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Original Research Article

Conventional and Microwave-Assisted Synthesis, Antimicrobial and Antitumor Studies of Tridentate Schiff Base Derived from *O*-vanillin and Phenyl Urea and its Complexes

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ABSTRACT

A tridentate Schiff base ligand (E)-1-(2-hydroxy-3-methoxybenzylidene)-3-phenylurea derived from *o*-vanillin and phenyl urea in 1:1 molar ratio. Also, the metal complexes of Mn (II), Co (II), Ni (II), Cu (II), Zn (II) and Zr (IV) were synthesized using the microwave-assisted irradiation and conventional methods. The ligand and its complexes 1-6 were characterized using the elemental analysis, FT-IR, UV-vis, ^{1}HNMR, mass spectroscopy as well as thermo-gravimetric analysis (TGA). The geometry structures of the complexes were confirmed using electronic spectra, electron spin resonance (ESR) and magnetic moment. Study of the thermal dehydration and decomposition of the Co (II), Ni (II), and Zn (II) complexes kinetically using the integral method applying the Coats–Redfern and Horowitz Metzger equation. The ligand and its metal complexes were screened for the antimicrobial activity against the gram-positive, gram-negative bacteria and fungi also, the cytotoxic activity was evaluated against two cell lines; human colon carcinoma (HCT-116) and breast carcinoma cells (MCF-7).

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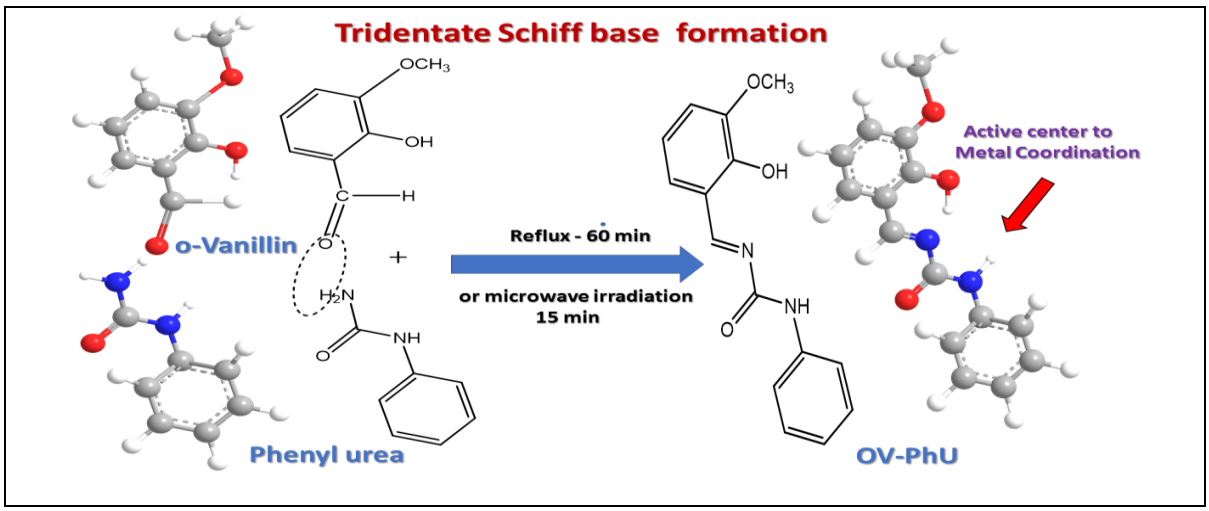
GRAPHICAL ABSTRACT



Introduction

Chemotherapy is the treatment of disease in which a chemical is specifically targeted for a microbial agent or a specific tissue. Chemotherapy science began with Paul Ehrlich in the late 1890s. Paul defines chemotherapy as the use of drugs to injure the invading organism without injury to the host [1]. Ehrlich understood that like human and animal cells, certain bacteria cells colored with certain dyes while others did not. Paul Ehrlich supposed that it might be possible to make certain dyes or chemicals that would kill bacteria not harming the host tissue. Schiff bases are compounds containing azomethane group, mostly synthesized from the amine and aldehyde or ketone. Schiff bases have great importance due to their application in many fields, Schiff bases and its metal complexes have been used as bactericidal, fungicidal, antiviral, antitumor, anti-inflammatory, analgesic, tuberculostatic, anticataract, pesticidal, insecticidal, and herbicidal [2-6]. Azomethines employed in many other fields paints and pigments, catalysis, organic semiconductors, cross-linked polymers, and corrosion inhibitors [7-9]. *O*-vanillin is also the most prominent principal flavor and aroma compound in vanilla which is used as a food flavoring agent in foods, beverages and pharmaceuticals [10]. Due to its numerous biological activities such as anti-inflammatory, analgesic, antiviral activities, it is extensively studied in the medicinal field [11-14]. It also can be used as an efficient herbicide, pesticide bactericides [15] and Schiff bases containing *o*-vanillin moiety form the stable complexes with various metal ions [16-18]. Therefore, *o*-vanillin is an optimal candidate for synthesizing various aromatic Schiff bases with significant bioactivities. In the present study, a novel Schiff bases ligand was synthesized using the microwave and condensation of *o*-vanillin with phenyl urea in 1:1 molar ratio under microwave condition. The Schiff base metal complexes of Zn (II), Ni (II), Mn (II), Co (II) and Cu (II) were prepared using the Schiff base ligand and metal ions. Using the metal acetate salts and except Zr (IV) Schiff base complex was prepared from Zirconyl oxychloride. Free Schiff base ligand and the complexes 1-6 have been fully characterized using the elemental analysis UV-vis, IR spectra, TGA, mass spectroscopy, ^{1}HNMR, ESR, magnetic susceptibility. Schiff base ligands and their metal complexes 1-6 were screened for *in vitro* antibacterial and antifungal activity against the gram-negative bacteria (Escherichia coli and Salmonella

typhimurium), gram-positive bacteria (Staphylococcus aureus and Bacillus subtilis) and Fungi (Candida albicans and Aspergillus fumigatus). the proposed structures were evaluated by Cytotoxic activity test (*In vitro* bioassay on human tumor cell lines) against HCT-116 cells (human colon cancer cell line) and breast carcinoma cells (MCF-7).

