

DETERMINATION OF ANTIOXIDANT ACTIVITY FOR METAL IONS COMPLEXES

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ABSTRACT

The synthesis and characterization of Schiff base and the complexities of metal ions and the evaluation of its antioxidant activities against 1,1-Diphenyl-2-picrylhydrazyl (DPPH) will compared with standard natural antioxidants and ascorbic acid. These prepared materials gave results are due exhibit excellent radical scavenging activities for all complexes with new prepared from reaction Schiff base and metal ions for [Mn(II), Zn (II), Cd(II), Pd(II) and Hg(II)]. The complexes prepared characterization by spectral methods (ultraviolet visible, infrared and mass spectra) will be identified in addition to the element micro analysis, (F.A.A.), magnetic sensitivity. The molar conductance values indicated that the complexes derived from Mn(II), Zn(II), Cd(II), Hg(II) chloride and Pd(II) bromide have nonelectrolytic nature. IR spectra indicated that the ligand acts as neutral bidentate moiety in all complexes. According to the results of diagnosis and analysis gave the all complexes form tetrahedral except in Pd (II) complex square planar. The antioxidant activities of the metal complexes will be examined using the DPPH radical scavenging method the compounds exhibited antioxidant properties of scavenging free radicals.

KEY WORDS: Schiff base Complexes, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Antioxidant activities, synthesis.

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1. INTRODUCTION

Antioxidants of Schiff base and its complexes are in general donors to hydrogen or electron donors into interactive site on neutralizing free radicals [1, 2]. Scavenging efficacy of various organic compounds can be evaluated utilizing DPPH free radical, And also ABTS⁺ tests. Many organic molecules have already been notified that act as anti-oxidants are very good, so it is paramount to understand the method of action as well efficacy from these anti-oxidants. There are large numbers of normal as well synthetic anti-oxidants whom will explored; also their anti-oxidant ability will be evaluated through different ways [3]. A search through literature [4-6] reveals that no work has been done on the ions metal complexes of the Schiff base 6-((4-Dimethylaminobenzylidene aminopyridine-2, 4 diol. In order to investing anew antioxidant agent, which may be applied later in pharmaceutical uses. We examined the antioxidant activity and the inhibition effect of four complexes

from Manganese, Zinc, Cadmium, Palladium and Mercury the prepared from reaction with Schiff base, using the UV-visible method and the standard natural antioxidant (DPPH) radical, ascorbic acid.

2. EXPERIMENTAL

2.1 Material and methods

Element microanalyses (C.H.N.) through employing, Conductivity measurements utilizing a Philips PW-digital, Magnetic moments have been measured for a magnetic susceptibility balance (Sherwood balance magnetic susceptibility model MSB-MK). UV-Visible spectra will recorded using (Shimadzu1800A) spectrophotometer. IR spectral has been acquired employing Shimadzu, (8400S) FT-IR spectrometer. Mass spectra to all complexes will recorded on MS Model 5973 Network Mass Selection Technology (HP) with Triple -Axis Detector by the analyzer Quadrupole at 230 °C. Melting points byusing stuart-

10capillary. Metals% will be determined by a Shimadzu (F.A. A) 620 G

All other organic chemicals, solvents and inorganic salts will be available from multiple companies, Fluk, B.D.H, Merck, sigma and Alderich and used without further purification.

2.2 Synthesis of Ligand: 6-((4-Dimethylaminobenzylidene aminopyridine-2, 4 diol(H₂L) (7)

4- Dimethylaminobenzaldehyde (1g or 6.8 mmole) will be dissolved in ethanol (20ml) with stirring. 4-amino-2,6-dihydroxypyrimidine (0.8643 g or 6.8 mmole) was dissolved in ethanol (25 ml) and added 3 drops with hydrochloride acid as a catalyst. The mixture solution will be heated under reflux at temperature (60 -70 °C)for six hours until the precipitation of the red solid will be complete. Then, the solid collected by filtration and washed by water (10 cm³×3), dried at temperature (50°C) for three hours and then recrystallized from absolute methanol. The red light crystals will collect by filtration and then

dried at temperature 50 °C for six hours. After that, the product precipitated (m.p.=241°C), (yield 84.4%),Scheme-1. ¹H-NMR-DMSO-d₆, ppm: (single, N=C-H azomethine) 9.623, (molty, aromatic-C-H) 8.135-7.838, (single, O-H phenol) 6.623, (molty, N-(CH₃)₂) 3.512-3.337. (Fig. 1) and Mass spectrometry will successfully utilized to explore molecular types at solution. Mass spectrum pattern from H₂L ligand confirms the probable formula and gives an impression of the successive fragmentations to the aim compound for a chain from peaks corresponding into diverse fragments. LC-Mass spectrum to the ligand appeared delicate parent molecular ion peak on m/z (257.9 amu) consistent for molecular weight theoretically. Spectrum predicted the monomeric nature of ligand. The series from peaks corresponding to degeneration for ligand appeared at m/z 234.9, 204.9 and 73.4 amu characterized to [C₁₁H₁₄N₄O₂]⁺, [C₁₁H₁₄N₃O]⁺ and [C₃H₉N₂]⁺, respectively (Fig. 2).

