Synthesis, Characterization, DNA Binding, Antimicrobial activity, Cytotoxicity, DFT and Molecular Docking of Copper (II) Complex Containing L-Phenylalanine and 2, 9-dimethyl-1, 10-phenanthroline

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Abstract-New copper (II) complex, [Cu(dmp)(L-phe)(H_2O)](NO_3)($3H_2O$), where dmp. = 2, 9-dimethyl-1, 10-phenanthroline and L-phe = L-phenylalanine, has been synthesized and characterized by elemental analysis, UV-visible, FT-IR, ESI-MASS, EPR spectroscopic techniques. The optimized structure of present copper (II) complex was obtained by using the DFT/UB3LYP/6-31G (d.p)/LANL2DZ level of theory. Binding interactions of the copper (II) complex with calf thymus DNA (CT-DNA) was investigated by UV–Visible absorption titration, fluorescence, cyclic voltammogram studies, and viscometric titration experiments. The copper (II) complex was subjected to *in-vitro* cytotoxicity studies against breast cancer cell lines (MCF-7). The anticancer activity of copper (II) complex IC_{50} values are less than that of Cis-platin against MCF-7 cell lines. The antimicrobial activity of copper (II) complex was carried out using disc diffusion method against different species of pathogenic bacteria and fungi. Binding ability of the synthesized compound results was showen in molecular docking studies.

Keywords- Copper (II) complex, L-phenylalanine, 2, 9-dimethyl-1, 10-phenanthroline, DNA binding, Cytotoxicity, Antimicrobial activity, DFT and Molecular docking studies.

I INTRODUCTION

Copper plays a prominent role in proper functioning of organs and metabolic processes and also acts as an essential co-factor for tumor angiogenesis related processes [1]. Copper is biologically an important element as it was identified that it activates many enzymes. It is an attractive prospect in therapeutics, since it plays a vital role in a biological process such as electron transfer, oxygen transport and endogenous oxidative DNA damage associated with aging and cancer [2]. Copper complexes of 1, 10-phenanthroline and derivatives are of great interest since they exhibit numerous biological activities such as antitumor, [3] anti-Candida, [4] antimycobacterial [5] and antimicrobial activity [6] etc.,

Deoxyribonucleic acid (DNA) plays a very important role in life processes as it delivers the inheritance of the information and instructs the biological synthesis in living cells. DNA particularly offers a variety of potential metal binding sites [9–11]. “Chakravarty et al. recently explored the transition metal-based chemistry towards the cleavages of DNA under the Physiological conditions by oxidative as well as photochemical means of charge transfers (or) d–d band excitation” [14-20]. Applications of metal complexes were incorporated with 1, 10-phenanthroline are numerous [21-23].

Amino acids are the basic structural and functional units of proteins that recognize a specific base sequence of DNA. This amino acid in a terminal –C(=O)–NH_2 groups are of potential significance to the hydrogen bonding interactions in the double-stranded DNA [24]. Copper (II) complexes were previously reported as potential anticancerous agents and have been found to be active in both *in-vitro* and *in-vivo* conditions [25].

The current study piques our interest in examining the structural and functional characteristics of copper (II) complexes contain bio-essential amino acids and N, N-donor heterocyclic bases. The efficiency of the DNA strand scission can be enhanced by increasing the binding affinity of metal complex. Thus, keeping these points in mind, it brings an interest to study the

interaction of these complexes with DNA and throw more light on their possible applications as new chemotherapeutic agents.

Taking into account the intriguing features of this phenanthroline-based metal complex in continuation of research [26-30], we report the synthesis of new copper (II) complex containing amino acids used as L-phenylalanine and 2, 9-dimethyl-1, 10-phenanthroline ligands. Further more insight into the bonding and possible geometrical structure has been made by elemental analysis, FT-IR, UV-visible, EPR studies, ESI-mass spectra. We have explored the DNA binding behavior of this complex by absorption titration with CT-DNA, viscosity, fluorescence and cyclic voltammogram studies. The antimicrobial activites of copper (II) complex against some selected Gram-positive and Gram-negative bacteria and fungi were also reported. Copper (II) complex demonstrated strong cytotoxic effects against MCF-7 human breast cancer cell line. The stability of the prepared copper (II) complex has been evaluated through the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energy gap. Binding interaction ability of the copper (II) complex was confirmed by molecular docking studies. From this study the knowledge gained can be applied for the development of potential probes of DNA structure and new therapeutic agents for tumors and other diseases.

II EXPERIMENTAL

Materials and Instrumentation

The most common reagent such as ethanol, methanol, copper nitrate trihydrate, 2, 9-dimethyl-1, 10-phenanthroline are all analytical grade were used as received. L-phenylalanine (amino acids) was purchased from Sigma Aldrich. Disodium salt of calf thymus DNA (CT-DNA), Tris-(hydroxyl methyl) amino methane-HCl (Tris-HCl) and Ethidium Bromide (EB) purchased from Sigma Aldrich. The Infrared spectra recorded on a Perkin Elmer Spectrometer as KBr Pellets (4000-400 CM^{-1}) and elemental analysis was performed on a Perkin Elmer 240C analytical Instruments, UV-Visible and Fluorescence spectra of the complex were recorded on Shimadzu UV-2450 Spectrometer and Jobin young Fluorolog3 Spectrometer respectively. Molar conductivity measurements were done using a Control Dynamics (India) conductivity meter. Electron paramagnetic resonance (EPR) spectra for polycrystalline copper (II) complex was obtained on a JEOL FA 200 ESR spectrometer at room temperature. ESI-MS spectra were obtained from thermo-Finnigan LCQ6000 advantage max ion trap mass spectrometer. Cyclic Voltammograms were recorded using a three electrode cell using DMSO solution of TBAP (0.1 M) as the electrolyte of support on the CH1602D (CH Instruments co., USA) electrochemical analyzer in an oxygen free environment. A platinum wire, glassy carbon, and the Ag/ AgCl electrode (saturated KCl solution) were used as counter, working and reference electrode respectively.

Synthesis of $[Cu(dmp)(L\text{-}phe)(H_2O)](NO_3)(3H_2O)$

An aqueous solution of copper nitrate trihydrate (0.480 g, 2 mM) was reacted with L-phenylalanine (0.330 g, 2 mM) which was pre-treated with NaOH (0.080 g, 2 mM) in water (10 ml) and stirred for 2 hours at room temperature. 10 ml ethanol solution of the heterocyclic base of 2, 9-dimethyl-1, 10-phenanthroline (0.416 g, 2 mM) was added in drops using a syringe at room temperature. The solution was filtered and the filtrate upon slow evaporation gave green coloured product in two weeks time. The product was isolated, washed with cold aqueous methanol and ether.

