Integrative Study on Synthesis, Antioxidant Potential, Molecular Docking and DFT Analysis of Dinuclear Copper (II) Complexes

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Abstract
Schiff base metal complexes originated by the condensation reaction of 2,6-diformyl-4-methylphenol and 4-methyl-o-phenylenediamine with copper ions. Several physico-chemical and spectroscopic methods were used to comprehensively describe this Schiff base and copper ion complexes, revealing the pentadentate chelation of the ligand. Around the Cu(II) center, the resultant complexes exhibit a square pyramidal geometry. The6-311++G(d,p) basis set was used to determine the ligand's optimal geometry parameters. Additionally, calculations were carried out for Mullikan atomic charges, total energy, and the energy of HOMO and LUMO. By interacting with the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), newly synthesized ligands and their metal complexes have been shown to have free radical scavenging activity at concentrations between 50 and 250 μg/ml. All the compounds has demonstrated promising antioxidant properties. Molecular docking revealed the type, position, energy account, and distance of the linkages influencing how these complexes interact with cancer proteins.

Keywords: *Schiff base ligand, DFT, HOMO – LUMO, _invitro_ antioxidant and Molecular Docking.*

1. INTRODUCTION

Coordination chemistry combines organic ligands with hetero atoms like nitrogen, oxygen as coordination sites, resulting in several fascinating beneficial molecules. Ligands with multiple donor hetero atoms have gained widespread interest in coordination chemistry due to their high chelating capacity to create mononuclear, dinuclear or multinuclear metal complexes [1-2]. Schiff base has been referred to by Hugo Schiff's name. Schiff bases, discovered in coordination chemistry about a century ago, it play a vital role as ligands and are widely used in organic compounds. They have several applications, including polymer stabilizers, catalysts and organic synthesis intermediates [3-6]. Schiff bases have a distinct imine group which leads to their biological action. Schiff base metal compounds have gained prominence as analytical reagents due to their broad application and simplicity of fabrication [7]. Keeping in view the importance of Schiff base metal complexes, The current work is a step forward in the hunt for new copper(II) Schiff bases compounds. Three novel copper (II) Schiff base complexes derived from 2, 6-diformyl-4-methylphenol and 4-methyl-o-phenylenediamine are reported here and in addition, DFT calculations of ligand (SBL), Molecular docking studies of metal complexes and antioxidant of ligand and metal complexes were studied.

2. MATERIAL AND METHODS

The electronic spectra of the ligand and its complexes were measured in liquid phase in 10^{-3} M solution using a Lambda-35 UV-Vis double beam spectrophotometer between 200 and 800 nm. Shimadzu FTIR spectrometer experimental scan was used for obtaining FTIR spectra using the KBr disc method. The range in which the spectra were taken in 400–4000 cm^{-1}. Using DMSO-d_6 as the solvent and TMS as the internal reference, the ligands' ^1H and ^{13}C-NMR spectra were obtained using a Bruker Advance II 400 MHz NMR instrument model.

2.1 DPPH Assay

A Shimadzu UV-1601 spectrophotometer model was used to record the antioxidant activity. The stable free radical used was 1,1-Diphenyl-2-picrylhydrazyl (DPPH), which has a red colour. The DPPH (0.1 mM) was added at predefined concentrations of 50, 100, 150, 200, 250 μg/mL to DMSO solution to make the resulting mixtures. The absorbance at 520 nm was measured following a 20 minute inhibitory period at room temperature. The standard used was ascorbic acid. When DPPH produces free radicals, the substance's red colour turns yellow [8]. The following equation can be used to compute the percentage of radical scavenging activity.

$$\textbf{Scavenging activity } (\%) = \left[\frac{\left(A_{\text{Control}} - A_{\text{Sample}}\right)}{A_{\text{Control}}}\right] \text{x } 100$$

2.2 Computational Details

The computational program Gaussian09 is used to calculate the molecular structure and chemical reactivity properties. Due of Gaussian's ability to forecast better levels of accuracy in chemical property predictions, most researchers have recently employed it to calculate [9,10]. Chemical natures are examined using the DFT/B3LYP method with the 6-311++G(d,p) basis set [11,12]. According to the current study, the techniques are employed to compute the following attributes: HOMO-LUMO, MEP, and Mulliken atomic charge chemical reactivity calculations, as well as chemical structure optimization.