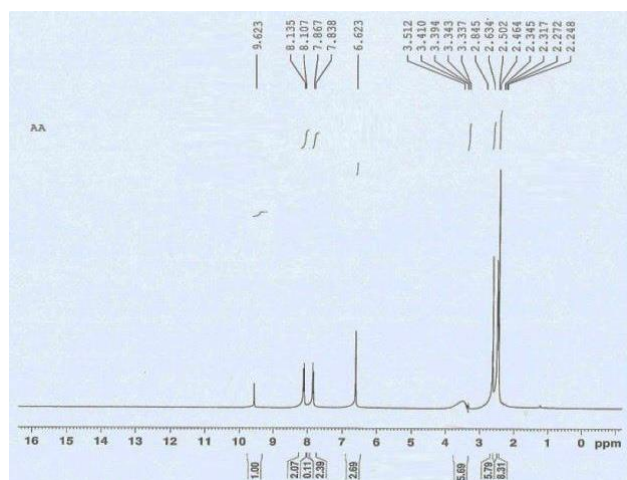


Fig. (1): ¹H-NMR spectrum for (H₂L)

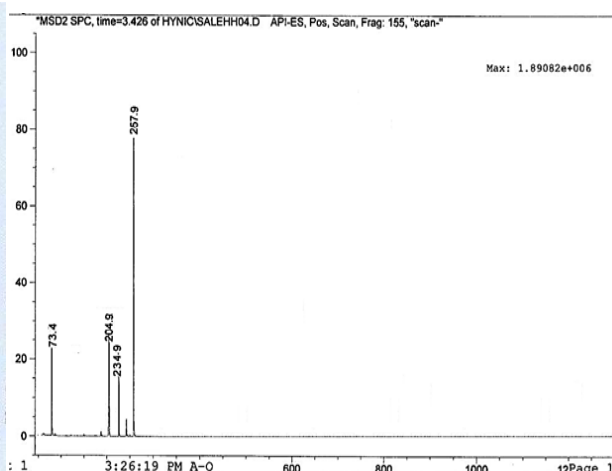


Fig. (2): Mass spectrum for ligand (H₂L)

2.3 Synthesis of Complexes

The complexes will be prepared by addition solution (H₂L) (0.516 g, 2mmol) with methanol to stirred aqueous solution of the respective metal(II) chloride(0.136g,1mmole) ZnCl₂, (0.201g,1mmole) CdCl₂.H₂O, (0.197 g, 1mmol) MnCl₂.4H₂O, (.0.176.g,1mmole) PdCl₂ and (0.271g,1mmole) HgCl₂ in the stoichiometric ratio metal:ligand (1:2) after the mixture will refluxed for (6 hrs.) Leaving the solution in the laboratory. Then will filtered off and washed by acetone followed by drying at room temperature and analyzed

employing standard methods. Deposits have appeared in colors for complexes, red, yellow and pale brown for Mn(II), Pd(II)and Hg(II) complexes, respectively. But the Zinc and Cadmium complexes will brown. The all complexes are non-soluble in water.

2.4 Study of Antioxidant Activity using DPPH Method

Each of the metal complexes will be dissolved in DMSO and ethanol to obtain concentration of (10 mmol l⁻¹). These stock solutions will then diluted to (0.2, 0.4, 0.6, 0.8 and

1 mmol.l-1). While gallic acid was used as standard. Then, 100 μ L of each sample solution will mix with 6ml of 45 μ g ml⁻¹DPPH that will dissolve in ethanol. The reaction mixture will be incubated in the dark place for (30 and 60 min) at room temperature while DPPH reacts with anti-oxidants compounds. These compounds will donate hydrogen, it is reduced. Changes in color (from deep violet into light yellow). DPPH radical scavenging efficiency has been determined through measuring the absorbance on 517 nm utilizing the UV-Vis spectrometer the percentage from DPPH radical scavenger has been calculated employing Equation 1. [8]

