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Research Article

Metal Complexes For Cancer

Experimental and Insilico Evaluations of Transition Metal Complexes with L-Histidine (N, N Donor) Schiff Base Ligand and Its Biological Applications in Ovarian and Melanoma Cancer Cell Lines

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Abstract: Schiff base metal complexes are essential in modern coordination chemistry. It owns modern structural features and finds several uses in various fields, including pharmaceuticals, agriculture, dyeing, polymer science, food packaging, O_2 detection, various biological systems, catalysis, and birth control. The Chemistry of Schiff base ligands and their metal complex increased, encompassing a wide range of organometallic compounds and bio-inorganic chemistry. Schiff bases belong to the class of organic compounds regarded as particularly significant. They have been investigated extensively in the past few years and have attracted much attention due to their appealing chemical and physical features. They are versatile and have a broader range of applications, such as anti-cancer, anti-fungal, anti-microbial, antiulcer, anti-tumor, anti-diabetic, herbicidal, anti-proliferative, and anti-inflammatory actions. The aim and objective of our research are to synthesize a series of five new Zn (II), Ni (II), Cu (II), Mn (II), and Co (III) of bis-3-(4.5-Dihydro-3 H-imidazol-4-yl)-2-[(4-methoxy-benzylidine)-amino]-propionic acid Schiff base metal complexes and to characterize those complexes by elemental analysis, UV-Visible, FT- IR, and Mass spectral studies. Bis-3-(4.5-Dihydro-3 H-imidazol-4-yl)-2-[(4-methoxy-benzylidine)-amino]-propionic acid ligand coordinates to metal ions as bidentate N and O donors. Biological studies of the synthesized Schiff base complex have also been investigated by anti-microbial, radical scavenging, docking study, anticancer analysis, and cytotoxic studies. Based on the analytical and spectral results, tetrahedral geometry has been tentatively proposed for all complexes except that of cobalt. The antioxidant studies of the Schiff base ligand and Schiff base complexes exhibit significant free radical scavenging activity against DPPH. *Invitro* cytotoxic activity of the Schiff complexes was evaluated against the over3 (ovarian) and melanoma cell lines (MCF14). The docking studies have been carried out using BSA protein and DNA biomolecules with synthesized metal complexes. Antimicrobial studies demonstrated the effectiveness against gram-positive and gram-negative bacteria.

Keywords: L-Histidine, Anisaldehyde, Molecular Docking, Anti-cancer activity, Schiff base, Ligand, DPPH Radical Scavenging Activity, Anti-microbial activity, Spectral Studies.

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1. INTRODUCTION

Coordination complexes have gained importance in recent years due to their technological applications.[1,2] Enhancement of duration of action[3,4], particularly long-acting drugs, is being developed. These properties directed the researchers to appraise the impact of metal-based compounds in treating several diseases, including various types of cancers in humans. Schiff base (named after Hugo Schiff) is a compound with the general structure $R^1R^2C=NR^3$. The term is often synonymous with azomethine, which refers specifically to secondary aldimines (i.e., R−CH=NR' where R' ≠ H.) Schiff bases can be synthesized from an aliphatic or aromatic amine and a carbonyl compound by nucleophilic addition forming a hemiaminal, followed by dehydration to generate an imine. Schiff bases are generally used in coordination because of their great potential to form solid complexes with numerous metallic ions. Transition metal complexes derived from bidentate Schiff base ligands have recently received much attention. The transition metals are necessary for the proper functioning of living organisms and hold great promise as potential drugs[5]. The coordination complexes of nitrogen donor ligands are a contentious issue. Complexes made by 3d metals with bidentate ligands with two nitrogens have received much attention in this field[6,7]. Schiff bases are a type of ligand that is important in coordination chemistry. For the past few decades, the applications of transition steel complexes with N and O-donor ligands have drawn significant interest among researchers. The practice of N, O-donor ligands via Schiff base condensation reaction is widely applied by scientists. Understanding the complex biological process can be aided by investigating various Schiff base complexes' structural and binding properties. Schiff bases derived from anisaldehyde are known for their unique ligation properties and broad applications [8-10]. Several details are available about the various metal complexes derived from SBs which are well known for their applications in the food and dye industry, analytical chemistry, catalysis, polymers, antifertility, agrochemical, anti-inflammatory activity, antiradical activities, and biological systems as enzymatic agents. Several reviews have established their antimicrobial, anti-bacterial, anti-fungal, anti-tumor, and cytotoxic activities. The complexation of amino acid with metal ions and its functionality enlarges the selective effect of DNA binding and a few different therapeutic targets. It also gives key recognition for synthetic nuclease activity on the molecules. Amino acids are biologically important natural molecules that play an essential role in the production of proteins and are intermediates in metabolism. All the amino acids having one-of-a-kind functional groups, including amine and carboxylic acid agencies, can act as potential donor ligands to shape solid complexes with steel ions. The amino acid derivatives of Schiff base complexes play an essential role in mutating the gene expression due to their binding capacity with DNA double helix. Among various amino acids, L-histidine is one of the important amino acids for human vitamins, and its miles have an imidazole ring. Schiff bases derived from L-Histidine are highly attractive for forming metal complexes, bestowing to the literature[11]. The interaction of these donor ligands and metal ions results in complexes of varying geometries, and a literature search indicates that these complexes may be more physiologically active. Because of their broad biological activity, Schiff bases and their metal complexes have received much attention in recent years[12,13]. We aim to synthesize Schiff's base ligand derived from anisaldehyde and L-Histidine. The newly prepared Schiff's base ligand is bis-3-(4.5-Dihydro-3 H-imidazol-4-yl)-2-[(4-methoxy-benzylidine)-amino]. In light of this, propionic acid Schiff base (L) was created to follow the previous work on transition metal complexes with Schiff bases[14,15]. Schiff base ligands having L-Histidine and complexation with Cu (II), Co (III), Ni (II), Mn (II), and Zn (II) metallic ions were characterized by diverse spectral techniques like elemental analysis, FT-IR, UV-Vis, and Mass spectrum[16-18]. The structural elucidation of the synthesized metal complexes and the theoretical interactions of metal complexes with DNA and BSA protein molecules were carried out using docking stimulation, which helps predict the interaction mechanism. The binding interactions help to study the biological activities like antimicrobial activity, DPPH activity, anticancer activity, and cytotoxic study with the newly synthesized Schiff's base compounds. The antimicrobial activities of the compounds were evaluated in opposition to Gram-advantageous and Gram-poor bacteria. The antioxidant studies indicated that the compounds had good antioxidant properties. The cancer studies were carried out using Ovar-3 and MCF-14 cell line cells, which prove that the complexes have great potential for cancer cells.

2. EXPERIMENTAL

2.1. *Materials and reagents*

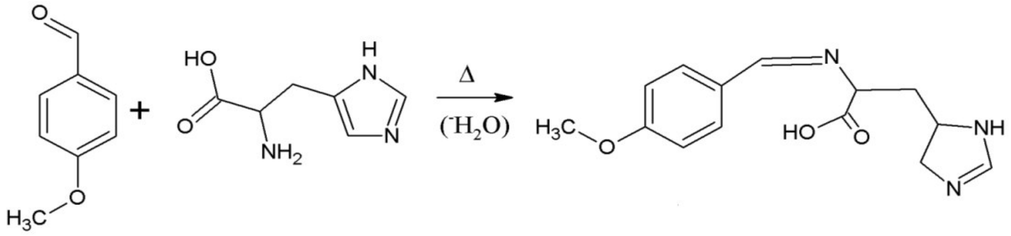
All the reagents were chemically pure and of AR grade. The solvents were purified and dried according to standard procedures.

2.2. *Physical measurements*

The electronic spectra of metal complexes in DMF were recorded using a Perkin-Elmer UV Win Lab Spectrophotometer at Periyar University in Salem, Tamil Nadu, India. The IR spectra of the Schiff base ligand and metal complexes were recorded using KBr pellets using a Perkin-Elmer FT-IR spectrophotometer in the range 4000–400 Cm^{-1} at Sastra University, Thanjavur, Tamil Nādu, India. Elemental analyses were performed at Karunya University, Coimbatore, using a PerkinElmer model 240 elemental analyzer. Using standard methods, the metal content was determined gravimetrically. Conductivity measurements were performed in anhydrous DMF using a Systronics model 305 Conductivity Bridge (Coimbatore, Tamil Nadu, India). On a capillary melting point apparatus, the melting points of the ligand and complexes were measured in open capillaries. The mass spectra were collected using a 410 Prostar Binary LC with a Mass spectrometer with 500 MS IT PDA detectors.

