

Synthesis of 2[N-Cinnamylideneamino]-5-Nitro Phenol and Its Metal(II) Complexes and Evaluation of Their Antitumor Activities

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The preparation of a series of metal(II) complexes (Zn, Cu, Co and Ni) with 2[N-(cinnamylidene)amino]-5-nitro phenol as a novel ligand and their biological evaluation against six species of pathogenic yeasts (*Candida albicans*, *C. fructus*, *C. glabrata*, *C. oleophila*, *C. parapsilosis* and *C. tropicalis*) are described. All new compounds were fully characterized by elemental analyses, spectroscopic measurements (IR, UV-vis, NMR, ESR and EIMS) as well as thermal and magnetic susceptibility measurements. Studies revealed that HL ligand acts as bidentate through N and O atoms. Zn(II) and Co(II) complexes exhibited tetrahedral geometry while Cu(II), Ni(II) are octahedral. Both Co(II) and Zn(II) complexes exhibited potential antitumor activities and the latter showed promising cytotoxicity (70-82 % cell mortality) and it may be considered a new antitumor agents.

Key Words: Schiff base, Complexes, Antitumor, Cytotoxicity.

INTRODUCTION

Mucosal human fungal infections have increased at an alarming rate in the last 30 years. Opportunistic fungal infections are responsible for increased mortality among patients immunocompromised as a result of cancer chemotherapy and organ transplantation, being treated with immunosuppressive drugs¹. Yeast cell is the leading primary agent causing superficial and often fatal infections, where the cell wall mannoproteins of the human pathogenic yeast is instrumental in virulence^{2,3}. HIV-positive patients strongly contribute to this problem since they have developed resistance to treatment with fungicides like fluconazole, which is the most currently used antifungal⁴. *C. albicans* is a dominant natural flora in the mouth and on other mucous membrane in different parts of the human body due to its ability to adsorb on the mouth hollowing⁵. *C. tropicalis* and *C. glabrata* are the non-*albicans* *Candida* strains currently emerging in fungal infections⁶. For this reason the elaboration of new types of antifungal agents is presently an urgent task.

Of particular note is the pressing need for new, more effective and safe antifungal agents to replace those that are losing their effectiveness in relation to drug-resistant

fungal strains because of the fast development of the microorganisms' resistance to the drugs that are currently used to treat different fungal infections⁷. A promising field for this search is the transition metal complexes of Schiff bases due to their significant antifungal⁸, antibacterial, anticancer and catalytic activities⁹. Also their interactions with microbial DNA and inhibiting tumor growth have been reported¹⁰.

Metal complexes of N- and O-chelating ligands have attracted considerable attention due to their physico-chemical properties, pronounced biological activities and as models of metalloenzyme active sites¹¹. The well known type of these ligands is the Schiff bases that possess donor sites like phenoxo oxygen atoms as well as imine nitrogen atoms¹². Such ligands have unusual configuration, structural lability and are sensitive to molecular environment. As a continuation of our interest in the field of the chemistry of Schiff base complexes¹³, we report in this communication the synthesis of a new type derived from condensation of cinnamaldehyde with 5-nitro-2-aminophenol and its complexes with Zn(II), Cu(II), Co(II) and Ni(II). The biological activities of the ligand and its complexes such as antitumor activity, *in vitro* against yeast species and cytotoxicity are described.

EXPERIMENTAL

All chemicals and solvents used were of reagent grade and used as received. The metal salts [CoCl₂.6H₂O, Ni(CH₃COO)₂.4H₂O, Cu(CH₃COO)₂.2H₂O and Zn(CH₃COO)₂.2H₂O] were of extra pure products (BDH) and have been used without further purification.

Elemental analysis (C, H, N) were performed on Element Analyzer 2400 Perkin-Elmer, infrared spectra of solids as KBr discs were recorded on a Jasco FT/IR 460 plus Fourier-transform spectrometer. Electronic absorption spectra were recorded on a Jasco V-530 UV/Vis spectrophotometer. ¹H NMR spectra were recorded on Joel ECA-500 spectrometer, TMS was used as an internal standard in DMSO-*d*₆ and the chemical shifts are given in ppm, Alexandria University. ESR spectra of the complexes were recorded at Elexsya E500 Bruker Company, National Research Center, Dokki, Cairo, Egypt. Electron impact mass spectra (EIMS) measurements were determined using a Finnigan SSQ 700 spectrometer attached to digital DEC 300 work station at the central scientific services unit, National Research Center, Dokki, Cairo, Egypt. Metal analysis was determined by atomic absorption technique. Magnetic susceptibility was measured at room temperature on a Magway Sherwood products Model MK1 magnetic susceptibility balance using a sealed-off sample of MnCl₂ solution as a calibrant. Melting points were determined with a digital melting point apparatus using capillary technique. The molar conductance measurements were carried out using a FisherAP75 conductometer at 25 °C from 10⁻³ M solution in DMF. Thermal analysis measurement was performed by using a dynamic nitrogen atmosphere with a Perkin-Elmer thermogravimetric analyzer TGA7, flow rate of 30 mL min⁻¹. The heating rate was 20 °C min⁻¹.