Experimental

Materials and methods

All chemicals used were of annular AR grade. *O*-vanillin and phenyl urea were purchased from the Sigma Aldrich and all the metals salts were purchased from the ADWIC except zirconyl oxychloride purchased from Acros organic. The microwave-assisted synthesis was carried out in a domestic microwave energy output 900 W. Purity of Schiff base ligand and its complexes were detected by using thin-layer chromatography (TLC) technique [19]. Melting points were recorded in open capillaries with Barnstead Thermolyne Mel-temp 1001D electrothermal melting point. Elemental analysis was done on a Perkin Elmer PE 2400 CHN elemental analyzer. Metals content were determined by complexometric titration using xylenol orange (XO) as an indicator and hexamine as a buffer (pH = 6). Existence of the acetate ion confirmed by the appearance of the odor of vinegar when a reaction of the small amount of solid complex Cu-L (4) with drops of hydrochloric acid. Chloride content was confirmed by titration of solid complex Zn-L (6) with silver nitrate in the present of potassium chromate as an indicator [20]. The UV-vis spectra samples carried out by using Jenway 6715 UV/vis spectrophotometer in the range (9090-52631 cm^{-1}), and using DMSO as solvent before measurement. The FT-IR spectra samples were ground with potassium bromide (KBr) powder. then pressed into a disk and recorded on Agilent Cary 630 FTIR spectrometer. ^{1}HNMR spectra for Schiff base ligand was recorded in Deuterated chloroform ($CDCl_3$) solution using the Bruker's high-performance Avance III NMR spectrometer 400 MHz, Electron impact mass spectrometric spectrum for Zn (II) complex was carried out using direct inlet unit (DI-50) in the Shimadzu QP-5050 GC-MS. Magnetic susceptibility of complexes at room temperature were carried out at Faculty of Science, Al-Azhar University. Thermal analysis measurements (TGA) were carried out with Shimadzu thermal analyzer model 50. The calculated the Thermodynamics parameter by two methods (Hawetezmetzegar and Coats Redfern). The ESR spectra of the powdered Cu (II) complex recorded at room temperature by X-band EMX spectrometer (Bruker, Germany) using a standard rectangular cavity of ER 4102 with 100 KH_Z frequency. Schiff base ligand and their metal complexes were screened for *in-vitro* antibacterial activity against two species of gram-positive bacteria and two species of gram-negative bacteria as well as two species of fungi. In addition, the Cytotoxicity evaluation was applied against two cell lines; human colon carcinoma (HCT-116) and breast carcinoma cells (MCF-7).

Microwave and conventional synthesis procedure for Schiff base ligand. (E)-1-(2-hydroxy-3-methoxybenzylidene)-3-phenylurea (OV-PhU)

Conventional

Preparation of Schiff base ligand was carried out from phenyl urea (1.36 g, 10 mmol) was dissolved in 30 mL of absolute methanol before mixing it with 30 mL of methanolic solution of *ortho*-vanillin (1.52 g, 10 mmole). Then, 2 mL of glacial acetic acid was added in the reaction mixture. The mixture was heated and stirred under reflux for 60 min (Scheme 1). The solution was allowed to cool to room temperature for 30 min. The solution was filtered and dried at room temperature; A yellowish white

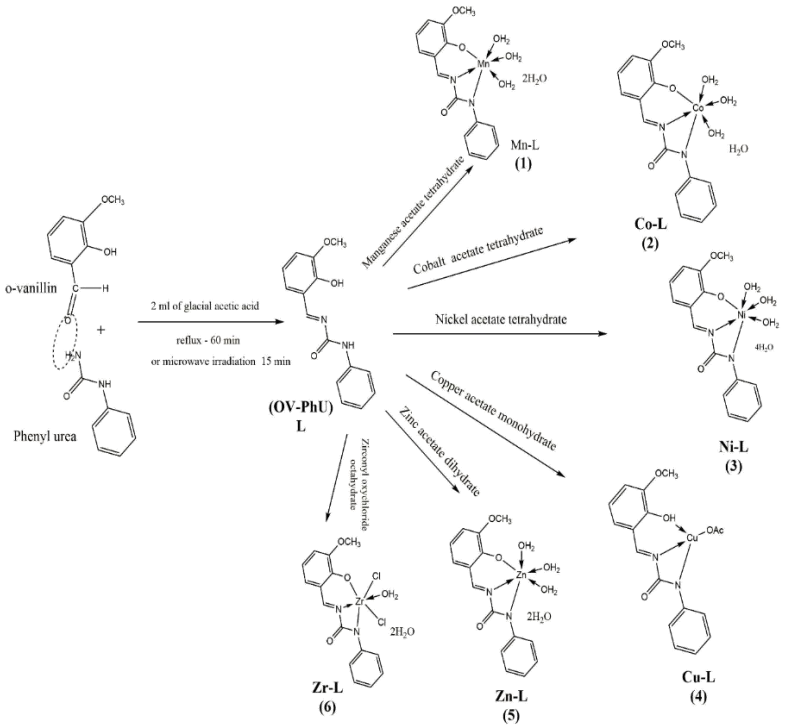
precipitate appears that can be increased by adding diethyl ether to the solution.

Microwave preparation of Schiff base was carried out by equimolar (1:1) ratio (0.136 g, 1 mmol) phenyl urea with (0.152 g, 1 mmol) 2-hydroxy-3-methoxy benzaldehyde, was mixed thoroughly in a grinder. The reaction mixture was then irradiated by the microwave oven by taking 1-2 mL of methanol; the reaction was completed in 15 min at 450 watts. The products were then recrystallized with hot methanol and finally dried under the reduced pressure over anhydrous $CaCl_2$ in a desiccator. The progress of the reaction and purity of the product was monitored by TLC technique. MP: 178-179 °C. the prepared Schiff base is then characterized IR (KBr): showed the bands at 1645 cm^{-1} corresponding to carbonyl group (C=O), and 1598 cm^{-1} for azomethine (C=N), 1255 cm^{-1} (C-O methoxy), 3060 cm^{-1} (C-H, aromatic), 2974 cm^{-1} (C-H, aliphatic) and 3508 cm^{-1} for OH group [21-23]. ^{1}HNMR (DMSO-d6, δ, ppm) Figure 3: 2.51 (m, 6H, DMSO), 3.85 (s, 3H, O-CH3), 5.82 (w, 1H, phenolic proton -OH) [24-26], 6.87 -7.75 (aromatic C-H), 8.5 (w, 1H Ph-NH-C), and 9.38 (m, 1H, azomethine CH=N) [27-29]. Elemental analysis; C% (found=67.1, calc.=66.66), H% (found=5.31, calc.=5.22), N% (found=10.81, calc.=10.36), Anal. Calcd. for ($C_{15}H_{14}O_3N_2$, MWt. +270.28).

General procedure for synthesis complexes using conventional and microwave-assisted Irradiation

A. Conventional preparation of the complexes was carried out by a methanolic solution of 10 mmole of $M(CH_3CO_2)_2$ nH_2O, where M= Mn (II), Co (II), Ni (II), Cu (II), and Zn (II), and 10 mmole Zr (IV) oxychloride octahydrate was added to an methanolic solution of (2.73 g, 10 mmole) of the Schiff base ligand (OV-PhU). The mixture was heated and stirred under the reflux for 60-180 min. On standing overnight, the precipitated product was obtained which was filtered, washed with methanol, then with diethyl ether and recrystallized from hot methanolic solution.

Scheme 1. The proposed structures of the prepared ligand OV-PhU and its metal complexes



B. Microwave preparation of the complexes was carried out by the equimolar (1:1) ratio (1.36 g, 5 mmole) Schiff base ligand (OV-PhU) with 5 mmole of $M(CH_3CO_2)_2$. nH_2O where M=Co (II), Ni (II), Cu (II), Zn (II) and Mn (II), and 5 mmole Zr (IV) oxychloride octahydrate was mixed thoroughly in a grinder. The reaction mixture was then irradiated by the microwave oven by taking 1-2 mL of methanol. The reaction was then completed in a short time with higher yields. The power and time are presented in Table 1. The products were then recrystallized with hot methanol and finally dried under the reduced pressure over the anhydrous $CaCl_2$ in a desiccator. The progress of the reaction and purity of the product was monitored by the TLC technique. Physical, analytical, and spectra data are tabulated in Tables 1-3.