2.3 Molecular Docking

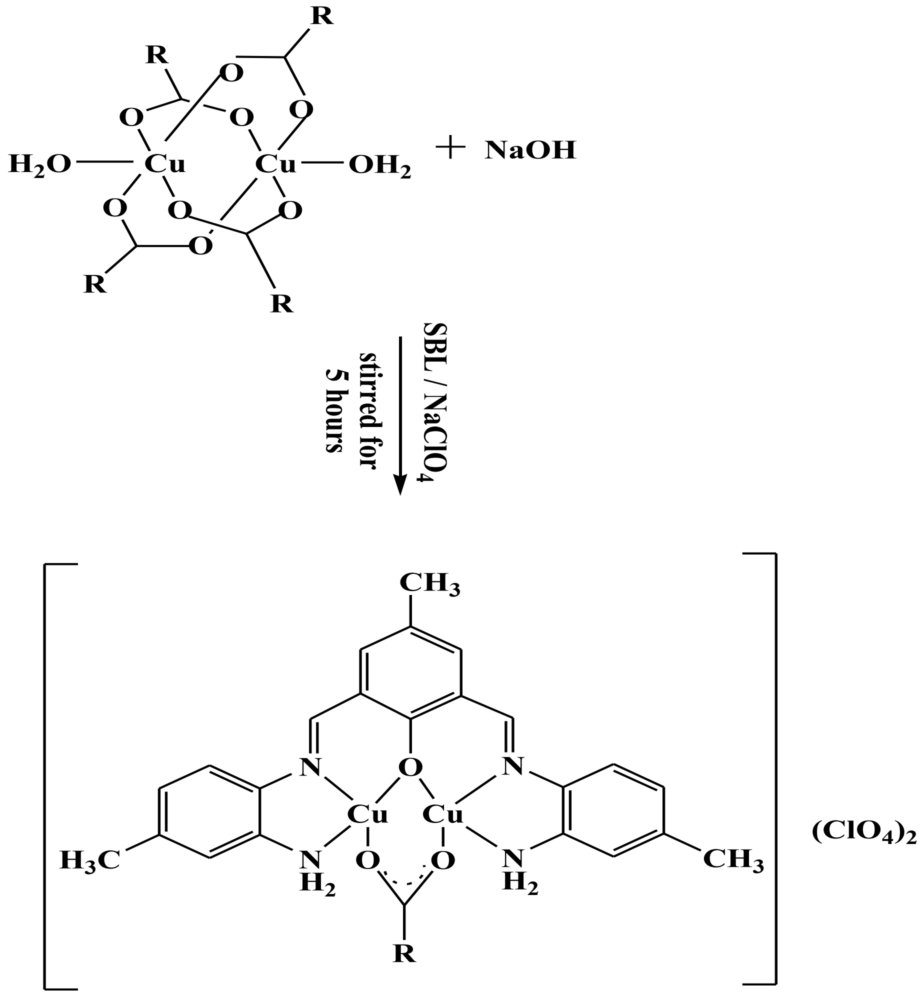
In order to increase pharmaceutical manufacturing while reducing time and cost, the Molecular Docking technique has been employed in medication development. It is a relatively recent method for pinpointing where a ligand and a protein bind [13]. An efficient tool for examining interactions and their three-dimensional structure as seen via Pymol software is Auto-Dock suite 4.2.6 [14].The five complexes have anticancer capabilities, and a suitable target protein has been discovered from the literature. The protein data bank is used to download the requested protein 5GIX. The following methods were used to create the protein for molecular docking:This protein was stripped of co-crystalline ligands, water, and cofactors using the Auto Dock Tools graphical user interface. The Kollman and Gasteiger technique was used to identify the atomic charges and allocate the protein polar hydrogen [15-17].

2.4 Synthesis of Schiff Base Ligand (SBL) :

2, 6-diformyl-4-methylphenol (0.328 g, 2.0 mmol) was dissolved in 15 ml ethanol and then mixed with 4-methyl-o-phenylenediamine (0.488g, 4.0 mmol) in 15ml of ethanol and the mixture was refluxed at 60 °C for 30 minutes with constant stirring. Then the reaction mixture was left to cool and the reddish-orange precipitate obtained were filtered, dried and recrystallised with ethanol.