2.3. *Synthesis of the Schiff Base Ligand*

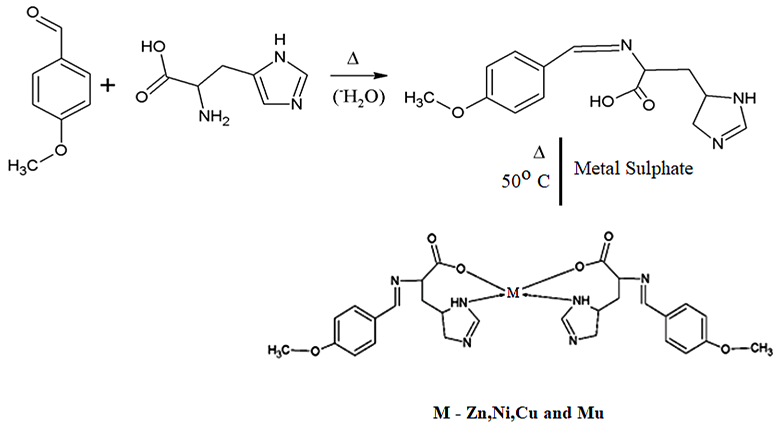
The Schiff base ligand was prepared by condensation of equimolar amounts of anisaldehyde (1 mmol) and L-Histidine (1 mmol) in hot water and ethanol (1:1) under reflux for 5 hr. After the reaction mixture was cooled to room temperature, the dark brown solid was separated, filtered off, washed with ethanol, and dried in a vacuum. The general structure of the Schiff base ligand used in this study is given below (Scheme 1)



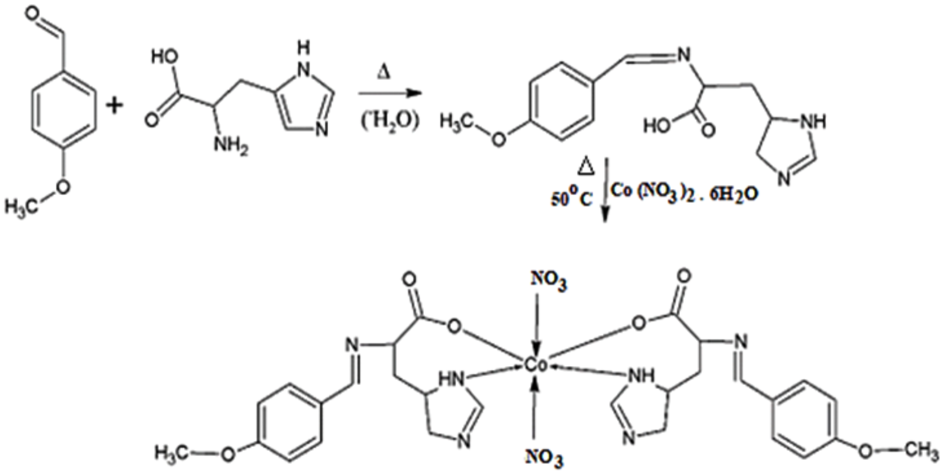
Scheme 1. Synthesis of 3-(4,5-dihydro-3 H-imidazole-4-yl)-2-[(4-methoxy-benzylidine)-amino]-propionic acid ($C_{14}H_{16}N_3O_3$)

2.4. *Synthesis of new Metal (II / III) Schiff base Complexes*

All the new metal complexes were prepared according to the following general procedure. Ethanol: hot water solution (50/50) of (20ml) Schiff base ligand (1 mmol) was added to a hot aqueous ethanolic solution of (20ml) sulfates of Zn (II), Ni (II), Cu (II), Mn (II) and Co (III) (1 mmol). The mixture was refluxed for four hours, and the precipitate formed was separated, filtered and washed with acetone, and dried in a vacuum. (Scheme 2 and 3).



Scheme 2. Synthesis of bis-3-(4.5-Dihydro-3 H-imidazole-4-yl)-2- [(4-methoxy- benzylidine)-amino]-propionic acid metal complexes (M- Zn (II), Ni (II), Cu (II), and Mn (II),)



Scheme 3. Synthesis of bis-3-(4.5-Dihydro-3 H-imidazole-4-yl)-2-[(4-methoxy-benzylidine)-amino]-propionic acid Cobalt (III) metal complex

2.5. *Antimicrobial Activity*

Schiff base metal complexes revealed their antimicrobial activity and were found to be active inhibitors of different bacterial strains [27,36]. In this study, the newly synthesized Schiff base ligands and metal complexes (Zn (II), Ni (II), Cu (II), Mn (II), and Co (III)) were tested against two bacterial strains: gram-positive bacilli (Staphylococcus Aureus) and gram-negative bacilli (Escherichia Coli).

2.6. *DPPH Radical Scavenging Activity*

Using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay described by[19-26], different concentrations of Schiff bases ligand and the synthesized Schiff base metal complexes were analyzed in vitro. DPPH$^•$ (2, 2-diphenyl-1-picrylhydrazyl) is a purple-colored stable free radical. The basic idea behind this assay is that when DPPH$^•$ is scavenged with antioxidants (AH), its purple color is reduced to yellow (DPPH)[28]. Schiff base ligands and their synthesized metal complexes at different concentrations (25, 50, 100, 200, and 400 M) were assorted with an alcoholic solution comprising 85 M DPPH radical. After 30 minutes of incubation at room temperature, the absorption maximum was measured at 518 nanometers in a UV spectrophotometer. At the same absorptions as the drugs, ascorbic acid was cast off as a constructive control [29]. The percentage of inhibition of the drugs as well as ascorbic acid, was calculated using the following equation:

$$\text{Effect of DPPH Inhibition (\%)} = [(A_c - A_s)/A\ \text{Control}] \times 100$$

where
Ac = Absorbance value of the control
As = Absorbance value of the sample.

2.7. *Molecular Docking*

The molecular action situation is a programme used in molecular docking[30] (MOE). The crystal arrangements of cancer object proteins from ovarian and melanoma biomolecule receptors (PDB codes: 6E3X (DNA) and 3V03 (BSA)) were downloaded from the Protein Information Bank (http://www.rcsb.org/pdb/, accessed on April 25, 2022). Based on previous research, these types were chosen[31]. MOE was cast-off to conclude the collaboration methods among the protein receptor and complexes.

2.8. *Anti-Cancer Activity*

Schiff base metal complexes show significant anticancer activity. Based on the literature survey, Schiff base ligands have been reported to have superior anti-cancer activity against cancer cell lines [32]. Photodynamic therapy is another application of Schiff base metal complexes in medicinal chemistry. Transition metals such as Ni, Zn, Co, Cu, and Mn are among the most studied in cutting-edge coordination of light-sensitive arrangements such as phthalocyanines. Metal centers through photo-active N-donor ligands cast-off in PDT, like porphyrins and phthalocyanines, received extensive attention in modern days and are not discussed in this review. Metal-phthalocyanine complexes, in general, have less toxicity and more chemical and photochemical permanence. Despite the fact that chemo toxicity is occasionally related to selected metal byproducts, in any case, lesser cytotoxicity is commonly required for genetically used photosensitizers. As the mechanism of action does not rely on the metal's biological involvement, the phototoxicity of the described complexes is commonly very highly active (IC50 standards in the micro-nano molar range). Therefore, it cannot stand to be associated with the harmfulness shown by the additional families of metal complexes discussed [33,34].

2.9. *Cytotoxic activity*

Schiff base metal complexes are widely used for their cytotoxic activity[35,38,40]. The cytotoxicity of the MCF7-Human breast adenocarcinoma cell line (from NCCS, Pune) and Ovra3 were analyzed by MTT assay. To maintain the high glucose - (#AL111, Himedia) - MCF7, DMEM was used. About 25 cm^2 of the cell line was grown in DMEM accompanied by ten percent FBS, L-glutamine, sodium bicarbonate in tissue culture flasks and then antibiotic solution comprising Penicillin (100 g/mL), Streptomycin (100 g/mL), and Amphoteric in B (2.5 g/mL) were added. Cultured cell lines were maintained in an incubator moistened with 5% CO_2 at 37 °C for two days. The two days long-standing confluent monolayer of cells which deferred in 10% growth medium, were seeded in 96 well tissue culture dish (5104 cells/well) and kept warm at 37 °C in an incubator moistened with 5% CO_2[33,34]. Using a cyclomixer, 1 milligram of complexes was precisely weighed and liquefied in 1 mL of DMEM. The complexes were filtered over a 0.22 m Millipore syringe strainer to confirm sterility. After 24 hours, the growth medium was disinterested, and 5% DMEM was successively adulterated five times in 500 L of 5% DMEM, with concentrations of 100, 50, 25, 12.5, and 6.25 g added three times to the corresponding wells and kept warm at 37 °C in an incubator moistened with 5% CO_2. Normal cells were not treated, and the complete dish was examined under the reversed segment difference tissue culture microscope after 24h. Every detectable change in cell morphology was observed as cytotoxic gauges. 15 mg of MTTs were dissolved in 3 mL of PBS before filtration and sanitized. The contents of the wells were removed after 24 hours of incubation, and 30 L of reconstructed MTTs were added to entire tests and cell control wells. The dish was gradually stunned, and the plate was kept warm at 37 °C in an incubator moistened with 5% CO_2 for 4 hours. Following the incubation period, the liquid was detached, and 100 L of MTT solubilization solution in DMSO was added to the wells, which were gently assorted by pipetting up and down to dissolve the formazan crystals. A microplate reader was used to measure the absorbance values at 540 nm, and then the cell capability (percentage) was intended.