Antimicrobial activity

The obtained metal complexes were screened for their activities as antibacterial, against Gram-positive of Staphylococcus aureus (ATCC 25923) and Bacillus subtilis (ATCC 6635); gram-negative species of Escherichia coli (ATCC 25922) and Salmonella typhimurium (ATCC 14028) as well as anti-fungal of Candida albicans (ATCC 10231) and Fungus: Aspergillus fumigatus. Antimicrobial activity was evaluated using the disc diffusion method [30]. The cephalothin, chloramphenicol, and cycloheximide were used as standard references for the gram-positive, gram-negative bacteria, and fungi, respectively, serving as positive controls. Nutrient agar was prepared then autoclaved at 121 °C for 15 min, cooled and finally poured in Petri dishes. The tested compounds were dissolved in dimethyl sulfoxide (DMSO) solvent and prepared in two concentrations; 100 and 50 mg/mL and then 10 µL of each preparation was dropped on a disk of 6 mm in diameter and the concentrations became 1 and 0.5 mg/disk, respectively. The bacterial cultures were grown in nutrient broth medium at 30 °C. After 16 h of growth, each microorganism, at the concentration of 108 cells/mL, the tested compounds were inoculated on the surface of the Mueller-Hinton agar plates using a sterile cotton swab. Subsequently, uniform size filter paper disks (6 mm in diameter) were impregnated by equal volume (10 µL) from the specific concentration of the dissolved compounds and carefully placed on surface of each inoculated plate. The plates were incubated in the upright position at 36 ºC for 24 h. Three replicates were carried out for each extract against each of the test organisms. Simultaneously, addition of the respective solvent instead of dissolved compound was carried out as negative controls. After incubation, the diameters of the growth inhibition zones formed around the disc were measured with a transparent ruler in millimeter, averaged and the mean values were recorded (as seen in Table 6).

Cytotoxic evaluation

The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% *L*-glutamine, HEPES buffer and 50 µg/mL gentamycin. All the cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and were sub cultured two times a week. Cytotoxicity evaluation using viability assay: for cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of l x10^4 cells per well in 100 µL of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37 °C in a humidified incubator with 5% CO_2 for a period of 48 h. Three wells were used for each concentration

of the test sample. Control cells were incubated without the test sample and with or without the DMSO. The little percentage of the DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for at 37 °C, various concentrations of the sample were added, and the incubation was continued for 24 h and viable cells yield was determined by a colorimetric method [31,32]. The results for the ligand and their metal complexes are demonstrated in Table 7.

Result and discussion

The Schiff base ligand was prepared using the condensation reaction of 2-hydroxy 3-methoxy benzaldehyde "*o*-vanillin" with phenyl urea under microwave irradiation and conventional synthesis, as shown in Scheme 1. Schiff base ligand and their metal complexes obtaining from microwave-assisted preparation have the same physical properties (color, shape, melting point) compering with those synthesized by conventional preparation. ligand and their metal complexes isolated from both methods exhibited one spot in the TLC chart indicating the high purity of these compounds.

Table 1. Melting points, yields, reaction time, analytical and physical properties of the ligand and its complexes

Molecular Formula	Symbol	Conventional		Microwave		M.P. C°	Color	Elemental Analysis Found. / (Calc) %				M⁺ Calc./ (Found)
		Time	Yield	Time	Yield			C	H	N	M	
$C_{15}H_{14}O_3N_2$	OV-PhUL	60 min	61%	15 min	89%	179	Light yellow	67.1 (66.66)	5.31 (5.22)	10.81 (10.36)	-	270.28
$C_{15}H_{22}O_8N_2Mn$	Mn-L (1)	75 min	67%	13 min	87%	141	Olive	44.17 (43.59)	5.78 (5.37)	6.42 (6.78)	12.78 (13.29)	413.28
$C_{15}H_{20}O_7N_2Co$	Co-L (2)	60 min	82 %	17 min	94%	84	Brown	44.42 (45.12)	4.73 (5.05)	7.24 (7.02)	14.41 (14.76)	399.26
$C_{15}H_{26}O_{10}N_2Ni$	Ni-L (3)	60 min	78%	14 min	93%	104	Greenish yellow	39.67 (39.76)	5.03 (5.78)	6.58 (6.18)	12.47 (12.95)	453.07
$C_{17}H_{20}O_5N_2Cu$	Cu-L (4)	135 min	83%	12 min	90%	114	Green	53.37 (52.11)	5.05 (4.12)	7.22 (7.15)	15.85 (16.22)	391.87
$C_{15}H_{22}O_8N_2Zn$	Zn-L (5)	60 min	77%	12 min	96 %	109	Yellow	42.59 (42.52)	4.92 (5.23)	6.62 (6.61)	15.67 (15.43)	423.72
$C_{15}H_{18}O_6N_2Cl_2Zr$	Zr-L (6)	75 min	80%	7 min	91 %	99	Crimson	37.77 (37.19)	4.14 (3.75)	7.23 (7.58)	17.91 (18.83)	484.44

Table 2. Characteristic FT-IR absorption bands (cm^{-1}) of Schiff base ligand and its metal complexes

Symbol	ν (OH)	$\nu(CH)_{aromatic}$	$\nu(CH)_{aliphatic}$	ν (C=O)	ν (C=N)	ν (C-O)	ν (M–O)	ν (M–N)
OV-PhU	3508	3060	2935	1646	1598	1271	-	-
Mn-L (1)	3427	3060	2973	1649	1595	1245	667	414
Co-L (2)	3443	3061	2938	1663	1593	1245	663	414
Ni-L (3)	3430	3060	2955	1655	1614	1246	674	410
Cu-L (4)	3431	3060	2940	1655	1612	1247	665	410
Zn-L (5)	3430	3063	2940	1653	1595	1245	663	414
Zr-L (6)	3440	3063	2940	1645	1612	1254	656	432

The comparison between the productivity and time spent on the preparation of Schiff base ligand and its complexes are presented in Table 1. The results demonstrated that the microwave preparation was faster and more productive and consumes less solvent, making it more suitable for principles of green chemistry. The synthesized Schiff base ligand was found to be soluble in methanol, acetone, acetonitrile, chloroform, DMF and DMSO at room temperature, also soluble in hot ethanol. Free Schiff base ligand and its metal complexes (1–6) have stability at room temperature, Melting points are listed inTable 1. They were prepared by the stoichiometric reaction of the corresponding metal salts and the respective ligand in the molar ratio M:L of 1:1 for all complexes. Physical measurements and analytical data of the complexes 1–6 are tabulated in Tables 1-3. The potential sites (N and O) of the prepared Schiff base ligand coordinate with the metal ions of Mn (II), Co (II), Ni (II), Cu (II) Zn (II) and Zr (IV) producing metal complexes. Characterization of the prepared metal complexes helped in more understanding of the mode of chelation of the ligand towards metals.