2.5 Synthesis of Copper(II) Complexes:

The synthesis of copper(II) complexes were achieved by taking the Schiff base ligand (SBL), NaOH, various copper(II) precursors and sodium perchlorate in the stoichiometric ratio of 1:1:1:2. The Schiff base ligand (SBL) (1.0 mmol; 0.372g) and NaOH (1.0 mmol; 0.056g) was stirred well thoroughly for deprotonation. To the above mixture copper(II) p-aminobenzoate (1.0 mmol; 0.3236g), was added and stirred well for 45 minutes. To the resulting mixture, $NaClO_4$ (2.0 mmol, 0.280g) was added (Caution to be taken while using perchlorate salts) and stirred well for 4 hours. Similarly the complexes (C2 - C3) was synthesised with various copper(II) precursors like copper(II)p-chlorobenzoate, copper(II)p-methylbenzoate respectively.



C1 : R = C_6H_4(p-NH_2)

C2 : R = C_6H_4(p-Cl)

C3 : R = C_6H_4(p-CH_3)

Schematic representation of synthesis of dinuclear copper(II) complexes

3. RESULTS AND DISCUSSION

3.1 UV-Visible Spectra:

The electronic spectrum of the ligand exhibit a band at 278 nmwhich is due to π-π* transition of the benzene ring and it is shifted to higher wavelength (red shift) upon complexation [18]. The band at 355 nmwhich is due to π-π* transition of the azomethine group present in the ligand, upon complexation it undergoes a red shift in the complexes. This supports the coordination of metal with the azomethine nitrogen. In all the complexes, bands observed in the range around 400 nmis due to n-π* transitions. Several absorption bands, including ligand absorption bands and the d–d transitions of the metal ions, were seen in the electronic spectra of the complexes.

3.2 FT-IR Spectra:

Examination of the H-C=N, -C-OH regions of an infrared spectrum provided valuable structural information related to the structure of Schiff bases. The peak that appeared at 1613 cm^{-1} is due to azomethine stretching while the peak at 3391 cm^{-1} is attributed to OH stretching and the band at 1263 cm^{-1} is due to phenolic (C-O) stretching frequency.IR spectra of all complexes show a peak around 1612-1610 cm^{-1} which is attributed to the azomethine ʋ(HC=N) group [19]. The azomethine group stretching frequency is shifted to the lower frequency suggesting the coordination of metal with the azomethine group compared to free ligand.All the complexes exhibit the peaks in the range 600-500 cm^{-1} and 500-400 cm^{-1} indicating the presence of ʋ(Cu-N) and ʋ(Cu-O) respectively.

3.3 NMR Spectra:

The^{1}H-NMR spectra exhibited a signal at 13 ppm due to the protons in the hydroxyl group (-OH). The singlet signal appeared at 9.02 ppm is due to the protons of the azomethine group (-HC=N). The multiplet signal observed at 6-8 ppm is due to the methine protons present in the aromatic group. The singlet peak observed at 2.5 ppm is due to protons present in the methyl group.The ^{13}C-NMR spectra showed the (-C-OH) carbon signal at 165.05 ppm. The signal at 159.52 ppm is due to the azomethine group carbon (-HC=N) present in the ligand. The signal at 117 - 139 ppm is due to the carbons of the aromatic group. The signal observed at 20.18 ppm is due to the methyl carbon (-CH$_3$) present in the methyl group.

4. In-vitro ANTIOXIDANT ACTIVITY

The antioxidant data demonstrated that the percentage radical scavenging activity of synthesised Schiff base ligands (SBL) and their copper(II) complexes (C1-C3) was precisely proportional to the sample concentration (concentrations of 50, 100, 150, 200, and 250 μg/mL).The highly acidic azomethine group in Schiff base ligands is very reactive to produce radicals during complexation. The antioxidant activity of the complexes increases as a result of the hydroxyl group's negative charge delocalizing to the phenyl ring. Due to the nature of the electron-donating or electron-withdrawing groups with synthesised moiety, the scavenging activity values of Schiff bases ligands and their copper(II) complexes exhibit the trend **C1 > C3 > C2>SBL**

Table 1: Absorbance of Synthesized compounds

Compound	Concentration (μg/mL)					
	0	50	100	150	200	250
Ascorbic acid	1.062	0.765	0.602	0.435	0.318	0.244
SBL	1.062	0.804	0.642	0.498	0.430	0.272
C1	1.062	0.846	0.702	0.698	0.540	0.513
C2	1.062	0.815	0.659	0.520	0.442	0.286
C3	1.062	0.832	0.675	0.640	0.453	0.389

5. MOLECULAR DOCKING

The Kollman and Gasteiger technique was used to identify the atomic charges and allocate the protein polar hydrogen. With ligand contact, 5GIX's inhibition constants (ki) are 497.40, 178.65 and 180.24 nm. The inhibition constant (ki), which is also a measure of the ligand's binding affinity to protein, gives information about how the ligand interacts with an enzyme. The ligand and the protein 5GIX formed a stable complex with binding energies of -8.60, -9.21 and -9.20kcal/mol for each complex. As seen in Figures (1-3), the protein interacts with several amino acid residues during ligand binding, indicating a variety of binding mechanisms and interaction patterns.