Yield: 70 %. Melting point: 130-132 °C, Analytical calculation for $C_{23}H_{30}CuN_4O_9$ (%) C, 48.46; H, 5.30; N, 9.83. Found (%) C, 48.39; H, 5.37; N, 9.72. FT-IR (KBr cm^{-1}) 3397br, 3264br, 3064w, 3011m, 1621s, 1594s, 1565s, 1503s, 1382vs, 1222s, 1025s, 857s, 721s, 655s, 542s and 458w (br.-broad; vs.-very strong; s-strong; m-medium; w-weak). UV-Visible (H_2O), λ/nm ($\varepsilon/M^{-1}\,cm^{-1}$): 225 (9150) and 272 (6430) ($\pi\rightarrow\pi^*$), 452 (13) due to ligand–metal charge transfer and 697 (33) ($d\rightarrow d$) transition. ESI-MS: 569.13 g/m, obtained m/z at 568.06 g/m. A molar conductance measurement of the complex is 82 $\Omega^{-1}\,cm^2\,mol^{-1}$ indicating 1: 1 electrolytes, μ_{eff}: 1.82 BM at 298 °K.

Mass spectra

The robust method is one of the mass spectral techniques for identifying the unknown molecules of the samples, with the structure and chemical properties. The process triggers multiple ions from the

solid samples to segregate based on mass to charge ratio. Copper (II) complex was analyzed for their identification from the mass spectral data.

Electron Paramagnetic Spectrum of the Copper (II) complex

In DMSO, the EPR spectrum was recorded at both room temperature (RT) and liquid nitrogen temperature (LNT). EPR spectrum of copper (II) complex is a characteristic of species, d^9 electronic configuration and having axial type of dx^2-y^2 ground state which is the most common Cu (II) complex [31]. To understand the metal ion environment in the complex is very important, ie., the geometry, nature of the donating atoms from the ligand and degree of covalency of the Cu (II)-ligand bonds.

Spectroscopic Studies on DNA interaction

Electronic spectra

The complex is one electron paramagnetic at room temperature, corresponding to d^9 electronic configuration of the copper (II) center. The complex displays copper (II) centered d-d bands 697 nm in addition to the ligands centered bands in the UV region of the electromagnetic spectra. The electronic spectra of the complex are in good agreement with the previously reported square pyramidal geometry of the copper complex [32-34]. In the UV–visible region, band that appeared around 272 nm and 225 nm is assigned to attribute to intra-ligand transitions [35]. The binding affinity between copper (II) complex and CT-DNA is investigated by the absorption spectral titrations, performed at room temperature with Tris-HCl/NaCl buffer (5 mM/50 mM buffer, pH 7.2). The electronic spectrum of complex was recorded before the addition of CT-DNA. A fixed concentration of the complex (1 X 10^{-5} M) was titrated by increasing the concentration of DNA. The interaction between intrinsic binding constant of copper (II) complex with DNA was obtained from absorption data. The observed "hyperchromic effect" could be attributed to $\pi \rightarrow \pi^*$ stacking interaction between the aromatic chromophore of complex and DNA base pairs suggestive of intercalative binding mode. The binding strength of complex with CT-DNA were quantified and determined from the following equation [36].

$[DNA]/ (\varepsilon a-\varepsilon f) = [DNA]/ (\varepsilon b-\varepsilon f) + 1/ (kb (\varepsilon b-\varepsilon f)$…(1)

Where, [DNA] is the DNA concentration in nucleotides and the apparent absorption co-efficient, ε_a, ε_b and ε_f corresponds to $A_{observed}$/ [Cu]. ε_f refers to the extinction coefficient of the free compound and ε_b is the extinction coefficient of the compound when fully bound to DNA. The plot of [DNA]/ (εa-εf) versus [DNA] gave a slope and intercept which is equal to 1/ (εb-εf) and 1/ kb(εb-εf), respectively; k_b is indicated by the ratio of the slop to the intercept.

Fluorescence spectra

Fluorescence spectra were recorded with excitation at 480 nm, experiment was carried out by titrating complex (5 mM Tris-HCl/50 mM NaCl buffer) into the solution of CT-DNA (8 X 10^{-5} M) and ethidium bromide (1 X 10^{-4} M). The quenching constant was calculated from Stern-Volmer by using the given equation [37].

$I_0/I = 1 + K_{sv}$ X r ……. (2)

Where I_0 and I are the fluorescence intensities in the absence and presence of complex, respectively. Ksv is a linear Stern-Volmer and r is the total concentration of complex to that of DNA. In the plot of I_0/I versus [complex] / [DNA], Ksv is given by the ratio slop to intercept.

Viscosity

Viscosity experiment was carried out in an Ostwald viscometer immersed in a thermostated water-bath maintained at a constant temperature at 28.0 ± 0.1 °C. CT-DNA samples of approximately 1.5 X 10^{-5} M, was prepared by sonication in order to minimize the complexities arising from CT-DNA flexibility [38]. Flow time was measured with a digital stopwatch three times for each sample and the average flow time was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ versus the concentration of the metal (II) complex. Where η is the viscosity of CT-DNA solution in the presence of complex and η_0 is the viscosity of CT-DNA solution in the absence of complex. Viscosity values were calculated after correcting the flow time of buffer alone (t_0), η-$(t-t_0)/t_0$ [39].

Cyclic voltammetry

The biophysical techniques of electrochemical studies are complementary to interaction between redox active molecules and biomolecules. To prepare the buffer solutions double distilled water was used. The cyclic voltammetry (CV) were performed on a three electrode system consisting of a glassy carbon (GC) electrode. Before the each experiment, solutions are

deaerated by purging dry N_2 for 15 minutes and nitrogen was kept over the solution during the experiments [40].

Antimicrobial activity

Antibacterial activities of the copper (II) complex were tested on Gram-positive bacteria (*Staphylococcus faecalis, Staphylococcus epidermis*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) bacteria grown in a nutrient agar medium and incubated at 37 °C for 24 hours followed by frequent subculture to fresh medium and used as test bacteria [41]. The fungi *Candida albicans, Aspergillus niger* grown as a sabouard dextrose agar medium and were incubated at 27 °C for 48 hours followed by periodic sub-culturing to fresh medium and used test fungus. Then the petriplates were inoculated with a loop full of bacterial and fungal cultures and spread throughout the petriplates and uniformly with a sterile glass spreader. To each disc, the test samples (25 µg/well), reference ciprofloxacin (20 µg/disc for bacteria) and reference clotrimazole (20 µg/well) was added with a sterile micropipette. The plates were then incubated at 35 ± 2 °C for 24-48 hours and 27 ± 1 °C for bacteria and fungus, respectively. The plates were with a disc containing respective solvents that served as a control. The zone of inhibition was recorded by measuring the diameter of the inhibitory zone after the period of incubation. All the experiments were repeated thrice and the values are observed.

Cytotoxicity assay

Cytotoxicity of the copper (II) complex was evaluated to human breast cancer (MCF-7) cell lines. The MTT [3-(4, 5-DIMETHYLTHIAZOL-2-YL)-2, 5-diphenyltetrazolim bromide] assay method is used to determine the cell viability. The cells were treated (100 µl) of MTT at 37 °C for 3 hour with mild shaking. The 200 µl of PBS was added and left overnight in dark conditions. Absorbance was read at 650 nm in a microtitre plate reader. Optical density of the oxidant-induced cells was fixed as 100 % viability of the cells in the other treatment groups were calculated relative to this [42].