Synthesis of Schiff base ligand (HL): The Schiff base ligand was prepared by the condensation of cinnamaldehyde (0.01 mol) with 2-amino-5-nitrophenol (0.01 mol) in absolute ethanol (25 mL). The mixture was refluxed for 1 h and the resulting brick-red precipitate was separated out and washed with EtOH followed by Et₂O, then dried in vacuum to give 85 % yield of the HL; m.p. 160 °C; Anal. calcd. (%) for C₁₅H₁₂N₂O₃: C, 67.16; H, 4.47; N, 10.44. Found (%): C, 67.42, H, 4.85, N, 10.22; IR (KBr, ν_{\max} , cm⁻¹): 3392 (OH phenolic), 3050 (C-H aromatic), 1270 (C-O), 746 (aromatic ring vibrations); ¹H NMR (500 MHz, DMSO-*d*₆, δ / ppm): 11.03 (s br, 1H, OH), 9.09 (s, 1H, -CH=N-), 8.59 (d, 1H, *J* = 8.4 Hz, Ar), 8.16 (d, 1H, *J* = 7.6 Hz, Ar), 7.13-7.57 (m, 6H, Ar), 6.57 (d, 1H, *J* = 9.9 Hz, -CH = CH), 6.37 (d, 1H, *J* = 9.9 Hz, HC=CH-). ¹³C NMR (125 MHz, DMSO-*d*₆, δ / ppm): 172.67 (C=N), 154.19, 150.27, 147.23, 146.17, 143.11, 136.89 (C=C), 135.99 (C=C), 132.37- 126.64, 118.78, 113.64, 111.67, 109.13; MS [*m/z*, (relative abundance,%)]: 268 (M⁺, 13) 267, 241, 221, 167, 165, 154, 149 (BP, 100) 124, 91; UV-Vis (DMF) (λ_{\max} , nm): 273, 415.

Synthesis of metal(II) complexes: The complexes were prepared by refluxing (1.0 mol) of metal acetates [Zn(II), Cu(II), Ni(II)] or Co(II) chloride with (1.0 mol) of the ligand in absolute EtOH for 1-2 h. The coloured complexes precipitates were filtered washed carefully with EtOH then Et₂O and dried in vacuum. The following data were recorded:

Zn(II) complex: Yield 65 %; m.p > 300 °C; Anal. calcd. (%) for C₁₇H₁₆N₂O₆Zn: C, 49.81; H, 3.90; N, 6.83, Zn, 15.99. Found (%): C, 49.65, H, 3.86, N, 6.73, Zn, 16.20; IR (KBr, ν_{\max} , cm⁻¹): 3427 (H₂O), 1586 (C=N), 1281(C-O), 442 (Zn-N), 520 (Zn-O); ¹H NMR (500 MHz, DMSO-*d*₆, δ /ppm): 9.64 (d, 1H, *J* = 8.4 Hz, -CH=N), 8.54 (s, 1H, Ar), 7.98-7.00 (m, 7H, Ar), 6.56 (dd, 1H, *J* = 11.0, 8.4 Hz, CH=CH), 6.16 (d, 1H, *J* = 11.0 Hz, CH=CH), 1.77 (s, 3H, CH₃). ¹³C NMR (125 MHz, DMSO- *d*₆, δ /ppm): 176.90 (CH₃COO), 162.16 (C=N), 148.27, 147.33, 136.89 (C=C), 135.66 (C=C), 129.66, 129.30, 129.02, 128.18, 117.40, 109.15, 22.90 (CH₃COO); MS (*m/z*): 385, 382, 322, 266, 245, 240 (78 %), 210, 178, 166, 149, 115, 109, 91; UV-Vis (DMF) (λ_{\max} /nm): 280, 415; magnetic moment, diamagnetic; molar conductance (Ω^{-1} cm² mol⁻¹): 10.75.

Cu(II) complex: Yield 75 %; m.p > 300 °C; Anal. calcd. (%) for C₁₇H₁₈N₂O₇Cu: C, 48.00; H, 4.23; N, 6.56; Cu 14.89. Found (%): C, 48.33, H, 4.25, N, 7.08, Cu, 14.83; IR (KBr, ν_{\max} , cm⁻¹): 3359 (H₂O), 1553 (C=N), 1281 (C-O), 432 (Cu-N), 520 (Cu-O); MS (*m/z*): 412, 395, 381, 333, 292, 278, 268, 240 (100 %) 220, 210, 191, 166, 164, 139, 115, 91; UV-Vis (DMF) (λ_{\max} /nm): 450, 585; magnetic moment, μ_{eff} , μ_{B} : 1.90; molar conductance (Ω^{-1} cm² mol⁻¹): 9.69.