Table 2 : Hydrogen bonding and molecular docking with protein targets.

Complex	Protein (PDB ID)	Bonded residues	Bond distance (Å)	Bond Interaction	Estimated inhibition constant (nm)	Binding energy (kcal/mol)	Intermolecular energy (kcal/mol)	Reference RMSD (Å)
I	5GIX	GLU’100	2.2	H-O	497.40	-8.60	-8.90	41.60
		GLU’252	2.7	H-O				
		GLU’244	3.4	Cu-O				
		GLU’244	3.0	Cu-O				
II		ASP’107	2.0	H-O	178.65	-9.21	-9.50	60.63
		GLN’104	2.6	H-O				
		LYS’106	2.3	H-O				
		LYS’106	3.3	Cu-O				
III		GLU’61	2.4	H-O	180.24	-9.20	-9.80	37.74
		HIS’67	2.2	O-H				
		GLU’252	2.4	H-O				

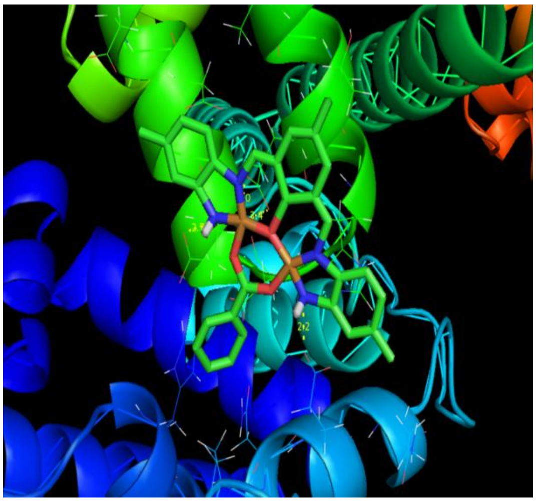


Figure 1: Protein interaction of C1

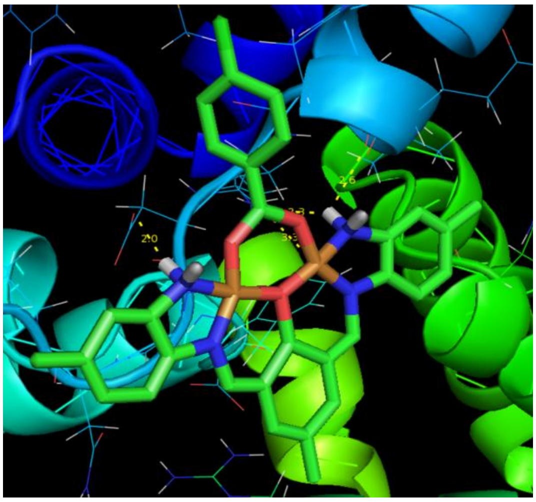


Figure 2: Protein interaction of C2

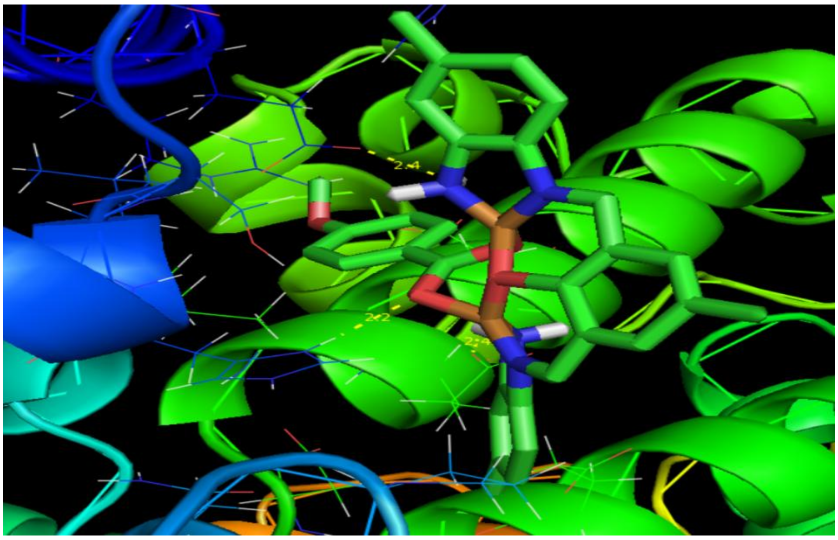


Figure 3: Protein interaction of C3

6. DFT STUDIES

6.1 Molecular structure

The average difference between the SBL theoretical attributes and the published XRD bond length and bond angle was determined to be a little divergence since the computational approach was used in the gaseous phase, while the experimental values in the literature were done in the solid phase. C6-C8 bonds have the longest bond lengths, measuring 1.503 (exe) and 1.510 (Å) (theo), while C-H bonds have the shortest bond lengths, ranging from 0.930 to 0.960 (Å) in experiments and from 1.084 to 1.086 (Å) in theory.

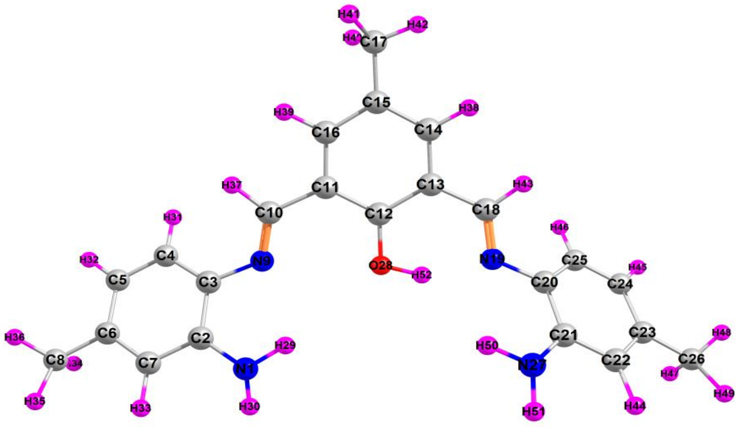


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Figure 4: Molecular structure of SBL

6.2 Molecular Electrostatic potential

