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SYNTHESIS, CHARACTERIZATION AND MOLECULAR DOCKING STUDIES OF PYRROLE-2-CARBOXALDEHYDE WITH GLYCINE

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ABSTRACT
Schiff base ligand was prepared by condensation of pyrrole-2-carboxaldehyde with Glycine. Cu(II), Co(II), Mn(II), Zn(II) and Ni(II) complexes of above ligand was synthesised as well. The synthesised ligand and complexes have characterized by elemental analysis, IR, UV, ^{1}HNMR, EPR, TG/DTA, Powder XRD, SEM and EDAX. All the evidences suggested that the complexes have octahedral geometry.The conductivity data showed that the complexes are non-electrolytic in nature. The antimicrobial activity of the synthesized ligand and its complexes have been tested for well diffusion method. The result found that the metal complexes were more active than the ligand. The DNA cleavage activity of the ligand and its metal complexes have been studied.The result found that the Cu(II) complex effectively cleave pUC 18 DNA. The molecular docking study of the ligand and Cu(II) complex have been carried out, the copper complex have more binding energy than the ligand.

KEYWORDS:
Pyrrole-2-carboxaldehyde, Glycine, XRD, SEM.

INTRODUCTION

Schiff bases are important due to carbon nitrogen double bond (C=N) which can coordinate with metal[1]. Schiff bases having azomethine group and their metal complexes are widely used for industrial purposes and also reveal a wide range of biological applications. The transition metal ions play significant roles in various enzymes functions. Certain drugs play a vital role as bio-ligands in the biological systems. Similarly, nitrogen containing bases such as derivatives of pyrrole, pyridine, pyrimidine, pyrazine and purine have a vital role as bio-ligands. In general, the nature of metal ion and its oxidation states, types, number of ligands and isomers can utilize a critical influence on the biological activity of transition metal complex systems [2]. As an understanding of these factors affecting biological activity, enables the design of metal complexes with specific medicinal properties. Transition metal complexes obtained from Schiff base have still received a great deal of attention worldwide in view of their potential biological significance[3] Amino acids have importance in Schiff base complexes formation in which these can act as ligands due to their physiological and pharmacological activities. Moreover, these metal chelates appear to be involved in many of biological processes, such as transamination, racemization and carboxylation[4]. Heterocyclic Schiff bases are used for synthesis of pyrazolines, azetidinones, oxazolines which are potent anti-microbial molecules. Schiff bases and their co-ordination compounds have been gained importance now-a-days as they are useful in biochemical, anti-cancer, anti-inflammatory and antipyretic activities. Some of them have been used as complexing agent and powerful corrosion inhibitors. In recent years, because of new interesting applications found in the field of pesticides and medicine, the metal complexes with tridentate O, N, N types of alternative structures have attracted the attention of chemist. Various metal complexes with bi- and tridentate Schiff bases containing nitrogen and oxygen donor atoms play important role in biological system and represent interesting models for metalloenzymes, which efficiently catalyze the reduction of dinitrogen and dioxygen[5].
Pyrrole derivatives have been extensively explored in medicinal chemistry studies of DNA groove binders[6], cytotoxic agents[7], DNA intercalators[8], and anti-inflammatory agents, to mention a few. This 5-member heterocycle has been used as a bioisosteric replacement moiety for pyrazoles and amides[9]. Pyrrole and its derivatives are recognized to inhibit reverse transcriptase (human immunodeficiency virus type 1 (HIV-1)) and cellular DNA polymerases protein kinases. They exhibit wide range of biological activities such as antibacterial,

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anti-fungal, anti-viral, anti-inflammatory[10], anticancer and antioxidant activity[11]. The Schiff base N, N[1]bis(pyrrole-2 - Carbaldehyde) ethylenediamine and its Mn(II), Co(II), Ni(II) and Cu(II) complexes were characterized by spectral, magnetic and electrochemical studies. The spectral studies of the complexes, the ligand has coordination through the azomethine nitrogen atoms, pyrrole atoms and anions, supported by octahedral geometry. Mass spectrum explains the successive degradation of the molecular species in solution and justifies ML Complex. The bio-efficacy of the ligand and their complexes has been examined through *invitro* antibacterial studies[12]. The synthesized four new and two known amino acid Schiff base compounds. The compounds were derived through the condensation reaction of benzaldehyde, Salicyaldehyde, pyrrole-2-carbaldehyde, pyridine-2-carbaldehyde, fluorene-2-carbaldehyde and terephthalaldehyde with 2-phenylglycine methyl ester hydrochloride by both conventional method and microwave irradiation protocol. The new compounds were characterized by FTIR, ^{1}HNMR, LCMS and electronic spectral studies. DPPH radical scavenging effect were performed to determine antioxidant activities of the new compounds. All the compounds exhibited significant activities in DPPH radical scavenging[13].

In this paper the metal complexes of Cu(II), Co(II), Mn(II), Zn(II) and Ni(II) with the Schiff base derived from pyrrole-2-carboxaldehyde with glycine have been synthesized. The ligand and the metal complexes have been characterized by IR, UV, ESI-mass, TG/DTA, powder XRD, SEM and EDAX. The ligand and their metal complexes have been screened for their antimicrobial activities using the well diffusion method against the selected bacteria and fungai. The DNA cleavage activity of the ligand and its metal complexes have been studied.The molecular docking study of the ligand and copper complex have been studied.

EXPERIMENTAL

Material and Methods

All reagents were analytical grade producers (Aldrich) and used without further purification. Chemical analysis of Carbon, Hydrogen and Nitrogen was performed using a Elemental vario EL III CHN analyzer.IR spectra of the ligand and complexes were recorded in the range of 4000-400cm-1 on JASCO FTIR-4100 Infrared spectrophotometer by KBR disc technique. UV-visible spectra was recorded using spectrophotometer with DMSO as the solvent in the range of 200-700 nm. X-ray diffraction study of some metal complexes were obtained on Rigaka D-Max C-Xray diffractometer. Scanning Electron microscope images were recorded in JEOL model JSM -6390V electron microscopy.