Co(II) complex: Yield 60 %; m.p > 300 °C; Anal. calcd. (%) for C₁₅H₁₂N₂O₃Cl₂Co: C, 45.23; H, 3.01; N, 7.03; Co 14.80. Found (%): C, 45.54, H, 2.98, N, 7.02, Co, 14.46; IR (KBr, ν_{\max} , cm⁻¹): 3392 (OH phenolic), 1587 (C=N), 1287 (C-O), 436 (Co-N), 510 (Cu-O); MS (*m/z*): 398 (M⁺, 3 %), 385, 384, 365, 322, 307, 266 (100 %) 220, 192, 165, 154, 115, 91; UV-Vis (DMF) (λ_{\max} /nm): 415, 590, 675; magnetic moment, μ_{eff} , μ_{B} : 4.32; molar conductance (Ω^{-1} cm² mol⁻¹): 2.57.

Ni(II) complex: Yield 60 %; m.p > 300 °C; Anal. calcd. (%) for $C_{19}H_{22}N_2O_9Ni$: C, 47.43; H, 4.57; N, 5.83; Ni, 12.19. Found: C, 47.55, H, 4.91, N, 5.63, Ni, 12.25; IR (KBr, ν_{max} , cm^{-1}): 3399 (OH/H₂O), 1585 (C=N), 1286 (C-O), 432 (Ni-N), 510 (Ni-O); MS (m/z): 480 (M⁺, 3 %), 414, 393, 323, 322, 282, 279, 223, 181, 167, 149 (100 %) 104, 91; UV-Vis (DMF) (λ_{max}/nm): 415, 590; magnetic moment, μ_{eff} , μ_B : 3.10; molar conductance ($\Omega^{-1} cm^2 mol^{-1}$): 4.65.

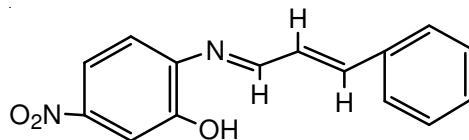
Microbiological assay: Biological studies were performed at the Department of Botany, Faculty of Science, Cairo University, Egypt. The free ligand and its metal(II) complexes were tested against six different pathogenic yeast species (*Candida albicans*, *C. fructus*, *C. glabrata*, *C. oleophila*, *C. parapsilosis* and *C. tropicalis*) using microplate dilution method. The antitumor activity was detected by biochemical induction assay (BIA). The activity of the new complexes were tested against *Escherichia coli* induced β -galactosidase in response to DNA damaging compound and this enzyme can be visualized by the addition of a chromogenic substance (6-bromo-2-naphthyl- β -galactopyranoside). The toxicity of the new compounds was recorded on yeast cells by measuring the activity of dehydrogenase enzyme.

RESULTS AND DISCUSSION

Characterization of the organic ligand: The Schiff base ligand, HL, is soluble in organic solvents and its structure was elucidated by elemental analyses, electronic, IR, ¹H NMR, ¹³C NMR spectra and electron impact mass spectra. The results of the elemental analyses are in good agreement with the proposed formula. A broad band at 3393 cm^{-1} is assigned to $\nu(OH)$, followed by aromatic (C-H) band at 3050 cm^{-1} . The medium intensity band at 1571 cm^{-1} is attributed to $\nu(C=N)$ and a strong phenolic $\nu(C-O)$ band exhibited at 1270 cm^{-1} . The intense band located at 746 cm^{-1} is typical aromatic ring vibrations.

The electronic spectrum of the ligand in DMF showed bands at 272 and 415 nm. The higher energy band is assigned to $\pi-\pi^*$ transitions of the azomethine linkage and the aromatic benzene ring, however the lower energy band assigned to $n-\pi^*$ electronic transition that involves the non-bonding electrons of the azomethine nitrogen atom with charge transfer (CT) transition within the molecule.

