Synthesis, Characterization, and Biological Activity of some Mixed-Ligands Vital Metal Complexes of Levofloxacin as Potential Medical Agents

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ABSTRACT

Two series of mixed-ligands metal complexes of Fe(III), Ni(II), Cu(II) and Zr(IV) based on levofloxacin as primary ligand, 8-hydroxyquinolene and 1,10-phenanthroline as secondary ligands were prepared and characterized by elemental and thermal analysis, magnetic susceptibility measurements, IR, UV-Vis. and ESR spectroscopic techniques. The study showed that levofloxacin interacted with the metal ions as a mono basic bidentate ligand bound to the metal through the pyridone and one carboxylate oxygen atoms, 8-hydroxyquinoline bonded to metal ion with its phenolic oxygen and ring nitrogen while 1,10-phenanthroline interacted through two ring nitrogen atoms thus producing stable mixed ligand complexes. The antibacterial activity of the complexes was tested against four different microorganisms where it was found that the complexes exhibited either comparable or increased antibacterial profile in comparison to the parent drug. The cytotoxic activities of some selected mixed ligands complexes were tested against HEPG2 cell line and compared to that of Vinblastine as a standard drug. The structural and molecular orbital calculations of the compounds were investigated theoretically by performing density functional theory (DFT) to investigate the structure, dynamics, surface properties, and thermodynamics of the inorganic systems. The calculated HOMO and LUMO energies showed that intramolecular charge transfer occurs within the molecules.

KEYWORD:

mixed ligand complexes, Levofloxacin, antibacterial activity, cytotoxic activity, molecular molding, DFT theory.

I. INTRODUCTION

Levofloxacin (Levo) is a member of third generation fluoroquinolones, which is a synthetic antibacterial agent containing a 4-oxo-1,4-dihydroquinoline skeleton (Turel, 2002). DNA gyrase is the enzyme essential for all bacteria and is therefore an excellent target for antibiotics. Quinolones turn the action of gyrase against the bacteria by blocking the strand passage and thus hindering proper replication of DNA which ultimately leads to cell death (Kampranis and Maxwell, 1998). The antibacterial activity of fluoroquinolones depends not only on the bicyclic hetero aromatic pharmacophore but also on the nature of the tangential substituents and their spatial relationship (Palumbo *et al*., 1993). These substituents exert their influence on antibacterial activity by providing additional affinity for the bacterial enzymes and enhancing the cell penetrations. The carbonyl group and carboxylic acid functionalities of quinolones provide an excellent site for chelation with divalent or trivalent metal ions. They can also act as bridging ligands and thus are capable of forming polynuclear complexes (Ruiz *et al*., 1998). 8-hydroxyquinoline (Oxin) is well known in many processes for the preparation of mixed ligand complexes (Wankhede et.al, 2013). Its ability to form coordination bond through its phenolic oxygen and ring nitrogen results in forming stable chelates with metal ions in combination with some other ligands, thus producing stable mixed ligand complexes. 1,10-Phenanthroline (Phen) forms strong complexes with most metal ions. Many of its complexes are known to be bioactive, for example the ferroin analogue $[Ru(Phen)_3]^{2+}$. It is an inhibitor of metallopeptidases, with one of the first observed instances reported in carboxypeptidase A (Felber et al, 1962). In recent years, computational chemical models are playing an ever increasing role in chemical research. HF and DFT methods are the common used methods in many reported references (Arockiadoss *et al* 2015). Moreover, it is known that the DFT (B3LYP) method adequately takes into account electron correlation contributions, which are especially in systems containing extensive electron conjugation and/or electron lone pairs (Savithiri *et al* 2015). In the present study and in continuity to our previous work (**Islam and Magda**, 2017), we prepare, characterize and investigate the biological and antitumor activities of some levofloxacin, 8-hydroxyquinoline and 1,10-phenanthroline mixed ligands complexes with transition metals of biological interest and to investigate significant enhancement of their antimicrobial activity. DFT theory was applied to study the structural and vibrational properties theoretically.

II. EXPERIMENTAL

Materials :

In the present study, chemicals of highest quality (Merck, Aldrich or Fluka Research Laboratories) and Levofloxacin (Egyptian Company for Chemicals and Pharma-ceuticals of purity 98%) were used without further purification. Mixed ligands complexes were prepared from hydrated chlorides of Fe(III), Ni(II), Cu(II) and zirconyloxychloride ($ZrOCl_2$). Freshly bidistilled water was used whenever water is necessary.

Preparation of the mixed ligands solid complexes

Aqueous solution of 1.00 mmol of metal salts was added with stirring to an ethanolic solution of 1.00 mmol of each of 8-hydroxyquinoline or 1,10-phenanthroline as secondary ligands and the mixture was refluxed for ≈ 3 hours. To the above mixture, an ethanolic solution of levofloxacin (1.00 mmol), as a primary ligand was added and the mixture (1 : 1 : 1 molar proportion) was again refluxed in a water bath for 3 hours. The mixture was cooled, and solid complexes obtained were filtered off and washed with distilled water followed by ethanol and dried under vacuum.

Physical measurements

Elemental analysis and all physical measurements were carried out as mentioned in our previous work (**Islam and Magda**, 2017).

Antimicrobial assay

The antimicrobial susceptibility was tested by the disk diffusion technique developed by Bauer *et al*.(Bauer *et al* 1966). For this purpose 50 µg mL^{-1} stock solution of levofloxacin and its complexes were prepared. The stock solution was diluted to 3 different concentrations i.e., 5, 10, and 20 µg mL^{-1}. Commercially available filter paper disks were soaked in the prepared drug and complex solutions, dried, and applied on the surface of solid culture media (Nutrient Agar), which had been streaked with standardized bacterial inoculums and incubated at 37 °C for 24 h. This method is based on the determination of an inhibited zone proportional to the bacterial susceptibility to the antimicrobial present in the disk. The results were compared with the parent against Gram – positive bacteria (*Streptococcus pyogenes* and *Staphylococcus epidermidis*) and Gram – negative bacteria (*Proteus vulgaris* and *Klebsiella pneumonia)*. Three replicas were made for each treatment to minimize error.

Antitumor activity assay

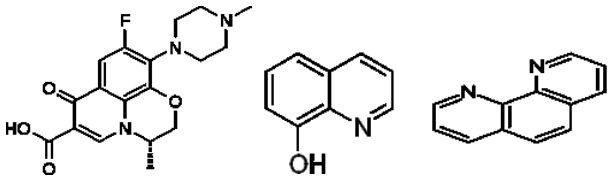
The antitumor activity of some selected complexes was tested against Human Hepatocellular Carcinoma (HepG2) cell lines and compared to that of Vinblastine as a standard drug. The later were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The optical densities of the treated and untreated samples were measured at 590 nm with the microplate reader (SunRise, TECAN, Inc. USA) and the number of viable cells and the percentage of viability were calculated as

$$(1 - \frac{ODt}{ODc}) \text{ x100}$$

where ODt is the mean optical density of the wells treated with the tested samples and ODc is the mean optical density of the untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), (the concentration required to cause toxic effect in 50% of intact cells), was estimated from graphic plots of the dose response curve for each concentration (Mosmann, 1983 and Gomha et al., 2015). All measurements were carried out at the Regional Center for Mycology and Biotecnology, Al-Azhar University, Cairo, Egypt.