3. STATISTICAL ANALYSIS

Statistical analysis of Schiff base ligands and their synthesized metal complexes at different concentrations (25, 50, 100, 200, and 400 M) were assorted with an alcoholic solution comprising 85 M DPPH radical. Statistical Data are presented as the mean ± standard deviation.

4. RESULTS AND DISCUSSION

Tetra-coordinated Zn (II), Ni (II), Cu (II), Mn (II), and octa-coordinated Co (III) Schiff base complexes of general formula [MSO_4 (L). L= bis-3-(4.5-Dihydro-3 H-imidazol-4-yl)-2-[(4-methoxy-benzylidine)-amino]-propionic acid] were synthesized in quantitative yield from the reaction of MSO_4 with Schiff base ligand in hot aqueous ethanolic solution in 1:2 molar ratio (Scheme 2 and Scheme 3). In all the reactions, it was observed that the Schiff base behaved as mono-negative bidentate ligands. Table 1 displays the compositions and colors of the complexes supported by elemental analysis[37].

Table 1: Analytical statistics and molar conductance ethics for Schiff base ligand and synthesized metal complexes Zn (II), Ni (II), Cu (II), Mn (II), and Co. (III).

S.No	Ligand/Metal complexes	Elemental Analysis				Melting Points °C	Color	Molar Conductance Ω^{-1} cm^{2} mol^{-1}
		C	N	O	M			
1	$C_{14}H_{16}N_3O_3$ [L]	36.19	8.76	4.14	-	255	Brown	--
2	$C_{28}H_{32}ZnN_6O_6$ [ZnL_2]	52.38	17.52	8.28	6.51	243	White	12.9
3	$C_{28}H_{32}NiN_6O_6$ [NiL_2]	51.43	17.42	8.43	6.23	238	Green	12.4
4	$C_{28}H_{32}CuN_6O_6$ [CuL_2]	54.87	18.12	7.98	6.17	241	Blue	13.6
5	$C_{28}H_{32}MnN_6O_6$ [MnL_2]	53.94	17.36	7.34	6.85	234	White	12.8
6	$C_{28}H_{32}CoN_8O_{12}$ [CoL_2]	55.24	19.39	9.47	7.41	248	Pink	13.5

All the complexes were analyzed for C, N, O, and M; the results are presented in the above table. Ligand and all the metal complexes agree with the theoretical data of C, N, O, and M analysis. Melting point, color, and molar conductance have been determined for all the metal complexes in the table. The conductivities of the complexes were dignified in DMSO using complex solutions containing 10^{-3}M. The molar conductivities of the transition metal compounds were 12.9 (Zn), 12.4 (Ni), 13.6 (Cu), 12.8 (Mn), and 13.5 (Co) Ω^{-1} Cm^{2} mol$^{-1,}$ respectively. Their low conductance values show that the complexes are nonionic.

4.1. UV-Visible Spectral Studies

The UV-Visible Spectrum of the synthesized metal complexes has been recorded in dimethylsulphoxide, as shown in table 2. The result shows two individual bands in the 300-400 nm and 600-700 nm ranges, which are attributed to the DPPP and APY moieties, respectively. However, the visible region of the Spectrum is distinct groups accredited to d-d transitions intended for Zn (II), Ni (II), Cu (II), Mn (II), and Co (III). The Zn (II) band is located in the 307.5 and 376 nm regions, dispersed to the d-d transition. This group is characteristic of tetrahedral Zn (II) metal complexes. Fig.1. shows that the d-d groups in the visible spectra of Ni (II) (313, 386.8, and 660.9), Cu (II) (253.4), Mn (II) (309.4 an383.2), and Co (III) complexes are observed at (309.44 and 379.5), as expected for tetrahedral Ni (II), Cu (II), Mn (II), as well as octahedral Co (III).

UV-Visible Spectrum of synthesized complexes

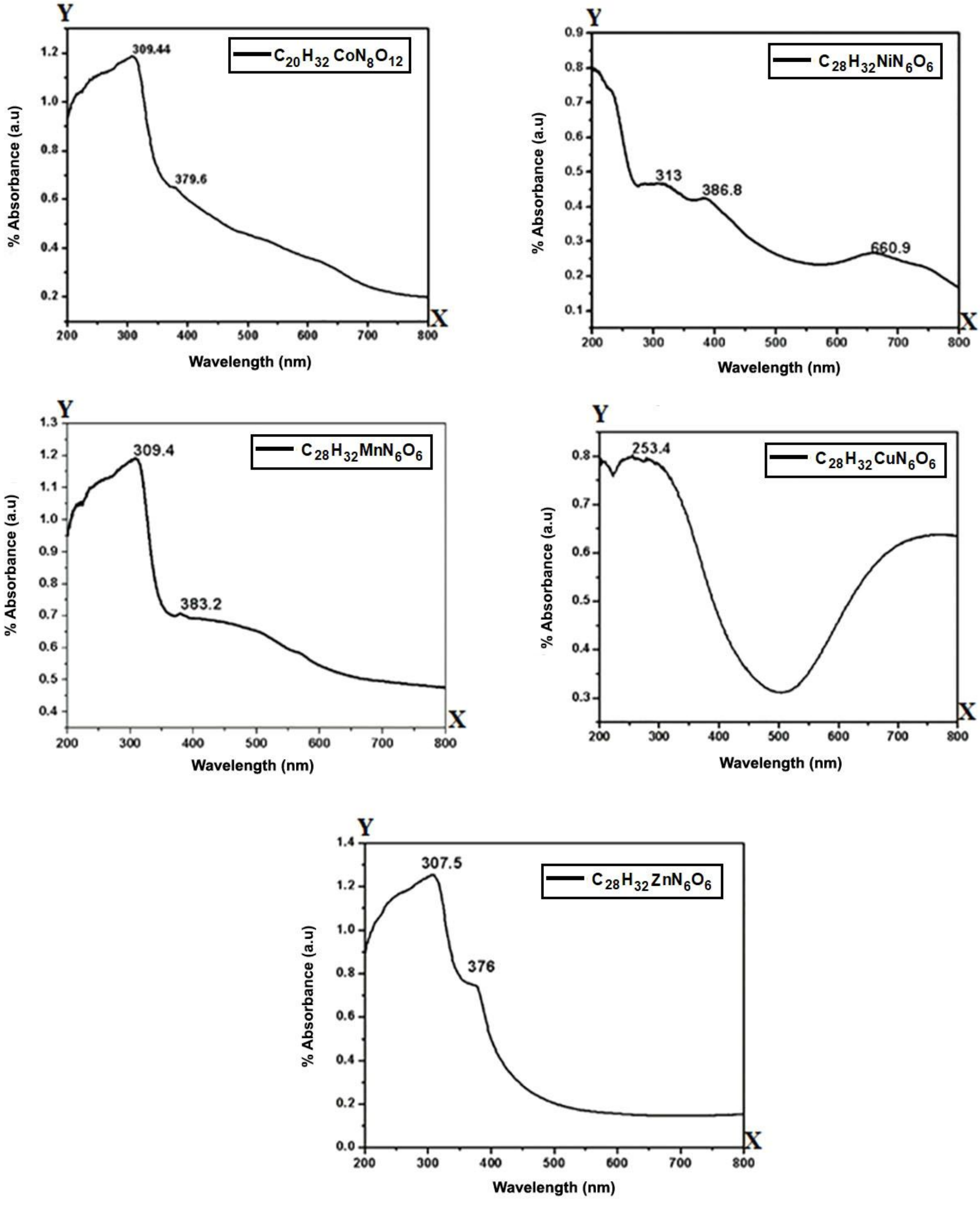


Fig.1.Shows the UV-Visible Spectrum of Schiff base metal complexes.

Table 2: Shows the UV-Visible Spectrum of Schiff base ligands and metal complexes with Schiff base ligands in DMSO.