¹H NMR spectral data (δ ppm) of the ligand relative to TMS in DMSO-*d*₆ demonstrated further support of the ligand structure (**Scheme-I**). The spectrum exhibited the olefinic protons at δ 6.37 and 6.57 ppm as two doublet with coupling constant $J = 9.9$ Hz, a singlet at δ 9.09 ppm corresponds to the imine proton (-CH=N-) while the signal of the OH proton appeared at 11.03 ppm as a broad singlet. The aromatic protons resonated, respectively, at δ 8.16 ppm (d, 1H, $J = 7.6$ Hz), 8.59 ppm (d, 1H, $J = 8.4$ Hz) and 7.13-7.57 ppm (m, 6H). The ¹³C NMR (125 MHz) spectral pattern exhibited the azomethine carbon (C=N) at δ 172.67 ppm, the olefinic carbon (C=C) at 136.89, 135.99 ppm and the aromatic carbons appeared at δ 154.19-143.11, 132.37-126.64, 118.78-109.13 ppm, respectively. Thus ¹³C NMR clearly indicated the presence of two stereo isomers of the ligand as *s-cis* and *s-trans* with (1:1) ratio.



Scheme-I: Structure of Schiff base ligand HL

The EI mass spectrum of the Schiff base ligand HL (Table-1) exhibited a molecular ion M^+ at m/z 268.1 (13 %) which supplements the proposed composition for the ligand $C_{15}H_{12}N_2O_3$. Other peaks were found at m/z 267 (32), 241 (26), 221 (1), 167 (26), 165(30), 154 (87), 149 (100), 124 (33) and 91(34).

Characterization of the metal complexes: All of the metal(II) complexes are coloured, stable in air and light, soluble in DMF and DMSO insoluble in water. The low molar conductance values of the complexes reveal their non-electrolytic nature¹⁴.

Infrared spectroscopy: In comparison with the spectra of the Schiff base ligand, all the spectra of metal complexes showed a downward shift ($14-19\text{ cm}^{-1}$) of $\nu(C=N)$ indicating the participation of the azomethine nitrogen in chelation. The band at 1270 cm^{-1} assigned to $\nu(C-O)$ in the free ligand was shifted to $1292-1280\text{ cm}^{-1}$ in the complexes and the intensity of the band has decreased showing the involvement of the phenolic oxygen in coordination. Zinc and copper complexes exhibited a broad band around 3427 and 3359 cm^{-1} , respectively, assignable to $\nu(OH)$ of coordinated water, which is confirmed by elemental analyses.

Nickel complex showed bands corresponding to $\nu_{\text{asym}}(\text{COO})$ and $\nu_{\text{sym}}(\text{COO})$ around 1559 and 1476 cm^{-1} , respectively, where the separation between the two $\nu(\text{CO})$ values is of the order of 100 cm^{-1} , which is indicative of bidentate chelating behaviour of acetate group. Support for the above interpretation is the appearance of the non-ligand bands at $520-509$ and $442-432\text{ cm}^{-1}$ assigned to stretching frequencies of (M-O) and (M-N), respectively.

Magnetic measurement data: The copper(II) complex exhibits an effective magnetic moment value of 1.90 BM, slightly higher than the spin only value (1.73 BM) and corresponding to the presence of one unpaired electron. The μ_{eff} value indicates the absence of spin-spin interaction in the complex and offers the possibility of an octahedral geometry¹⁵.

Cobalt(II) complex has μ_{eff} value of 4.32 BM in agreement with high spin tetrahedral geometry. For tetrahedral cobalt(II) complex, the state acquires orbital angular momentum only indirectly through the mixing of the 4T_2 state by a spin-orbit coupling perturbation¹⁶.

Nickel(II) complex was found to be paramagnetic, with a room temperature magnetic moment value of 3.10 BM suggesting consistency with its octahedral environment^{15,16}. Zinc(II) complex is found to be diamagnetic as expected for d^{10} configuration, according to the empirical formula of the complex, a tetrahedral geometry is proposed.

TABLE-1
 MASS SPECTRAL DATA OF HL AND ITS METAL COMPLEXES

Compound	M/z	(%)	Compound	M/z	(%)
[HL]	268	13	[(L)Cu(OAc)(H ₂ O) ₂]	412	1
	267	32		395	2
	241	26		381	3
	221	15		333	2
	167	26		292	2
	165	30		278	3
	154	87		268	8
	149	100		266	62
	124	33		240	100
	91	34		220	32
[(L)Zn(OAc).H ₂ O]	385	1	210	75	
	382	5	191	6	
	322	8	166	75	
	266	28.	164	31	
	245	67.	139	19	
	240	78.	115	28	
	210	62	91	30	
	178	40	480	3	
	166	59	414	2	
	149	59	393	1	
[Co(HL)Cl ₂]	115	40	323	1	
	105	48	322	10	
	91	59	282	5	
	[Ni(HL)(OAc) ₂].2H ₂ O]	398	3	279	5
		385	10	223	8
		384	37	181	5
		365	37	167	15
		322	13	149	100
		307	45	104	66
		266	100	91	32
220		67			
192		51			
165		60			
154	86				
115	51				
91	72				

Electronic spectra of the metal complexes: The electronic spectrum of the copper(II) complex in DMF displayed a band centered at 585 nm, assigned as 10Dq band and corresponding to ${}^2E_g \rightarrow {}^2T_{2g}$ transition in distorted octahedral structure¹⁷. The band at 450 nm can be attributed to ligand-metal charge transfer transition.