Infrared spectra

The infrared spectrum of the Schiff base ligand and its metal complexes are shown in Figures 1,2, demonstrating the azomethine symmetric stretching frequency strong bands at the range of 1598-1614 cm^{-1} are assigned to the imine, ν (C=N) group [21]. The phenolic ν (C-O) frequencies for the ligand and its complexes were observed at 1245-1271 cm^{-1} [23]. The ligand and its complexes shows strong bands between 1645-1663 cm^{-1} due to ketone ν (C=O) group [22]. The appearance of a weak band at 410–432 cm^{-1} due to ν (M–N) vibrations, further supported the evidence of the metal–nitrogen (M–N) linkage. The coordination of metal to oxygen is further justified by the appearance of a new band at 656–674 cm^{-1} due to the M-O [33]. The positions of other bands assigned to $\nu(CH)_{aliphatic}$ near 2940 cm^{-1}, $\nu(CH)_{aromatic}$ near 3060 cm^{-1} and ν(OH) near 3430 cm^{-1} are demonstrated in Table 2. The structure of the Schiff-base ligand confirmed by the absence of aldehydic band (CHO) at 1715 cm^{-1} while the azomethine (C=N) band appeared as a strong band at 1598 cm^{-1} [34] (Figure 1).

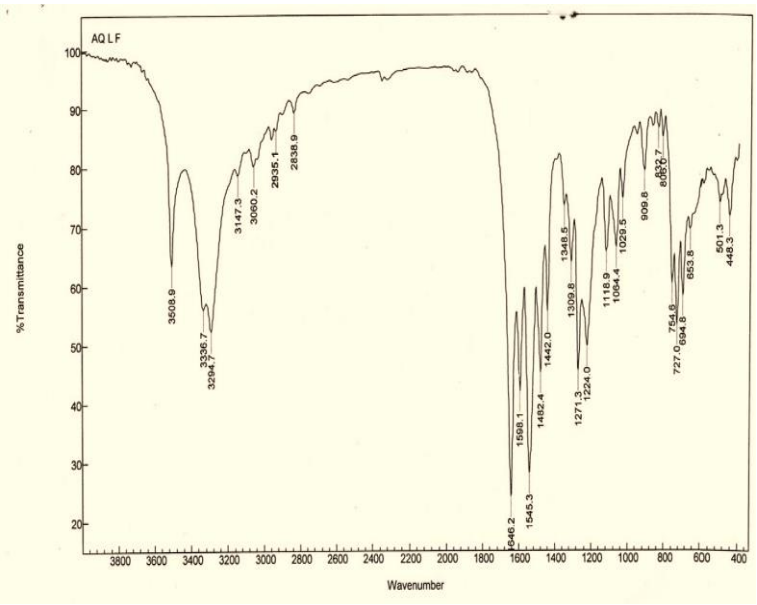


Figure 1. FTIR spectrum of Schiff base ligand

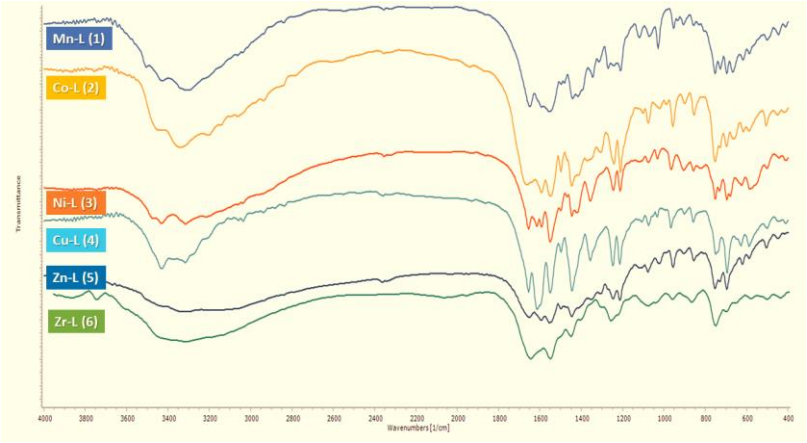


Figure 2. FTIR spectrum of Mn (II), Co (II), Ni (II), Cu (II), Zn (II) and Zr (IV) complexes

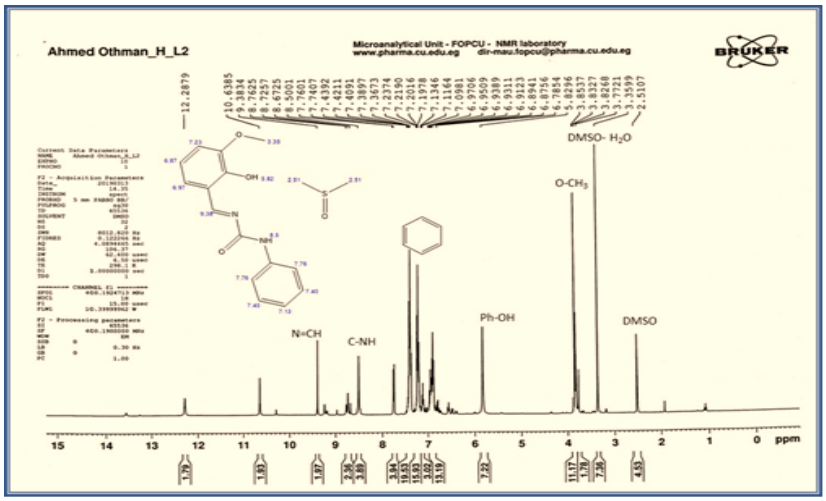
^{1}H NMR spectra

The ^{1}HNMR spectra of ligand (OV-PhU) were recorded in DMSO-d6, the characteristic absorption ^{1}H NMR signals (Figure 3). Chemical shifts of the methoxy group protons (-OCH_3) as a singlet at δ 3.85 ppm [28-29]. The peaks at δ 2.51, 3.35 ppm were attributed to the DMSO and DMSO-H_2O protons [35-38]. Chemical shifts of the Phenolic proton -OH appeared at δ 5.82 ppm [24-26]. A broad multiple was observed at δ 6.87-7.75 ppm were assigned to aromatic protons [39]. Chemical shifts of the aromatic amine proton Ph-NH-C, Azomethine proton-CH=N appeared at δ 8.5, δ 9.38 ppm, respectively [27-29].