III. REASULTS AND DISCUSSION

Two series of mixed ligand complexes of Fe^{3+}, Ni^{2+}, Cu^{2+} and ZrO^{2+} were prepared and characterized. The first series formed from levofloxacin (Levo) (primary ligand) and 8-hydroxyquinoline (Oxin), the second series between Levo and 1,10-phenanthroline (Phen). The structures of the ligands have the following formula:



Levo **Oxin** **Phen**

Structure Elucidation of the Mixed Ligands Metal Complexes:

Elemental analysis and molar conductivity

All complexes are nonhygroscopic in air and have high decomposition points. They are insoluble in common organic solvents but readily soluble in DMSO and DMF. The results of elemental analysis and molar conductivities (*c.f.* Table 1) are in in good agreement with the calculated values of the proposed

formulae. The values reveal that the stoichiometry of the complexes is 1:1:1: (levofloxacin: metal: secondary ligand). The molar conductance values (in DMF) for Fe^{3+} complexes are 21.12 and 33.31 $ohm^{-1}cm^2mol^{-1}$ for Levo-Oxin and Levo-Phen complexes, respectively indicating the electrolytic nature with number of ions equal two and three, respectively. The Levo-Oxin complexes of Ni^{2+}, Cu^{2+} and Zr^{4+} showed molar conductivity within the range 8.22- 10.47 $ohm^{-1}cm^2mol^{-1}$ indicating their non-ionic nature, while those corresponding to Levo-Phen Ni^{2+} and Cu^{2+} complexes are 19.21 and 19.88 ohm^{-1} cm^2mol^{-1} indicating the ionic nature with number of ions equal two. The presence of the anion (Cl^-) outside the coordination sphere is confirmed by the precipitation of Cl^- as AgCl by the addition of $AgNO_3$ solution to the solubilized chelates in DMF.

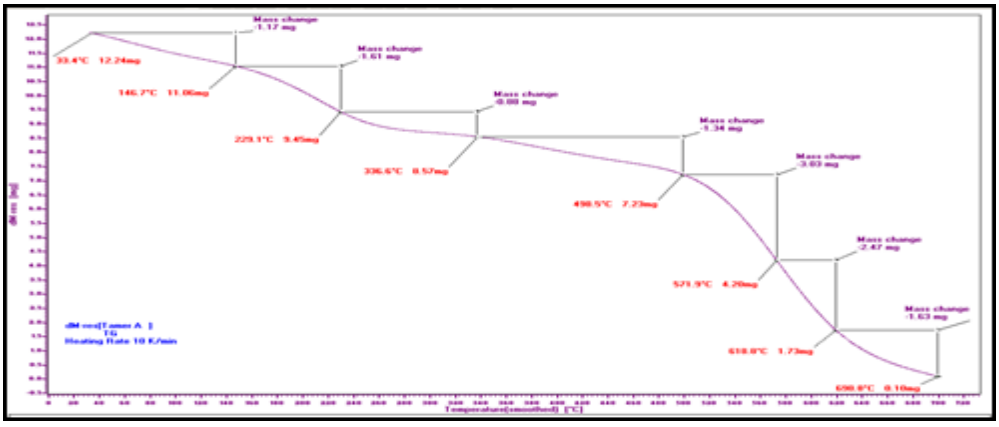
Table (1): Elemental analysis and molar conductivity of the mixed ligand complexes with Fe^{3+}, Ni^{2+}, Cu^{2+} and ZrO^{2+} complexes

Complex	Tentative formula	M.Wt.	Elemental analysis*				Λ_m**
			%C	%H	%N	%M	
Levo-Oxin- Fe	$[C_{27}H_{29}N_4O_7F\text{-}Fe]^+Cl^-$	631.85	51.33 (51.10)	4.63 (4.61)	8.87 (8.04)	8.84 (9.11)	21.12
Levo-Oxin- Ni	$[C_{27}H_{29}N_4O_7F\text{-}Ni]$	599.21	54.11 (54.31)	4.87 (4.38)	9.34 (10.05)	9.79 (9.15)	8.22
Levo-Oxin- Cu	$[C_{27}H_{29}N_4O_7F\text{-}Cu]$	604.08	53.68 (49.23)	4.83 (3.62)	9.27 (11.56)	10.52 (8.44)	10.47
Levo-Oxin- Zr	$[C_{27}H_{27}N_4O_7F\text{-}Zr]$	629.73	51.49 (51.03)	4.32 (4.11)	8.89 (8.66)		8.14
Levo-Phen- Fe	$[C_{30}H_{31}N_5O_6FFe]^{2+}2Cl^-$	758.20	47.52 (48.03)	4.12 (4.32)	9.24 (10.06)	7.35 (7.04)	33.31
Levo- Phen. Ni	$[C_{30}H_{31}N_5O_6F\text{-}Ni]^+Cl^-$	706.17	51.03 (51.31)	4.42 (4.31)	9.91 (9.34)	8.31 (8.92)	19.21
Levo- Phen. Cu	$[C_{30}H_{31}N_5O_6FCu]^+Cl^-$	711.02	50.68 (51.33)	4.39 (3.89)	9.85 (10.11)	8.94 (8.42)	19.88
Levo- Phen. Zr	$[C_{30}H_{29}N_5O_6F\text{-}Zr]$	701.23	51.38 (51.32)	4.16 (4.66)	9.98 (9.43)		10.33

***values between parentheses are found values ** $ohm^{-1}cm^2mol^{-1}$**

Thermal analysis

The thermogravimetrical behavior of some selected solid complexes is studied and representative thermograms are given in Fig. (1). Inspection of the TG – DT curves shows that the mixed complexes degrade thermally, more or less, within the temperature range 30°C – 570°C through three main steps: i- Dehydration of physically adsorbed and coordinated water molecules from the coordination sphere takes place within the temperature range 119.5°C-143.1°C, ii- The unhydrated complexes began to decompose thermally through the second step at 229.1°C – 305.6 °C range leading to evolution of N_2 and CO_2 gases, finally, iii- Full thermal decomposition takes place through the third step (usually composed of multiple successive steps) started at 305.6°C leading to the metal oxides as final products. The overall degradation steps are summarized numerically in Table (2).