A highly helpful descriptor for identifying the regions for electrophilic and nucleophilic reactions as well as hydrogen bonding interactions, the MEP is correlated with the electronic density [20,21]. Different colors correspond to the various electrostatic potential values at the surface (Fig. 2). The majority of positive and electronegative zones are indicated by the colors blue and red, respectively.Thecolor green denotes areas with zero potential. According to the color grading scheme, the most electronegative (electron-rich) region is represented by the color red, while the most electropositive (electron deficient) region is represented by the color blue. It is clear from the MEP that the methyl and phenyl ring groups are covered by the positive region, while the oxygen and nitrogen atoms are covered by the negative charge. The hydroxyl group is the most reactive component of the molecule due to its higher electronegativity. These findings provide insight into the area in which the molecule may interact intramolecularly.

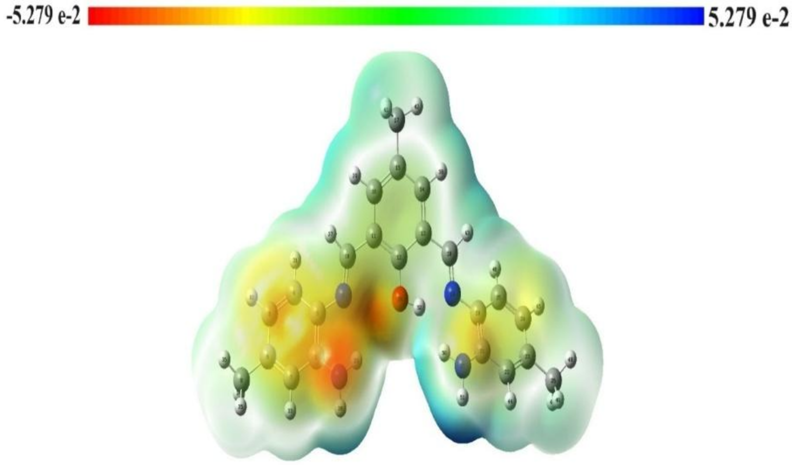

Figure 5: Molecular Electrostatic potential of SBL

6.3 Frontier molecular orbitals

The Schiff base ligand (SBL) computed high ionization potential value demonstrates its capacity for nucleophilic attack. The title molecule has a stable structure, according to the computed band gap value of 3.4015eV. The stability of the molecule is shown by the higher hardness and lower softness values. The bioactive compounds were identified by their decreased electrophilicity index and chemical potential values.