Synthesis of Schiff base ligand potassium(E)-2-(((1H-pyrrol-2-yl) methylene)aminoacetate [PMAA]

Pyrrole-2-carboxaldehyde (0.01 mol) is dissolved in 20 ml MeOH and added 20 ml methonic solution of Glycine (0.01 mol) containing KOH (0.01 mol). The solution obtained was heated at 60° for 9 hours. Brownish yellow solution was formed. The volume of the solution is reduced to half. Filtered the precipitate, washed with ether followed by ethanol and dried in desiccators [12]. Brown solid; m.p: 160°C; Anal. (Cacld.) for $C_7H_7KN_2O_2$: C, 44.25 (44.19), H, 3.23 (3.71), N,14.91 (14.73); LC-MS (m/z): 190.3 [M+]; IR (cm^{-1}): 1690 ν(C=N), 1589 $\nu_{as}(COO^{-})$, 1480 $\nu_{sy}(COO^{-})$; 1H NMR (DMSO-d6, ppm); -CH=N- (1H, s, 8.31); UV-Vis. (λmax, nm): 343,456 ($\pi\rightarrow\pi^{*}$), ($n\rightarrow\pi^{*}$).

Schiff base metal complexes

To the hot methanolic solution of Schiff base ligand (0.01 mol), and the methanolic solution of metal ions ($CoCl_2.6H_2O$, $CuCl_2.2H_2O$, $NiCl_2.2H_2O$, $MnCl_2.2H_2O$, $ZnCl_2$) was added drop by drop at 60° in 1:2 (metal:ligand) molar ratio. The mixture was then refluxed for 1 hour the intensity of the colour becomes translucent. The resulting mixture was filtered out, washed repeatedly with ether and dried in desiccator. $[Cu(PMAA)_2(H_2O)_2]$: blue solid; m.p: 205°C; Anal. (Cacld.) $C_{14}H_{18}CuN_4O_6$: C, 41.50(41.84), H, 4.30 (4.51), N, 13.70 (13.94), M, 15.70(15.81); LC-MS (m/z): 402. [M+]; IR (cm^{-1}): 1627 ν(C=N), 1595 $\nu_{as}(COO^{-})$, 1435 $\nu_{sy}(COO^{-})$, 516ν(M-O), 478 ν(M-N); UV-Vis. (λmax,nm):313,580,625; $\Lambda c(\wedge^{-1}\ cm^2\ mol^{-1})$: 1.9; μeff (B.M): 1.79

$[Co(PMAA)_2(H_2O)_2]$: pink solid; m.p: 166°C; Anal. (Cacld.) $C_{14}H_{18}CoN_4O_6$: C, 42.38(42.33), H, 4.53 (4.57), N, 14.08 (14.10), M,14.60 (14.84); IR (cm^{-1}): 1635 ν(C=N), 1597 $\nu_{as}(COO^{-})$, 1388 $\nu_{sy}(COO^{-})$, 535ν(M-O), 470 ν(M-N); UV-Vis. (λmax,nm): 310,780,855; $\Lambda c(\wedge^{-1}\ cm^2\ mol^{-1})$: 9.60; μeff (B.M): 4.74

$[Mn(PMAA)_2(H_2O)_2]$: Dirty white solid; m.p: 200°C; Anal. (Cacld.) $C_{14}H_{18}MnN_4O_6$: C, 42.91(42.76), H, 4.42 (4.61), N, 15.28 (14.25), M,13.76 (13.97); IR (cm^{-1}): 1604 ν(C=N), 1404 $\nu_{as}(COO^{-})$, 1303 $\nu_{sy}(COO^{-})$, 550 ν(M-O), 435 ν(M-N); UV-Vis. (λmax, nm): 320,480,854; $\Lambda c(\wedge^{-1}\ cm^2\ mol^{-1})$: 7.05; μeff (B.M): 5.74

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$[Zn(PMAA)_2(H_2O)_2]$: Dirty white solid; m.p: 250°C; Anal. (Cacld.) $C_{14}H_{18}ZnN_4O_6$: C, 41.86(41.65), H, 4.08 (4.49), N, 12.96 (13.88), M,15.66 (16.20); IR (cm^{-1}): 1604ν(C=N), 1550 $\nu_{as}(COO^-)$, 1481 $\nu_{sy}(COO^-)$, 540ν(M-O), 478 ν(M-N); 1H NMR (DMSO-d6, ppm); -CH=N- (1H, s, 9.47); UV-Vis. (λmax, nm): 433,540,625; $\Lambda c(\wedge^{-1} cm^2 mol^{-1})$: 13.5

$[Ni(PMAA)_2(H_2O)_2]$: pink solid; m.p: 166°C; Anal. (Cacld.) $C_{14}H_{18}NiN_4O_6$: C, 42.40(42.35), H, 4.14 (4.57), N, 14.08 (14.11), M,14.56 (14.78); IR (cm^{-1}): 1612 ν(C=N), 1533 $\nu_{as}(COO^-)$, 1419 $\nu_{sy}(COO^-)$, 509 ν(M-O), 478 ν(M-N); UV-Vis. (λmax, nm): 433,655,723; $\Lambda c(\wedge^{-1} cm^2 mol^{-1})$: 18.0; μeff (B.M): 3.18

Antimicrobial activity

Test organisms

The Schiff base ligand and its metal complexes were screened against bacterial species such as Bacillus subtilis, Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Pseudomonos aeruginosa, Staphylococcus aureus and fungal species like Candida albicans and Aspergillus niger in agar well diffusion method. The Solvent used for dissolving the synthesised compounds was DMSO.

Experimental methods

Muller hinton agar medium (20ml) was poured into each petri plate, and plates were swabbed with 100 µl inoculated of the test microorganisms and kept for 15 minutes for adsorption. Using sterile cork borer of 8mm diameter, wells were bored into the seeded agar plates, and these were located with a 100µl solution of each compound in DMSO. All the plates were incubated 37° for 24 hrs .After incubation, the inhibition growth was analysed and the results were recorded.

DNA cleavage

Agarose gel electrophoresis

Agarose gel electrophoresis is a method for separating and visualizing DNA fragments. The fragments are separated by charge and size and movement through agarose gel matrix, when subjected to an electric field. The electric field is generated by applying potential across an electrolyte solution (buffer). When boiled in an aqueous buffer, agar dissolve and upon cooling solidifies to a gel. 1.5% agarose gel was prepared in 1x TE buffer and melted in hot water bath at 90 ˚C. Then the melted agarose was cooled down to 45 ˚C. 6 µl of 10 mg/ml of ethidium bromide was added and poured in to gel casting apparatus with the gel comb. After setting, the comb was removed from the gel. The electrophoresis buffer was poured in the gel tank and the platform with the gel was placed in it so as to immerse the gel. The gel was loaded with the samples and run at 50 V for 30 minutes. The stained gel was visualized using a gel documentation system (E gel imager, Invitrogen).