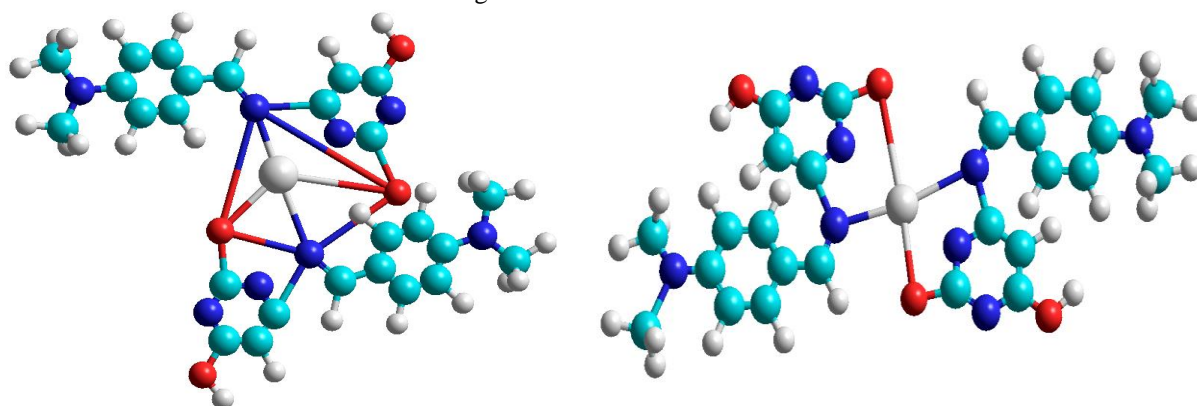
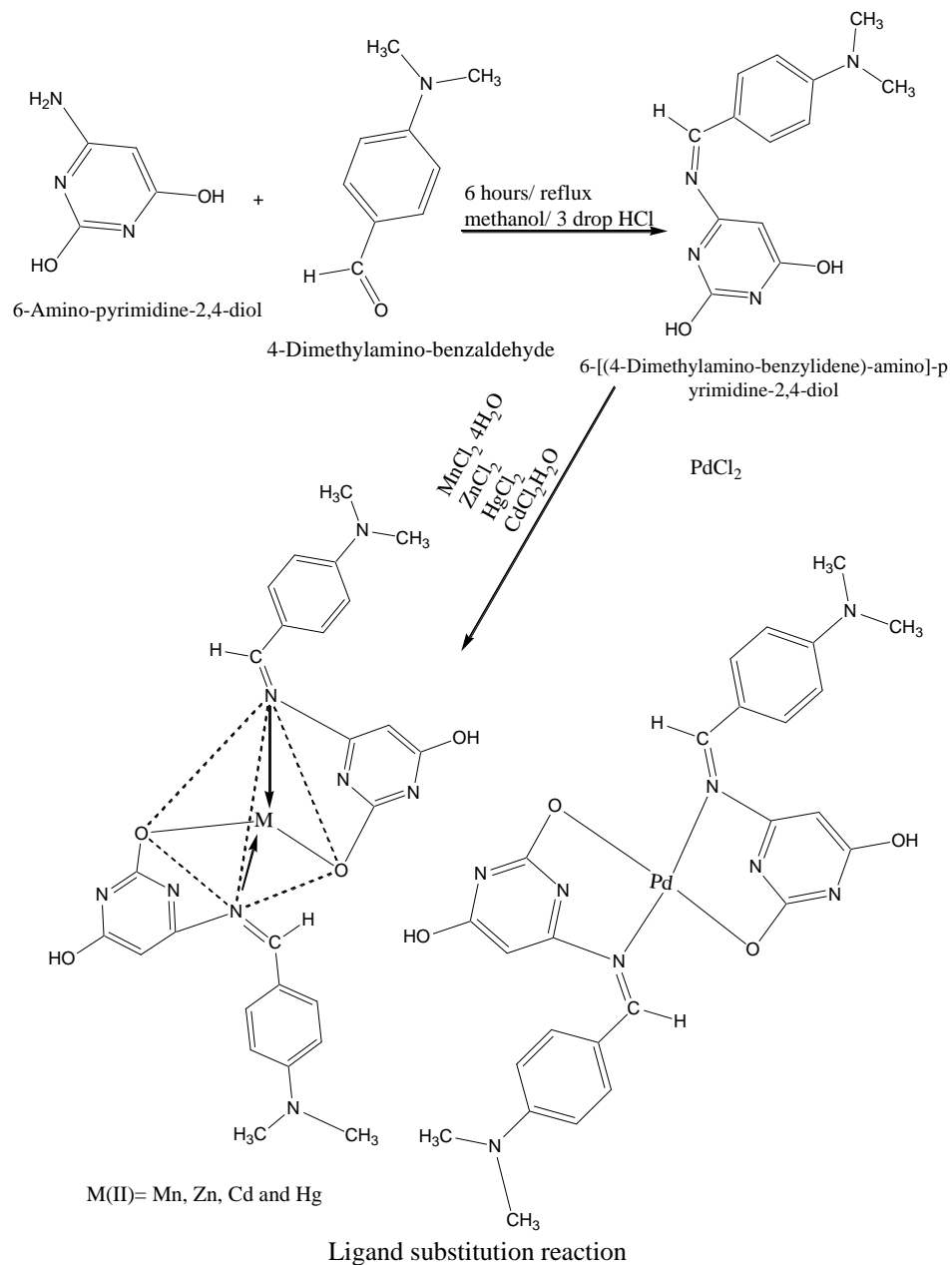
$$\% \text{ Inhibition} = \frac{A_{\text{Control}} - A_{\text{Test}}}{A_{\text{Control}}} \times 100$$

3. RESULTS AND DISCUSSION

All metal complexes have been synthesized during mole reaction. The formative complexes are stable, non-hygroscopic also partly soluble on most organic solvents like CHCl₃, CH₃OH, C₂H₅OH, C₂H₃N, as well as fully dissolved on DMSO also DMF. A set from molar conductivity values registered on Table (1) elucidates that the complexes from manganese(II), zinc(II), cadmium(II), palladium(II) also Mercury(II) ions have non-electrolytic nature elucidating which each anions are involved in the chelation.