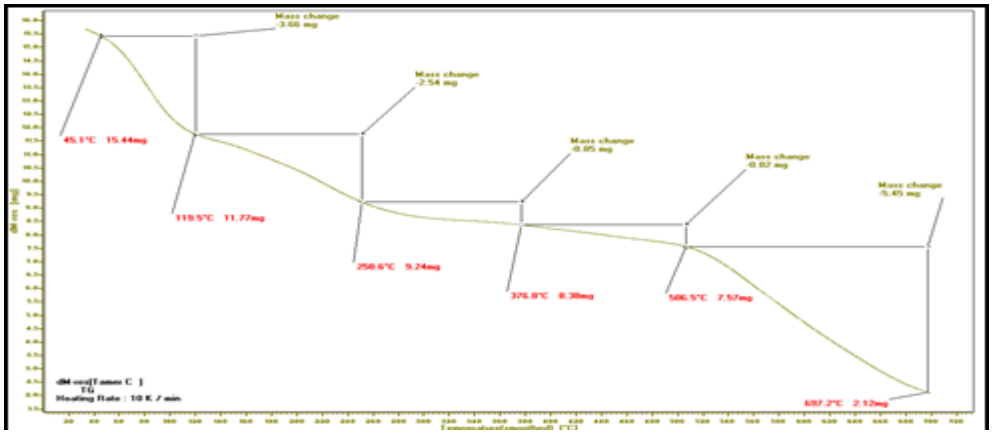


Fig.(1): TGA curve of the complexes Levo – Fe^{3+} – Oxin (A) and Levo – Ni^{2+} – Phen (B)

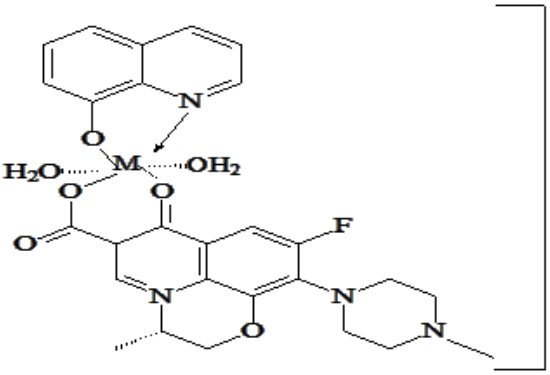
Table (2): Thermogravimetric data for levofloxacin – M – Oxin (-Phen) complexes.

Complex	Temp.(°C)	Assignment
Levo-Fe-Oxin	146.7 229.1 336.6	Removal of coordinated H_2O molecule. Beginning decomposition of Oxin, HCl and CO_2 gases Decomposition of Levofloxacin Moiety leading to NiO_2; 17.30 %. Ni% = 13.59 (15.22)
Levo-Ni-Oxin	119.5 250.6 376.8	Removal of hydrated H_2O molecule. Beginning decomposition of Oxin HCl and CO_2 gases Decomposition of Levofloxacin Moiety leading to Cu_2O; 149 %. Cu% = 13.17 (14.44)
Levo-Cu-Oxin	133.2 254.2 375.3	Removal of hydrated H_2O molecules. Beginning decomposition of Oxin, HCl and CO_2 gases Decomposition of Levofloxacin Moiety leading to Zr_2O_3; 20.73 %. Zr% = 17.64 (16.41)
Levo-Fe-phen	142.5 267.4 406.3	Removal of coordinated H_2O molecule. Beginning decomposition of Phen, HCl and CO_2 gases Decomposition of Levofloxacin Moiety leading to NiO_2; 17.30 %. Ni% = 13.59 (15.22)
Levo-Ni-phen	132.4 305.6 442.8	Removal of hydrated H_2O molecule. Beginning decomposition of Phen, HCl and CO_2 gases Decomposition of Levofloxacin Moiety leading to Cu_2O; 149 %. Cu% = 13.17 (14.44)
Levo-Cu-phen	128.9 294.6 458.6	Removal of hydrated H_2O molecules. Beginning decomposition of Phen, HCl and CO_2 gases Decomposition of Levofloxacin Moiety leading to Zr_2O_3; 20.73 %. Zr% = 17.64 (16.41)

Values between parentheses are theoretical values.

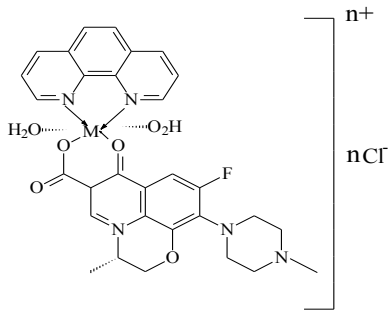
Infrared spectra

The IR spectra of the complexes under study along with their free ligands were studied to get knowledge concerning the mode of bonding. The absorption frequencies (cm^{-1}) of the most important bands are listed in Table (3). The spectrum of the free drug levofloxacin (Levo) exhibits the V_{OH} band at 3262.3 cm^{-1} and the $V_{C=O}$ band at 1725 cm^{-1}, while the V_{OH} of 8-hydroxyquinoline (Oxin) appears at 3410.3 cm^{-1} and the $V_{C=N}$ of Phen appears at 1597.1 cm^{-1}. These bands suffer notable broadness and shifting to lower frequencies in the spectra of complex species indicating their contribution in coordination process. This is accompanied by the appearance of new broad bands within the range 3435.3-3173.5 cm^{-1} on complexes due to the stretching vibrations of coordinated water molecules. A new two sets of bands appear in the spectra of the mixed ligand complexes within the ranges 642.1 – 777 cm^{-1} and 467.2 – 592.7 cm^{-1} due to the stretching vibrations of the M – N and M – O bonds, respectively. Elemental analysis confirmed the formation of Levo: M: Oxin and Levo: M: Phen complexes with stoichiometric ratio 1:1:1, accordingly the mode of bonding of such complexes can be represented as follows:



n= 0 for Ni(II) and Cu(II) and = 1 for Fe(III)

Scheme 1: Structure of Fe^{3+}, Ni^{2+} and Cu^{2+} Levo – Oxin complexes



n = 1 for M = Ni(II) and Cu(II) and = 2 for M = Fe(III)

Scheme 2: Structure of Fe^{3+}, Ni^{2+} and Cu^{2+} Levo – Phen complexes

Table (3): IR vibrational frequencies (cm^{-1}) of some function groups of Levo – M – Oxin and Levo – M- Phen complexes

Compound	V_{OH}	$V_{C=O}$	$V_{C=N}$	V_{COO^-}	δ_{OH}	V_{M-N}	V_{M-O}
Levo.	3262.3	1725	1453.1	1164	982	---	---
Oxin.	3410.3	---	1593.3	---	---	---	---
Levo-Oxin- Fe	3173.5	1624	1516.4	1115	1098	711.1	567.2
Levo-Oxin- Ni	3372.3	1784	1607.4	1133	1099	756.3	592.7
Levo-Oxin-Cu	3387.4	1786	1545.3	1363	1117	777.3	588.7
Levo-Oxin- Zr	3391.2	1613	1437.6	1096	770	719.2	476.6
Phen.		---	1597.1	---	---	---	---
Levo-Phen- Fe	3435.3	---	1578.6	---	935.5	692.5	520.8
Levo-Phen- Ni	3425.8	---	1556.4	---	947.5	685.3	518.2
Levo-Phen-Cu	3387.5	---	1592.3	---	984.2	68 2.5	520.9
Levo-Phen- Zr	3394.2	---	1590.3	---	952.3	642.1	508.4