Molecular Docking study

Molecular docking is one of the important drug design tools to understand the binding pattern of investigated compounds toward target protein PDB ID : 1JIJ from Staphylococcus aureus. Molecular docking of the drug molecule with the protein provides vital information about protein-ligand interactions and is usually used to examine the binding course of drug molecules to their protein targets in order to predict the affinity, activity and to rationalize the experimental results. The binding confirmations between the synthesized compound and the protein complex are analyzed with the help of Auto Dock 4.0 Tools[14]. The grid map of dimension and grid spacing of were selected so as to ensure appropriate accessible space for the compound and displayed in Table 4. The stable confirmation between the protein and the ligand were selected based on Lamarckian Genetic algorithm (LGA). The hydrogen bond interaction between the protein and ligand were visualized using PyMOL viewer[15].

RESULTS AND DISCUSSION

The analytical data and some physical properties of the ligand and its metal complexes were reported in experimental sections.They are showed that the complexes are formed in the ratio 1:2 (M : L). The resulting metal complexes were found to be stable at room temperature and insoluble in common solvents such as EtOH, MeOH but soluble in DMF and DMSO. The low molar conductivity values of the complexes suggest their non-electrolytic nature.

Electronic Spectra

The electronic spectrum of Schiff base ligand, there are two absorption bands at 343, 456 nm assigned to □ - □* and n - □* transitions. These transitions are also found in the spectra of the complexes, but they are shifted towards longer wavelength from ligand to complex, indicating coordination of ligand to metals through the azomethine moiety. The electronic spectrum of copper (II) complex showed two absorption band in the range of 313, 580, 625 nm is assigned to $^2B_{1g}$ □ $^2A_{1g}$, $^2B_{1g}$ □ $^2B_{2g}$ and $^2B_{1g}$ □^2E_g transition. These values suggested

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octahedral geometry. It has been further confirmed by the magnetic moment of Cu(II) complex is 1.79 B.M corresponding to the presence of one unpaired electron and it supports an octahedral geometry[16]. The Co(II) complex contains a d-d band at 780, 855 nm which corresponds to $^4T_{1g}(F) \rightarrow ^4A_2g(F)$ and $^4T_{1g}(F) \rightarrow ^4T_{2g}(F)$ transitions. These transitions correspond to the octahedral geometry and further supported by their magnetic susceptibility values are 4.74 BM. The spectrum of Mn(II) complex contains bands at 320, 480 and 854 nm. Those absorption bands may be assigned to the $^6A1_g \rightarrow ^4A_1g$, $^6A1_g \rightarrow ^4A_{2g}$, $A1_g \rightarrow ^4Eg$ respectively. These bands suggest that the complex possess an octahedral geometry and further supported by their magnetic susceptibility values are 5.74 BM. Zn(II) complex shows the bands at 655, 723 nm due to charge transfer transition and an octahedral geometry was suggested for this complex. The Zn(II) complex is found to be diamagnetic as expected for d^{10} configuration. Ni(II) complex display three bands at 443, 655,723 nm describe to $^3A_{2g}(F) \rightarrow ^3T_{2g}(F)$, $^3A_{2g}(F) \rightarrow ^3T_{1g}(F)$, $^3A_{2g}(F) \rightarrow ^3T_{1g}(P)$ transitions respectively these bands indicate that the complex have also an octahedral geometry[17]. Ni (II) complex showed the magnetic moment value of 3.18 B.M, in the range of 2.8-3.5 B.M suggesting the octahedral structure[18].

Infrared Spectra

Infrared spectrum of the ligand and copper(II) complex are given in figure 1 and 2. A comparison of IR stretching frequency of the ligand and its metal complexes gave an idea about the mode of bonding in complexes on complexation with metal ion, the characteristics IR frequencies of the coordinating groups are influenced by the force constant on the metal ligand bond resulting in the shift of the group frequencies. These shifts are useful in identifying the coordination sites.

The band at 1690 cm^{-1} is due to azomethine group of the ligand undergoes a shift to lower frequency by about ~ 50 cm^{-1} in the IR spectra of the metal complexes. The shift of this group frequency in the spectra of the metal complexes suggested the coordination of azomethine nitrogen atom to metal ion[19]. The band at 1480 and 1589 cm^{-1} can be respectively ascribed to symmetric carboxyl $V_s(COO^-)$ and asymmetric carboxyl groups[20,21]. During complexation these bonds were shifted to lower frequency by ~40-30 cm^{-1} range indicating the linkage between the metal ion and carboxylato oxygen atom. The large difference between the $V_{as}(COO^-)$ and $V_s(COO^-)$ values of ~ 200cm^{-1} indicates the monodentate binding nature of the carboxylato group in the complexes. The aqua complexes contain weak to medium a broad band at (3556-3209) cm^{-1} due to stretching vibration OH of water and a sharp shoulder at (1512-1550) cm^{-1} may be assigned to bending vibration of water. Water molecules are coordinated, confirmed by occurrence of additional strong and sharp band at 802-864 cm^{-1} due to OH rocking vibrations[22]. A characteristic band at ~3250 cm^{-1}, due to (N-H) of pyrrole, was observed in the spectra of the ligand and the complexes[23]. In the lower frequency region the weak bands observed at 509 - 555 and 435 - 478 cm^{-1} have been assigned respectively to the (M-O) and (M-N) vibrations[24]. Accordingly, one can deduce the ligand binds the metal ion as bidentate fashion. The bonding sites are the azomethine nitrogen and the carboxylato oxygen atoms.