Table (1): Analytical and physical data of the ligand and its complexes

Compounds	Formula M. wt	Color	m.p °C	yield	Elemental micro analysis experimental (theoretical) %			
					C	H	N	M
H ₂ L	C ₁₃ H ₁₄ N ₄ O ₂ 258.28	Light red	241	84.4	61.31 (60.45)	4.49 (5.46)	22.75 (21.69)	
Mn(HL) ₂	C ₂₆ H ₂₆ N ₈ O ₄ Mn 569.47	red	289	86	53.12 (54.84)	4.05 (4.60)	21.45 (19.68)	10.11 (9.65)
Zn(HL) ₂	C ₂₆ H ₂₆ N ₈ O ₄ Zn 579.93	Brown	282	76	53.60 (53.85)	5.16 (4.52)	21.01 (19.32)	9.66 (11.28)
Cd(HL) ₂	C ₂₆ H ₂₆ N ₈ O ₄ Cd 626.95	Brown	195	71	50.121 (49.81)	3.98 (4.18)	19.45 (17.87)	18.09 (17.93)
Pd(HL) ₂	C ₂₆ H ₂₆ N ₈ O ₄ Pd 620.96	Yellow		69	49.11 (50.29)	4.01 (4.22)	20.41 (18.05)	18.44 (17.14)
Hg(HL) ₂	C ₂₆ H ₂₆ N ₈ O ₄ Hg 715.13	Pale brown	110	81.5	43.00 (43.67)	4.01 (3.66)	17.03 (15.67)	29.32 (28.05)



Scheme (1): synthesis of the ligand and complexes and 3D for complexes

3.1 IR spectra

The IR spectrum of (H₂L) ligand (Table 2 and Figure 3) shows characteristic bands at 3425 and 1404, 923 cm⁻¹ due to stretching and bending modes vibrations of OH group, respectively. Also, the spectrum bands observed in the regions 3067, 2866, 2959, 1608 and (1713, 1655) cm⁻¹ may be ascribed to $\nu(\text{C-H})_{\text{aromatic}}$, $\nu(\text{C-H})_{\text{aliphatic}}$, $\nu(\text{C-H})_{\text{aldehyde}}$, (C=C) and $\nu(\text{C=N})_{\text{azomethine}}$, respectively.[9, 10]. The IR spectra of metal complexes are represented. These spectra exhibit ligand bands with appropriate shifts due to complex formation (Table 2). The data refer to the following observations. The stretching vibration

of (C=N) in all complexes exhibits change in shape and undergoes downshift via 13- 44 cm⁻¹, indicating its contribution in chelation. The IR spectra of metal complexes depicted weak spectral bands at 401-476 and 490-506 cm⁻¹, which may be due to $\nu(\text{M-O})$ and $\nu(\text{M-N})$, respectively. [10-13]. the aforementioned infrared studies indicated that the ligand acts as neutral ON bidentate moiety in all complexes. The metal ions chelated to the ligand through ON donor sites of nitrogen atom of azomethine group and oxygen atom of phenol group to confirm the tetrahedral all complexes except in Pd(II) complex square planar.

Table (2): FT-IR spectra data (wave number $\hat{\nu}$) cm⁻¹ for the ligand and its complexes

compounds	$\nu(\text{O-H})$ phenol	C-H aromatic C-H alipatic	C-H aldehyde C=C	C=N	M-N	M-O
H ₂ L	3507	3067 2866	2959 1608	1713 1655		
Mn(HL) ₂	3444	3012 2888	2984 1624	1704 1644	506	460
Zn(HL) ₂	3509	3044 2879	2974 1623	1689 1650	497	467
Cd(HL) ₂	3487	3100 2974	2977 1616	1694 1649	490	476
Pd(HL) ₂	3486	3086 2900	2984 1625	1694 1650	501	401
Hg(HL) ₂	3489	3079 2940	2973 1631	1708 1647	497	455

3.2 Atomic absorption spectra and magnetic susceptibilities:

The electronic spectral to the ligand (Figure 4) show intensive absorption on 465,336nm also 268 nm attributed into $n \rightarrow \pi^*$ also $\pi \rightarrow \pi^*$ respectively. The electronic spectral for Mn(II) complex reveals bands at 490 and 615 nm, assignable to ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{2g}$ (4G) overlapped with LMCT and ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{1g}$ (4G) transitions, respectively [14,15]; confirming the tetrahedral geometry around Mn(II) ion. The magnetic moment value (5.33 B.M) was an evidence for high spin tetrahedral Mn(II) complex. The diamagnetic Zn (II), Cd (II) and Hg (II) complexes has an tetrahedral geometry based on analytical, conductance and spectral data. [15,16]. The spectrum of diamagnetic Pd(II) complex gives three bands at 514, 552 and 665 nm corresponding to LMCT overlapped with ${}^1\text{A}_{1g} \rightarrow {}^1\text{E}_{1g}$, ${}^1\text{A}_{1g} \rightarrow {}^1\text{B}_{1g}$ and ${}^1\text{A}_{1g} \rightarrow {}^1\text{A}_{2g}$ transitions, respectively, which is consistent with square planar geometry around Pd(II) metal ion.[15,17].