UV-vis spectra and magnetic susceptibility of complexes

Electronic spectra and magnetic moment (B.M) of the Schiff base ligand and its metal complexes of Mn (II), Co (II), Ni (II), Cu (II), Zn (II) and Zr (IV) in DMSO are shown in Figure 4, Table 3, Schiff base ligand and its metal complexes were scanned in the region 9090-52631 cm^{-1} at concentrations between 50 µM and 1 mM at room temperature. the electronic data of the studied for Schiff base ligand exhibited strong absorption broad band at λ_{max} near 35715 cm^{-1} due to π-π* conjugated system Fig. 4 between two benzene rings, azomethane, carbonyl group and lone pair of nitrogen atom. and shows a weak band at 28635 cm^{-1} assigned to n-π* transition. the electronic spectra of Mn (II) complex exhibited bands at 36101, 28818, 25510, and 17762 cm^{-1} are assigned to π-π*, n-π*, $^6A_{1g} \rightarrow {}^4T_{2g}$, d-d transitions respectively suggesting an octahedral geometry around Mn (II) ion with magnetic moment value 7.44 B.M [4]. For the Co (II) complex exhibited bands at 35715, 29155, 24875 and 16129 cm^{-1} are refers to π-π*, n-π*, $^4A_{2g}(F) \rightarrow {}^4T_{2g}(F)$, $^4A_{2g}(F) \rightarrow {}^4T_{1g}(F)$ transitions respectively suggesting an octahedral geometry with magnetic moment value 6.89 B.M [4, 42]. The electronic spectra of Ni (II) complex showed the bands of appreciable intensity at 35715 , 24450 and 17605 cm^{-1} these transitions have tentatively been assigned to π-π*, ligand to metal transfer (LMCT), $3A_{2g} \rightarrow 3T_{1g}$ (F)(v2) transitions respectively suggesting an octahedral geometry with magnetic moment value 4.02 B.M [39]. for Cu (II) complex shows three bands at 35715, 25445 and 20202 cm^{-1} are assigned to π-π*, n-π*, MLCT transitions respectively. The magnetic moment is 1.93 B.M. Therefore, the tetrahedral geometry [7, 40] has been suggested for the Cu (II) complex. The electronic spectra of the Zn (II) complex shows bands at 35461, 30120, 26667, and 17064 cm^{-1} are assigned to π-π*, n-π*, ligand to metal transfer (LMCT) and d-d transitions respectively with diamagnetic properties suggesting an octahedral geometry around Zn (II) ion [39]. The Zr (IV) complex shows bands at 35715, 30960, and 15649 cm^{-1} are assigned to π-π*, n-π*, $^2B_{1g}$- $^2E_{2g}$ transitions, respectively, with the diamagnetic properties suggesting an octahedral geometry around Zr (IV) ion [41].

Figure 3. ^{1}H NMR spectra of Schiff base ligand



Scheme 2. Conjugated system in Schiff base ligand OV-PhU

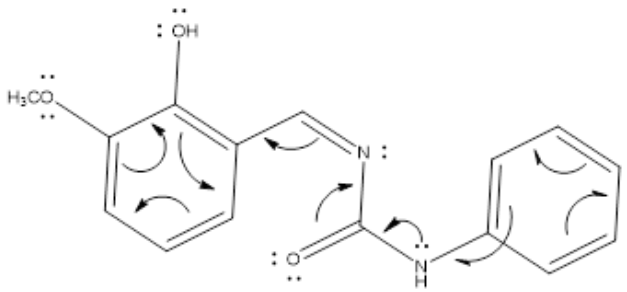
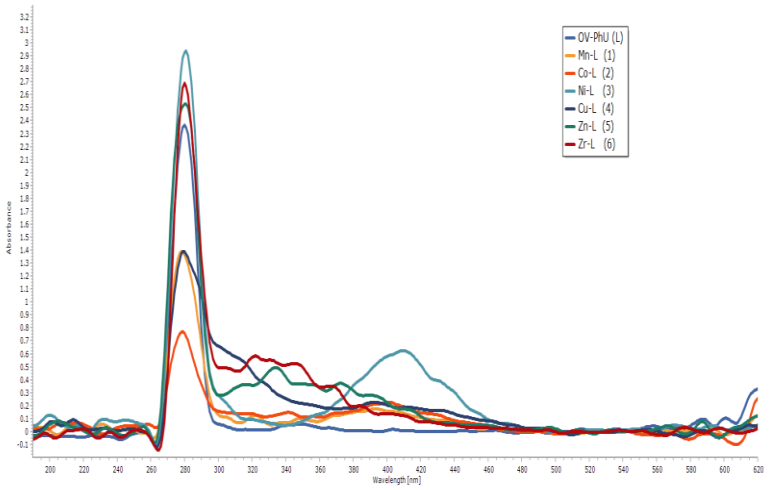


Figure 4. Comparative electronic spectra of the Schiff base ligand (OV-PhU) and its complexes in DMSO



Mass spectra

The mass spectrum of the Schiff base OV-PhU showed the ion peak at m/z=270 as the molecular peak of ($C_{15}H_{14}N_2O_3$), as demonstrated in Figure 5. The highest ion peak at m/z 93 is due to the M^+(C_6H_7N), while the other characteristic peaks are observed at m/z values 151, 150, 136, 122, 119, 108, 106, 92, 91, 90, 77, 66, 65, 52, 51, and 40 suggested the structural assignments of the fragments (Scheme 3).

ESR spectrum

The ESR spectra of Cu (II) complex showed broad signal (Figure 6) with two "g" values ($g_{\parallel}$, $g_{\perp}$) depicted in Table 3. The $g_{\parallel} < g_{\perp} < 2.3$, characteristic of complexes with $^{2}B_1(d_{x^2-y^2})$ orbital ground state. The

average g values were calculated according to the equation $g_{av.} = 1/3[g\backslash\backslash + 2g\perp]$.

Cu (II) complex exhibited g\\ at 2.06 which are less than 2.3, suggesting a covalent character of copper-ligand bonds in the present complexes, where, g\\ < 2.3 concerns ionic metal-ligand bond [42-43]. Fairly high values of g are in conformity with the oxygen, nitrogen coordination in these compounds [42]. This coupling is known as the hyperfine interaction. The above complex has a tetrahedral structure as indicated by the electronic absorption spectra.

Table 3. Magnetic moment and electronic spectral data of Schiff base ligand and their metal complexes

Compounds	λ_{max} cm^{-1}	Assignments	μeff (BM)	g⊥	g\\	Suggested Structure
OV-PhU	35715, 28653	*π-π*, n-π**	-	-	-	-
Mn-L (1)	36101, 28818 25510 17762	*π-π*, n-π** $^6A_{1g} \rightarrow {}^4T_{2g}$ d-d	7.44	-	-	Octahedral
Co-L (2)	35715, 29155 24875 16129	π-π*, n-π* $^4A_{2g}(F) \rightarrow {}^4T_{2g}(F)$ $^4A_{2g}(F) \rightarrow {}^4T_{1g}(F)$	6.89	-	-	Octahedral
Ni-L (3)	35715, 24450 17605	π-π*, LMCT $3A_{2g} \rightarrow 3T_{1g}$ (F)(v2)	4.02	-	-	Octahedral
Cu-L (4)	35715, 25445 20202	*π-π*, n-π** MLCT	1.93	2.17	2.06	Tetrahedral
Zn-L (5)	35461, 30120 26667 17401	*π-π*, n-π** LMCT d-d transition	Di	-	-	Octahedral
Zr-L (6)	35715, 30960 15649	*π-π*, n-π** $^2B_{1g}$- $^2E_{2g}$	Di	-	-	Octahedral