Table 1. IR spectral data of PMAA and its complex (cm^{-1})

Compound	C = N	$\{_s COO^-$	$v_{as}COO^-$	H_2O	N-H	M-O	M-N
PMAA	1690	1480	1589	-	3250	-	-
$[Cu(PMAA)_2 2H_2O]$	1627	1435	1595	3350	3240	516	478
$[Co(PMAA)_2 2H_2O]$	1635	1388	1597	3556	3220	555	470
$[Mn(PMAA)_2 2H_2O]$	1604	1303	1404	3425	3240	550	435
$[Zn(PMAA)_2 2H_2O]$	1604	1481	1550	3340	3250	540	478
$[Ni(PMAA)_2 2H_2O]$	1612	1419	1555	3209	3225	509	478

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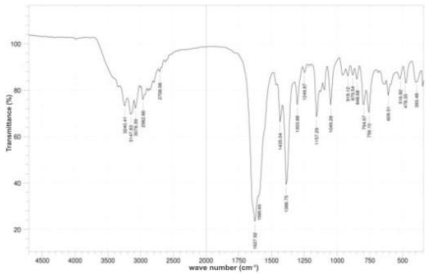

Figure: 1 IR spectrum of PMAA

Figure: 2 IR Spectrum of $[Cu(PMAA)_2(H_2O)_2]$

^{1}HNMR Spectra

The ^{1}HNMR spectrum of the ligand and Zn(II) complex was recorded in DMSO-d_6. The spectrum of the complex showed the multiplet signals at δ 6.90-7.26 ppm denote aromatic protons and these remains almost at the same position in the spectra of the ligand[25]. The peaks in the region δ 6.18 - 6.28 ppm were assigned to pyrrole hydrogen.

The coordination of the azomethine nitrogen was assigned by the downfield shifting of the azomethine proton signal from 8.31 ppm in free ligand to 9.47 ppm in their zinc complex. The downfield shifting of azomethine proton in Zn(II) complexes was attributed to the de-shielding is probably due to the donation of the lonepair of electrons on the azomethine nitrogen to metal resulting in the formation of a coordinate bond. These observations suggest that the ligand coordinate to the Zn(II) through the azomethine nitrogen and carboxylato oxygen atom. The appearance of signals due to NH protons at the same positions in the ligand and its complex, show the non involvement of this group in coordination[26](13.0 ppm). A peak in the region 2.9 ppm characteristic of methylene group is present in the spectrum of the complex. Appearance of a new singlet at 3.8 ppm with an integration corresponding to four protons may reasonable be ascribed to coordinated water molecule in the complex[27]. The ^{1}HNMR spectrum of the ligand and Zinc complex is shown in Figure.3, 4.

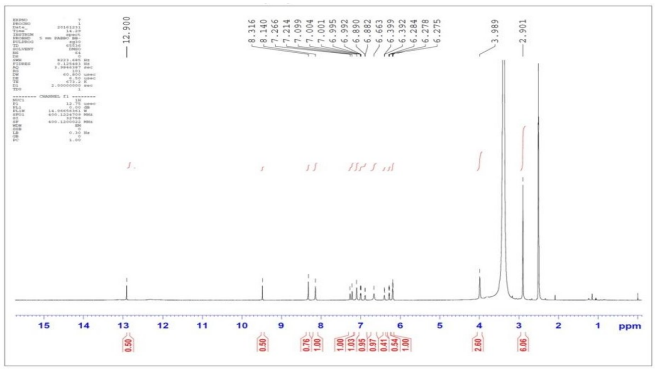

Figure 3. ^{1}HNMR spectrum of PMAA

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Figure 4. ^{1}HNMR spectrum of $[Zn(PMAA)_2(H_2O)_2]$

Mass spectra

The ESI mass spectra of Schiff base ligand was performed to determine their molecular weight and study their fragmentation. The mass spectrum of Schiff base ligand showed of a molecular ion peak at m/z 190.3 and it corresponds to $[M^+]$ ion. The other fragments of the ligand show the peaks at different m/z values at 104 (C_3KNO), 174 ($C_7H_5KN_2O$). The mass spectrum of copper(II) complex showed molecular ion peak at m/z = 402 $[M^+]$ ion. which is also supported by the Nitrogen rule, since the compound possesses the even number of nitrogen atoms[28]. Elemental analysis values are in close agreement with the values calculated from molecular formula assigned to these complexes, which are further confirmed by the ESI- mass spectral study of the complexes. The ESI mass spectrum of the ligand and copper complex are shown in Figure. 5, 6.

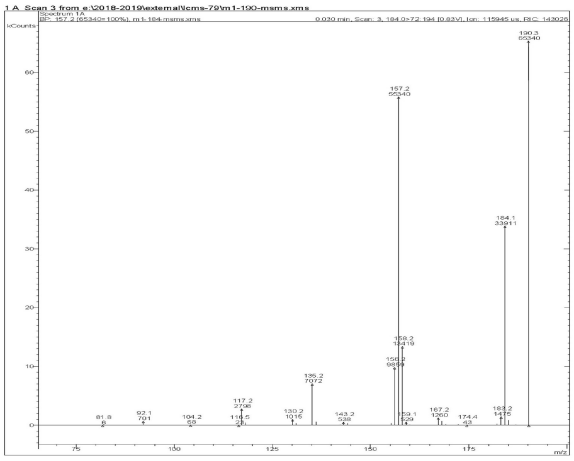

Figure 5. Mass spectrum of PMAA

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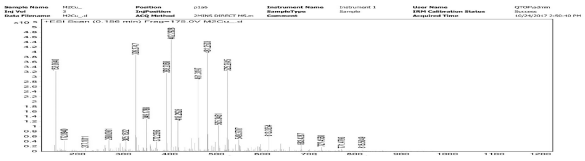

Figure 6. Mass spectrum of $[Zn(PMAA)_2(H_2O)_2]$

EPR spectra

The X-band EPR spectra of Cu(II) complex were recorded in DMSO at liquid nitrogen temperature and at room temperature. The spectrum of Copper(II) complex was given Figure. 7.