3.3 Mass spectra

Mass spectrometry will be successfully applied to examine molecular types on solution. Pattern from mass spectral for complexes proves the possible formula as well gives an idea to the successive fragmentations to the target compound for a series from peaks corresponding into diverse fragments. Mass spectral from PdHL complex (Figure 6), displayed delicate parent molecular ion peak on m/z (621 amu) consistent with the theoretical molecular weight (620.96). The spectrum predicted the monomeric nature of Pd complex. The series of peaks corresponding to degradation of the ligand appeared at m/z 488, 329 and 189 amu characterized to [C₂₂H₁₄N₄O₃Pd]⁺, [C₁₈H₉N₄O₃]⁺ and [C₈H₃N₃O₃]⁺, respectively.

Mass spectral from ZnHL complex (Figure 7) offered delicate parent molecular ion peak on m/z (580 amu) proportionate for the molecular weight theoretically (579.93). The spectrum predicted the monomeric nature of Zn complex. The series of

peaks corresponding to degradation of the ligand appeared at m/z 429, 300, 204 and 76.5 amu characterized to $[C_{22}H_{19}N_7O_3]^+$, $[C_{14}H_{14}N_5O_3]^+$, $[C_8H_6N_5O_2]^+$, and $[C_6H_4]^+$, respectively.

Mass spectral from HgHL complex (Figure 8), appeared delicate parent molecular ion peak on m/z (715 amu) proportionate for the molecular

weight theoretically. The spectral prophesied the monomeric nature from Hg complex. The chain of peaks corresponding to the degeneration to the ligand appeared at m/z 430, 429, 205, 204 and 79 amu characterized to $[C_{22}H_{19}N_7O_3]^+$, $[C_{22}H_{19}N_7O_3]^+$, $[C_8H_7N_5O_2]^+$, $[C_8H_6N_5O_2]^+$ and $[C_6H_7]^+$, respectively [18, 19].