The cobalt(II) complex showed absorption bands at 590 and 675 nm assigned to ${}^4A_2 \rightarrow {}^4T_1(P)$ transition. The existence of spin-orbit coupling also allows some

quartet \rightarrow doublet spin transition to occur. Based on the electronic spectral and magnetic data, nickel(II) complex has the octahedral geometry with its characteristic features. The electronic spectrum of the complex showed a band at 590 nm corresponding to the ${}^3A_{2g} \rightarrow {}^3T_{2g}$ electronic transition and is consistent with an octahedral geometry of the complex¹⁸. The spectrum of Zn(II) complex shows no $d-d$ bands which is in accordance with the d^{10} electronic configuration of Zn(II).

Electron paramagnetic resonance spectroscopy: The EPR spectrum of copper(II) complex has been studied at room temperature and depicted in Fig. 1. The spectrum showed an axial symmetry where the g_{\parallel} and g_{\perp} values have been found to be 1.977 and 2.09, respectively. The g_{av} value was calculated from the relation: $g_{av} = 1/3 (g_{\parallel} + 2g_{\perp})$ and found to be 2.05. The trend $g_{\parallel} < g_{\perp}$ indicates that the electron is delocalized in dz^2 orbital of the ground state of Cu(II). In this case ($g_{\parallel} < g_{\perp}$) distortion occurs by compression¹⁹. The axial symmetry parameter $G = g_{\parallel} - 2/g_{\perp} - 2$ is much less than 4 suggesting considerable interaction in the solid state²⁰. The deviation of the g_{av} from that of the free electron (2.0023) is due to the covalence property²¹. This is supported by Kivelson and Neiman²² where g_{\parallel} is less than 2.3 indicating considerable covalent character of metal-ligand bond in the complex.

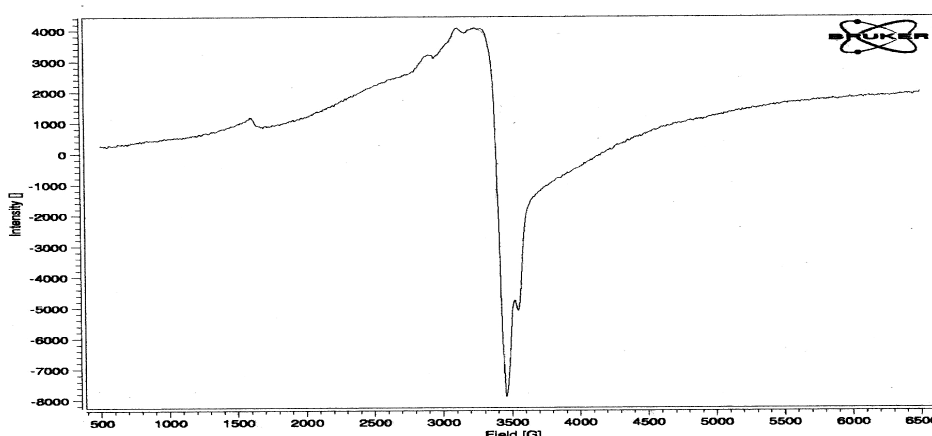


Fig. 1. EPR spectrum of $[(L)Cu(OAc)(H_2O)_2]$ at room temperature

Electron impact mass spectra (EIMS): The data of mass spectral fragmentation of the metal complexes are presented in Table-1. As it is seen from Table-1 cobalt and nickel complexes exhibited a molecular ion M^+ at m/z 398 and 480 (although with low intensity) corresponding to $[Co(HL)Cl_2]$ and $[Ni(HL)(OAc)_2] \cdot 2H_2O$ respectively. However the M^+ peak was not observed in zinc and copper complexes which may attribute to the instability of these complexes under the drastic EI condition. The electron impact mass spectra for zinc and copper showed a series of peaks, Table-1, corresponding to various fragments their intensity gives an idea of the stability of fragments.

Nuclear magnetic resonance spectroscopy: The ^1H NMR spectrum of Zn(II) complex, exhibited an azomethine proton at δ 9.64 ppm as doublet with $J = 8.4$ Hz, a singlet aromatic proton at δ 8.54 ppm and olefinic protons at 6.56 and 6.16 ppm as two doublet each has $J = 11.0$ Hz. The aromatic protons resonated at δ 7.13-7.57 ppm (m, 7H). The spectrum showed a singlet at δ 1.77 ppm for CH_3 , the disappearance of OH signal indicates the loss of phenolic proton on complexation forming metal-oxygen bond.