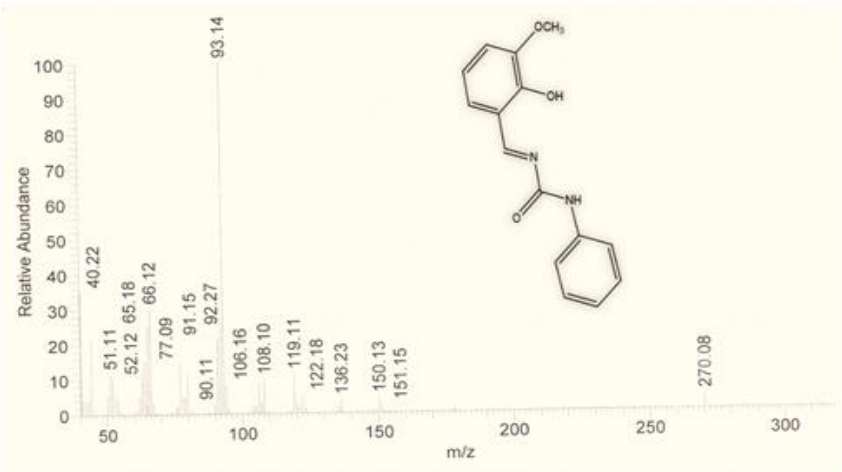
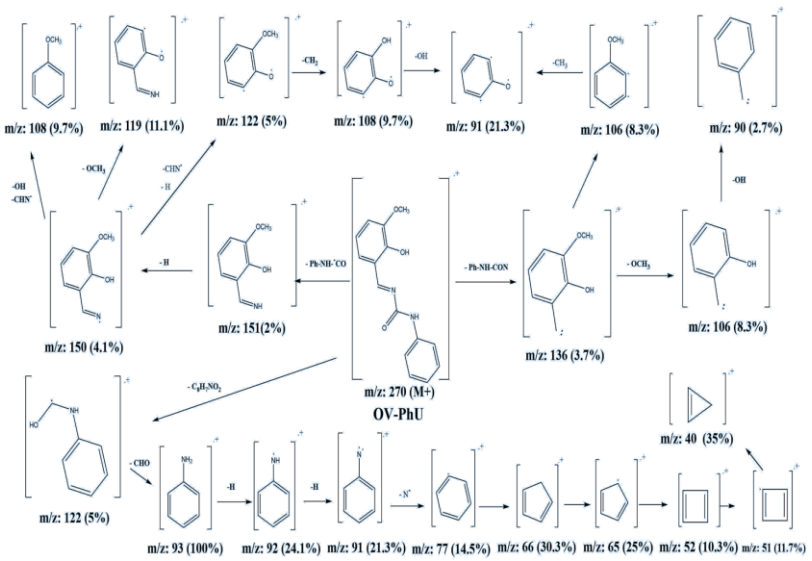


Figure 5. Mass spectra of Schiff base OV-PhU

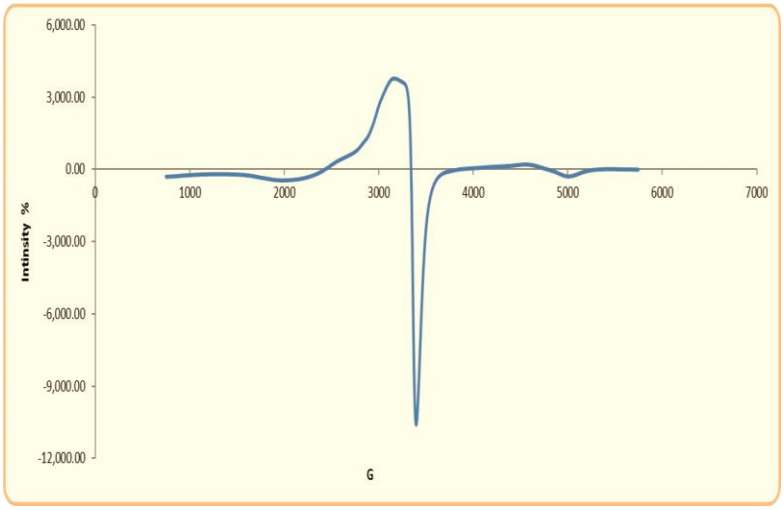
Scheme 3. Mass fragmentation of Schiff base ligand OV-PhU



Thermal analysis and thermo-kinetic parameters of metal complexes

Thermodynamic activation parameters of the decomposition of complexes mostly enthalpy (ΔH*), activation energy (E*), entropy (ΔS*) and Gibbs free energy change of the decomposition (ΔG*) are evaluated graphically by employing the Coats–Redfern relation [44] and Horowitz-Metzger [45]. Kinetic parameters (entropy of activation (ΔS*), enthalpy of activation (ΔH*) and the free energy change of activation (ΔG*) were calculated. The data are calculated for the first stages by employing the Coats-Redfern and Horowitz-Metzger equations, for three complexes of ligand and summarized in Table 5. The thermogram of Co (II) complex showed three decomposition steps; the first step at 53–208 ºC, corresponds to the loss of H_2O (4.5%) molecule. The second step at 215-417 ºC, corresponds to the loss of two molecules of $2H_2O$ (9%). The third step at 505-669 ºC, corresponds to the loss of organic part with MF of ($C_{15}H_{12}O_2N_2$) (73.8%), leaving (20.8%) cobalt (III) oxide Co_2O_3 for every two molecules of complex as a residue [46]. The thermogram of Ni (II) complex shows three decomposition steps; The first step at 56–208 ºC, correspond to the loss of five molecules of water $5.H_2O$ (19.86%). The second step at 211-326 ºC, corresponds to the loss of two molecules of water $2H_2O$ and an organic part with MF of (C_6H_5N) (28.04%). The third step at 328-640 ºC, corresponds to the loss of organic part with MF of (C_9H_7ON) (35.5%), leaving 16.5% of NiO as a residue. The thermogram of Zn (II) complex revealed four decomposition steps; the first step at 56–135 ºC, corresponds to the loss of one molecule of water H_2O (4.3%). The second step at 167-369 ºC, corresponds to the loss of three molecules of water $3H_2O$ (12.8%). The third step, at 370-724 ºC, correspond to the loss of one molecule of water H_2O and an organic part with MF of (C_7H_5ON) (32.33%). The last step at 725-999 ºC, correspond to the loss of organic part with MF of (C_8H_7ON) (31.4%), leaving 19.17% of ZnO as a residue. Many studies indicated that the lattice water, free ions and organic fragments that are not directly coordinated to the metal ions were found to leave the complex at earlier stages compared with coordinated fragments. The heat range of 50-800 °C, formed incomplete decomposition of metal complexes and the final decompositions of metal complexes under nitrogen gave metal oxide. The results obtained from the thermal analysis gave supportive evidence to the suggested structures of the studied complexes.