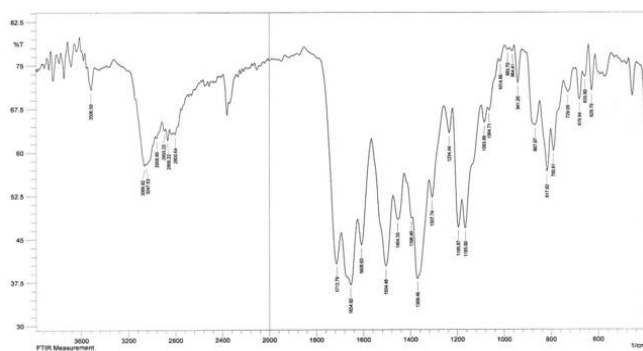


Fig. (3): FT-IR Spectrum of ligand H₂L

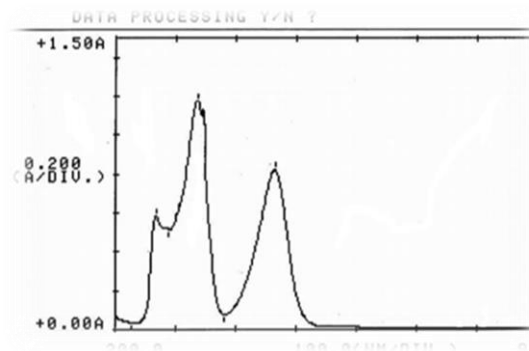


Fig. (4): (UV-Vis) Spectrum of Ligand H₂L

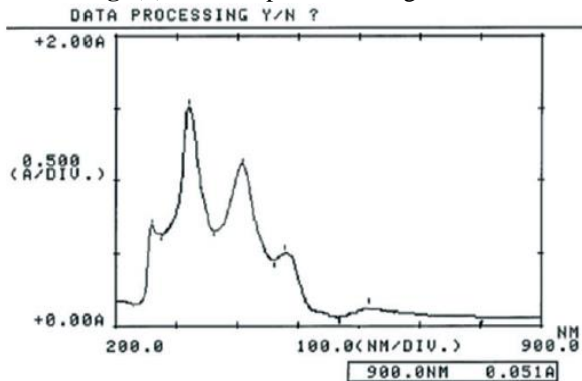


Fig. (5): (UV-Vis) Spectrum of complex Mn (HL)₂

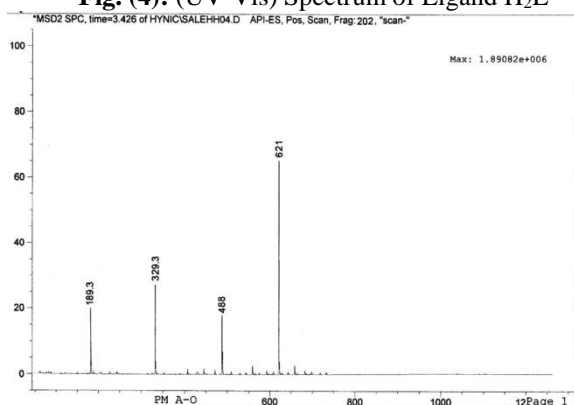


Fig. (6): Mass Spectrum of complex Pd (HL)₂

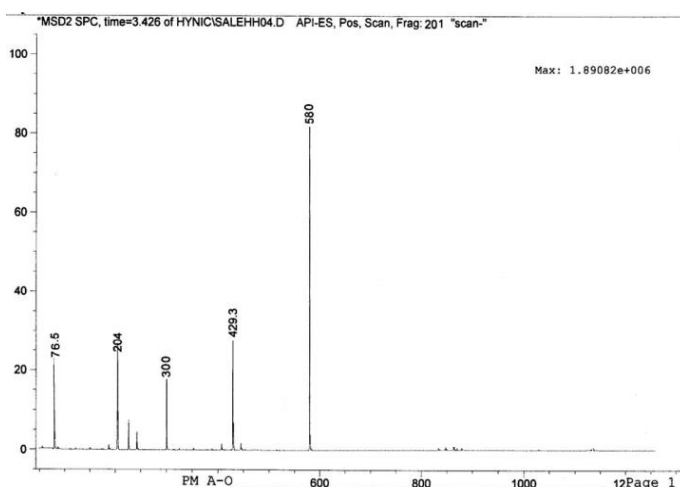


Fig.(7): Mass Spectrum of complex Zn (HL)₂

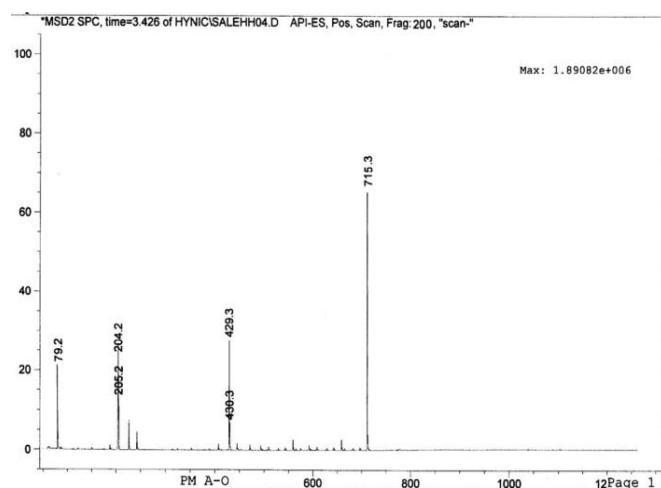


Fig. (8): Mass Spectrum of complex Hg (HL)₂

3.4 Statistical Analysis

All the analysis will be carried out in Excel and expressed as mean Standard deviation, Coefficient

of variation %, Correlation coefficient and IC50 were showed in table (3) to (6), see Figure 9.

Table (3): Means, standard deviations, coefficients of variation, Correlation coefficient and IC 50 of antioxidant activity in percentage (aa%) of the tested samples at 30. Minute

Tested sample	Mean	Standard deviation	Coefficient of variation %	Correlation coefficient	IC50
H ₂ L	48.5	23.4512451	34.45126522	0.97845784	0.334
Mn(HL) ₂	82.41	4.5123546	5.412451464	0.99842175	
Zn(HL) ₂	71.11	20.1452194	27.41576542	0.984075954	
Cd(HL) ₂	41.12	8.10214875	19.74186452	0.999918755	0.948
Pd(HL) ₂	55.43	16.8731974	20.45718542	0.99745812	
Hg(HL) ₂	23.41	26.8271648	28.415765485	0.880997451	1.78

Where IC50: the half maximal inhibitory concentration

Table (4): Means, standard deviations, coefficients of variation, Correlation coefficient and IC50 of antioxidant activity in percentage (aa%) of the tested samples at 60. minute

Tested sample	Mean	Standard deviation	Coefficient of variation %	Correlation coefficient	IC50
H ₂ L	59.632	28.798547	49.78421	0.99445	0.334
Mn(HL) ₂	87.832	5.4972183	4.978462	0.98875	
Zn(HL) ₂	72.19	11.8731543	16.46152	0.99227	0.47
Cd(HL) ₂	56.784	9.7194318	19.74124	0.97775	
Pd(HL) ₂	79.784	8.419752	11.57164	0.89748	
Hg(HL) ₂	26.487	4.527198	4.99147	0.99974	0.741

Where IC50: the half maximal inhibitory concentration

Table (5): Means, standard deviations, coefficients of variation, Correlation coefficient and IC50 of antioxidant activity in percentage (aa%) of the Gallic acid at 30. minute.