The g values of $g_{\|} = 2.32$ and $g_{\infty} = 2.03$, respectively, which is characteristic for the axial symmetry of Cu(II) complexes[29,30]. The $g_{\|}$ and g_{∞} values are closer to 2 and $g_{\|} > g_{\infty}$. This suggests a tetragonal distortion around Cu(II) corresponding to elongation along the four-fold symmetry Z-axis[31]. The trend $g_{\|}$ (2.32) > g_{∞} (2.03) > ge (2.0023) shows that the unpaired electron is localized in the $dx^2 - y^2$ orbital of the Cu(II) ions in the complex[32]. In addition, an exchange coupling interaction between two Cu(II) ions is explained by Hathaway expression[33]$G = (g_{\|} - 2) / (g_{\infty}-2)$. If the value G> 4.0, the exchange interaction is negligible and if G< 4.0, a considerable exchange coupling is present in the solid complex. In the present Cu(II) complex, the 'G' value (11.11) is >4 indicating that there is no interaction in the complex. Moreover, the absence of a half field signal at 1600 G corresponding to DM=±2 transitions indicates the absence of any Cu–Cu interaction in the complex[34]. Kivelson and Neiman have shown that for an ionic environment $g_{\|}$ is 2.3 or larger, but for a covalent environment $g_{\|}$ is less than 2.3. The $g_{\|}$ value for the present complex is 2.32, indicating a significant degree of covalency in the metal–ligand bond[35]. The empirical distortion in copper (II) complexes, if the ratio is close to 100 cm^{-1}, it indicates rough by a square planer structure around the copper (II) ions and the values from 170 – 250 cm^{-1} are indicative of distorted tetrahedral geometry . If the ratio is between 110 and 170 cm^{-1}. It indicated nearly and octahedral environment around copper (II) on with small distortion. For the present copper complex the $g_{\|}/A_{\|}$ value is 127 cm^{-1} which indicate the complex have distorted octahedral geometry.

The covalency parameter $\langle^2$ and $®^2$ have been calculated using the following equations. If $\langle^2 = 1.0$ it s indicated complete ionic character whereas $\langle^2 = 0.5$ denotes 100% covalent bonding , with assumption of negligible small values of the overlap integral.

$$\langle^2 = (A_{\|}/0.036) + (g_{\|} - 2.0027) + 3/7\ (g_{\infty} - 2.0027) + 0.04$$

$$®^2 = (g_{\|} - 2.0027)\ E/\ (-8\lfloor\langle^2)$$

The covalency parameter (α^2=0.50) indicates considerable covalent character for the metal ligand bond. Thus, the EPR study of the copper (II) complex has to provide supportive evidence to the conclusion obtained on the basis of electronic spectrum and magnetic moment value.

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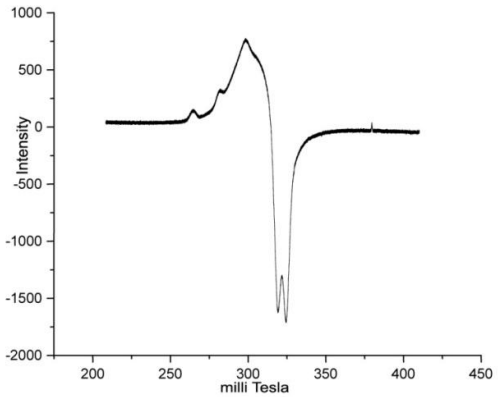

Figure 7. EPR spectrum of $[Cu(PMAA)_2(H_2O)_2]$

Thermo gravimetric studies

The thermal behaviour of the copper(II) complex was examined using TG and DTA methods, in the temperature range of 40 °C to 750 °C at a heating rate of 20 °C/min. The thermogram indicates that the complex is stable upto 250 °C and then begins to decomposed in three stages. Water molecules present in the complexes are of two types lattice water and coordinated water, the lattice water will be lost 60-120 °C and coordinated water molecules will be lost at high 120-250 °C. In the present study Cu(II) complex decomposes in three stages, in the first stage (230 °C) it shows weight loss (18.75%) corresponding to high temperature range this indicates that coordinated water molecules[36]. In the second stage (249 °C) it shows the weight loss upto (7.60%) and that corresponding to the ligand glycine. In the third stage (329°C) the weight loss (21.66%) corresponds to complete decomposition of the complex and oxidation of CuO as shown in Figure.8.

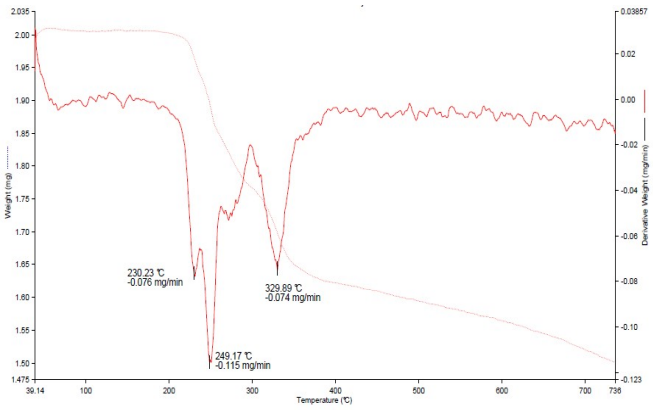

Figure 8.TG/DTA Spectrum of $[Cu(PMAA)_2(H_2O)_2]$

Thus from the various physiochemical and spectral studies, the proposed structure of the Schiff base ligand PMAA and metal complexes are given in Figure 9, 10.

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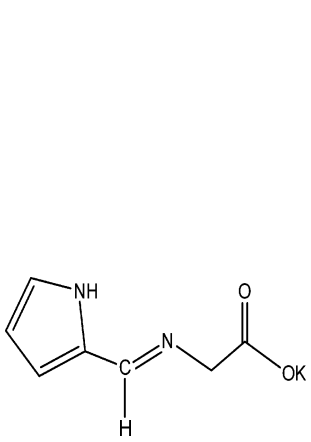

Figure 9.Structure of ligand PMAA

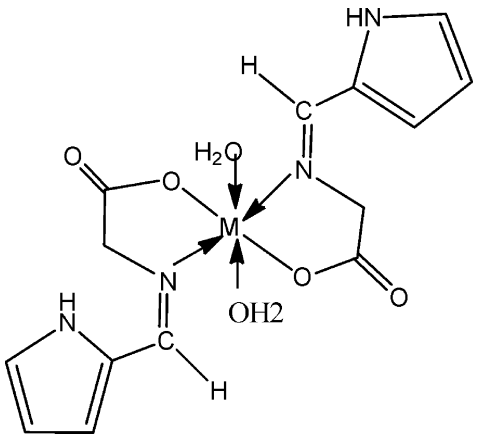

Figure10. Structure of Schiff base metal complexes where M= Cu(II), Co(II), Mn(II), Zn(II) and Ni(II)

XRD

The powder XRD patterns of ligand was recorded over the $2^{\theta\theta}$ = 0-80A° range and are shown in Figure 11. The Ligand have an average size is 26.38 nm that the ligand is a nano crystalline phase. The average crystalline size of the complex d_{XRD} was calculated using schorrer's formula[37].

$$d_{XRD} = 0.9\lambda/\beta \cos \theta$$

The Cu(II) complex have an average size of 29.61 nm, suggesting that the complex are in a nanocrystalline phase[38]. From the data, the complexes show sharp peaks indicating their crystalline nature. The powder XRD pattern of Cu (II) complex is shown in Figure.12.