Magnetic susceptibility and electronic absorption spectra

Magnetic susceptibility measurements at room temperature exhibit paramagnetism for Fe(III), Ni(II) and Cu(II) complexes and diamagnetism for Zr(IV) complexes. The μ_{eff} values, term symbols and ground state symbols along with the spectral data of the mixed ligands complexes (in nujol mull) are cited in Table (4). Inspection of the data obtained shows that:

i- The high spin Fe (III) complexes with d^5 configuration have the ground state $^6A_{1g}$ and all the d-d transitions are spin and laporte forbidden. The electronic spectra of Fe(III) complex display three bands at 95238.1, 10204.1 and 23809.9 cm^{-1} assignable to $^6A_{1g}(S) \rightarrow {}^4T_{1g}(G)$, $^6A_{1g}(S) \rightarrow {}^4T_{2g}(G)$ and $^6A_{1g}(S) \rightarrow {}^4E_g, {}^4A_{1g}(G)$ transitions, respectively, indicating that the complex possesses a high spin octahedral configuration (Pranita et.al, 2015).

ii- The electronic spectra of Ni (II) complexes show the three spin allowed bands at 23255.8, 30303.0 and 33333.3cm^{-1} which are assigned to electronic transition type $^3A_{2g(F)} \rightarrow {}^3T_{1g(P)}$, $^3A_{2g(F)} \rightarrow {}^3T_{1g(F)}$ and $^3A_{2g(F)} \rightarrow {}^3T_{2g(F)}$ respectively.

iii- Copper (II) complexes show two spin allowed transition bands at 28985.5 cm^{-1} and 33333.3 cm^{-1} due to the $^2a_{1g(D)} \rightarrow {}^2b_{1g(D)}$ and $^2e_{g(D)} \rightarrow {}^2b_{1g(D)}$ transitions, respectively. It was reported that Cu (II) complexes showed a broad asymmetric band in the region 20576 cm^{-1} expected for a d-d transition of an octahedral Cu(II) complex (Kopel et al, 2001). The broadness of the band could be attributed to the overlapping of several bands as a result of strong Jahn-Teller distortion expected in a d^9 ion (Shaker et al, 2009).

iv- Finally, the electronic configuration of Zr (IV) complexes (d^{10}) confirms the absence of any d→d transitions. The absorption bands in its spectra are due to n→π* or CT interaction (Zr → L).

The spectral data are fully assigned in Table (4).

Table (4): electronic absorption spectral data (in Nojol mull) and magnetic moments of the mixed ligands complexes

Complex	wavenumber (cm^{-1})	Assignment	Term symbol	Ground state	μ_{eff} (BM)
Levo-Oxin-Fe^{3+}	95238.1 10204.1 23809.9	$^6A_{1g}(S) \rightarrow {}^4T_{1g}(G)$, $\rightarrow {}^4T_{2g}(G)$a $\rightarrow {}^4E_g$,	$^6S_{5/2}$	$^6A_{1g}(S)$	5.52
Levo-Oxin-Ni^{2+}	23255.8 30303.0 33333.3	$^3A_{2g(F)} \rightarrow {}^3T_{1g(P)}(\nu_3)$ $\rightarrow {}^3T_{1g(F)}(\nu_2)$ $\rightarrow {}^3T_{2g(F)}(\nu_1)$	^3F	$^3A_{2g}$	2.87
Levo-Oxin-Cu^{2+}	30303.0 33333.3	$^2a_{1g(D)} \rightarrow {}^2b_{1g(D)}$ $^2e_{g(D)} \rightarrow {}^2b_{1g(D)}$	^2D	^2E_g	1.79
Levo-Oxin-Zr^{4+}	29850.75 23529.41	M→L charge transfere	---	---	Dia
Levo-Phen-Fe^{3+}	10162.6 23529.4 33333.3	$^6A_{1g}(S) \rightarrow {}^4T_{1g}(G)$, $\rightarrow {}^4T_{2g}(G)$a $\rightarrow {}^4E_g$,	$^6S_{5/2}$	$^6A_{1g}(S)$	5.46
Levo-phen-Ni^{2+}	29850.75 22471.91	$^3A_{2g(F)} \rightarrow {}^3T_{1g(P)}(\nu_3)$ $\rightarrow {}^3T_{1g(F)}(\nu_2)$ $\rightarrow {}^3T_{2g(F)}(\nu_1)$	^3F	$^3A_{2g}$	2.88
Levo-phen-Cu^{2+}	28985.5 33333.3	$^2a_{1g(D)} \rightarrow {}^2b_{1g(D)}$ $^2e_{g(D)} \rightarrow {}^2b_{1g(D)}$	^2D	^2E_g	1.80
Levo-phen-Zr^{4+}	31250.0 40000.0	M→L charge transfere			Dia

Electron spin resonance (ESR) spectra

The X-Band ESR spectra of the powder [Levo-Oxin-Cu] and [Levo-Phen-Cu] complexes (*c.f.* Fig.'s 2 and 3) were recorded at room temperature using DPPH as reference standard. One unpaired electron in Cu (II) complex with $^2B_{1g}$ as ground state lies in dx^2-y^2orbital and follows the trend $g_{II} > g_{\perp} > g_e$ (g_e = 2.0036 free ion value). The ESR spectra of the complexes show anisotropic spectra with $g_{II} > g_{\perp} > g_e$ characteristic for distorted elongated tetragonal Cu (II) complexes with d_{x2-y2} ground state (Sandipan and Biswajit, 2014) and (Yuzo et al.,1980). Analysis of spectra gave the g_{II} and $g_{\perp}$ values as cited in Table (5). The observed g_{II} values (2.588 and 2.134) and $g_{\perp}$ values (2.196 and 2.080) of the Cu(II) complexes under study followed the same trend $g_{II} > g_{\perp} > g_e$ which suggest the presence of unpaired electron in dx^2-y^2 orbital giving distorted elongated tetragonal geometry (Vivekanand et al 2013). The observed g_{av} values for the complexes evidenced the monomeric nature of the complexes;

$$g_{av.} = 1/3 g_{II} + 2/3\ g_{\perp}$$

Table (5): ESR spectral parameters for Cu(II) complexes

Complex	g_{II}	$g_{\perp}$	g_{av}
[Levo-Oxin-Cu]	2.588	2.196	2.327
[Levo-Phen-Cu]	2.134	2.080	2.098