Test sample	Mean	Standard deviation	Coefficient of variation %	Correlation coefficient
Gallic acid	89.741	3.4175421	2.974851	0.97845718

Table (6): Means, standard deviations, coefficients of variation, Correlation coefficient and IC50 of antioxidant activity in percentage (aa%) of the Gallic acid at 60. Minute

Test sample	Mean	Standard deviation	Coefficient of variation %	Correlation coefficient
Gallic acid	90.0041	2.44152	2.07415741	0.9700045

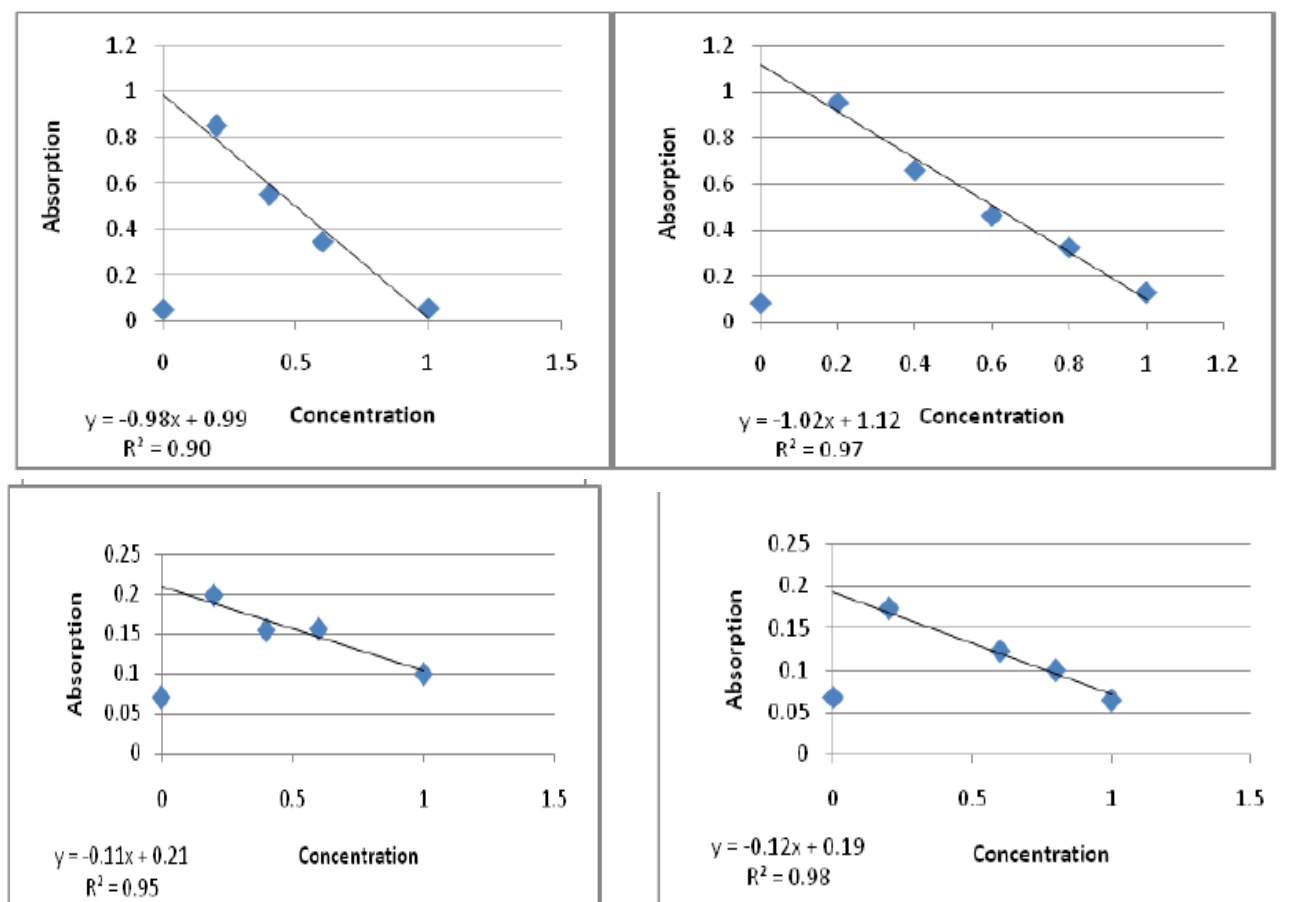


Fig. (9): Standard curve of ligand and Ni complex at 30 and 60min.

3.5 Spectrophotometric determination of DPPH radical scavenging efficiency

Most investigations are conducted like DPPH are conducted to assess the anti-oxidant efficiency of their aims because of its simple proceeding accuracy. The DPPH radical results of the scavenging efficacy to the compounds are depicted depending on the Table 1. Lower Depress IC50 value reflects better DPPH radical-scavenging efficacy. According to the table, it will be evident that nearly all the compounds display radical scavenging efficacies on DPPH test. It will be important to note that Schiff base of the complex displayed better anti-oxidant efficacy than Schiff base (an according to the Table 8). The existence for Schiff base as well -OH groups too has impact at DPPH radical scavenging efficiency also ethylene spacer does not have any influence to the characteristic of the anti-oxidant. So while a check the sample solution is added, free radical is equalized through the exam sample which, either donate hydrogen or an electron whom outcome

neutralization from free radical. While the free radical is neutralized during screening sample less free radical will be obtainable [6, 7].