S.No	Compounds	Wavelength Range (nm)	Assigned transition
1	$C_{14}H_{16}N_3O_3$ [L]	376 and 342	π → π*, n → π*
2	$C_{28}H_{32}ZnN_6O_6$ [ZnL$_2$]	307.5 and 376	π→π*, *n*→π* $^2B_2 \rightarrow {}^2A_1$ L→Zn(C.T)
3	$C_{28}H_{32}NiN_6O_6$ [NiL$_2$]	313, 386.8 and 660.9	π→π*, *n*→π* $^4A_2 \rightarrow {}^4T_2(F)$, $^4A_2 \rightarrow {}^4T_1(F)$, $^4A_2 \rightarrow {}^4T_1$ L→Ni(C.T)
4	$C_{28}H_{32}CuN_6O_6$ [CuL$_2$]	253.4	π→π*, *n*→π* $^1A_{1}g \rightarrow {}^1B_{1}g$, L→Cu(C.T)

5	$C_{28}H_{32}MnN_6O_6$ [MnL_2]	309.4 and 38 3.2	$\pi\rightarrow\pi^*$, $n\rightarrow\pi^*$ ${}^1A_1g\rightarrow{}^1B_1g$, ${}^1A_1g\rightarrow{}^1Eg$ L→Mn(C.T)
6	$C_{28}H_{32}CoN_8O_{12}$ [CoL_2]	309.44 and 379.5	$\pi\rightarrow\pi^*$, $n\rightarrow\pi^*$ ${}^1A_1g\rightarrow{}^3B_1g$, ${}^1A_1g\rightarrow{}^1B_1g$, ${}^1A1g\rightarrow1Eg$, L→Co(C.T)

Electronic spectra of the complexes are shown in fig.2, and the corresponding λmax values are presented in the respective table. The cobalt complexes are diamagnetic and must be in an octahedral environment with a CoN_6 chromophore. The intra ligand $\pi\rightarrow\pi^*$ transition is mainly associated with the N=O group, an intense band in the 300nm-379nm region is due to either metal ligand or the charge transfer from ligand to metal. Similarly, the same transition is possible for Zn (II), Ni (II), Cu (II), and Mn (II). In the case of the Ni complex, the bands at 386.8 nm, 660.9 nm, and the band observed below 350 nm are due to intra ligand $\pi\rightarrow\pi^*$ mainly associated with the Ni-N=O group. Several authors ascribe intense bands in the 350-425 nm region to either metal-ligand or ligand-metal charge transfer bands. Bands observed in the 425-550 nm region are due to the d-d transition.

4.2. *FT-IR Spectra*

Newly synthesized Schiff base metal complexes were characterized using an FTIR spectrophotometer[39]. Table 3 shows the FT-IR Spectrum of Schiff base ligands and metal complexes. A comparison of the FT-IR spectra of the synthesized Schiff base ligand as well as metal complexes Zn (II), Ni (II), Cu (II), Mn (II), and Co (III) reveals interesting metal-ligand (M-L) interactions. The C-H band, which appears at 1415 Cm^{-1} in the Spectrum of the Schiff base ligand, is moved to a lower wave number (1415–1490 Cm^{-1}), showing the distribution of this group in the attachment through metal ions. According to the FT-IR spectra, in all complexes, the stretching frequencies of (NH_2 and NH) are detected at 3425.36 cm^{-1} to 3256.39 Cm^{-1}; the Spectrum almost does not shift, representing that this assembly does not contribute to the coordination. The stretching vibration of the L - histidine group, which is located at 1640 cm1 (v) C = C 1415.56 Cm^{-1} C = N 1640.26 Cm^{-1} in the Schiff base ligand, displays a significant transferal to a wave number (1640.26 – 1591.75) and (1490–1415 Cm^{-1}) in the entire complex. The C-N stretching in the Schiff base ligand's ring bands is moved to lesser wave numbers in the 1090.20–1048.21 Cm^{-1} range. (C-NH_2) occurs at 1090.20 cm1 in the FT-IR Spectrum of the Schiff base ligand, with no shift in the spectra of the complexes. These findings approve that the L- histidine groups are mono-dentate coordinated with metal ions via the nitrogen atom. The sturdy band for the M-N pulsation of Schiff base ligand-free forms shifts from 505.83 cm^1 to a lower wave number (555.32 –505.83 cm1). The bands between 2348–2331 and 3362–3351 cm^{-1} are caused by phenyl and CH_2 group absorption. These discussions conclude that metal complexes coordinate to transition metal ions in a bidentate ligand via nitrogen atoms. In all metal complexes, v(OH) in the lattice H_2O can be assigned an extensive spread band with standard intensity in 3465–3454 Cm^{-1}. The FT - IR spectra of the metal complexes show a band at 999.33–965.43 cm^{-1} assigned to (NO_3), indicating the presence of a coordinated nitro group in the cobalt complex. Vibration bands appear at 555.32–503.20 Cm^{-1} and 487.92–481.76 Cm^{-1}, metal-oxygen, and M-nitrogen, respectively, as presented in Fig.2.

Table 3 shows the IR spectrum of Schiff base ligands and synthesized metal complexes with Schiff base ligands.

S.NO.	Compounds	Wave Number cm^{-1}			
		C=N	C-O	M-O	M-N
1	$C_{14}H_{16}N_3O_3$ [L]	1640.26	1145.89	-	-
2	$C_{28}H_{32}ZnN_6O_6$ [ZnL_2]	1594.85	1090.20	505.83	485.45
3	$C_{28}H_{32}NiN_6O_6$ [NiL_2]	1635.86	1136.28	515.84	487.98
4	$C_{28}H_{32}CuN_6O_6$ [CuL_2]	1602.34	1120.65	503.20	481.76
5	$C_{28}H_{32}MnN_6O_6$[MnL_2]	1594.85	1048.21	518.40	484.46
6	$C_{28}H_{32}CoN_8O_{12}$[CoL_2]	1591.75	1120.40	555.32	488.12

All the Spectrum shows two characteristic bands due to V_{C-N} and $V_{C=O}$ vibrational modes. The V_{C-N} is used to measure the contribution of C-N stretching to the structure of the complex. Energy associated with the C-O and C-N stretching in all the complex lies intermediate between the stretching frequency region assigned to single carbon bond 1050cm^{-1}- 1150cm^{-1} and double bond 1590cm^{-1} -1650cm^{-1}. As shown in the table, V_{C-O}, $V_{M=O}$, and V_{M-N} stretching vibrations were observed for all the metal Schiff base complexes.