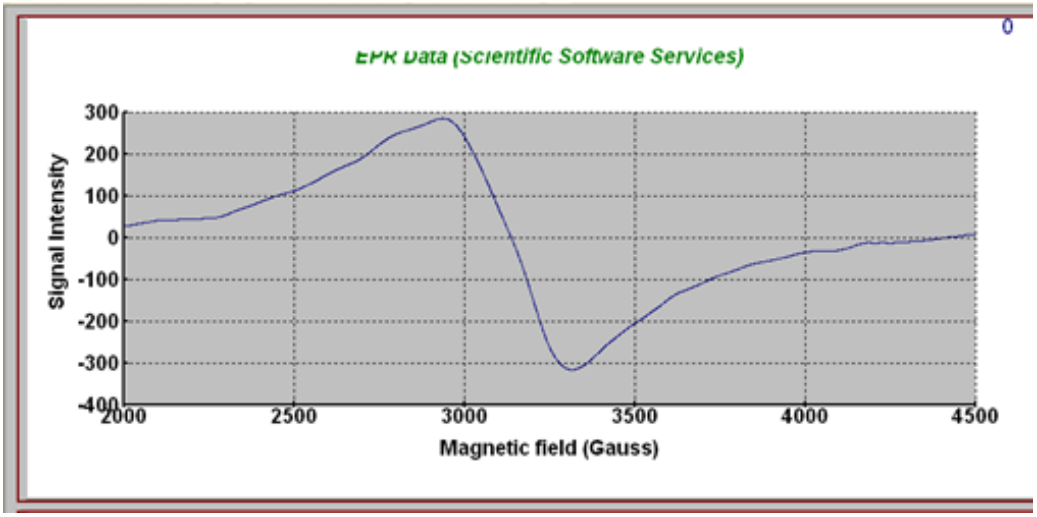


Fig.(2): Esr spectra of [Levo-Oxin-Cu] complex

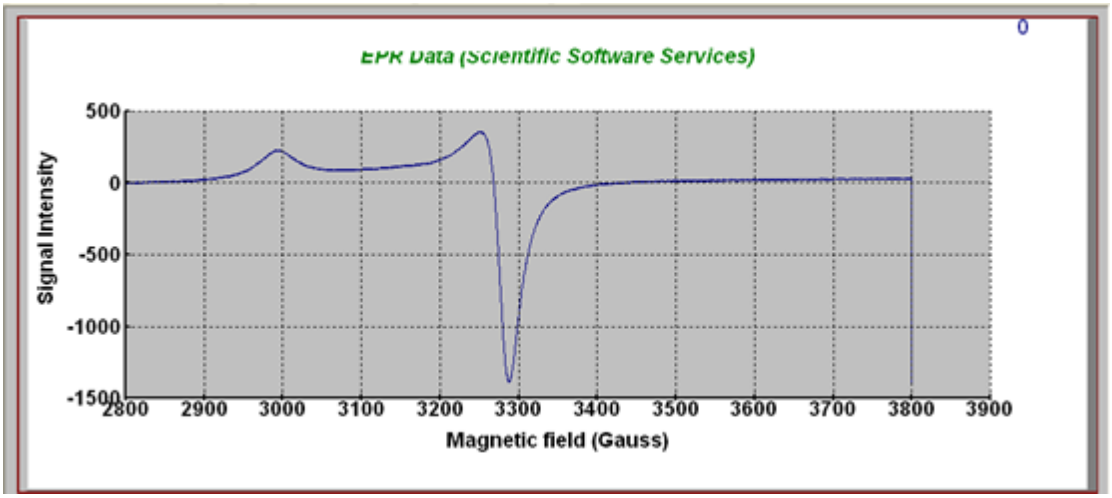


Fig.(3): ESR spectra of [Levo-Phen-Cu] complex

Antimicrobial activity

The antimicrobial activity of the mixed ligands – drug complexes is tested against representatives of Gram – positive bacteria (*Streptococcus pyogenes* and *Staphylococcus epidermidis*) and Gram – negative bacteria (*Proteus vulgaris* and *Klebsiella pneumonia)*. Standard drug; levofloxacin and DMF solvent control were screened separately for their antibacterial activity. The antibacterial results (*c.f.* Table 6) suggest that the mixed ligands complexes show high activity against the tested organisms compared to levofloxacin taken as a standard drug. Although there is no general trend concerning the antimicrobial effect of the tested complexes, but the activity is, more or less, in the order Ni^{2+}> Fe^{3+} >Cu^{2+}> Zr^{4+}.

Increased activity on metal chelation can be explained on the basis of chelation theory. Many factors are capable to enhance the antimicrobial property such as the nature of the metal ion and the ligand, the geometry of the metal complex, the lipophilicity, the steric, and the pharmacokinetic factors (Raman et al, 2010). Such increased activity of metal chelates had been explained by Overtone's concept (Jayabalakrishnan and Natarajan (2001) and the Tweedy's theory (Tweedy, 1964), according to which chelation reduces the polarity of the ligand due to partial sharing of its negative charge with the metal, favoring transportation of the complexes across the lipid layer of the cell membrane. The positive results suggest the very diffusion of the complexes into the bacterial making them able to kill the bacterium as indicated by the zones of inhibition of bacterial growth. On the other hand, the negative results can be attributed either to the inability of the complexes to diffuse into bacteria cell membranes and hence unable to interfere with its biological activity or they can diffuse but inactivated by unknown cellular mechanism.

Table (6) Inhibition zone diameter (mm) of levofloxacin and its metal complexes against various microorganisms

Organism	*Strept. Pyog.*			*Staph. Epid.*			*Prot. Vulgaris*			*Kleb. Pne.*		
Conc. (µg/mL)	5	10	20	5	10	20	5	10	20	5	10	20
Levofloxacine	***12***	***16***	***22***	***15***	***18***	***25***	***12***	***15***	***25***	***15***	***18***	***25***
Levo-Oxin-Fe	10	17	23	14	20	27	13	17	26	14	20	27
Levo-Oxin-Ni	14	17	24	16	20	28	14	20	28	16	21	27
Levo-Oxin-Cu	13	18	23	16	20	26	14	18	25	17	20	28
Levo-Oxin-Zr	10	12	18	12	17	24	11	14	21	14	16	21
Levo-Phen-Fe	13	17	22	16	21	28	13	17	28	16	17	26
Levo- Phen Ni	14	17	24	16	18	26	12	18	27	14	19	27
Levo- Phen –Cu	14	15	23	16	19	26	11	16	26	14	20	27
Levo- Phen –Zr	11	15	20	13	15	23	11	13	22	13	16	23

Antitumor activity

The cytotoxic activities of some selected mixed ligands complexes were tested against HEPG2 cell line and compared to that of Vinblastine as a standard drug. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the mixed ligands complexes. The 50% inhibitory concentration (IC_{50}) was estimated from graphic plots of the dose response curve for each concentration. Representative example of the survival curve of of Levo – Fe(III) – Oxin complex is shown in Fig. (4) While the lethal concentrations (IC_{50}) values compared to that of the standard Vinblastine drug are listed in Table (7). Inspection of the cytotoxic data, it is found that mixed Levo. – Oxin complexes are, in general, more effective than those of Levo. – Phen complexes and the metal ions are arranged according to their cytotoxicity as Fe (III) > Cu (II) > Ni (II) > Zr (IV) for mixed ligand complexes with both Oxin and Phen. The enhanced activity of metal complexes may be attributed to the increase in conjugation in the ligand moiety takes place in complexation process.

Table (7): Lethal concentration (IC_{50}) of the mixed Levo. - M complexes with ligands Oxin and Phen on HEPG2.

