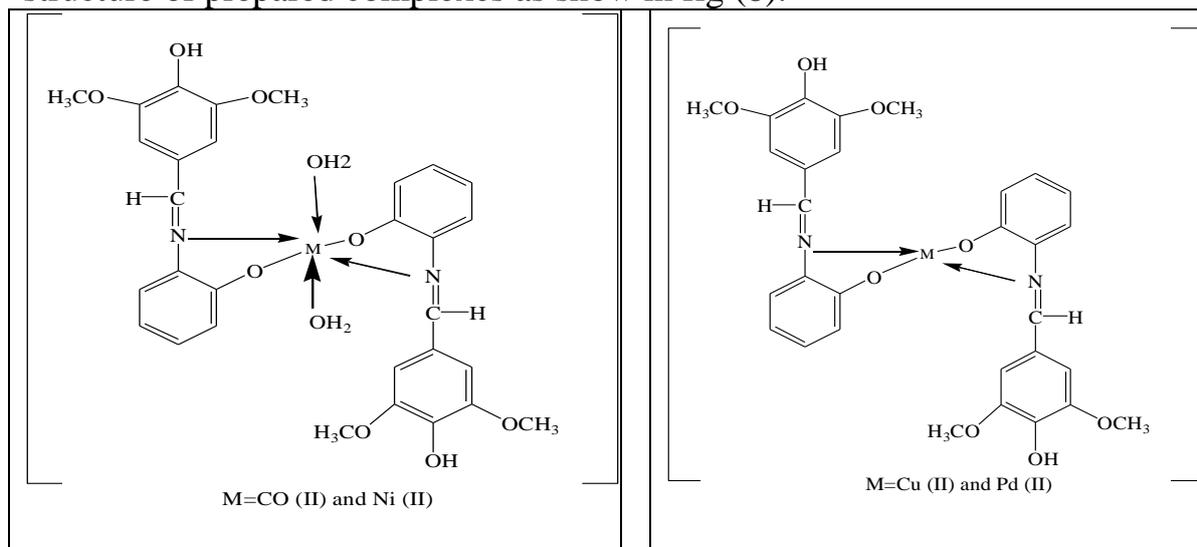


Fig (8): proposed structures for the prepared complexes

Biological Activity

A) Studying of Enzyme Activity

Methods

Determination of AST activity

Human serum AST activity was determined using Colorimetric method. ^[37]

Determination of Biological Activity of ligand and its metal complexes and type of Inhibition.

The Inhibition percentage was calculated by comparing the activity between with and without Inhibition under the same condition according to the following equation ^[38].

$$\% \text{ inhibition} = 100 - \frac{\text{the activity in the presences of inhibitor}}{\text{the activity in the absence of inhibitor}} * 100$$

Results and Discussion

Present work determined the activity of human AST in the absence and presence of ligand and its metal complexes under different substrate concentrations and designed to investigate the biological activity and effects of series of compounds listed in table (4). First experiment tried to study the effect of solvent DMSO which didn't show any Inhibitory effect. Then examine the ligand and complexes in the mixture at different concentration

(10^{-2} , 10^{-4} , 10^{-6} , 10^{-8})M. Before each set of Inhibition experiments were conducted, The AST activity was measured by using four different concentrations of substrate (0.02, 0.06, 0.06, 0.06) gm as shown in fig (9). The biochemical tests indicated that all compounds have caused noticed Inhibitory effects on enzyme activity compared with the measured normal values of enzyme activity, Table(4). Table (4) showed that the greater Inhibition percent was found at concentration(10^{-2})M for *Cu(II)*, *Pd(II)* and *Ni(II)* complexes^[38]. it has been observed that the nature of these metals to chelate with ligand make ales steric hinders compared to other complexes which substrate.

Table (4): the effect of different concentration of ligand and its metal complexes on the human serum AST activity

compounds	Inhibition conc (M)	AST activity(IU/L)	%Inhibition
control	Zero	360	-
$[L^2]$ $C_{15}H_{15}NO_4$	10^{-2}	315	12.5
	10^{-4}	330	8.4
	10^{-6}	360	0
	10^{-8}	360	0
$[Co (L^2)_2 (H_2O)_2]$	10^{-2}	310	13.9
	10^{-4}	360	0
	10^{-6}	360	0
	10^{-8}	360	0
$[Ni (L^2)_2 (H_2O)_2]$	10^{-2}	285	20.8
	10^{-4}	360	0
	10^{-6}	360	0
	10^{-8}	360	0
$[Cu (L^2)_2]$	10^{-2}	300	16.7
	10^{-4}	335	7
	10^{-6}	360	0
	10^{-8}	360	0
$[Pd (L^2)_2]$	10^{-2}	290	19.5
	10^{-4}	340	5.6
	10^{-6}	360	0
	10^{-8}	360	0