FTIR Spectrum of synthesized Schiff base complexes

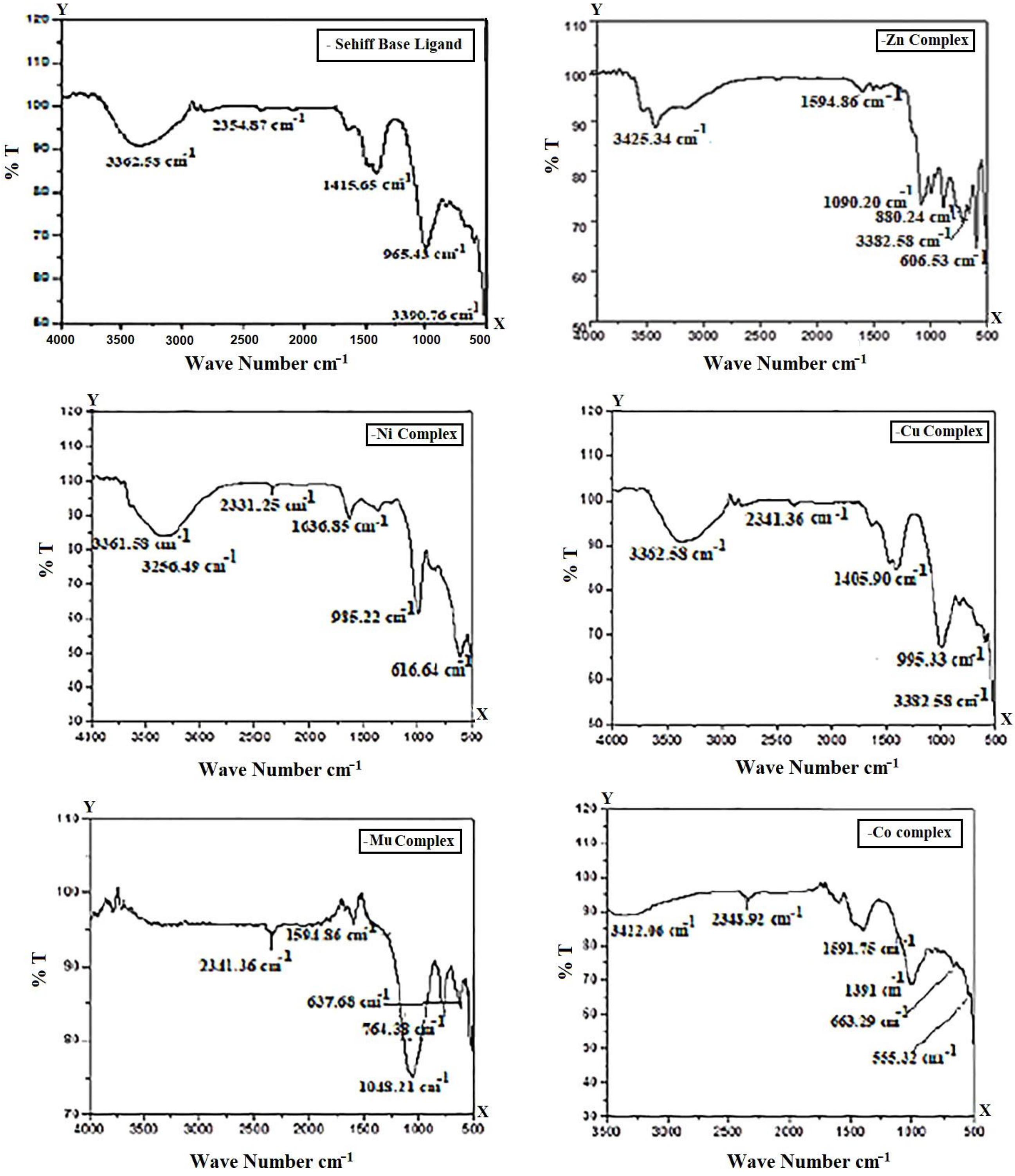


Fig.2. IR spectral data of ligand Schiff base ligand and Zn (II), Ni (II), Cu (II), Mn (II), and Co (III) metal complexes.

4.3. *Mass spectra*

The mass spectral data of the Schiff base ligand ($C_{14}H_{16}N_3O_3$) shows a molecular ion peak at m/z 307, which is consistent with the suggested molecular formula of the Schiff base. The m/z 136 and 122 mass fragmentation peaks are caused by the loss of [C_4H_4O] and [C_6H_6N] moieties commencing the molecular ion peaks. The Mass Spectrum of $C_{28}H_{32}ZnN_6O_6$ [ZnL_2] displays a molecular ion peak on m/z 590 due to $[Zn(L)_2]^+$, which is consistent with the complex's suggested formula. The fragments $(C_9H_{11}NO)^+$ and $[Zn(C_6H_4COH)NH]^+$ may be responsible for the additional peaks at m/z tenets 155 and 131. The zinc metal connected to the donor atoms of the ligand may be responsible for the base peak at m/z 113. At m/z 264, the molecular ion peak of $C_{28}H_{32}NiN_6O_6$ [NiL_2] and $C_{28}H_{32}CuN_6O_6$ [CuL_2] agrees well with the 1:2 (M: L) proportion stoichiometry. At m/z 366, the molecular ion peak of $C_{28}H_{32}MnN_6O_6$ [MnL_2] agrees well with the 1:2 (M: L) ratio stoichiometry, while that of C28H32CoN8O12 [CoL_2] agrees at m/z 266. The fundamental data for the newly synthesized compounds agrees with the investigational mass spectral values (Fig.3).

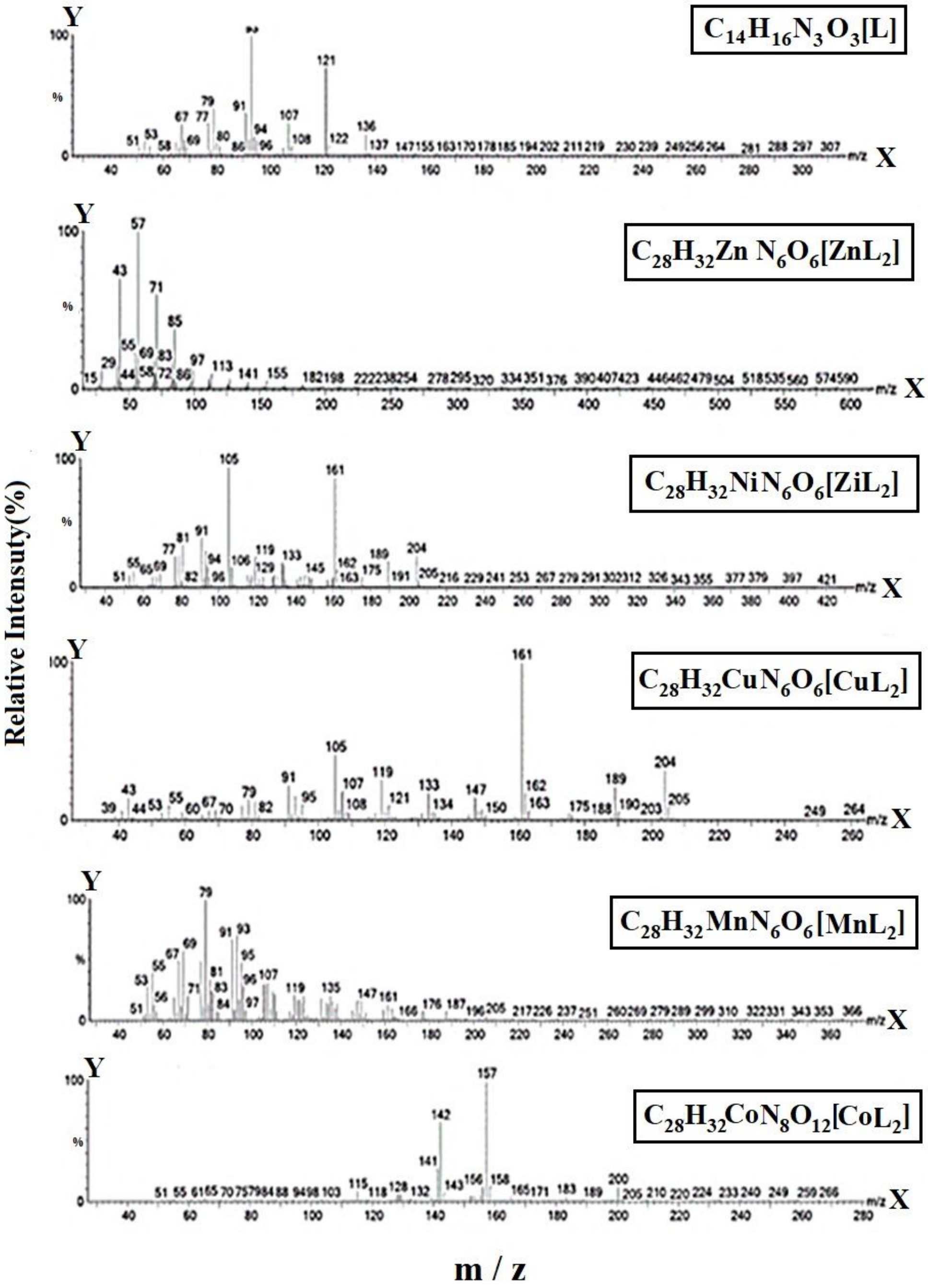


Fig.3. Mass spectral data of Schiff base ligand and Zn (II), Ni (II), Cu (II), Mn (II), and Co (III) metal complexes.

4.4. Biological Activity of Schiff base ligand and their metal complexes

Shebl, Magdy[44] (2014), in her study, focussed on the Schiff base and its copper (II) complexes and evaluated their antimicrobial activity against gram-positive bacteria (*Staphylococcus aureus*), gram-negative bacteria (*Escherichia coli*) and fungi (*Candida albicans* and *Aspergillus flavus*). So, this study was focused on the newly synthesized Schiff base ligand and their metal complexes of Zn (II), Ni (II), Cu (II), Mn (II), and Co (III). The results confirmed the in vitro antimicrobial activity in contradiction of various bacterial and fungal strains (Fig.4 and Fig.5). Gram-positive (Staphylococcus aureus) as well as gram-negative (Escherichia coli) bacilli were used. Gram-positive bacilli originated to be significantly extra penetrating to all tested excerpts, with considerably larger inhibition zones than gram-negative bacilli. A ruler measured the inhibiting zone around the wells in millimeters. Mamta Subhash et.al[42] (2022) reported the antimicrobial activity of the synthesized compounds against bacterial (*E. coli, B. subtilis*) and fungal strains (*C. albicans, F. oxysporum*). The results indicated that the macrocyclic Mn (II) complexes have more antimicrobial potential than macrocyclic ligands against tested pathogens.

Table 4. In vitro anti-bacterial (Gram-Positive) activities of Schiff Base metal complexes.