Complex	IC_{50} (µg/ml)	
	Levo-Oxin complex	Levo-Phen complex
Vinblastine	4.6	
Fe (III)	6.80	7.33
Ni (II)	8.24	8.57
Cu (II)	7.02	7.22
Zr (IV)	13.55	14.20

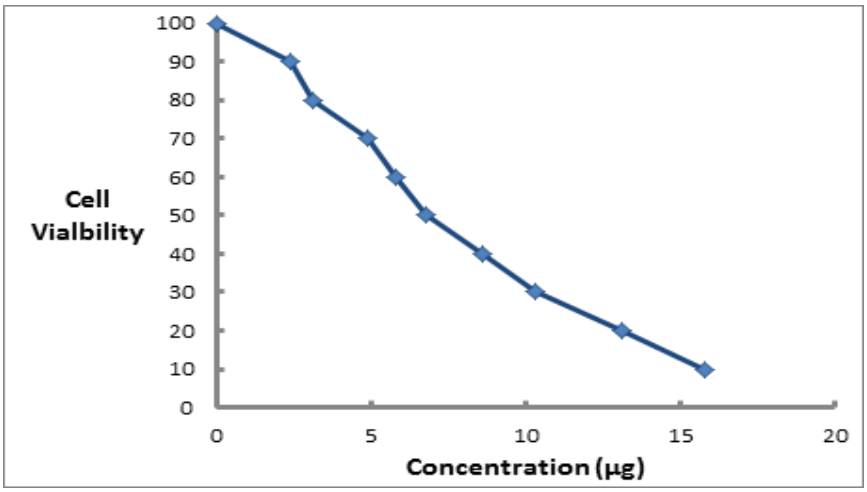


Fig. (4): Cytotoxicity effect of Levo – Fe(III) – Oxin complex against HEPG2 cell line (IC_{50} = 6.80 µg/mL)

It was reported that compounds having IC_{50} values 10 – 25 µg/mL are considered to have weak cytotoxic activities, while those having intermediate values (ranging from 5 – 10 µg/ml) are classified as moderately active (Shier; 1991). On the other hand, compounds with IC_{50} values less than 5µg/mL are considered to be very active. Consequently, the mixed ligands complexes under study are considered as moderately active except for ZrO complexes which was found to have weak activity with IC_{50} values equal 13.55 and 14.20 µg/mL for complexes with Levo - Oxin and Levo – Phen, respectively. In general, these complexes seem to be promising as an anticanser agent due to their high cytotoxic activity.

Molecular modeling of the free ligands and their complexes

A group of measurements involving $DMOL^3$ program in materials studio package which is shaped for the wide scale Density Function Theory (DFT) were applied. The Molecular modeling, Total density, Deformation density and 3D plots frontier orbital energies using DFT method for free ligands and representative example of (e.g Ni(II) complex) were determined and given in Fig.'s (5 - 8).

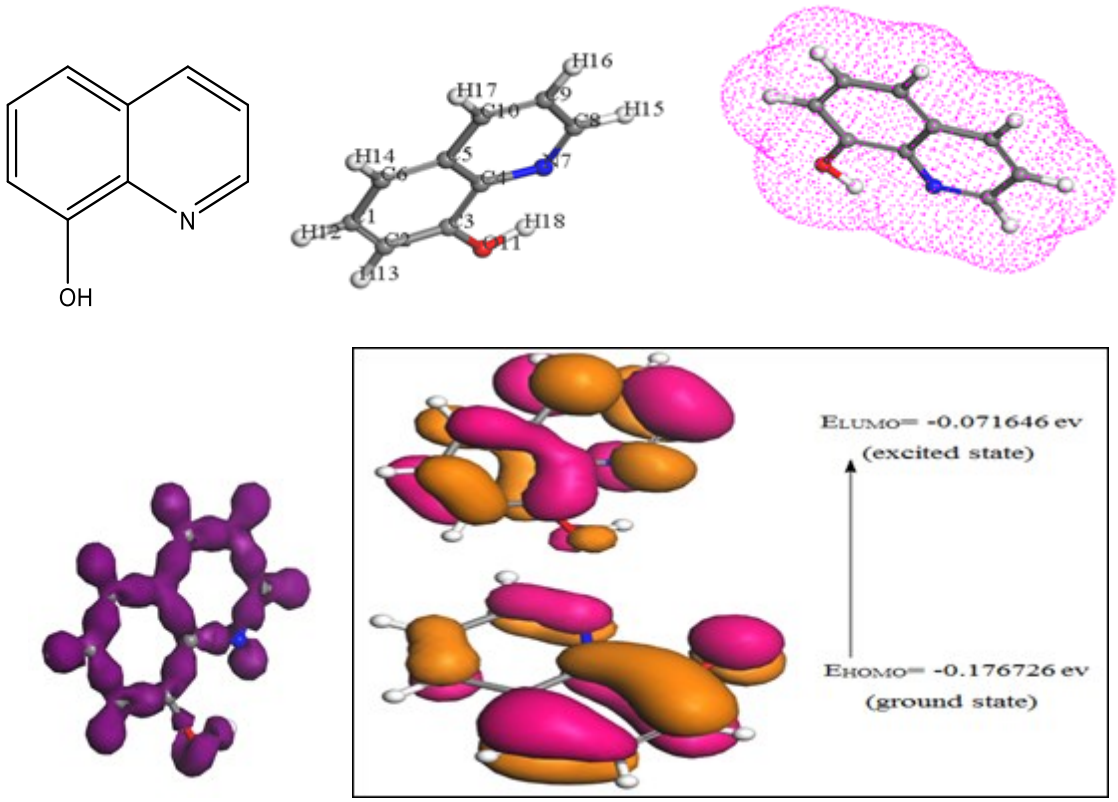


Fig. (5) Molecular modeling, Total density, Deformation density and 3D plots frontier orbital energies using DFT method for ligand 1 (Oxin)