^{13}C NMR spectral pattern of the Zn(II) complex displayed a signal at δ 22.90 ppm characteristics to a CH_3 and a $\text{C}=\text{O}$ signal at δ 176.90 ppm that indicated the presence of acetate group in the zinc(II) coordination sphere. In addition the spectrum exhibited a signal at δ 162.16 ppm diagnostics to the azomethine carbon, the olefinic carbon ($\text{C}=\text{C}$) at 136.89, 135.66 ppm and the aromatic carbons resonated at δ 148.27, 147.33, 129.66, 129.30, 129.02, 128.18, 117.40 and 109.15 ppm, respectively.

Thermal analysis: Thermogravimetric (TG) and the derivative thermogravimetric (DTG) plots of the copper(II) complex in the 25-657 $^\circ\text{C}$ range under nitrogen atmosphere is reproduced in Fig. 2 The complex shows four decomposition steps, within the temperature range (25-657 $^\circ\text{C}$). The first step of decomposition within the temperature range (25-250 $^\circ\text{C}$) corresponds to the loss of the two coordinated water molecule and loss of acetate (as $\text{CH}_4 + \text{CO}_2$ gases)²³ with mass loss of 23 % (calcd. 22.56 %). The second step (250-280 $^\circ\text{C}$) corresponds to the loss C_9H_8 moiety (mass loss 27 %; calcd. 27.26 %). The third step (280-310 $^\circ\text{C}$) corresponds to the loss of 0.5 NO_2 (mass loss 5 %; calcd. 5.4 %). The fourth step (310-490 $^\circ\text{C}$) corresponds to the loss of C_6H_6 , 0.5 N_2 and H_2 (mass loss 22 %; calcd. 22.09 %). The total mass loss up to 654 $^\circ\text{C}$ is in agreement with the formation of $\text{CuO} + 0.5\text{O}_2$ as the final residue (TG 23 %, calcd. 22.44 %). Thus the thermal behaviour of the studied copper complex confirmed the presence of one acetate group and two water molecules coordinated to copper ion.

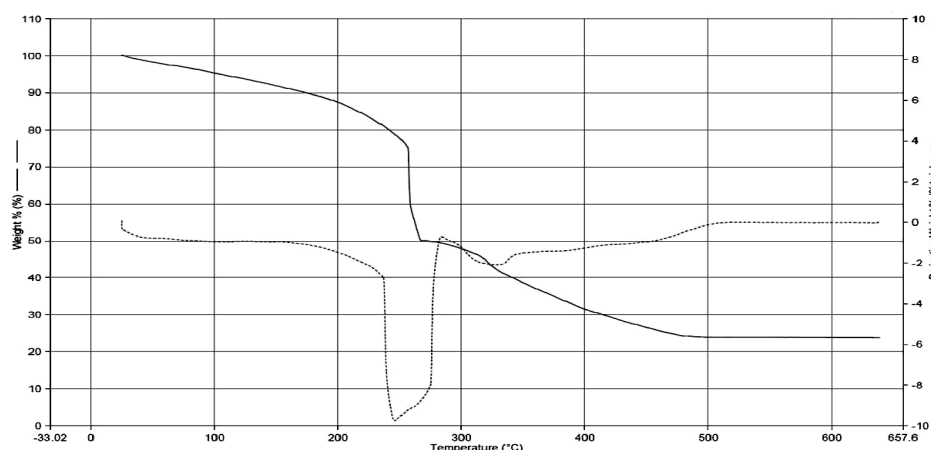


Fig. 2. Thermogravimetric (TGA/DTG) curves of $[(\text{L})\text{Cu}(\text{OAc})(\text{H}_2\text{O})_2]$