Organisms *Streptococcus*	$C_{28}H_{32}ZnN_6O_6$ [ZnL_2]	$C_{28}H_{32}NiN_6O_6$ [NiL_2]	$C_{28}H_{32}CuN_6O_6$ [CuL_2]	$C_{28}H_{32}MnN_6O_6$ [MnL_2]	$C_{28}H_{32}CoN_8O_{12}$ [CoL_2]
20 µl	0.5 ±0.3cm	0.6±0.4cm	0.6±0.3cm	0.4±0.2cm	0.5±0.2cm
50 µl	0.9±0.7cm	0.7± 0.5cm	0.8±0.6cm	0.7±0.4cm	0.7±0.5cm
100 µl	1.2±0.5cm	1.0±0.4cm	0.9±0.5cm	0.9± 0.6cm	0.9±0.7cm
Standard	1.5±0.3cm	1.4±0.5cm	1.0±0.4cm	1.1±0.5cm	1.2±0.4cm

The newly synthesized Schiff base complexes were screened for preliminary anti-bacterial activity against gram-positive strains of streptococcus by diffusion method in different concentrations of 20 µl, 50 µl, and 100 µl. These results show that all complexes are effective against bacterial strains. Inhibition of gram-positive bacteria by Zinc (II) and Nickel (II) complexes and other tested complexes are shown in table 4.

Table 5. In vitro anti-bacterial (Gram-Negative) activities of Schiff Base metal complexes.

Organisms E.Coli	$C_{28}H_{32}ZnN_6O_6$ [ZnL_2]	$C_{28}H_{32}NiN_6O_6$ [NiL_2]	$C_{28}H_{32}CuN_6O_6$ [CuL_2]	$C_{28}H_{32}MnN_6O_6$ [MnL_2]	$C_{28}H_{32}CoN_8O_{12}$ [CoL_2]
20 µl	0.3±0.2cm	0.4±0.2cm	0.5±0.3cm	0.5±0.4cm	0.2±0.1cm
50 µl	0.6±0.3cm	0.6±0.4cm	0.7±0.4cm	0.6±0.3cm	0.4±0.2cm
100 µl	1.0±0.5cm	0.9±0.4cm	1.0±0.5cm	1.2±0.6cm	0.9±0.4cm
Standard	1.3±0.5cm	1.0±0.4cm	1.0±0.5cm	1.6±0.4cm	1.1±0.6cm

Mohan RaoKollipara et al[43] (2020) reported the biological studies of Schiff base derivative complexes of ruthenium, rhodium, and iridium exhibiting efficient anti-bacterial activity against gram-positive and gram-negative bacteria. The newly synthesized Schiff base complexes were screened for preliminary anti-bacterial activity against gram-negative strains of E.Coli by diffusion method in different concentrations of 20 µl, 50 µl, and 100 µl. These results show that all complexes are effective against bacterial strains. In addition, manganese (II), Zinc (II), and Copper (II) complexes have a greater zone of inhibition in gram-negative bacteria than the further tested extract complexes, as shown in Table 5.

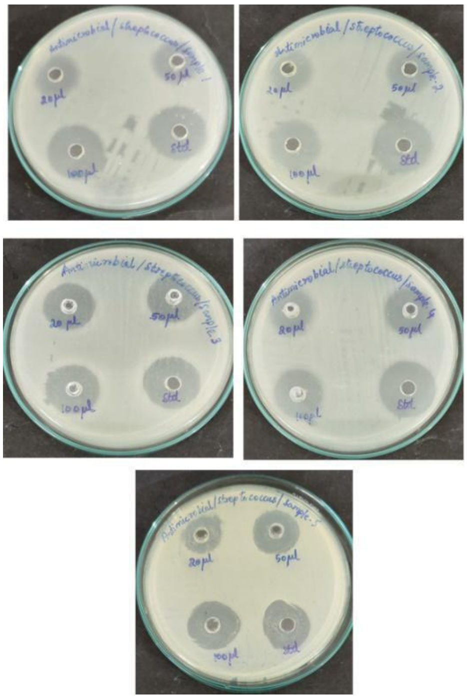


Fig.4. In vitro anti-bacterial Gram Positive (Streptococcus) activities of Schiff Base Metal Complexes (Zn (II), Ni (II), Cu (II), Mn (II), and Co (III))

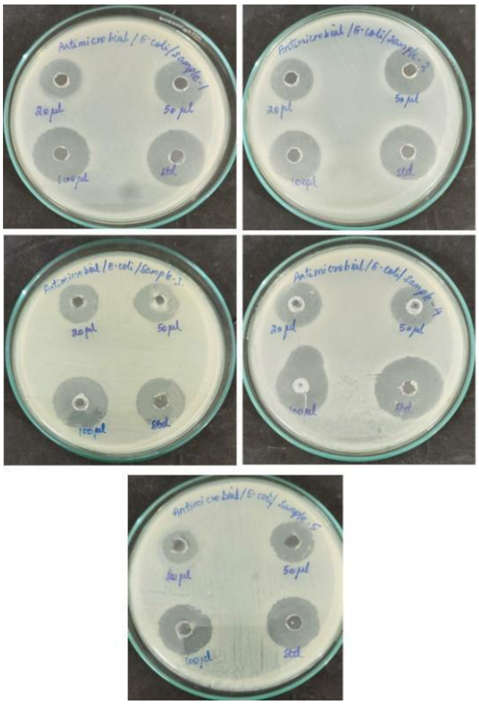


Fig.5. In vitro anti-bacterial Gram-Negative (E-Coli) activities of Schiff Base Metal Complexes (Zn (II), Ni (II), Cu (II), Mn (II), and Co (III))

4.5. DPPH radical scavenging activity

A.G.Bharathi Dileepan et al. [45] (2018) reported that the isatin-based macrocyclic Schiff base ligands show the radical scavenging potencies of the compounds, which was explored by employing DPPH, OH, and NO assays, in which ligand exhibited a highest inhibitory effect on the radicals (IC_{50} = 23.59 μM (DPPH), 26.14 μM (OH), 28.41 μM (NO). In this study, the Schiff base ligand and their metal complexes Zn (II), Ni (II), Cu (II), Mn (II), and Co (III) were associated with the well-known antioxidant ascorbic acid. According to the findings, Schiff base ligands and their metal complexes significantly scavenge DPPH (Fig.6). The percentage of scavenging activity increases significantly as the application of the compounds increases. The IC50 values for DPPH radicals with alcoholic solutions and their metal complexes were determined to be 229.29 ± 13.81M ($C_{14}H_{16}N_3O_3$), 358.31±6.051M (Std Ascorbic acid), 275.46±11.63M ($C_{28}H_{32}NiN_6O_6$), 302.67±10.63M ($C_{28}H_{32}CoN_8O_{12}$), 343.81±9.869M ($C_{28}H_{32}CuN_6O_6$), 328.05±10.11M ($C_{28}H_{32}ZnN_6O_6$) and 298.67±10.53M ($C_{28}H_{32}MnN_6O_6$) (Table.6). The free ligand is less effective than its metal complexes (Ni (II), Co (II), Cu (II), and Zn (II)) in decolorizing the pink color of the DPPH solution. Ikechukwu P. Ejidike et al. [46] (2016) reported that the scavenging ability of the Ru (III)-Schiff base complexes on DPPH radical can be ranked in the following order: [Ru(DEE)$Cl_2(H_2O)$] > [Ru(HME)$Cl_2(H_2O)$] > [Ru(DAE)$Cl_2(H_2O)$] > [Ru(MBE)$Cl_2(H_2O)$].

Table 6. **DPPH radical scavenging assay of Schiff base ligand and their metal complexes Ni (II), Co (III), Cu (II), Zn (II), Mn (II), as well as ascorbic acid % inhibition mean ± SEM, was cast-off to state the standards.**

S.No	Drugs	%Inhibition Mean (n=3)±SEM					IC50(μM) ±SEM
		12.5(μM)	25(μM)	50(μM)	100(μM)	200(μM)	
1	$C_{14}H_{16}N_3O_3$	1.42±0.71	4.77 ±1.16	10.86±1.65	19.76±1.27	35.13±1.79	229.29±13.81
2	Ascorbic acid	7.03±0.93	12.42±1.85	26.05±1.74	46.39±0.69	83.08±4.04	358.31±6.051
3	$C_{28}H_{32}NiN_6O_6$	3.18±0.73	7.93 ±1.24	15.76±1.86	28.28±1.28	58.33±1.90	275.46±11.63
4	$C_{28}H_{32}CoN_8O_{12}$	5.07±1.08	10.16±1.19	19.56±1.39	35.78±1.69	64.14±2.35	302.67±10.63
5	$C2_8H_{32}CuN_6O_6$	6.39±1.25	11.29±1.11	20.04±1.09	39.70±2.19	70.06±2.92	343.81±9.869
6	$C_{28}H_{32}ZnN_6O_6$	4.45±0.64	9.09 ±1.10	16.30±1.61	31.37±0.79	60.45±3.06	328.05±10.11
7	$C_{28}H_{32}MnN_6O_6$	4.09±1.06	9.16±1.13	18.56±1.30	33.78±1.64	62.14±2.37	298.67±10.53

DPPH Radical Scavenging assay of Schiff's base and their metal complexes are given in table 6. The antioxidant activity of the ligand and its metal Schiff's base complexes has been determined in vitro by the DPPH method, which indicated greater antioxidant activity against DPPH. Among the tested complexes, the Cu (II) complex had the highest scavenging activity (IC50=343.81±9.869M ($C_{28}H_{32}CuN_6O_6$).