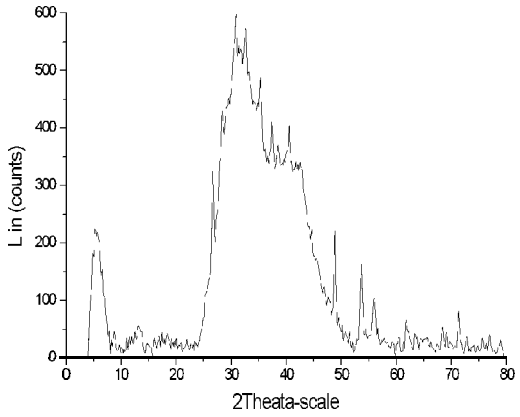

Figure 11. XRD spectrum of PMAA

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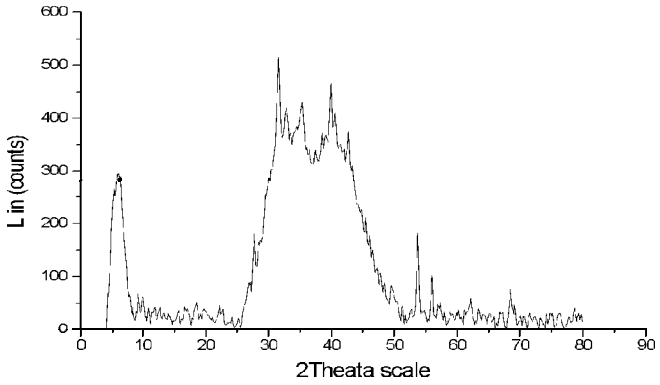

Figure 12. XRD Spectrum of $[Cu(PMAA)_2(H_2O)_2]$

Scanning Electron Microscope (SEM)

The surface morphology of ligand is shown in Figure 13. The SEM images of Schiff base ligand has stone like morphology. This arrangement showed a thickness in the submicron regime. The average size of particle is 68.64 nm. However particles with size less than 100 nm were also absorbed which groups to form agglomerate of larger size.

The surface morphology of Cu(II) complexes is shown in Figure 14. The SEM images of the complex have needle shaped species. The average size of the particle is 84.33 nm. The particle size is less than 100 nm which groups to form agglomerate of larger size. The average crystallite size obtained from XRD also shows that the particles were agglomerated that these complexes have polycrystalline with nano sized grains[39].

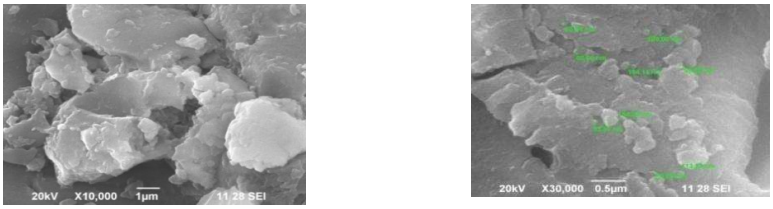

Fig 13.SEM Photograph of PMAA

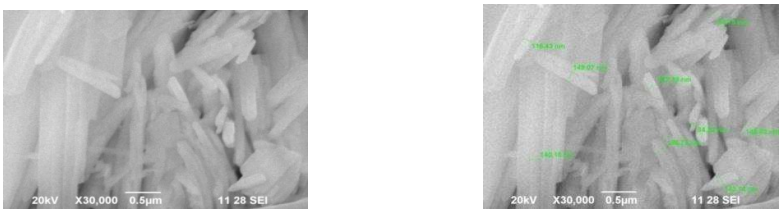

Figure14.SEM photograph of $[Cu(PMAA)_2(H_2O)_2]$

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EDAX Analysis

EDAX can be used to determine the chemical elements present in the sample, and can be used to estimate their relative abundance. Elemental maping of a sample and image analysis are also possible as showed in Figure 15 and 16. The EDAX result of ligand showed that the atomic percentage of carbon, nitrogen, oxygen and potassium are 48.00, 15.73, 24.62, 11.65% respectively while that of Cu(II) complex were found to be 40.86, 23.95, 13.22, 15.16% for carbon, oxygen, nitrogen and copper respectively. The result of EDAX data reveals the purity of the complex which indicates that there is no elemental contamination present in the complex.

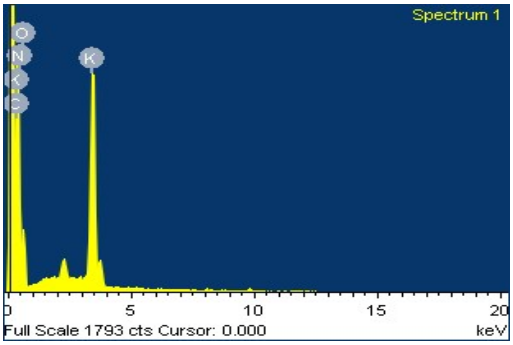

Figure 15. EDAX photograph of PMAA

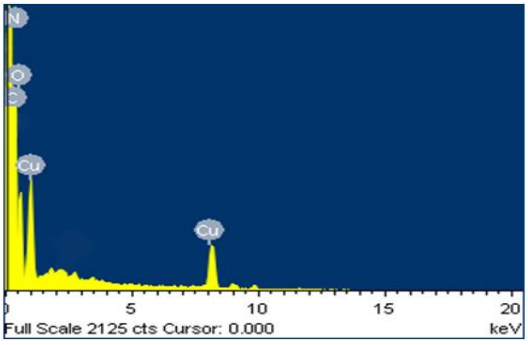

Figure 16. EDAX photograph of $[Cu(PMAA)_2(H_2O)_2]$

Antimicrobial activity

The ligand PMAA, and its metal complexes were screened against six bacterial species to defect their anti bacterial properties. The results were presented in the Table 2 and Figure 17. Cu(II) complex showed good activity against all the selected bacterial species. Mn(II) and Ni(II) complexes were less activity against all the bacterial species. Co(II) and Zn(II) complex showed moderate activity against *Pseudomonas aeruginosa* and inactive against *Staphylococcus aureus*. Cu(II) complex showed highest activity against *Pseudomonas aeruginosa*, and *Staphylococcus aureus* and the zone of inhibition was greater than the Schiff base ligand, other complexes and the standard Amikacin. Cu complex can be tested for *in vivo* studies and can be further used as drugs for *Pseudomonas aeruginosa* and *Staphylococcus aureus*. All the metal complexes showed less activity against the *Bacillus cereus*.