Microbiological studies

Determination of the minimum inhibitory concentration (MIC) using microplate susceptibility test: The anticandidal values of the free ligand and its metal complexes zinc(II), copper(II), nickel(II) and cobalt(II) were estimated against the tested yeasts by a minimum inhibitory concentration (MIC, $\mu\text{g/mL}$), listed in Table-2. The activities of all the metal complexes against the tested yeast strains are higher than that of the free ligand itself. A significant enhancement in anticandidal activities of the tested metal complexes ranged from 1.25 $\mu\text{g/mL}$ in the most sensitive yeast species (*C. glabrata*) using Zn(II) complex up to 150 $\mu\text{g/mL}$ in Ni(II) complex, compared with the standard fungicides (50->200 $\mu\text{g/mL}$) and the free ligand (75-100 $\mu\text{g/mL}$). The observed results was in accordance with Lippard²⁴ which stated that binding of a drug to a metallo-element enhances its activity and in some cases, the metal complex possesses even more healing properties than the parent drug. It is suspected that some factors such as solubility and cell permeability mechanism (influenced by the presence of metal ions) may be the possible reasons for the increase in the anticandidal activity. Moreover, coordination reduces the polarity of the metal ion mainly because of the partial sharing of its positive charge with the donor groups within the chelate ring system formed during chelation which in turn, increase the lipophilic nature of the metal atom and favours its permeation more efficiently, through the lipid layer of the microorganism wall, thus destroying them more aggressively²⁵. It has been suggested that the ligand and its metal(II) complexes with nitrogen and oxygen donor systems suppress the growth of microbe by inhibiting the microbial enzyme activity, since the enzymes which required for yeast activity appear to be especially more susceptible to deactivation by metal ions on coordination. It is noteworthy that the anticandidal activity decreases in the sequences: [LZn(OAc)(H₂O)] > [HLCuCl₂] > [LCu(OAc)(H₂O)₂] > [HLNi(OAc)₂].2H₂O suggesting that the lipophilic behaviour increases in the same order. The biological activity of the metal complexes is governed by the following factors²⁶: (i) the chelating effect

TABLE-2
MINIMUM INHIBITORY CONCENTRATION (MIC, $\mu\text{g/mL}$) OF THE TESTED LIGAND AND ITS METAL COMPLEXES ON THE TESTED PATHOGENIC *Candida* SPECIES

Compounds	Minimum inhibitory concentration (MIC, $\mu\text{g/mL}$)					
	<i>Candida albicans</i>	<i>Candida fructus</i>	<i>Candida glabrata</i>	<i>Candida oleophila</i>	<i>Candida parapsilosis</i>	<i>Candida tropicalis</i>
Ligand (HL)	100	100	75	100	100	100
Cobalt(II) complex	25	50	25	25	25	50
Copper(II) complex	75	75	70	70	75	70
Nickel(II) complex	150	150	150	150	150	150
Zinc(II) complex	12.50	6.25	1.25	12.50	12.50	12.50
Ketoconazole	>200	75	50	>200	>200	>200
Fluconazole	50	100	50	75	50	50
Amphotericin	50	50	50	>200	>200	>200

of the ligands, (ii) the nature of the donor atoms, (iii) the total charge on the complex ion, (iv) the nature of the metal ion, (v) the nature of the counter ions that neutralize the complex and (vi) the geometrical structure of the complex. Since all the complexes (i) have the same chelating effect (the Schiff base ligand forms one 5-membered chelating ring), of the same donating atoms which are N/O (ii) are neutral and there are no counter ions, non-electrolyte complexes (iii) have the same oxidation number in their complexes (M^{2+}), therefore, the more effective factors are the geometrical shape and the nature of the central atoms.

According to the spectral and magnetic studies, zinc and cobalt have a tetrahedral geometry, copper has a distorted octahedral geometry and nickel is associated with an octahedral geometry. Therefore, the higher antimicrobial activity of zinc and cobalt complexes can be referred to their similar structure which is the tetrahedral, the less steric structure compared to the other complexes. This tetrahedral structure increases the lipophilicity of the central atom by decreasing the effective nuclear charge (polarity) of the Zn(II) and Co(II) more than the octahedral structure of Cu(II) and Ni(II). On the other hand, the smaller size of Zn(II) ion may be taken as an additional reason for the higher activity, which favours its permeation through the lipid layer of the fungal membrane. This enhances the rate of uptake/entrance and thus the anticandidal activity of the zinc compound. However the lower anticandidal activity of nickel complex relative to copper complex may be referred to the nickel octahedral geometry which formed from the Schiff base ligand and the two bidentate acetate groups besides the two water molecules present in its formula which may decrease the lipophilic nature of nickel complex than copper complex.

Test for the antitumor activity of the ligand and its metal(II) complexes: A marked antitumor activity exhibited with application of Zn(II) and Co(II) complexes on the tested pathogenic yeasts (Table-3), which deserve further attention in search of anticancer compounds, however the parent ligand and the rest metal(II) complexes revealed to be totally inefficient. Our finding is in accord with literature data²⁷ that showed the metal complexes inhibit cellular growth by binding with DNA. Several mechanisms of antitumor action may stabilize cleavable complexes formed by DNA leading to apoptosis, and the stabilizing effect is mainly due to the alkylation of thiol residues on DNA complex.

Test for the cytotoxicity of the ligand and its metal(II) complexes: Moderate cell toxicity was detected with the tested Co(II) and Cu(II) complexes ranged from 22 % viable *C. tropicalis* cells to 50 % *C. glabrata* cell viability, while more than 50 % of the cells still alive and active with application of the less toxic metal complex (Ni(II) complex). Maximum toxicity (70-82 % cell mortality) was observed with addition of Zn(II) complex (Table-3).