Theoretical calculations

All calculations such as gas phase geometry optimizations, Highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbitals (LUMO) for $[Cu(dmp)(L\text{-}phe)(H_2O)]$ $(NO_3)(3H_2O)$ were carried out using the Gaussian 09 software [43] at B3LYP level [44]. Output log files were visualized by the Avogadro and chemcraft visualization program. Basis set, 6-311G + + (d, p) were used for C, H, N, O and Cu at moderate computational cost. The molecular orbital energy parameters such as the E_{HOMO} and E_{LUMO}, are often used for determining the reactivity of molecules.

Molecular docking studies

In order to examine the docking mechanisms of DNA, various types of enzymes and proteins molecular docking studies were conducted using the interactive molecular graphics programme Hex 8.0.0. The copper (II) complex structure was drawn using chemsketch and then converted to PDB format using Acdlabs (http://www.acdlabs.com). (http://www.vcclab,org/lab/babel/) OPENBABEL software was employed. The B-DNA dodecamer crystal structure (PDB ID: 1BNA) was obtained from the protein data bank (http://www.rcsb.org/pdb/). The docked images were visualized using the molecular graphics programmes CHIMAERA (www.cgl.ucsf.edu/chimera) and Pymol (http://pymol.sourceforget.net/).

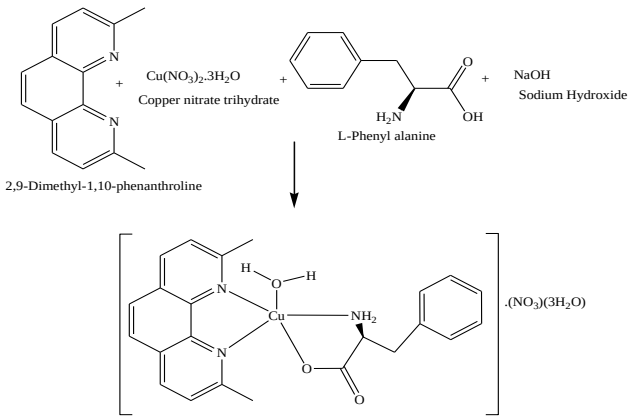
III RESULTS AND DISCUSSION

Synthesis and characterization

Newly synthesized Copper (II) complex of $[Cu(dmp)(L\text{-}phe)(H_2O)](NO_3)(3H_2O)$, Where dmp is 2, 9-dimethyl-1, 10-phenanthroline and L-phe is L-phenylalanine , has been synthesized by adding a ethanol solution of one equivalent amount of copper nitrate trihydrate to a mixture of one equivalent amount of the ligand 2, 9-dimethyl-1, 10-phenanthroline and L-phenylalanine in ethanol solution (Scheme-1). The copper (II) complex is soluble in 5 mM Tris-HCl/50 mM NaCl buffer at pH 7.1 in methanol; water mixed solvent (1:10 v/v). The complex is one electron paramagnetic at room temperature, corresponding to d^9 electronic configuration for the copper (II) center. The absorption spectra of copper (II) complex display copper (II) centred d-d band at 680 nm (Figure-1). Electronic spectra of copper (II) complex are in good agreement with the previously reported square-pyramidal geometry. [45, 46]. The intense absorption band observed in the UV region (225 and 272 nm) for complex is attributed to the intraligand $\pi \rightarrow \pi^*$

transition located on the coordinated bidenate of 2, 9-dimethyl-1, 10-phenanthroline, L-phenylalanine ligands.

The tentative assignments of the FT-IR bands are useful aids for determining the coordination behavior of the ligands with copper (II) ion. The broad centered band was obtained in the region 3397 cm^{-1} is due to O-H stretching of water molecules, an uncoordinated nitrate anion show the stretching vibration in the region of 1382 cm^{-1} [47]. The FT-IR spectra of the copper (II) complex was shows asymmetric $\nu_{asy.}(COO^-)$ and the symmetric stretching vibration $\nu_s(COO^-)$ fall in 1503 and 1290 cm^{-1} for complex (figure-2). The difference between $\nu_{as}(COO^-)$ and $\nu_s(COO^-)$ stretching frequencies is greater than 200 cm-1, which are indicates that the carboxylate groups are coordinated to the metal ion in a monodentate fashion [48]. The bands at 1621, 1594 cm^{-1} can be attributed to the stretching frequencies of [ν(C=N) and ν(C=C) of 2, 9-1, 10-phenanthroline. The medium to low intensity bands at 542 and 458 cm^{-1} are attributed to the coordination bonds of (Cu-N) and (Cu-O) respectively. From the elemental analysis and spectral arguments, the geometry of the copper (II) complex was concluded as a distorted square-pyramidal. The ESI-MS spectrum obtained for the complex are in good agreement with proposed molecular formulae.



Scheme 1: Synthetic route for the preparation of the Cu (II) complex of [Cu(dmp)(L-phe)(H_2O)](NO_3)($3H_2O$)

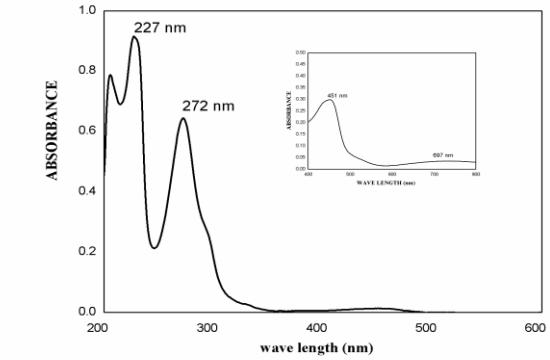


Figure-1: The electronic spectra of [Cu(dmp)(L-phe)(H_2O)](NO_3)($3H_2O$) in water, Inset shows the d-d band of complex.