4. CONCLUSION

In such paper synthesis as well description from novel complexes for generic formula $[M(H_2L)_2]$ for $(M = Mn(II), Cd(II), Zn(II), Pd(II)$ and $Hg(II)$ complexes will prepared from this ligand. The characterization of ligand and its metal complexes will done by elemental microanalyses and spectral techniques. The complexes of $Mn(II)$, $Cd(II)$, $Zn(II)$, $Hg(II)$ and $Pd(II)$ chloride will be nonelectrolytes. The ligand behaved as neutral ON bidentate moiety in all complexes. All complexes will be tetrahedral except that of $Pd(II)$ which has square planar geometry. The radical scavenging efficiency from Schiff base as well its complexes were scrutinized utilizing DPPH screening. Anti-oxidant mensuration's from attended compounds explained

that the OH functional groups as well the existence from electron donating significantly impact the radical scavenging efficiency from phenolic Schiff bases.

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ئهف فهكولينه يا گرڊايه ب نامادهكرن و پشكنينا وان ناوٲٲٲن ئالوزيٲن نوى يٲن Shiff base دگهل هندهك, ساخلهٲٲن فهگوهاسٲٲن و ههلسهنگاندنا چالاكيٲن دژه ئوكسان وى ل دژى (DPPH picrylhydrazyl 1.1-Diphenyl-2- دى هٲٲه بهراوردكرن دگهل دژه ئوكسانٲن ستاندارد و Ascorbic acid . فان ناوٲٲٲن ئالوزيٲن نامادهكرى ئهجمائٲن باش داينه ژبه ر چالاكيٲن رهديكهل كو ژ ئهجمائٲن كارليكا Shiff base دگهل فان كانزايا و ئايونان [Hg (II) (Mn (II), Zn (II), Cd (II), Pd (II) . ئهف ناوٲٲٲن ئالوزيٲن نامادهكرى هاتينه پشكنين ب كارئينانا ريكن روناھيٲن يان روناھيا تيشكا ل ژبر سوري و روناھيا (ديٲن - لسهر بنفشى) و روناھيا وهرگرتنا ئهتومى و پيفهريٲن موگناتيزى و پيفهريٲن پهيوهنديكرنيٲن ئهلكٲريكى يا مولارى.

پيفهريٲن موگناتيزى و پيفهريٲن روناھيا (ديٲن - لسهر بنفشى) سهلماند بو ناوٲٲٲن ئالوزيٲن نامادهكرى كو هندهك شيوٲن چوارينيٲن گونجاى (چوارگوشه يا ئاسوى) وهردگريٲن. ژبلى كو (Pd (II شيوٲن چوارگوشه وهردگريٲن.

پيفهريٲن پهيوهنديكرنيٲن ئهلكٲريكى يا مولارى دياركر كو هه مى ناوٲٲٲن ئالوزيٲن نامادهكرى پيكاھٲٲن نهٲٲت دگرڊاينه ب ئهلكٲريكى فه (ههفسهنگه).

و هاته سهلماندن ب هاريكاريا تيشكا ل ژبر سوري كو دناف ناوٲٲٲن ئالوزيٲن نامادهكرى ههف جووتيا ligand يا هه.

ل كوتايٲن دا چالاكيا دژه ئوكسان يا ناوٲٲٲن كانزاي هاته ههلسهنگاندن ب كارئينانا ريكا ليٲدانا DPPH radical وان ناوٲٲن ساخلهٲا دژه ئوكسانٲن دياركريبه ب ريكا ليٲدانا Free radicals.

الخلاصة

تم تحضير وتشخيص قاعدة شف ومعقدات الايونات الفلزية وتقييمها كمضادات للاكسدة -1,1, Diphenyl-2-picrylhydrazyl (DPPH) ومقارنتها مع مضادات الطبيعية ومنها حمض الأسكوربيك وهي مادة قياسية. حيث اعطيت المواد الجديدة المحضرة من تفاعل قواعد شف مع الايونات [(Mn(II), Zn (II), Cd(II), Pd(II) and Hg(II) نتائج ممتازة بازالة فعالية جذر الحر الموجودة في (DPPH) . شخصت جميع المركبات المحضرة من خلال استخدام الطرق الطيفية المعروفة مثل طيف الشععة فوق البنفسجية- المرئية وطيف الشععة تحت الحمراء وطيف الكتلة) بالإضافة إلى تحليل العناصر الدقيقة، (F.A.A)، الحساسية المغناطيسية. حيث اشارة قيم التوصيل المولي للمعقدات المشتقة من كلورايدات (Mn(II), Zn(II), Cd(II), Hg(II) و بروميد الرصاص لها طبيعة غير الكرونية. واطهرت نتائج طيف الاشعة تحت الحمراء (IR) ان لليكند المستخدم يسلك سلوك ثنائي السن في جميع المعقدات. وطبقا للنتائج المستحصلة تم تشخيص اشكال المعقدات بشكل رباعي السطوح (tetrahedral) ماعدا المعقد البلاديوم ياخذ شكل مربع مستوي (square planar). تم فحص أنشطة مضادات الأكسدة في المعقدات الفلزية باستخدام طريقة (DPPH) كسح الجذري للمعقدات.