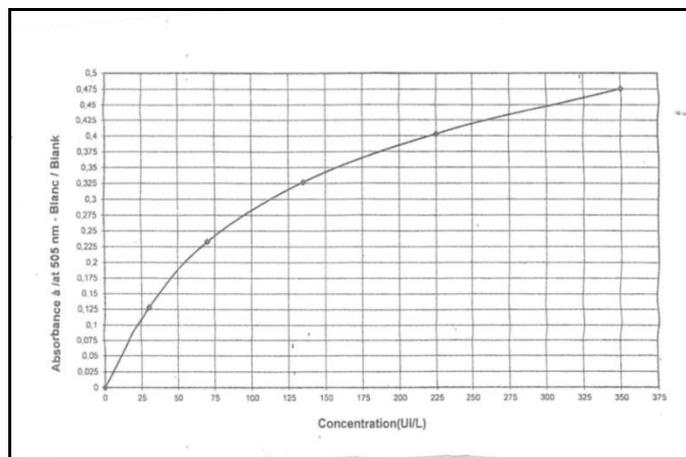


Fig (9): AST Standard Curve

B) the test of the anti- fungi activity

in this work the synthesized compounds were checked for anti – fungi activity (candida albicans)^[39] samples were dissolved in DMSO to provide a final concentration of (0.001) mg/ml. Fungi activity data against tested compounds are shown in table (5). Fig (10) display the inhibition capacity of the synthesized compounds on the tested fungi types.

Table (5): Fungi activity of the ligand $[L^2]$ and its complexes.

Compounds	C. albicans
$[L^2]C_{15}H_{15}NO_4$	16
$[Co (L^2)_2 (H_2O)_2]$	10
$[Ni (L^2)_2 (H_2O)_2]$	15
$[Cu (L^2)_2]$	13
$[Pd (L^2)_2]$	13



Fig (10): the effect of $[L^2]$ and its complexes on *C.albicans*.

Conclusion:

Condensation of 2-amino phenol with 4-hydroxyl-3,5-dimethoxy benzaldehyde products a new bases ligand having potential binding sites towards metal ions form five member chelate ring. Schiff base ligand acts as a bidentate ligand by coordination through azomethine nitrogen and phenolic oxygen atoms. Different geometries have been obtained from coordination of the prepared ligand with selected bivalent metal ions. DMSO has been used in preparation of solution in studying of enzyme activity which did not reveal any inhibition effect. The inhibition concentration was $(10^{-2})M$ for *Ni(II), Cu(II) and Pd(II)*.

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تشخيص، دراسة تركيبية و بابلوجيه لفعاليه انزيميه لمعدتات بعض العناصر الانتقاليه مع
4)-(2-هيدروكسي فنيل أمينو) مثل) -2، 6- داي ميثوكسي فينول

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مستخلص البحث:

تم تحضير الليكند الجديد لقواعد شف $[L^2]4-$ ((2- هيدروكسي فنيل أمينو) مثل) -6,2 داي ميثوكسي فينول والمشتق من تكاتف 2- أمينوفينول مع 4- هيدروكسي -3، 5- داي ميثوكسي بنزالديهايد وشخص بواسطة تقنيات الأشعة تحت الحمراء، الأشعة فوق البنفسجية – المرئية، طيف الرنين النووي المغناطيسي، طيف الكتلة وتقنية التحليل الدقيق للعناصر (كربون، هيدروجين، نيتروجين). كما حضرت وشخصت معدتات أملاح بعض ايونات الثنائية التكافؤ (كوبلت، نيكل، نحاس، وبلاديوم) باستخدام تقنيات الأشعة تحت الحمراء، الأشعة فوق البنفسجية – المرئية، الامتصاص الذري اللهي، التوصيلية الكهربائية والحساسية المغناطيسية ونسبة $(L:M), (2:I)$ واستنتج من التحاليل ان معدتات $Ni(II)$ و $Co(II)$ لها شكل ثماني السطوح حول ايون الفلز مع الليكاند $[L^2]$ ثنائي السن (O,N) بينما مع $Cu(II)$ و $Pd(II)$ معطياً الشكل المربع المستوي. كما أجريت دراسة الفاعلية الأنزيمية (AST) والحيوية لفطر $(Candida albicans)$ لليكند المحضر ومعدتاته وقد أظهرت النتائج امتلاكها سلوك تنبطي متفاوت.