The results indicated that Cu(II), Co(II) and Ni(II) complexes of the ligand PMAA showed medium antifungal activity against *C. albicans*. Mn(II) and Zn(II) complexes did not exhibit any significant effect against the fungal strains, *C. albicans* and *Aspergillus niger*. Co(II) and Ni(II) complexes showed moderate antifungal activity against *A. niger*. Also, the ligand showed moderate antifungal activity (Table 3and Figure 18). The enhanced activities of the complexes are due to the variation in the structure and reactivity of the PMAA upon coordination. The increase in biological activity of the metal chelates may be explained on the basis of overtone's concept and Tweedy's chelation theory[40,41].

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Table 2 Antibacterial activities of PMAA and its metal complexes

Compound	Zone of inhibition (mm)					
	B. subtilis	*B. cereus*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*
PMAA	7	4	7	8	8	7
$[Cu(PMAA)_2(H_2O)_2]$	10	6	10	24	32	20
$[Co(PMAA)_2(H_2O)_2]$	6	-	4	6	20	7
$[Mn(PMAA)_2(H_2O)_2]$	-	5	-	5	6	-
$[Zn(PMAA)_2(H_2O)_2]$	5	5	5	25	20	-
$[Ni(PMAA)_2(H_2O)_2]$	5	-	-	-	-	7
Amikacin	30	33	12	34	31	18

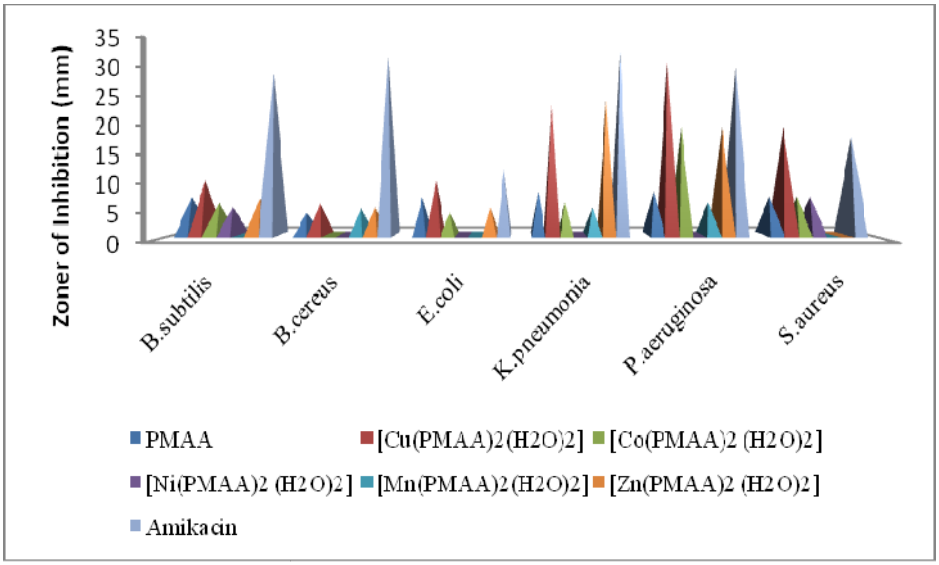

Figure 17. Antibacteriogram of PMAA and its metal complexes

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Table 3. Anti fungal activities of PMAA and its metal complexes

Compounds	Zone of inhibition (mm)	
	C. albicans	*A. niger*
PMAA	9	5
$[Cu(PMAA)_2(H_2O)_2]$	7	-
$[Co(PPMAA)_2(H_2O)_2]$	7	4
$[Mn(PMAA)_2(H_2O)_2]$	-	-
$[Zn(PMAA)_2(H_2O)_2]$	-	-
$[Ni(PMAA)_2(H_2O)_2]$	5	6
Nystatin	18	13

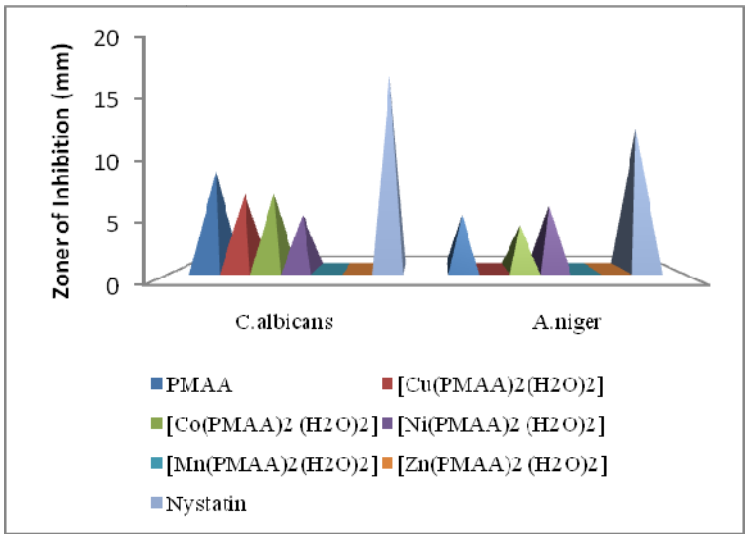

Figure 18. Antifungal activity of PMAA and its metal complexes

DNA cleavage

The results of the series of the ligand PMAA and its metal complexes are shown in the Figure .19 (Lane 1-8). From the result of this series of the ligand and its metal complexes it can be concluded that the copper (II) complex, Manganese (II) complex (lane 3,5) showed high DNA cleavage activity where as Zinc(II) complex, cobalt(II) complex, nickel(II) complex and ligand exhibited moderate DNA cleavage activity.