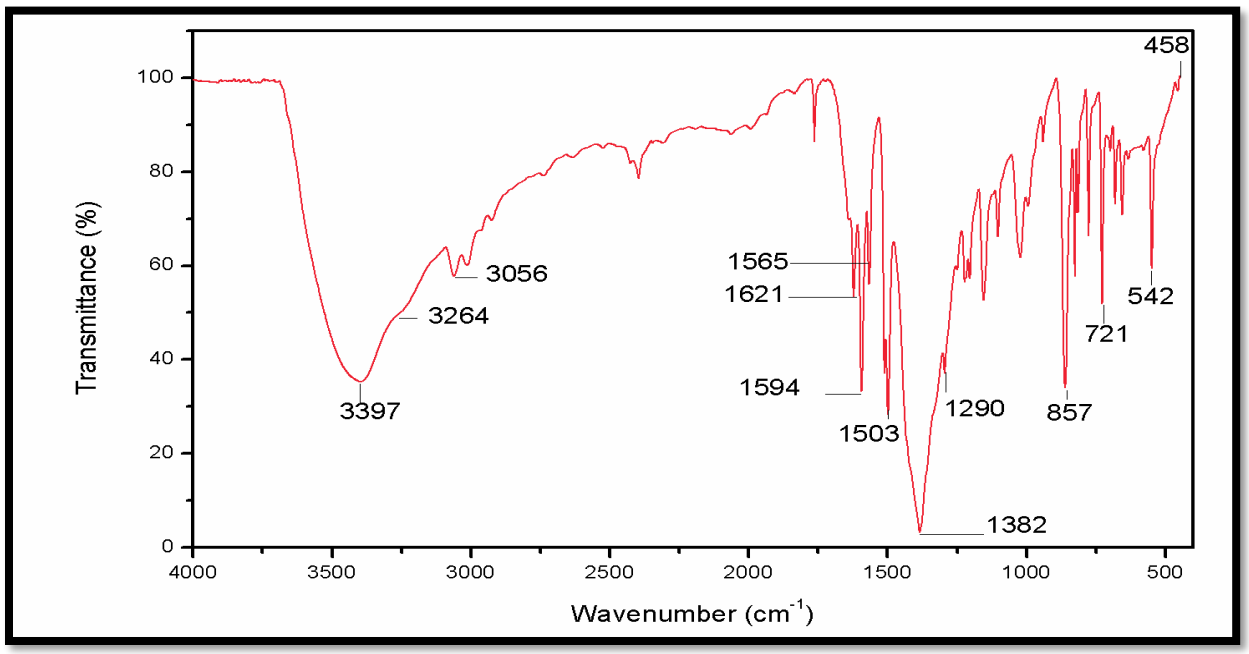


Figure-2: The FT-IR spectrum of the Cu(dmp)(L-phe)(H_2O)](NO_3)($3H_2O$) complex.

Mass spectra

The molecular structures of many complexes are being gradually elucidated using an ESI-MASS spectrum. The molecular ion peak (M^+) at m/z = 568 (78%) and a weak peak at m/z = 569 due to ^{13}C and ^{15}N isotopes may be seen in the complex ESI-MASS spectra. The peak at m/z = 209 (100%) is due to 2, 9-dimethyl-1, 10-phenanthroline ligands. Peaks for the complex are given by the other positive ions, which have mass numbers of 479, 439, 294, 231, and 172. Intensity of these peaks indicates the ions stability [49, 50]. All the fragmental ions of the mixed ligands of metal complex confirm the stoichiometry of the complex. The ESI-MS spectrum of the copper (II) complex is given in figure-3. From the above evidence the molecular formula of the copper (II) complex is $C_{23}H_{30}N_4O_9Cu$, accurate molecular weight is found to be 569.13 m/z.

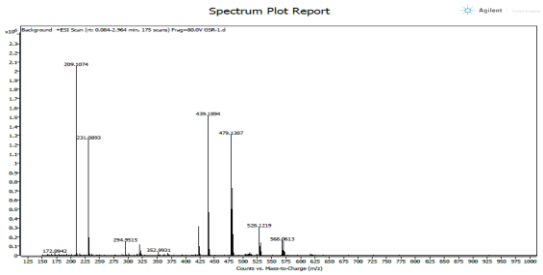


Figure-3: ESI-MASS spectrum of [Cu(dmp)(L-phe)(H_2O)](NO_3)($3H_2O$) complex.

EPR spectra of copper (II) complex

The solid-state EPR spectrum of copper (II) complex was recorded in X-band frequencies (figure-4). At liquid nitrogen temperature, complex exhibits well defined single isotropic feature near $g_{\parallel}$ = 2.12 and $g_{\perp}$ = 2.07. Such isotropic lines are usually the results of intermolecular spin exchange, which broaden the lines. This intermolecular type of spin-exchange is caused by the strong spin coupling which occurs during a coupling of two paramagnetic species. The one electron paramagnetic mononuclear copper (II) complexes display X-band EPR spectra in 100 % DMSO at 77 K giving $g_{\parallel} > g_{\perp} > 2.0023$ indicating a [dx2-dy2] ground state [51] in a square pyramidal geometry. The spectra revealed well-resolved EPR signals coming from the copper's M1 = +3/2 component, providing an $A_{\parallel}$ value of 185 ± 2G indicating a slight distortion in the square pyramidal geometry [52- 54].

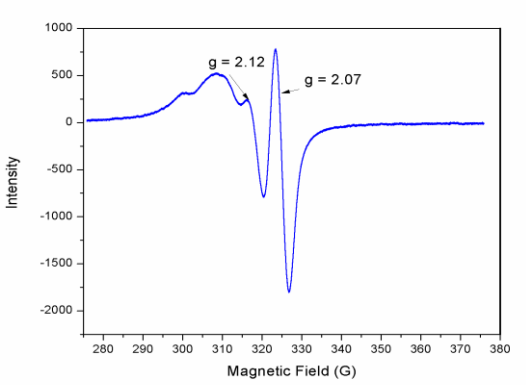


Figure-4: EPR spectrum of [Cu(dmp)(L-phe)(H_2O)](NO_3)($3H_2O$) complex.

Spectral studies of the interactions with DNA

The binding modes of the copper (II) complex with CT-DNA have been employed for analyzing the electronic absorption spectroscopy. The ligand based $\pi \rightarrow \pi^*$ spectral bands exhibit hypochromism (15-31%) with red shifts in band location CT-DNA to the complex. The absorption spectral traces with increasing concentration of CT-DNA are shown in (figure-5). The extent of hypochromism is commonly associated with the strength of CT-DNA interaction, observed decrease in hypochromism and reflects the decreasing DNA binding affinities of the complex. Copper (II) complex, can partially insert in their co-ligands into the DNA base pairs, if the coupling π orbital of co-ligands is partially with electrons. The results showed a decrease in the transition probabilities and concomitantly in hypochromism [55-56]. Copper (II) complex can bind with the double stranded DNA in different binding modes on basis of their structures, charges and types of ligands. The intrinsic binding constants (kb) for both complex calculated, using equation (1) [57], to be 4.88 ± (0.03) X 10^4 M^{-1} for complex. Copper (II) complex shows the least binding strength to DNA structure. Substitution at the position 2 and 9 of ancillary phenanthroline ligands may cause severe steric constraints near Cu (II) core, when the complex intercalates with DNA base pairs at the intercalation sites. These steric clashes prevent copper (II) complex from intercalating effectively, which are causes diminution of the intrinsic constant.

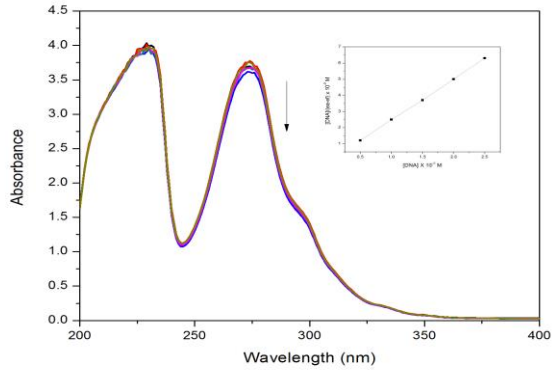


Figure-5: Electronic absorption spectrum of $[Cu(dmp)(L\text{-}phe)(H_2O)](NO_3)(3H_2O)$ in the absence and in the presence of increasing amounts of DNA Concentrations. [COMPLEX] = 15 μM, [DNA] = (0, 5, 10, 15, 20, 25) μM. The arrow shows the absorbance changes increasing DNA concentrations. Inset plot of [DNA]/ (εa-εf) versus [DNA] for absorption titration of CT-DNA.