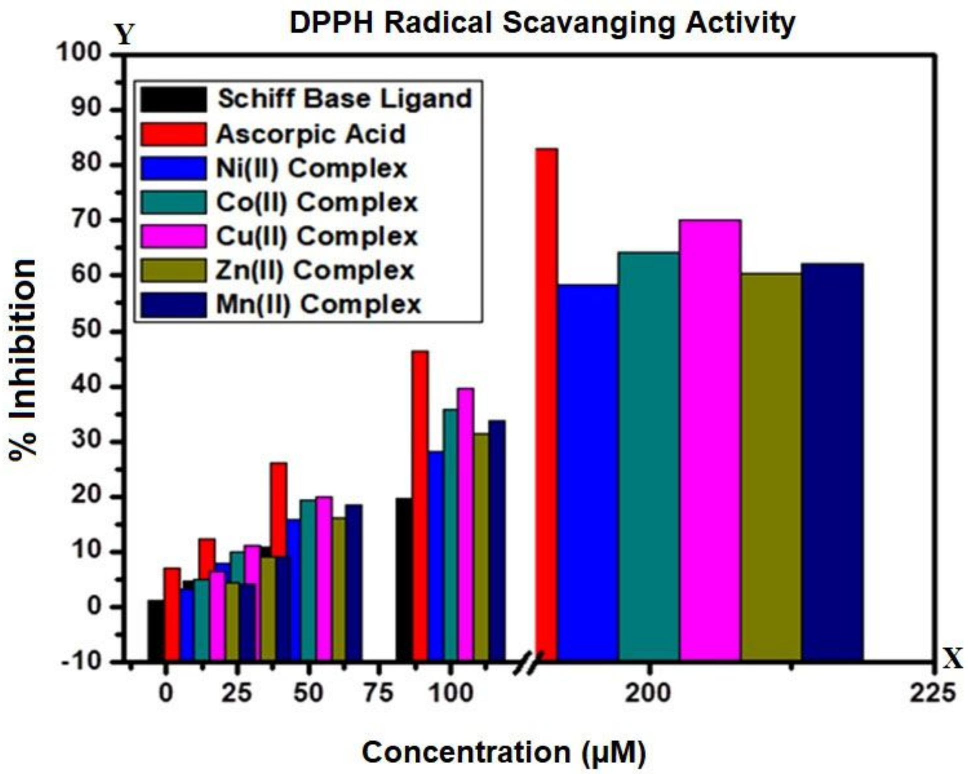


Fig. 6. DPPH radical scavenging assay of Schiff base ligand and their metal complexes Ni (II), Co (III), Cu (II), Zn (II), Mn (II), and ascorbic acid

4.6. *Molecular Docking Study*

A molecular docking study is a theoretical method used to examine drug-receptor communications. Ranjan-Mohapatra et al. [47] (2018) reported the docking analysis of the transition metal complexes with benzothiazole-based Schiff base ligands using the auto dock vina software. The study was carried out to understand the binding pattern of the investigated compounds towards target proteins CYP121 and CYP51 from mycobacterium tuberculosis. Shebl and Magdy[44] (2014) reported that the DNA binding affinity of copper (II) complexes to DNA *via* an intercalation binding mode with an intrinsic binding constant, K_b of 1.34×10^4 and 2.5×10^4 M^{-1}, respectively. Docking imitations on minor molecule drug applicants, known as ligands, and their object proteins exclusive for the human body are used to forecast the effectiveness of the ligand as a drug. After the docking replications, well-deducted protein–ligand complexes are created in investigational laboratories. They were simulating the connection of complexes with (M14) melanoma cancer cells (entry 6E3X (DNA) as well as in the Protein Data Bank) and ovarian cancer cells (entry 3V03 (BSA) in the Protein Data Bank were used to investigate the docking process. The purpose of this study was to regulate the space, binding energy, type, and location of links involved in the contact of cancer proteins as well as Schiff base ligands and their metal complexes Zn (II), Ni (II), Cu (II), Mn (II), and Co. (III) as indicated in Fig.7. Molecular docking activity of metal complexes Ni (II), Co (III), Cu (II), Zn (II), Mn (II) are summarized in tables 7 and 8.

4.6.1. *MOE- Simulation Strategy*

The molecular operating environment assists drug design through molecular simulation, protein structure analysis, data processing of molecule docking, and so on under the unified operation. Reem shah et.al[48] (2020) reported in their studies that by applying MOE, dynamic simulation was executed for the ligand towards different pathogen-proteins at top-scoring poses. The interaction affinity towards protein pockets and the electrostatic contour maps represent all interaction possibilities and confirm the *in-vitro* results. MOE gives a detailed database of non-reductant protein domains from the protein data bank by which the computational chemistry experts can adopt the best usage of MOE to assign molecular simulations. A solid foundation for molecular modeling and computation chemistry can be provided based on MOE's docking representation binding modes between small to medium-sized ligands [41]. MOE was cast-off to conclude the collaboration methods among the protein receptor and complexes. The newly synthesized Schiff base metal complexes containing an aromatic ring has higher binding energy. So it shows higher DNA binding affinity. Mohammad Shakir et al.,[49] (2011) reported that the Schiff base ligand L and its Co(II), Ni(II), and Cu(II) complexes exhibited significant and higher binding to calf thymus DNA than the free Schiff base ligand L. Synthesized metal complexes were through the interaction of their phenyl ring with DNA via insert that is stable through hydrogen bonding. The binding energy values of newly synthesized metal complexes are -97.61, -105.23, -96.84, -95.64, and -96.89 kcal mol^{-1}, respectively. The Ni (II) complex shows good binding energy with DNA molecules than other complexes. The newly synthesized metal complexes strongly interact with BSA protein via hydrogen bonding and hydrophobic interactions. The binding energy values of newly synthesized metal complexes were -20.17, -14.79, -17.18, -19.89, and -17.01 kcal mol^{-1}, respectively. The Zn (II) and Mn (II) complexes show good binding energy values with BSA molecules than other complexes.

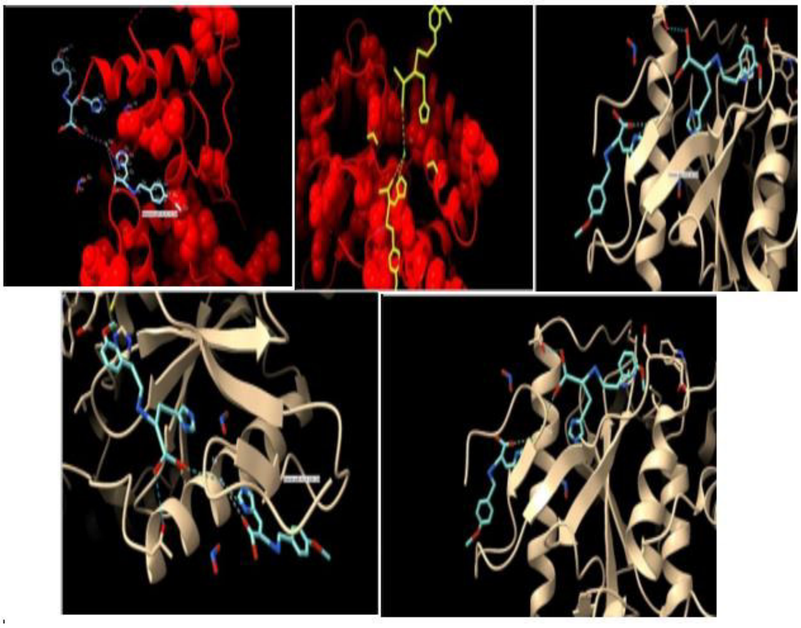


Fig. 7. Molecular Docking activity of metal complexes Ni (II), Co(III), Cu (II), Zn (II), Mn (II).

Table 7. Molecular Docking activity of metal complexes Ni (II), Co (*III*), Cu (II), Zn (II), Mn (II) with Ovarian Cancer Cells (BSA).

S.No	Compound with Ovarian cancer cells	Energy	H-Bond
1	$C_{28}H_{32}ZnN_6O_6$ [ZnL_2]	-20.17	-15.7
2	$C_{28}H_{32}NiN_6O_6$ [NiL_2]	-14.79	-12.36
3	$C_{28}H_{32}CuN_6O_6$ [CuL_2]	-17.18	-12.42
4	$C_{28}H_{32}MnN_6O_6$ [MnL_2]	-19.89	-16.09
5	C28H32CoN8O12 [CoL2]	-17.01	-13.49

Table 8. Molecular Docking activity of metal complexes Ni(II), Co(III), Cu (II), Zn (II), and Mn (II) with Melanoma Cancer Cells (DNA).