Effect of the ligand and its metal(II) complexes on respiration rate in the tested pathogenic yeast cells: Application of Zn(II) complex significantly decreased all tested Candidal cell respiration from 25 $\mu\text{L}/\text{mg}/\text{h}$ (*C. albicans*) to 11.2 $\mu\text{L}/\text{mg}/\text{h}$ in the most sensitive yeast cell (*C. glabrata*) (Fig. 3). This may suggest that Zn(II)

TABLE-3
EFFECT OF THE NEW LIGAND AND ITS METAL(II) COMPLEXES ON ANTITUMOR
ACTIVITY AND CYTOTOXICITY ON THE TESTED PATHOGENIC YEAST CELLS

Compound	<i>Candida albicans</i>		<i>Candida fructus</i>		<i>Candida glabrata</i>		<i>Candida oleophila</i>		<i>Candida parapsilosis</i>		<i>Candida tropicalis</i>	
	A	B	A	B	A	B	A	B	A	B	A	B
Ligand	-	62.0	-	75.0	-	80.0	-	85.0	-	80.0	-	60.0
Cobalt(II) complex	+	25.0	+	35.0	+	50.0	+	42.0	+	40.0	+	22.0
Copper(II) complex	-	33.0	-	40.0	-	45.0	-	20.0	-	25.0	-	35.0
Nickel(II) complex	-	55.0	-	70.0	-	85.0	-	65.0	-	60.0	-	55.0
Zinc(II) complex	+	20.0	+	20.0	+	30.0	+	22.0	+	28.0	+	18.0
Fluconazole	-	40.0		22.0		60.0		52.0		60.0		55.0
LSD at 5%		17.2		20.2		25.4		9.4		9.8		12.8

A = Antitumor activity; B = Cytotoxicity (% of cell viability).

complex may block the active site of certain cell enzyme included respiration process as the main target. The respiration range for the rest tested metal complexes was range from 17.8 $\mu\text{L}/\text{mg}/\text{h}$ in *C. fructus* with application of the Co(II) complex to 37 $\mu\text{L}/\text{mg}/\text{h}$ in *C. oleophila* with addition of Cu(II) complex. Zn(II) complex was the most effective complex against all pathogenic yeasts.

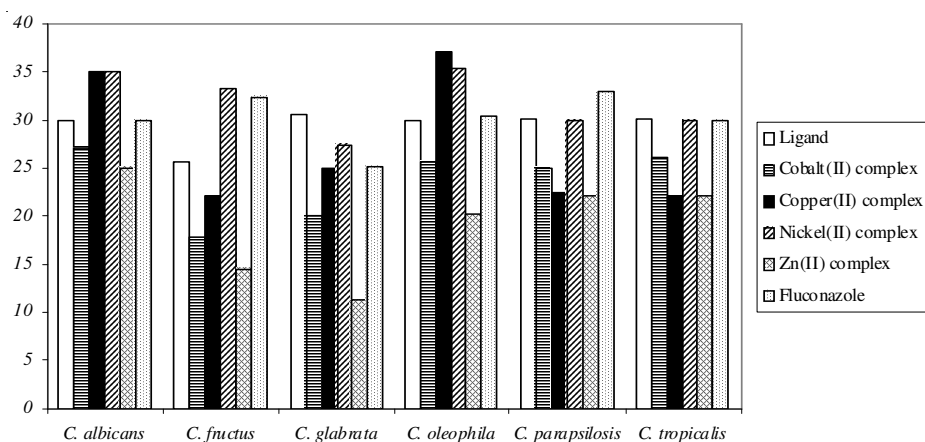


Fig.3. Effect of the new ligand and its metal(II) complexes on respiration of the tested pathogenic yeast cells (*C. albicans*, *C. fructus*, *C. glabrata*, *C. oleophila*, *C. parapsilosis* and *C. tropicalis*). LSD = 0.32, 0.51, 1.23, 2.76, 3.11, respectively

Conclusion

The interaction of the newly synthesized, Schiff base ligand HL with Zn^{2+} , Co^{2+} , Cu^{2+} and Ni^{2+} leads to the formation of neutral complexes. Their structures and formation are determined using microanalysis, magnetic, thermal and different spectral tools. The metal complexes exhibited different geometrical arrangements.

Zn(II) and Co(II) complexes are of a tetrahedral geometry whereas Cu(II) and Ni(II) complexes are associated with octahedral structure. The anti candidal tests showed that metal complexes are more active than the free ligand. Zinc(II) complex has shown promising activity and could be used in future for designing new chemotherapeutic agent capable of inhibiting human immunodeficiency against pathogenic yeasts.

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