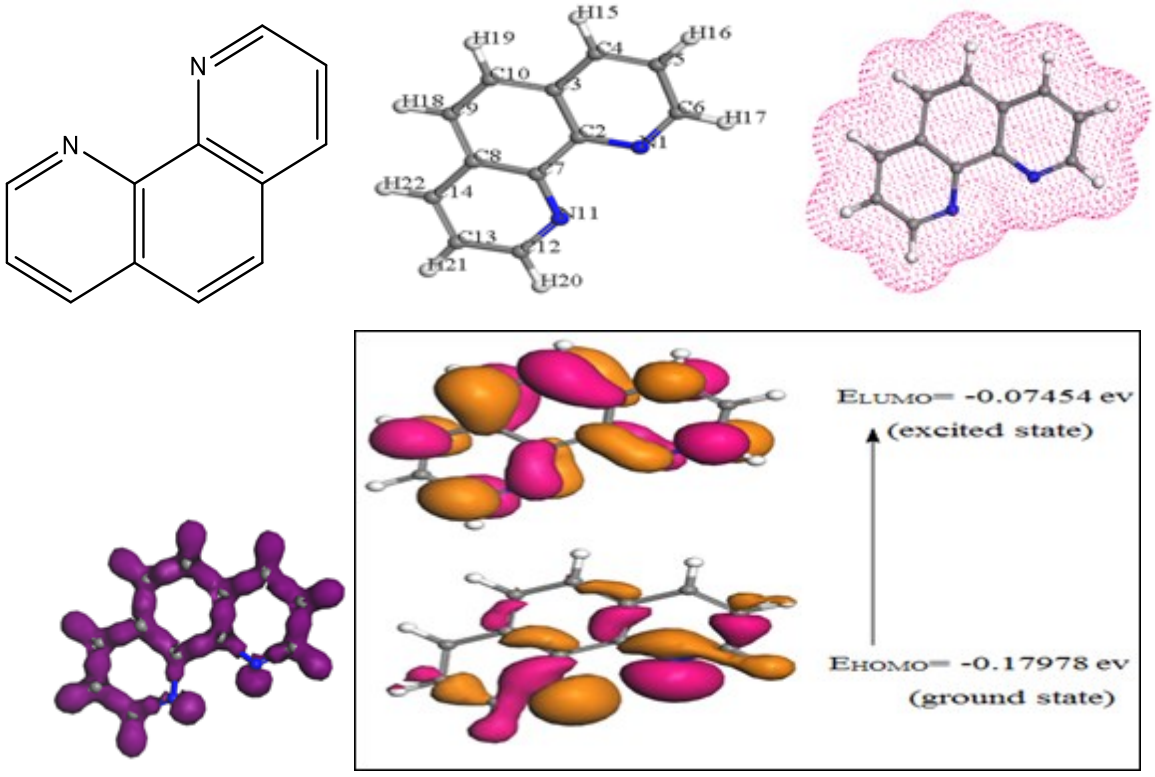


Fig. (6) Molecular modeling,Total density,Deformation density and 3D plots frontier orbital energies using DFT method for ligand 2(Phen)

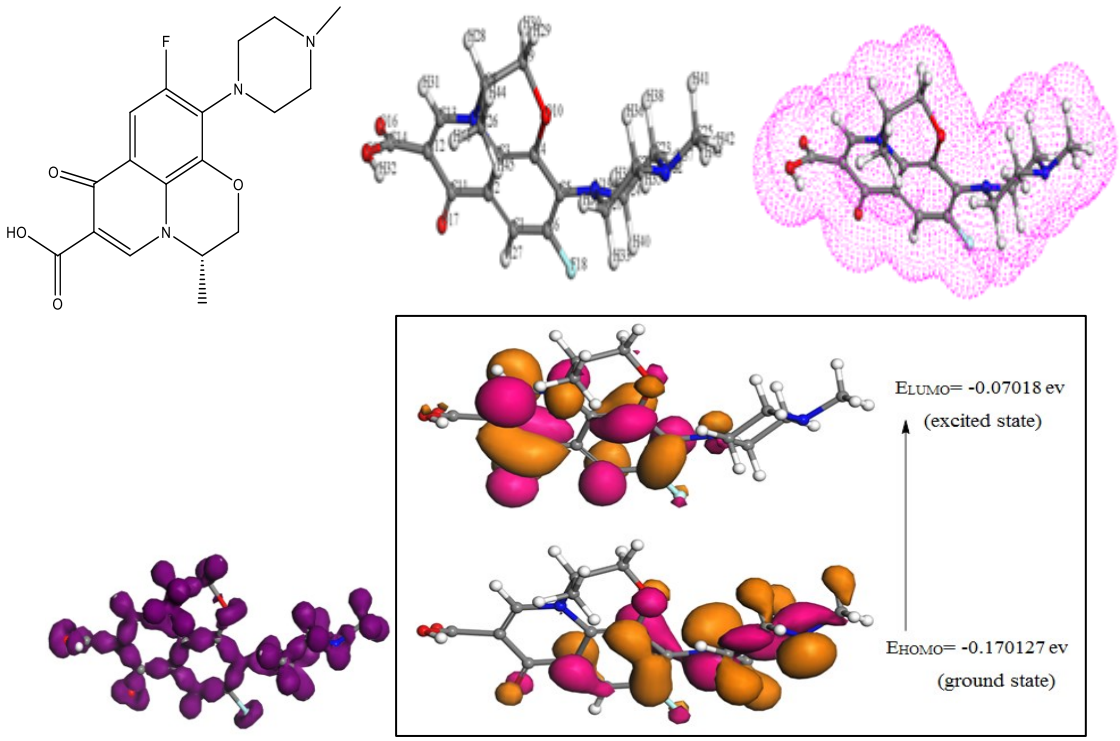


Fig. (7) Molecular modeling, Total density, Deformation density and 3D plots frontier orbital energies using DFT method for ligand 3 (Levo)

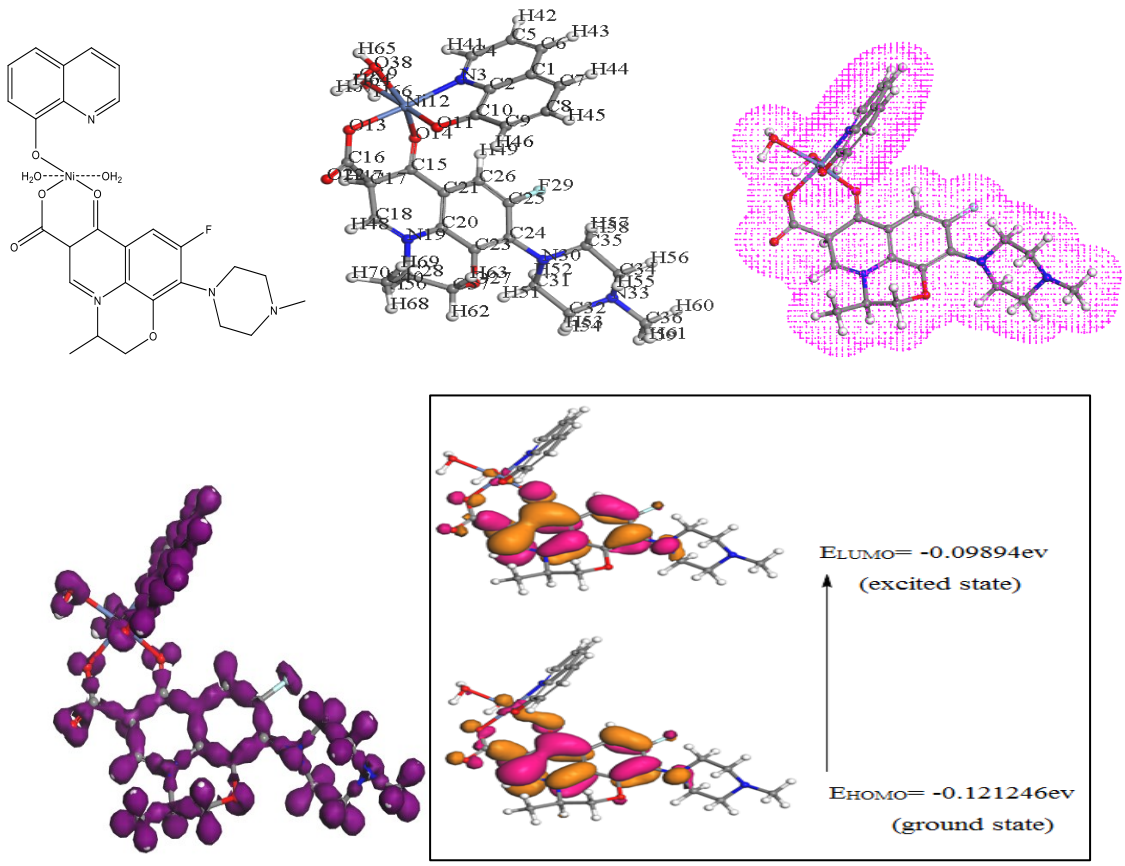


Fig. (8) Molecular modeling, Total density, Deformation density and 3D plots frontier orbital energies using DFT method for Ni(II) complex