S.No	Compound with Melanoma cancer cells	Energy	H-Bond
1	$C_{28}H_{32}ZnN_6O_6$ [ZnL_2]	-97.61	-3.5
2	$C_{28}H_{32}NiN_6O_6$ [NiL_2]	-105.23	-7.96
3	C28H32CuN6O6 [CuL2]	-96.84	-15.62
4	C28H32MnN6O6 [MnL2]	-95.64	-14.89
5	C28H32CoN8O12 [CoL2]	-96.89	-15.21

All Schiff's base-synthesized compounds showed better molecular docking binding energy results against the two kinds of cancer cells, namely ovarian cancer cells (BSA) and melanoma cancer cells (DNA). The results show the molecular Docking activity of metal complexes Ni (II) (-14.79), Co (III) (-17.01), Cu (II) (-17.18), Zn (II) (-20.17), Mn (II) (-19.89) with ovarian cancer cells (BSA) and that of Ni (II) (-105.23), Co (III) (-96.89), Cu (II) (-96.84), Zn (II) (-97.61), Mn (II) (-95.64) with melanoma cancer cells. Among the molecular docking activity of metal complexes, the melanoma cancer cells (DNA) had the highest binding energy. (Tables 7 and 8).

4.7. Anticancer and Toxicological Studies

The Schiff base ligands and their metal complex were tested for anti-cancer activity in contrast to two types of cancer. Table 9 displays the results. When both cells were examined, the results revealed that Schiff base metal complexes Zn (II), Ni (II), Cu (II), Mn (II), and Co (III) have better anticancer activities than ligands. NanZhangYu et al. [50] (2012) reported that the $C_{18}H_{16}N_3O_2$: 2-acetylpyridine-L-tryptophan with Cu (II), Zn (II), and Cd (II) complexes has been investigated against MDA-MB-231 breast cancer cell lines. $Cd(C_{18}H_{16}N_3O_2)_2 \cdot 2CH_3OH$ has the highest anti-proliferative activity among the three complexes. In addition, $Cd(C_{18}H_{16}N_3O_2)_2 \cdot 2CH_3OH$ can inhibit proteasomal chymotrypsin-like activity and induce apoptosis in human breast cancer MDA-MB-231 cells.

Table 9. In vitro anticancer activity of Ovra3 and MCF14, two cancer cell lines.

Compounds	Ovar3 (Ovarian)		MCF14-(Melanoma cell lines)	
	IC50 µg/ml	SD	IC50 µg/ml	SD
MCF-Std	7.13	0.08	6.73	0.09
Cu Complex	7.09	0.08	6.65	0.08
Ni Complex	7.63	0.08	7.14	0.08
Co Complex	6.65	0.05	6.56	0.05
Zn Complex	6.24	0.05	6.45	0.05
Mn Complex	6.20	0.04	6.41	0.05

Ikechukwu P. Ejidike et.al[51] (2016) reported that the Ru (III)-Schiff base complexes were investigated for the in vitro anticancer activity of the synthesized complexes against renal cancer cells (TK-10), melanoma cancer cells (UACC-62), and breast cancer cells (MCF-7) by the Sulforhodamine B assay. By comparing the action of metal complexes as anti-cancer mediators for several diverse cell lines in the literature, it remained discovered that Schiff's base metal complexes with Ovar3 (Ovarian), Std (6.73), Zn (II) (6.24), Ni (II) (7.63), Cu (II) (7.09), Mn (II) (6.20), Co (III) (6.65) and schiff's base metal complexes with melanoma cell lines, Std (7.13), Zn (II) (6.45), Ni (II) (7.14), Cu (II) (6.65), Mn (II) (6.41), Co (III) (6.56) arranged in this revision have higher effective IC50, indicating lower toxicity than earlier reported results, which may be because this is the first study that examined their result in contradiction of ovarian cancer, as well as melanoma cell lines (Fig. 8). Based on the result, Ni (II), Cu (II) complexes, shows more anti-cancer activity in over three cells and also melanoma cell lines than the other complexes.

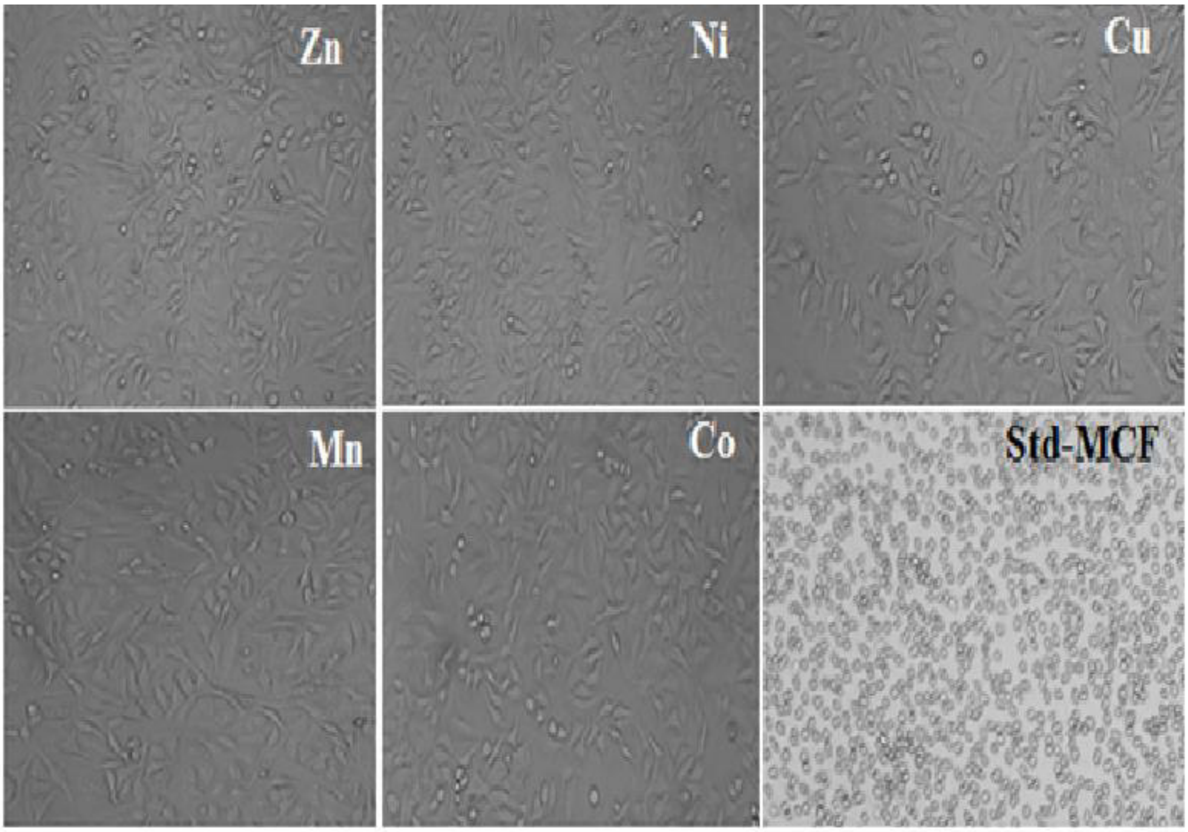


Fig.8. Anti-Cancer activity of metal complexes Ni (II), Co (III), Cu (II), Zn (II), Mn (II), and standard MCF Cell

5. CONCLUSION

The novel Schiff base is derived from L- Histidine amino acid and anisaldehyde, and its coordinated five metal complexes were synthesized in this investigation. The synthesized complexes were characterized by UV-visible, FT-IR, and ESI – MS spectral techniques. The antioxidant studies of the Schiff base ligand and five complexes reveal that the complexes exhibit significant free radical scavenging activity. The antimicrobial activities show that the Zinc (II) and Nickel (II) complexes inhibit gram-positive bacteria more than the other Schiff base complexes. Manganese (II), zinc (II), and copper (II) complexes have a greater zone of inhibition in gram-negative bacteria than the other Schiff base complexes. Among the five complexes Cu (II) complex shows greater antioxidant activity towards DPPH radical among the five complexes. In vitro cytotoxic activity of the five complexes was evaluated against the ovar3(Ovarian) and melanoma cancer cell lines, and these results reveal that complexes Cu (II) and Ni (II) exhibit higher cytotoxicity than any other synthesized metal complexes. In molecular docking study, Co (III) complexes have enhanced binding nature value compared with other metal complexes. BSA protein binding docking energy values explains the Co (III) complex exhibits improved activity towards the BSA molecule.

6. AUTHORS CONTRIBUTION STATEMENT

All authors have made a substantial contribution to the article's design concept. All authors have made acquisition, analysis, and interpretation of data for the article. Dr.D.Madheswari provided valuable input in designing the manuscript. All authors have approved the version to be published.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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