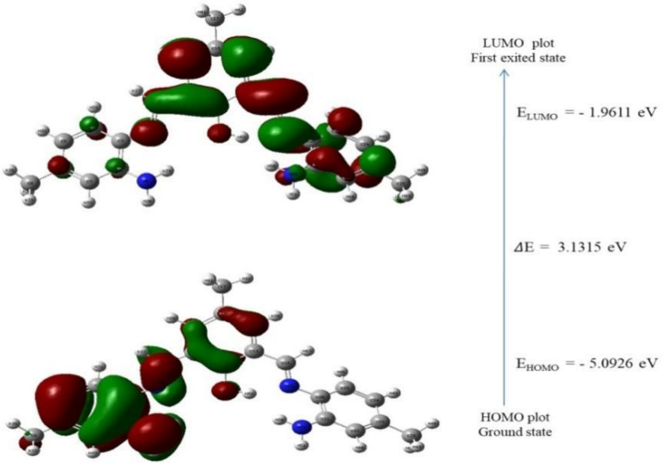

Figure 6: Frontier molecular orbitals structure of SBL

Table 3: The calculated Frontier molecular orbitals of SBL

Parameters	**Values**
HOMO(eV)	-5.0926
LUMO(eV)	-1.6911
Ionization potential	5.0926
Electron affinity	1.6911
Energy gap(eV)	3.4015

Electronegativity	3.3919
Chemical potential	-3.3919
Chemical hardness	1.7008
Chemical softness	0.2940
Electrophilicity index	3.3822

6.4 Mulliken atomic charges

According to the results, Fig. 4 illustrates the improved depiction. Because it is joined to an electronegative oxygen atom, the H52 (0.2581) atom has a greater positive charge than other hydrogen atoms, according to Mulliken atomic charges, which show that all hydrogen atoms have a net positive charge. Since the C27 atom shares a bond with the N27 (-0.5296) nitrogen atom, which has the lowest negative charge of all the nitrogen atoms in the title compound, it has the highest net positive charge (0.2268) among the carbon atoms.

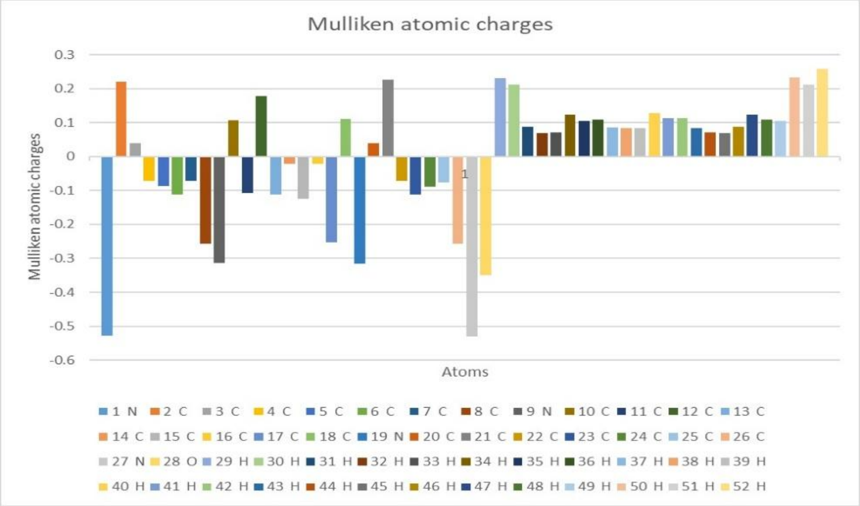

Figure 7: The histogram of Mulliken atomic charges of SBL

CONCLUSION

New pentadentate Schiff base ligand copper(II) complexes were synthesized and characterized utilizing FTIR, UV-Vis, 1H NMR, and 13C NMR, among other physicochemical techniques. The findings showed that the metal complexes utilize the nitrogen atom of the azomethine group and the deprotonated phenolic oxygen atom to yield square planar geometry with Schiff base ligands. ^{1}H and ^{13}C NMR supported the synthesis of the proposed compound. All of the synthesized copper(II) complexes had a percentage radical scavenging activity that was exactly proportional to the concentration of the sample (50, 100, 150, 200, and 250 µg/mL), according to the antioxidant data. The protein interacts with several amino acid residues during ligand binding, indicating a variety of binding mechanisms and interaction patterns.

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