Frontier molecular orbitals

The HOMOs and LUMOs are known as Frontier molecular orbitals (FMOs), which play an important role for evaluating molecular chemical stability, chemical reactivity and hardness-softness of the molecule. Representative picture of Frontier molecular orbitals and Deformation density are shown in Fig.'s. (5-8). The HOMO acts as an electron donor, while the LUMO is an electron acceptor. In the HOMO of all selected compounds; the electron density mainly delocalized over quinolone rings OH and C=N groups. While in the LUMO orbital this density is delocalized on the two phenyl rings. The energy gap (ΔE) represents the chemical reactivity of compound; lower value of ΔE makes the system more reactive or less stable. As depicted in Table 8, compound 2 (Phen) has a largest energy gap which decreases in the following order 2 (Phen) > 1 (Oxin) > III (Levo) while metal complexes decreases in the order Cu(II) > Ni(II) >Fe(III). The energy gap ΔE is directly involved with hardness/softness of a chemical species. The higher value of ΔE represents more hardness or less softness of a compound. Soft molecules are more reactive than hard ones because they could easily offer electrons to an acceptor. Thus compound 2 (Phen) referred as hard molecule when compared to other two ligands**.** Another global reactivity descriptor electrophilicity index (ω) describes the electron accepting ability of the systems quite similar to hardness and chemical potential. High values of electrophilicity index increases electron accepting abilities of the molecules. Thus, electron accepting abilities of the compounds are arranged in following order: 2 > 1 > 3. The electrophilicity index (ω) is positive, definite quantity and the direction of the charge transfer is completely determined by the electronic chemical potential (μ) of the molecule because an electrophile is a chemical species capable of accepting electrons from the environment and its energy must decrease upon accepting electronic charge. Therefore, the electronic chemical potential must be negative exactly as supported by the values in Table 8. The values of (E_{HOMO}-E_{LOMO}) energies of frontier molecular orbitals, energy band gap that explains the final charge transfer interaction inside the molecule, electronegativity (χ), chemical potential (μ), global hardness (η), global softness (σ), additional electronic charge (ΔN_{max}) and global electrophilicity index (ω) were calculated and listed in Table (8):

$\chi = -1/2\ (E_{LUMO}+E_{HOMO})$ $\mu = -\chi = 1/2\ (E_{LUMO}+E_{HOMO})$ $\eta = 1/2\ (E_{LUMO}-E_{HOMO})$

$\Delta N_{max} = -\mu / \eta$, $\omega = \mu^2/2\ \eta$, The inverse value of the global hardness is designed as the softness (σ); $\sigma = 1/\eta$. Also the calculated energy parameters of the free ligands and their complexes are cited in Table (9).

Table 8: The calculated quantum chemical parameters of free ligands and their complexes

Compound	HOMO(ev)	LUMO(ev)	ΔE (ev)	H	Σ	μ	X	Ω	ΔN_{max}
1	-0.17673	-0.07165	0.10508	0.05254	19.03312	-0.12419	0.124186	0.146766	2.363647
2	-0.17978	-0.07454	0.10524	0.05262	19.00418	-0.12716	0.12716	0.153646	2.416572
3	-0.17013	-0.07018	0.099947	0.049974	20.01061	-0.12015	0.120154	0.144445	2.404344
Cu complex	-0.14287	-0.08147	0.061399	0.0307	32.57382	-0.11217	0.112173	0.204933	3.653887
Ni Complex	-0.12125	-0.09894	0.022306	0.011153	89.66197	-0.11009	0.110093	0.543373	9.871156
Fe complex	-0.10989	-0.09196	0.017929	0.008965	111.5511	-0.10092	0.100921	0.568071	11.25779

Table 9: The calculated energy parameters of the free ligands and their complexes

Comp.	Energy components (Kcal/mol)						Binding energy (Kcal/mol) ($x10^3$)
	Sum of atomic energies ($x10^5$)	Kinetic energy ($x10^5$)	Electrostatic energy ($x10^2$)	Exchange-correlationenergy ($x10^2$)	Spin polarization energy ($x10^2$)	Total energy ($x10^5$)	
1	-2.973	$-3.165X10^3$	-4.28	7.322	7.045	-2.995	-2.157
2	-3.561	$-4.584X10^3$	1.80	9.01	9.011	-3.587	-2.602
3	-7.877	$-9.169\ X10^3$	2.80	2.028	17.58	-7.928	-5.102
Cu^{2+}	-13.199	$-7.820X10^3$	-53.57	3.159	23.53	-13.276	-7.663
Ni^{2+}	-13.017	$-6.767X10^3$	-65.75	3.229	23.77	-13.107	-7.735
Fe^{3+}	-12.702	$-7.486X10^3$	-59.41	3.275	23.98	-12.779	-7.754

IV. CONCLUSION

Two series of mixed ligand complexes were prepared and characterized using the following techniques: elemental analysis, Molar conductivity, thermal analysis, FTIR spectra, electronic absorption spectra and ESR spectra.

The first group concerning the Fe (III), Ni (II), Cu (II) and Zr (IV) metal ions with levofloxacin (Levo) as primary ligand and 8-hydroxyquinoline (Oxin) as secondary ligand while in the second group levofloxacin was kept as primary ligand but 1,10-phenanthroline (Phen) was used as secondary ligand.

The study showed that Levo bonded to metal ion through OO fashion acting as monobasic bidentate ligand, Oxin bonded through ON fashion acting also as monobasic bidentate ligand while Phen bonded in NN fashion acting as neutral bidentate ligand.

The antibacterial activity of the mixed ligand complexes was tested against two gram-positive (*Streptococcus pyogenes* and *Staphylococcus epidermidis*) and Gram – negative bacteria (*Proteus vulgaris* and *Klebsiella pneumonia)* where it was found that the complexes exhibited either comparable or increased antibacterial profile in comparison to the parent drug.

The cytotoxic activities of some selected mixed ligands complexes were tested against HEPG2 cell line and compared to that of Vinblastine as a standard drug. The complexes under study are considered as moderately active except for ZrO complexes which was found to have weak activity with IC_{50} values equal 13.55 and 14.20 μg/ml for complexes with Levo - Oxin and Levo – Phen, respectively.

A group of measurements involving $DMOL^3$ program in materials studio package using Density Function Theory (DFT) were applied. The Molecular modeling, Total density, Deformation density and 3D plots frontier orbital energies using DFT method for free ligands and their complexes were determined. Also the quantum chemical parameters and the energy parameters of the free ligands and their complexes were calculated.

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