Gel electrophoresis experiments were performed with complexes in the presence of H_2O_2 as an oxidant. The nuclease activity was greatly enhanced by the incorporation of metal ion in the respective co-polymer, it is evident from Figure 9.16**.** which shows that the complexes Lane 3 and 5 cleavage DNA more efficiently in the presence of oxidant, which may be due to the formation of hydroxyl free radicals. The production of hydroxyl free radical is due to the reaction between the metal complex and oxidant. These hydroxyl radicals participate in the oxidation of the deoxyribose moiety, followed by hydrolytic cleavage of the sugar phosphate backbone[42].

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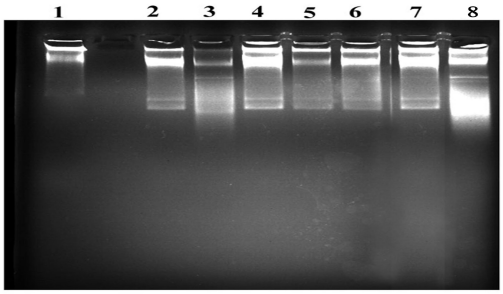
These results indicate that the metal ions play an important role in the cleavage of isolated DNA. As the compound was observed to cleave the DNA. The complexes which have feeble hydrogen abstraction from the sugar moiety show less cleavage activity. Many compounds were observed to cleave the DNA, it can be concluded that the compound inhibits the growth of the pathogenic organism by cleaving DNA.



Lane 1: DNA control; Lane 2: DNA+PMAA+ H_2O_2; Lane 3: DNA+ $[Cu(PMAA)_2(H_2O)_2]$ + H_2O_2; Lane 4: DNA+ $[Co(PMAA)_2(H_2O)_2]$ + H_2O_2; Lane5: DNA+ $[Mn(PMAA)_2(H_2O)_2]$ + H_2O_2; Lane6, DNA+ $[Zn(PMAA)_2(H_2O)_2]$ + H_2O_2; Lane7: DNA+ $[Ni(PMAA)_2(H_2O)_2]$ + H_2O_2; Lane 8: DNA+ H_2O_2

Figure .19.Gel electrophoresis for the DNA cleavage of PMAA and its metal complexes

Molecular Docking study

Influence of PMAA and $[Cu(PMAA)_2(H_2O)_2]$ complex against target protein

PMAA exhibited a binding affinity of -6.3 Kcal/mol towards the target protein with four hydrogen bond interactions at the residue positions ASN 124, THR 75, TYR 170, GLN 174. The carboxylate oxygen atoms form three hydrogen bond with the residues ASN 124, THR 75, TYR 170. The azomethine nitrogen atoms form hydrogen bond with the residue GLN 174. The $[Cu(PMAA)_2(H_2O)_2]$ complex structure exhibited highest binding affinity compared to the PMAA molecule. The carboxylato oxygen atoms form five hydrogen bond interations at residue position ASP 243, LYS 105, LEU 242 was observed and they contributed to a binding affinity of -9.2 Kcal/mol. Thus the $[Cu(PMAA)_2(H_2O)_2]$ complex structure had contributed significantly towards the binding of the compound with the target protein.

The best binding energy of the copper complex of $[Cu(PMAA)_2(H_2O)_2]$ were obtained as -9.2 kcal/mol. The water coordination of the copper complex of PMAA has high binding affinity. The higher binding affinity is apparently attributed to the formation of more and strong hydrogen bonds[43]. The more negative of binding energy indicated its strong binding ability to the protein.

Table 4 Autodock score of compound against target protein

S.No	Compound	Binding affinity (Kcal/mol)	No. of H bonds	Interacting Amino acid Residues
1	PMAA	-6.3	4	ASN 124,THR 75, TYR170,GLN 174
2	$[Cu(PMAA)_2(H_2O)_2]$	-9.2	5	ASP 243,LYS 105,LEU 242,LYS 105

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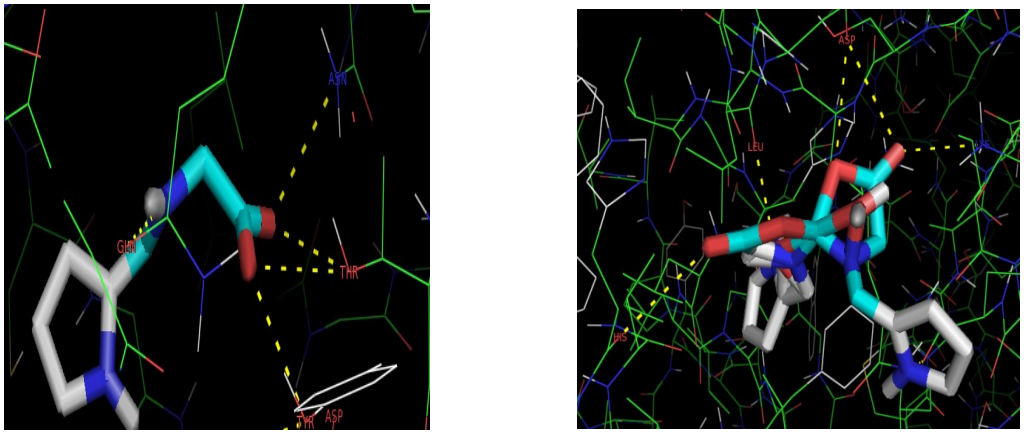

Fig 20.Binding mode of PMAA and $[Cu(PMAA)_2(H_2O)_2]$ complex with 1JIJ

CONCLUSION

Schiff base and its metal complexes were prepared and characterized using the elemental analysis, molar conductance, Magnetic measurement, SEM, XRD, IR and electronic spectral analysis. IR spectral data shows that the ligand act as bidentate, coordinating through azomethine nitrogen and carboxylato oxygen atoms. EPR, ESI-mass, Electronic spectral studies revealed that octahedral geometry for Cu(II), Co(II) ,Zn(II) and Ni(II). XRD and SEM analysis explains the crystalline and morphological structure of the complexes. The *in vitro* antimicrobial study revealed that all complexes exhibit significant activities as compared to ligand. The DNA cleavage activity of the ligand and its metal complexes have been found that the Cu(II) complex effectively cleave pUC 18 DNA. The molecular docking study of the ligand and Cu(II) complex have been carried out, the copper complex have more binding energy than the ligand. The higher binding affinity is apparently attributed to the formation of more and strong hydrogen bonds. The more negative binding energy indicated its strong binding ability to the protein.

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