Fluorescence spectral studies

To investigate the interactions between small molecules and DNA, fluorescence spectroscopy technique is employed [58-60]. To investigate the potential DNA with ethidium bromide (EB) was used for binding mode of the reported copper (II) complex. The intense fluorescence of EB molecules emits at 612 nm in the presence of CT-DNA due to its strong intercalation between the adjacent DNA base pairs. The DNA induced EB emission would be suppressed by the addition of a second molecule with greater DNA-binding affinity than EB [61-63]. The quenching of the fluorescence of EB bound to DNA would reflect the extent of the DNA binding of the second molecule. The emission spectra of DNA-bound EB in the absence and presence of different concentrations of the copper (II) complex are shown in (figure-6). The addition of the copper (II) complex to CT-DNA pretreated with EB was clearly seen in appreciable reduction in emission intensity, indicating that complex can bind to DNA at the sites occupied by EB. It is evident that the replacement of EB bound to DNA results were decrease in fluorescence intensity with the increase in concentration of the investigated compounds. This implies that the complex can strongly compete with EB in binding to DNA. The Ksv values were obtained from the slope of the linear plot of I_0/I versus Q. The quenching of EB bound to CT-DNA by copper (II) complex is good agreement with the linear Stern-Volmer equation. The Ksv value is 2.15 ± (0.01) X 10^5 M^{-1} of the copper (II) complex.

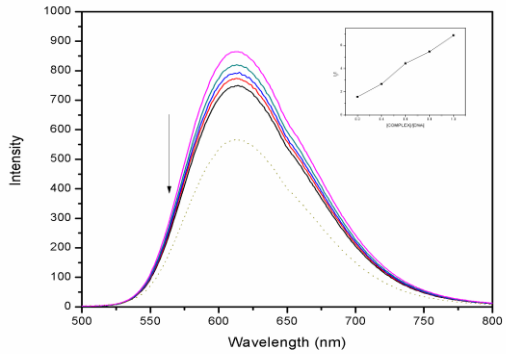


Figure-6: Emission spectra (excited at 480 nm) of EB bound to DNA in the absence (dotted line) and in the presence (solid line) of $[Cu(dmp)(L\text{-}phe)(H_2O)](NO_3)(3H_2O)$ [COMPLEX] = 8, 16, 24, 32, 40 X 10^{-6} M, [DNA] = 3 X 10^{-5} M, [Ethidium Bromide] = 3 X 10^{-5} M. The arrow shows the intensity changing upon increasing complex concentrations.

Viscosity measurements

The viscometric measurement is also an essential tool to find the nature and binding of metal complex to DNA. The relative specific viscosity of DNA is determined by varying the concentration of the added metal complex. The classical technique of measuring the viscosity of DNA is used to analyze the DNA binding mode in solution. Hydrodynamic techniques that are sensitive to changes in DNA length are thought to be the least ambiguous and most important evaluations of binding in solution in the absence of crystallographic structural data. The partial intercalation of 2, 9-dimethyl-1,10-phenanthroline in between the DNA base pairs leads to an increase in separation of the base pairs and hence an increase in overall contour length resulting in increase viscosity of copper (II) complex. A stacking intercalation model causes the DNA helix to extend when base pairs are split to make space for the complex, increasing DNA viscosity. Figure-7 depicts the effects of copper (II) complex on viscosity of CT-DNA at 25 °C. The results of Viscosity measurements clearly show that Cu (II) complex can stack between adjacent DNA base pairs, creating an expansion in the helix and hence raising the viscosity of DNA.

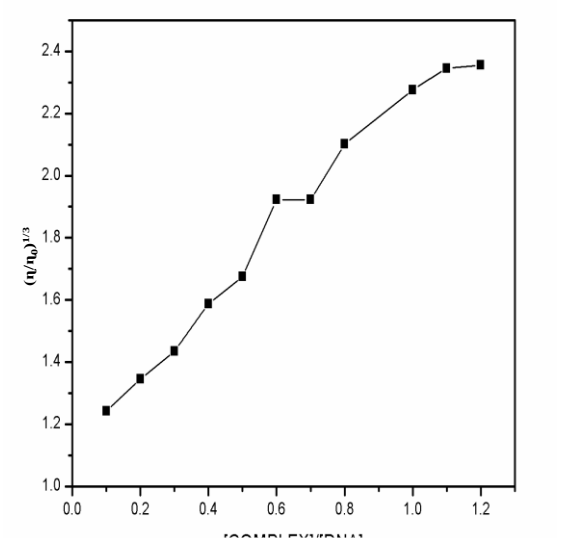


Figure-7: Effect of increasing amount of [Cu(dmp)(L-phe)(H_2O)](NO_3)($3H_2O$)(10,15,20,25, 30, 35,40, 45, 50 µM) on the relative viscosity of Calf-Thymus DNA (15 µM) in 5 mM Tris-HCl/50 mM NaCl buffer.

Cyclic Voltammetry

In order to further explore the DNA binding modes found from the previously mentioned spectrum and viscometric experiments, the interaction of a current redox-active metal complex with DNA has been studied using cyclic Voltammetric techniques. Figure-8 illustrates a typical copper (II) complex cyclic voltammetry (CV) response in both the presence and absence of CT-DNA in Tris-HCl buffer (pH 7.2). In the forward scan, a single cathodic and anodic peak were observed, which corresponds to the reduction and oxidation of complex, which indicates that the process is reversible. When CT-DNA is added to a solution of complex, marked decrease in the peak current and potential value was observed. Due to complex binding to the DNA, the addition of a very substantial excess of DNA had no effect on the cyclic Voltammetric behaviour [64-65]. Peak current and possible shifts become gradually as CT-DNA concentration increases. This indicates that the complex has interactions with the DNA of the calf thymus.

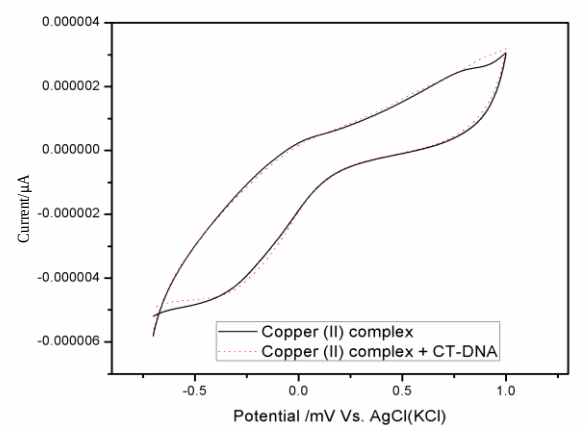


Figure-8: Cyclic voltammogram of [Cu(dmp)(L-phe)(H_2O)](NO_3)($3H_2O$) (1 X 10^{-3} M) complex in the absence (solid line) and presence (dotted line) of CT-DNA (1.5 X 10^{-5} M). 5 mM in buffer containing 50 mM NaCl-5 mM Tris-HCl, pH 7.2, scan rate: 100mVS^{-1}.