Figure 6. ESR: ESR spectra of Cu (II) complex



Scheme 4. The sequence of decomposition steps for Co (II), Ni (II) and Zn (II) metal complexes

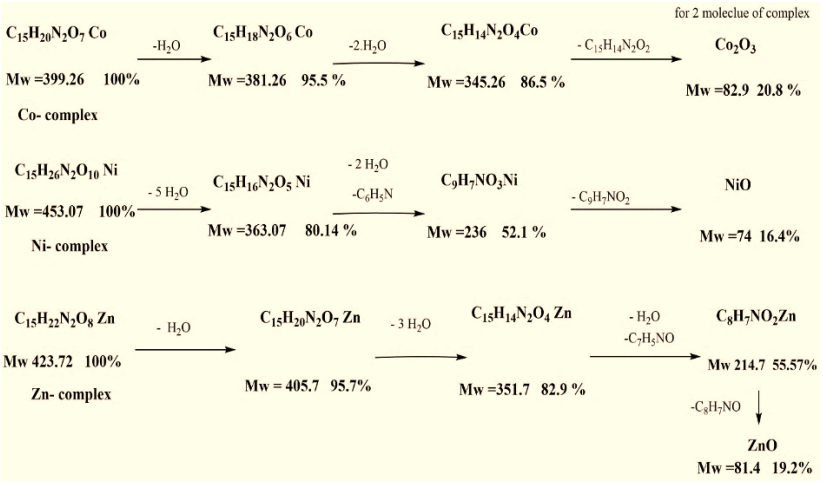


Table 4. Thermo-gravimetric data of complexes

Compd.	Molecular Formula	Molecular Weight	Steps	ΔT ºC		mass %		Assignment
				T_i	T_f	Calc.	Found	
Co-L (2)	$C_{15}H_{20}N_2O_7Co$	399.26	1st	53	208	4.5	4.39	H_2O
			2nd	215	417	9.0	8.24	2 H_2O
			3rd	505	669	63.1	62.32	$C_{15}H_{12}N_2O_2$
			Residue			20.8	21.5	Co_2O_3
Ni-L (3)	$C_{15}H_{26}N_2O_{10}Ni$	453.07	1st	56	208	19.86	19.03	5 H_2O
			2nd	211	326	28.04	29.94	$2H_2O$, C_6H_5N
			3rd	328	640	35.5	33.23	$C_9H_7NO_2$
			Residue			16.4	17.11	NiO
Zn-L (5)	$C_{15}H_{22}N_2O_8Zn$	423.72	1st	56	135	4.3	5.01	H_2O
			2nd	167	369	12.8	13.58	3 H_2O
			3rd	370	724	32.33	32.28	H_2O, C_7H_5NO
			4th	725	999	31.4	29.78	C_8H_7NO
			Residue	-	-	19.17	18.47	ZnO

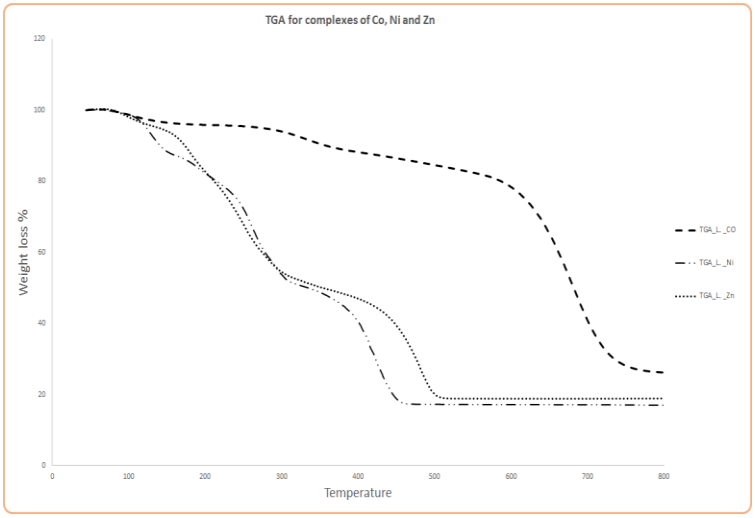


Figure 7. Thermographs of metals complexes of Co, Ni and Zn of OV-PhU

Table 5. Thermodynamic kinetic parameters data of the thermal decomposition of complexes

		R^2	E_a KJ mol^{-1}	A S^{-1}	ΔS^* mol^{-1} K^{-1}	ΔH^* KJ mol^{-1}	ΔG^* KJ mol^{-1}	R^2	E_a KJ mol^{-1}	A S^{-1}	ΔS^* mol^{-1} K^{-1}	ΔH^* KJ mol^{-1}	ΔG^* KJ mol^{-1}
Co-L (2)	1st	0.99	99	$2.4x10^6$	-125	96	143	0.98	43	$2.3x10^5$	-152	40	98
	2nd	0.99	150	$2.6x10^5$	-131	145	223	0.99	79	$1.9x10^6$	-146	74	161
	3rd	0.98	66	$8.1x10^7$	-166	59	203	0.98	40	$1.6x10^1$	-240	33	241
Ni-L (3)	1st	0.98	119	$1.2x10^7$	-112	115	163	0.97	64	$3.4x10^7$	-114	60	109
	2nd	0.97	413	$1.8x10^{34}$	157	409	328	0.98	190	$1.3x10^{19}$	105	186	132
	3rd	0.99	944	$1.3x10^{80}$	481	939	663	0.99	420	$3.2x10^{38}$	475	416	143
	4th	0.98	618	$5.3x10^{40}$	208	613	478	0.98	271	$4.6x10^{21}$	151	266	167
Zn-L (5)	1st	0.99	246	$2.6x10^{28}$	298	243	139	0.99	105	$5.4x10^{15}$	38	102	89
	2nd	0.99	279	$2.1x10^{25}$	66	276	246	0.99	132	$1.5x10^{15}$	29	128	115
	3rd	0.99	244	$5.4x10^{16}$	-52	240	270	0.98	123	$7.5x10^{10}$	-47	118	145
	4th	0.99	141	$8.5x10^8$	18	135	122	0.99	65	$1.2x10^4$	-184	59	188

Applications

Anti-microbial activity

Data of the antibacterial and antifungal activity of the isolated Schiff base ligand and their metal complexes were recorded (Table 6), and their antimicrobial activity taken as inhibition zone diameter is depicted in Figure 8. It was found that, the free Schiff base ligand (OV-PhU) is biologically inactive and its metals complexes more active upon chelation is attributed to Tweedy's chelation theory. According to Tweedy's chelation theory, the chelation reduces the polarity of the metal atom mainly because of the positive charge of the metal partially shared with donor atoms present on the ligand and there is electron delocalization over the whole chelate ring this, in turn, increases the lipophilic character of the metal chelate and favors its permeation through the lipid layers of the bacterial membranes [47]. Furthermore, the presence of the metal ions in complexes with azomethane derivatives are more effective in membrane destabilization than free ions. This leads to disturbing structural integrity of the cell and hence eradicating the microorganism [48]. Complexes of the Cu (II), Co (II), and Ni (II) achieved a highest inhibition zone diameter and antifungal activity against *C. albicans* (ATCC10231), leading to a possibility that complexes maybe act as antifungal, on the other hand, Zn (II), Co (II) complexes of Schiff base ligand achieved a highest inhibition zone diameter and antibacterial activity against *Staphylococcus aureus* (ATCC 25923).

Table 6. Antimicrobial result of Free Schiff base ligand and its related metal complexes.