Antimicrobial activity

Using the disc diffusion method, the antimicrobial activity of copper (II) complex was screened *in-vitro* against certain human pathogenic bacterial and fungal species. It was found that the Copper (II) complex demonstrated that significant effects against Gram-positive and Gram-negative bacteria. The test solutions were made in DMSO and (Table-1) presents the results of the study. According to the diameter, as indicated in (figure-9), inhibition zone of bacterial growth was measured in mm. The antibacterial activity results revealed that the metal complex is shown to have good activity when compared to control drugs (Ciprofloxacin) [66-67]. In our biological experiments, copper (II) complex, have high antibacterial activity against Gram-positive bacteria such as *staphylococcus epidermis* and *staphylococcus faecalis* than the Gram-negative *Escherichia coli* and *klebsiella pneumonia* compared to standard drugs (Ciprofloxacin). Copper (II) complex shows high antifungal activity in *Aspergillus niger* and *Candida albicans* compared to the standard drugs (Clotrimazole). This high activity may be due to an efficient diffusion of the metal complex to bacterial/fungal cells and/ or interaction with these organisms. The copper (II) complex is capable of inhibiting the growth of bacterial and fungal to a greater extent.

Table- 1: Antimicrobial activities (diameter of zone of inhibition, in mm) of copper (II) complex.

SL. NO.	Micro-organisms	Copper (II) complex	Positive control Ciprofloxacin/ Clotrimazole	Negative control (DMSO)	Copper nitrate tri hydrate
1.	Staphylococcus epidermis (G^+)	22	11	NA	NF
2.	Staphylococcus faecalis (G^+)	23	12	NA	12
3.	Escherichia coli (G^-)	03	23	NA	NF
4.	Klebsiella pneumonia (G^-)	12	22	NA	NF
5.	Candida albicans	28	20	NA	8
6.	Aspergillus niger	37	25	NA	NF

Note: NF-Minimum inhibition concentration (MIC) not found in the concentrations screened,
NA- Not Active.

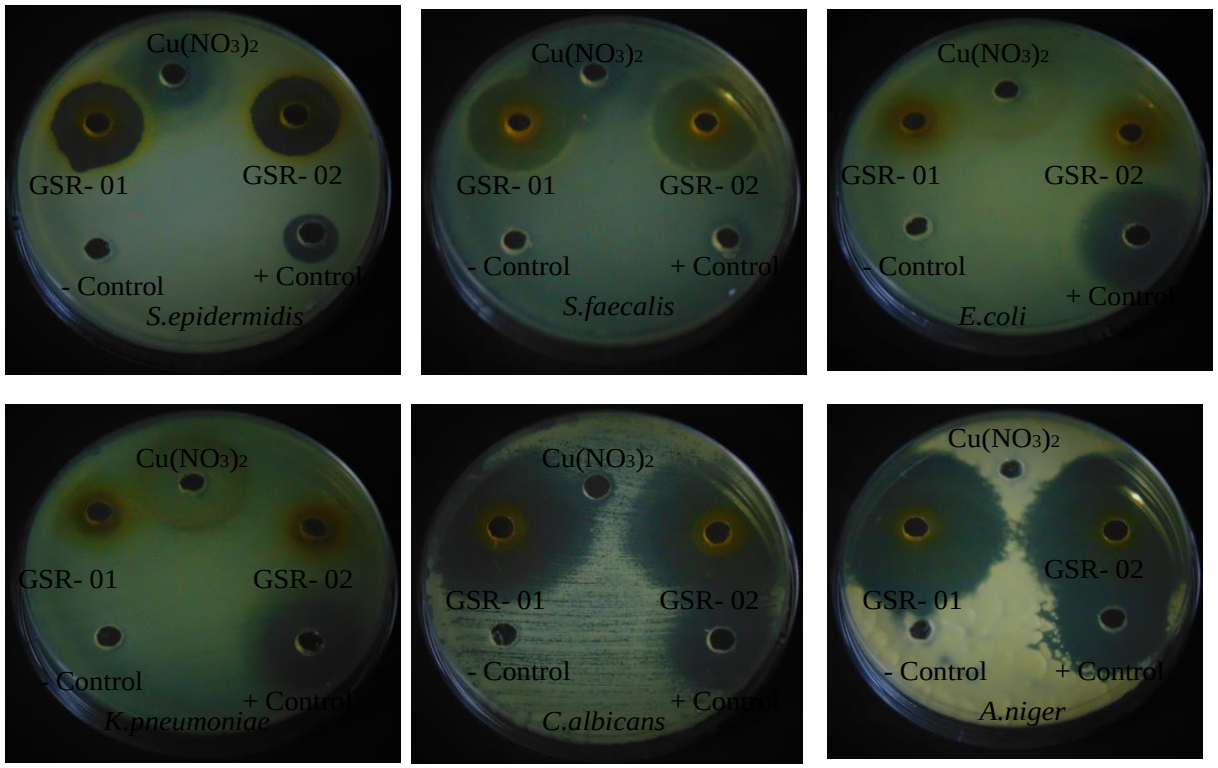


Figure-9: Antimicrobial activity of copper (II) complex

Cytotoxicity activity

Our main aim to examine the potential of antitumor activities, in vitro anticancer assay of copper (II) complex against human breast cancer (MCF-7) cell lines were conducted and Cis-platin was used as a control to assess the cytotoxicity of tested compound. The observed results are means of cell inhibition expressed as IC_{50} values, which are very close to that of control drugs (IC_{50} = 5.51 ± 0.3 µg/ml) and complex IC_{50} value found to be 7.73 ± 1.007 µg/ml. The cytotoxicity activity of tested compound of IC_{50} values indicates clinical antitumor drugs of cis-platin. Furthermore these compounds exhibit higher activity against MCF-7 cells. Comparatively control and treated MCF-7 cells of the morphological changes for an incubation period of 24 hours are also shown in figure-10. Copper (II) complex were exhibited with significant cytotoxic activity as shown in figure-11. Moreover, interestingly this observation is in accordance with their DNA binding abilities, suggesting that the antitumor activities of tested compounds against choosen cell lines may be closely related to intercalate base pairs of DNA [68].

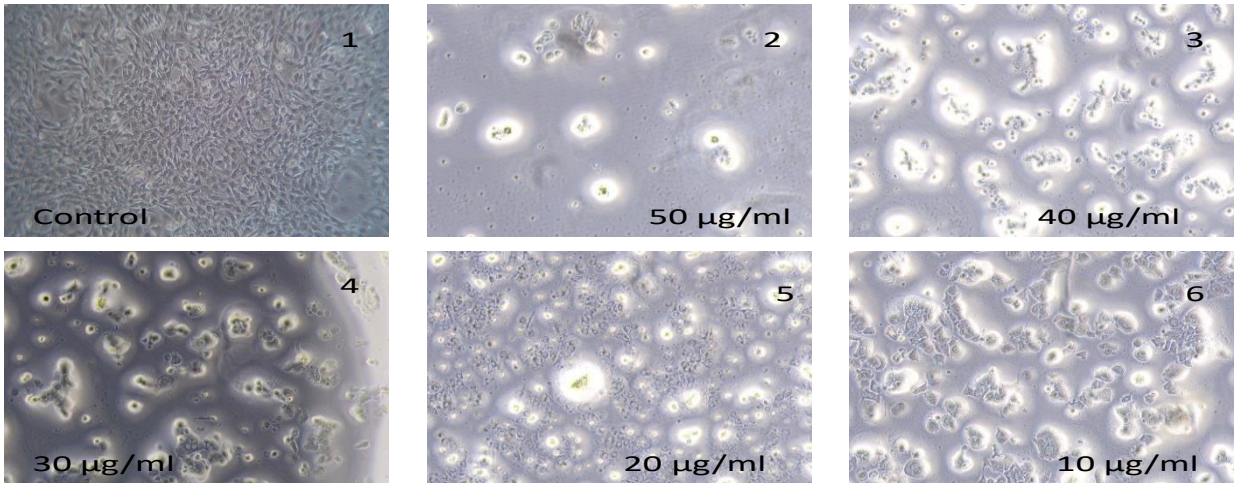


Figure-10: Morphological changes after treating with MCF-7 cell after an incubation period of 24 hours [A] Normal MCF-7 cell (control) [B] (2, 3, 4 & 5) Copper (II) complex treated with different concentrations with human breast cancer cells for 24 hours