Sample code	Gram - positive bacteria		Gram - negative bacteria		Yeasts and Fungi	
	Staphylococcus aureus (ATCC 25923)	*Bacillus subtilis* (ATCC 6635)	*Salmonella typhimurium* (ATCC14028)	*Escherichia coli* (ATCC25922)	*Candida albicans* (ATCC10231)	*Aspergillus fumigatus***
OV-PhU	NA	8	NA	NA	9	13
Co-L (2)	26	9	13	18	28	18
Ni-L (3)	10	18	NA	16	23	23
Cu-L (4)	14	14	10	15	31	NA
Zn-L (5)	29	21	18	11	15	12
Zr-L (6)	7	8	7	7	11	22
Control	35	35	36	38	35	37
DMSO	0	0	0	0	0	0

Figure 8. Antimicrobial activity of Schiff base ligand and their metal complexes against different strains

Anticancer activity

It is well known that the substance appears effective and exhibits cytotoxic activity when it is able to inhibit the growth of cancer cells at low concentrations. Data compiled in Table 7 and Figures 9-10 showed that the Co, Cu and Zn complexes exhibited the highest cytotoxic activity against MCF-7 and HCT-116. Free Schiff base ligand showed low activity against the MCF-7 and HCT-116 than their metals complexes. This was in accordance with [49] who reported that the metal complexes

possess higher cytotoxic activity against the selected human cancer cells. They have different antitumor mechanisms and have great potential as metal-based anticancer agents. This might attribute to the ability of these complexes to induce *S*-phase arrest in cancer cells at low concentration with increasing expression of tumor suppressors' genes like p^{21}, p^{27} and p^{53}. In addition, these complexes might exhibit the ability to induce apoptosis, production of ROS followed by increasing intracellular Ca^{2+} in cancer cells and finally caused complete apoptosis of cancer cells [50,51]. Furthermore, postulated that there are several potential mechanisms of antitumor activity of metal complexes, especially in the case of Cu-complexes [52]. This type of the activity could be associated with the transport of Cu (II) ions into the cell. The Cu (II) ions are introduced by specific copper transporters in the Cu (I) form. The presence of the natural copper transport system is crucial from the clinical point of view because no additional exogenous drug carriers are needed. Thus, the cellular effects of metal complexes activity might be determined by their interaction with proteins (receptors) or nucleic acids. Considering metal complexes activity not only the interactions with DNA but also with proteins should be analyzed as the targeted molecules in antitumor mechanisms. For comparison purposes, the cytotoxicity of cisplatin, as standard drug, was evaluated against MCF-7 and HCT, produced IC_{50} values (7.22 µg/mL, 6.9 µg/mL), respectively, under the same conditions. The results clearly show that, the entry of metals into organic compounds improves their effectiveness in killing cancer cells.

Table7. Cytotoxic activity of free Schiff base ligand and its related metal complexes against human colon carcinoma (HCT-116) and breast carcinoma cells (MCF-7)

Cell line	L	Mn-L (1)	Co-L (2)	Ni-L (3)	Cu-L (4)	Zn-L (5)	Zr-L (6)	Cisplatin
	The median inhibitory concentration (IC_{50}) in µg/ml							
MCF-7	219	70.3	90.4	149	61.1	61.3	111	7.22
HCT-116	168	49.9	60.6	76.8	52.7	51.9	87.1	6.9

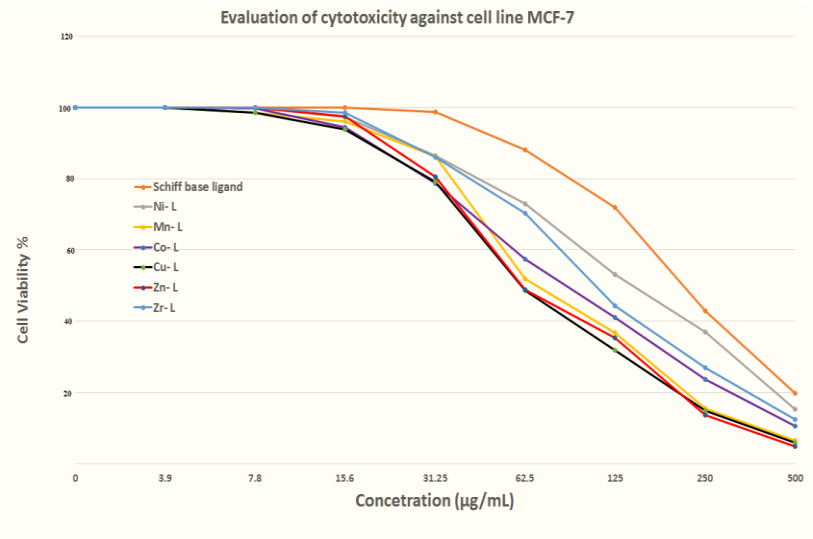


Figure 9. Cytotoxicity of Schiff base ligand and its metal complexes against breast carcinoma cells (MCF-7)

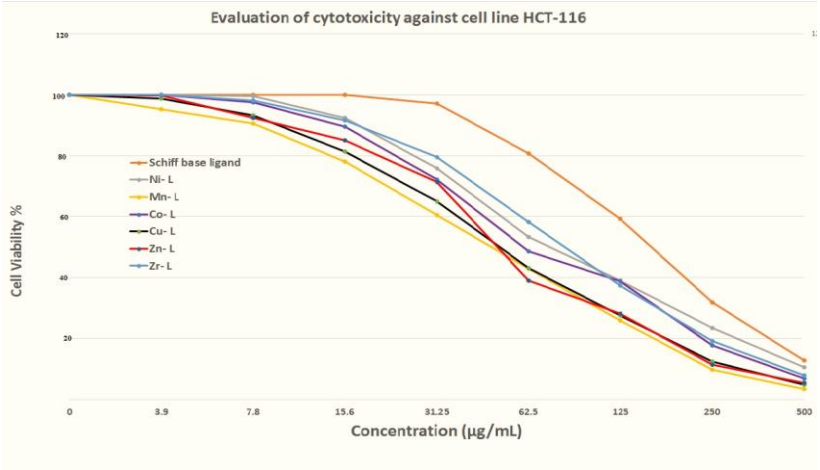


Figure 10. Cytotoxicity of Schiff base ligand and its metal complexes against human colon carcinoma (HCT-116)

Conclusion

A novel Schiff base ligand derived from the *o*-vanillin and phenyl urea using the microwave-assisted irradiation and conventional techniques can act as tridentate ligand, which coordinated through the azomethine-N, Ar-N and hydroxy -OH to the metal ions; Mn (II), Co (II), Ni (II), Cu (II), Zn (II) and Zr (IV). All the metal ions of complexes possessed an octahedral geometrical structure except Cu (II) is tetrahedral. The thermal dehydration and decomposition of Mn (II), Ni (II), and Zn (II) complexes showed dehydration of water, and elimination of acetate then organic content and metal oxide MO remained as a residue. The antimicrobial activity of the ligand and its metal complexes against the bacterial and fungal strains demonstrated that, the Schiff base ligand is biologically inactive and its metals complexes are more active upon chelation, which is attributed to the Tweedy's chelation theory. The complexes of the Cu (II), Co (II) and Ni (II) achieved a highest inhibition zone diameter and antifungal activity against *C. albicans* (ATCC10231), leading to a possibility that complexes maybe act as antifungal. The cytotoxicity activities tested against two cell lines; human colon carcinoma (HCT-116) and breast carcinoma cells (MCF-7). The result demonstrated that, the metals complexes more active than the free Schiff base ligand. The results clearly showed that, the entry of metals into organic compounds improved their effectiveness in killing the cancer cells.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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