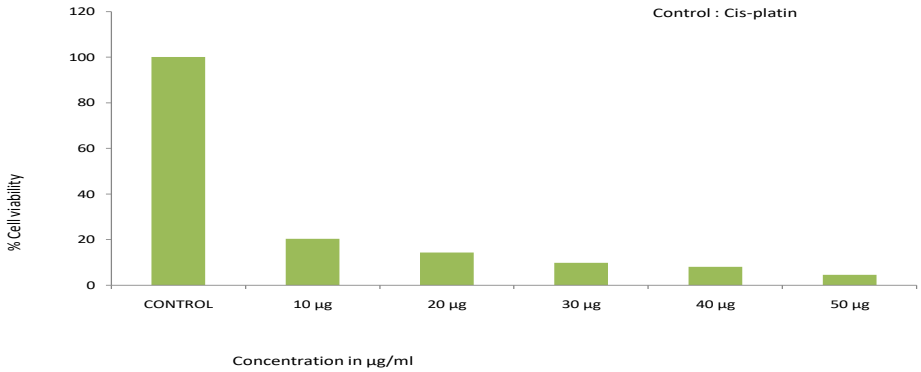


Figure-11: Graphical representation of % Cell viability of human breast cancer cells (MCF-7) (control) and after treatment with copper (II) complex at different concentration at 24 hours.

Frontier molecular orbital analysis (FMO)

Full optimization of copper (II) complex in the gas phase led to the final structure (figure-12). Nitrate ion (NO_3^-) of [Cu(dmp)(L-phe)(H_2O)](NO_3)($3H_2O$) is held to H_2O coordinated to Cu^{2+}. The slight change in Cu-O and Cu-N bond lengths are due to the electrostatic contribution from NO_3^-. The graphical surfaces of the HOMO and LUMO in the studied complex [Cu(dmp)(L-phenyl)(H_2O)](NO_3)($3H_2O$) are shown in figure-13. The measure of E_{HOMO} demonstrates the potential of a molecule to donate its loosely bound electron to the orbitals of an acceptor molecule. The E_{LUMO} measures the tendency of a molecule to accept electrons from the orbital of donor molecule. In this copper (II) complex HOMO and LUMO the energy gap is 1.1804 eV) (Table-2 & 3). The result shows that copper (II) complex reactivity is high and most stable form in nature. Electrostatic potential (ESP) is mapped onto the iso-electron density surface utilizing a method known as the MEP surface [69-70]. In accordance with the classification of colours, the MEP simultaneously provides molecular structure, size and ESP areas. Additionally, it is an essential component in the study of the association between structure-activity and physicochemical properties of compounds such as drugs and biomolecules [71-72]. The copper (II) complex of MEP surface is shown in figure-14 with this in mind. The MEP color code values between the deepest blue and red for the complex range 11.906e-2 to -11.906e-2 a. u. As can be seen in Figure-14, the negative potential are over the electronegative O

atoms belonging the carboxylate groups coordinated with Cu (II) ions, this negative region represented by red color indicate to electrophilic reactivity. The positive regions represented by blue color are related to nucleophilic reactivity, these regions cover on more C-H bonds.

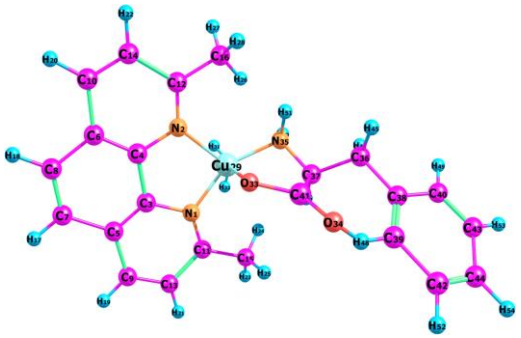


Figure-12: Optimized geometry of Copper (II) complex

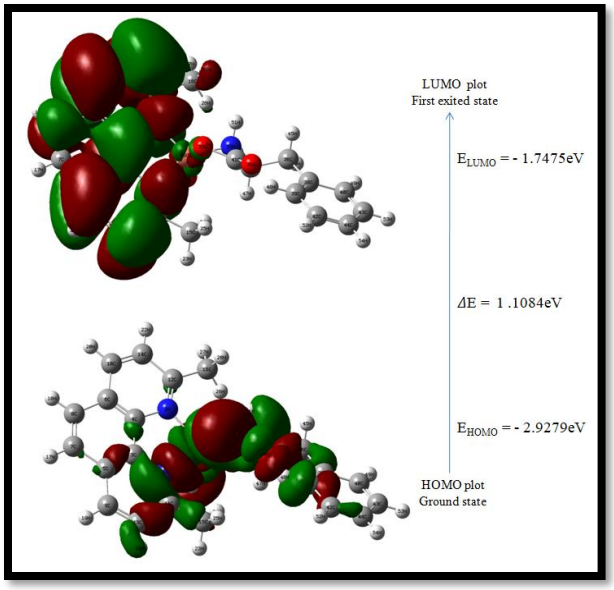


Figure-13: The frontier molecular HOMO-LUMO orbital's of the copper (II) complex.

Table-2: Theoretical calculations of selected bond lengths [Å] and angles [°] for [Cu(dmp)(L-phe)(H_2O)](NO_3)($3H_2O$)

Parameters	B3LYP/6-31G	Parameters	B3LYP/6-31G
Bond Length/[Å]			
N1-Cu29	1.879	Cu29-O33	1.871
N2-Cu29	1.886	Cu29-N35	1.880
Cu29-O30	1.832		
Bond Angle[°]			
N1-Cu29-N2	89.3	O30-Cu29-O33	153.1
N1-Cu29-O30	93.2	O30-Cu29-N35	82.4
N1-Cu29-O33	90.9	Cu29-O30-H31	109.7
N1-Cu29-N35	151.1	Cu29-O30-H32	109.2
C4-N2-Cu29	111.7	O33-Cu29-N35	81.2
C12-N2-Cu29	127.1	Cu29-O33-C41	108.7
N2-Cu29-O30	103.8	Cu29-N35-C37	105.4
N2-Cu29-O33	102.8	Cu29-N35-H50	112.0
N2-Cu29-N35	119.5	Cu29-N35-H51	109.5

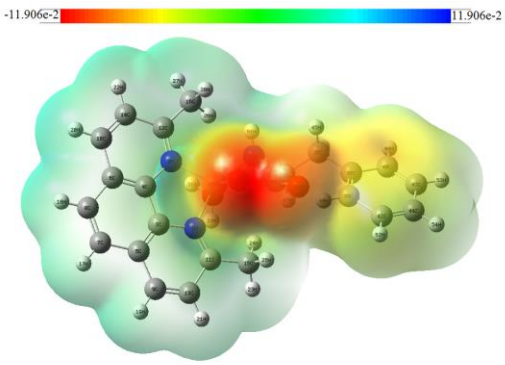


Figure-14: Molecular electrostatic potential (MEP) surfaces for copper (II) complex.

Table-3: Frontier molecular orbital parameters of synthesized copper (II) complex.

Frontier molecular orbital parameters	Values
HOMO (eV)	-2.9279
LUMO (eV)	-1.7475
Ionization potential (I)	2.9279
Electron affinity (A)	1.7475
Energy gap (eV)	1.1804
Electro negativity (χ)	2.3377

Chemical potential (μ)	-2.3377
Chemical hardness (η)	0.5902
Chemical softness (ξ)	0.8472
Electrophilicity index (ψ)	4.6297
Electronic charge	3.9609
Electron donating capability (w-)	5.8723
Electron accepting capability (w+)	3.5346

Molecular docking study

To understand how the synthesized compounds can interact with biological target, molecular docking is a fantastic method. In order to determine the preferred orientation of copper (II) complex inside the helix, a molecular docking experiment involving the Cu (II) complex was carried out employing a DNA duplex with sequence of $d(CGCGAATTCGCG)_2$ dodecamer (PDB ID: 1BNA). For the purpose of understanding drug-DNA interactions and the development of new medicinal products, molecular docking is a very advantageous, practical and attractive method. In order to determine the binding potential and orientation of copper (II) complex inside the DNA groove, molecular docking studies were carried out. The best docked positions identified during docking experiments of copper (II) complex with DNA duplex helix (PDB ID: 1BNA) are depicted in figure-15. The docking demonstrated that the Cu (II) complex is effectively bound to DNA receptors minor groove and exhibited free energy of binding (FEB) values of -8.74 k.cal/mole (Table-4) [73]. Stronger interaction ability of Cu (II) complex was with DNA reveals on the basis of highly negative binding energy. The results are in good agreement with obtained from absorption data. The more negative relative binding energy it binds more strongly to DNA receptor of Cu (II) complex. A molecular docking study indicated that the Cu (II) complex interacts with minor groove of DNA backbone through non-covalent interactions. This observation confirms that the docking studies may help in providing a better understanding of binding mechanism of compounds with DNA. Thus, molecular docking studies provide additional evidence for the preferred binding of complex at minor groove of DNA.

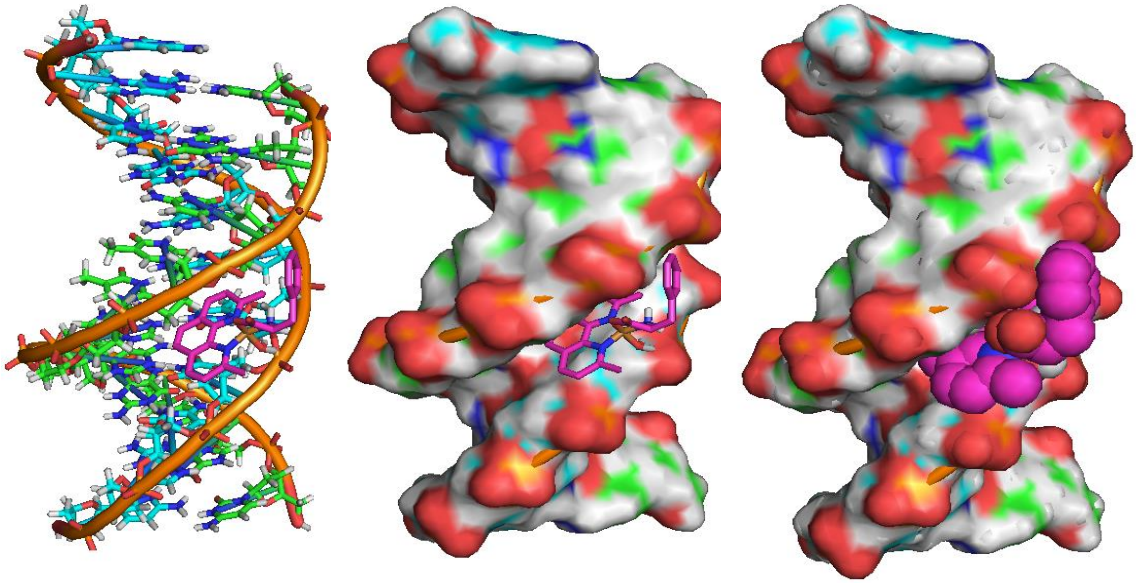


Figure-15: Molecular docking studies of $[Cu(dmp)(L\text{-}phe)(H_2O)](NO_3)(3H_2O)$ complex and DNA

Table-4: Molecular docking binding energy of the copper (II) complex

Parameters	Values
PDB ID	1BNA
Binding Energy (k.cal./mole)	-8.74
Inhibition constant, ki (μM)	388.89
RMSD [Å]	10.886
Intermolecular Energy (k.cal./mole)	-9.64
Vdw + Hbond + desolv Energy (k.cal./mole)	-8.44
Binding Nature	Groove Binding

IV CONCLUSION

We have reported the synthesis and characterization of new copper (II) complex using C, H, and N elemental analysis, EPR, ESI-MASS, FT-IR and UV-Visible. The compounds exhibit square pyramidal geometry around the central metal ion, consistent with these findings. The proposed geometry of copper (II) complex was further confirmed by DFT calculations. The binding interaction of CT-DNA with Cu (II)

complex was studied by electronic absorption, fluorescence, cyclic voltammetry and viscosity titrations. The result of EPR analysis was observed that the Cu-centered complex with five-center coordination is square pyramidal geometry. We can also say from EPR results that the base condition is dx^2-dy^2 and G value was found to be 2.12 and this value indicates the existence of spin exchange interactions between copper ions. DFT calculations for the Cu (II) complex are agreed well with the experimental observations. The results revealed that the Cu (II) complex can interact with DNA via groove binding. Molecular docking results were suggested that the prepared copper (II) complex can successfully bind in the cavity of the proteins. The cytotoxic potency of prepared copper (II) complex was determined against liver cancer cell lines (MCF-7) using Cis-platin as a reference drug control. Copper (II) complex have the most powerful anticancer effect with an IC_{50} value of 7.73 ± 1.007 µg/ml against human breast liver cancer cell line (MCF-7), which is significant and close to that of the reference drug (Cis-platin). The antimicrobial studies have shown that copper (II) complex have higher potent activities. Copper (II) complex may, therefore, be promising potential drugs for therapeutic intervention in various diseases.

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