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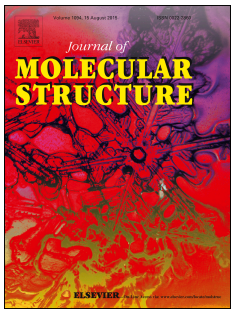

Synthesis, Structure Elucidation, Biological Screening, Molecular Modeling and DNA binding of Some Cu(II) Chelates incorporating Imines Derived from Amino Acids

Laila H. Abdel-Rahman, Ahmed M. Abu-Dief, Mohammed Ismael, Mounir A.A. Mohamed, Nahla Ali Hashem

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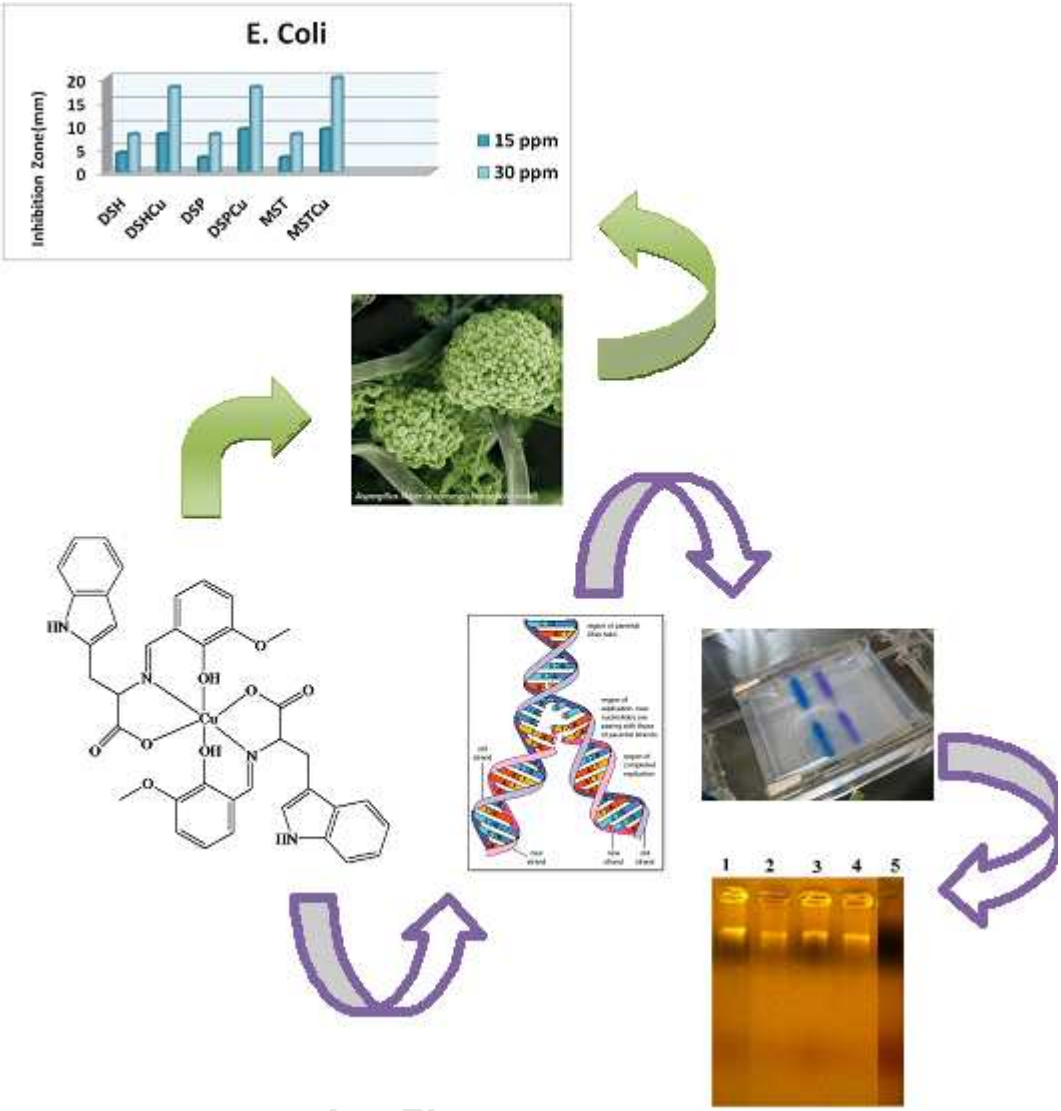
E. Coli
Inhibition Zone(mm)
20
15
10
5
0
DSH
DSHCu
DSP
DSPCu
MST
MSTCu
15 ppm
30 ppm
1 2 3 4 5

Synthesis, Structure Elucidation, Biological Screening, Molecular Modeling and DNA binding of Some Cu(II) Chelates incorporating Imines Derived from Amino Acids

Laila H. Abdel-Rahman, Ahmed M. Abu-Dief*, Mohammed Ismael, Mounir A. A. Mohamed and Nahla Ali Hashem

Chemistry Department, Faculty of Science, Sohag University, Sohag 82524, Egypt

Corresponding author: E- mail address: ahmed_benzoic@yahoo.com

Abstract:

Three tridentate Schiff bases amino acids were prepared by direct condensation of 3-methoxysalicylaldehyde (MS) or 4-diethylaminosalicylaldehyde (DS) with α-amino acid ligands [L-phenylalanine (P), L-histidine (H) and DL-tryptophan (T)]. The prepared Schiff bases amino acids were investigated by melting points, elemental analysis, ^{1}H NMR and ^{13}C NMR, IR, UV –Vis spectra, conductivity and magnetic measurements analyses. Subsequently, copper was introduced and Cu(II) complexes formed. These complexes were analyzed by thermal and elemental analyses and further investigated by FT-IR and UV/Vis spectroscopies. The experimental results indicating that all Cu(II) complexes contain hydrated water molecules (except DSPCu complex) and don't contain coordinated water molecules. The kinetic and thermal parameters were extracted from the thermal data using Coast and Redfern method. The molar conductance values of the Schiff base amino acid ligands and their Cu(II) complexes were relatively low, showing that these compounds have non-electrolytic nature. Magnetic susceptibility measurements showed the diamagnetic nature of the Schiff base amino acid ligands and paramagnetic nature of their complexes. Additionally, a spectrophotometric method was determined to extract their stability constants. It was found that the complexes possess 1: 2 (M: L) stoichiometry. The results suggested that 3-methoxysalicylaldehyde and 4-diethylaminosalicylaldehyde amino acid Schiff bases behave as monobasic tridentate ONO ligands and coordinate Cu(II) ions in octahedral geometry according to the general formula $[Cu(HL)_2].nH_2O$. To further understanding the structural and electronic properties of these complexes, Density Functional Theory (DFT) calculations were employed and provided a satisfactory description. The optimized structures of MST Schiff base ligand and its complex were calculated using DFT. The antimicrobial activity of the Schiff base ligands and their complexes were screened against some types of bacteria such as *B. subtilis (+ve), E. coli (-ve)* and *M. luteus (+ve)* and some types of fungi such as *A. niger*, *C. glabrata* and *S. cerevisiae*. The results of these studies indicated that the metal complexes exhibit a stronger antibacterial and antifungal efficiency compared to their corresponding ligands. The complexes were screened for antiviral activity against a panel of DNA and RNA viruses. Minimum cytotoxic and minimum virus inhibitory concentrations of these complexes were determined. The mode of interaction between complexes and CT-DNA was monitored using absorption spectra, viscosity measurements and gel electrophoreses.

Keywords: Amino acids, Imine, Molecular modeling, antimicrobial, antiviral, DNA

Introduction

The chemistry of metal-drug coordination compounds nowadays has a greater attention than before due to the importance needs particularly in designing more biologically active drugs [1]. Metal ions are known to affect the action of many drugs [2]. Several metal chelates are known to possess antimicrobial, antiviral and anticancer activity. In many cases, metal chelates have been found to be more antimicrobial than the chelating agents themselves [2-4]. Schiff bases ligands and their complexes have a variety of applications in clinical, analytical and industrial field [5].

Among these, heterocyclic Schiff base ligands and their complexes do have significant interest because of pharmacological properties [7]. Furthermore, the interaction of these complexes with DNA has gained much attention due to their possible applications as new therapeutic agents [8]. Some drugs show increased activity when administered as metal chalets and inhibit the growth of tumors [9]. The transition metal ions are responsible for the proper functioning of different enzymes. Certain drugs play a vital role as bio-ligands in the biological systems. Also, nitrogen bases such as (pyridine, pyrimidine and pyrazine) and amines such as (histamine, carbohydrates and different vitamins) have a vital role as bio-ligands. Metal complexes of Schiff base phenolates with favorable cell membrane permeability have been exploited in cancer multidrug resistance and used as antimalarial agents [10].

A number of diseases and their remedies are dependent on metabolism of inorganic constituents. The complexes of copper with Schiff bases have wide applications in food and dye industries, catalysis, fungicidal, agrochemical, antiradical activities and biological activities [11, 12].

Cu(II) complexes are also attractive since Cu(II) is known to play a significant role in naturally occurring biological systems as well as a pharmacological agent. Copper is a biologically relevant element and many enzymes that depend on copper for their activity have been identified. The metabolic conversions catalyzed by most of these enzymes are oxidative.

This paper describes the synthesis of binary Cu(II) Schiff base amino acid complexes and characterization by various physical methods. Moreover, antimicrobial studies of the investigated Cu(II) complexes were performed against many types of bacteria, fungi and viruses. Moreover, the interaction between DNA and the synthesized Cu(II) complexes was performed by using absorption spectra, viscosity measurements and gel electrophoresis. 3-methoxysalicylaldehyde (MS) or 4-diethylaminosalicylaldehyde (DS) is the aldehyde that used in this investigation, the amino acids are L-phenylalanine (P), L-histidine (H) and DL-tryptophan (T).

Materials

All chemicals reported here, such as 3-methoxysalicylaldehyde ($C_8H_8O_3$) (MS), 4-diethylaminosalicylaldehyde ($C_{11}H_{15}O_2N$) (DS), amino acids[L-Phenylalanine (P), DL-Tryptophan (T), L-Histidine (H)], Calf thymus DNA (CT – DNA), and the metal salt [copper acetate ($Cu(CH_3COO)_2$. H_2O), were purchased from Sigma-Aldrich and used as received.

Characterization of the prepared Schiff base amino acid ligands and their complexes

Melting points for the isolated ligands and decomposition points for their complexes were monitored on a melting point apparatus, Cimarec 3 Thermolque. The carbon, hydrogen, and nitrogen contents were determined on a Perkin Elmer (2400) CHNS analyzer. IR spectra (4000 – 400 cm^{-1}) were recorded on Shimadzu FT-IR model 8101 spectrometer using KBr pellets. ^{1}HNMR and ^{13}CNMR spectral measurements were determined by using a BRUKER, using DMSO as an internal reference. The TG/DT analyses were recorded on Shimdzu corporations 60 H at 10 degrees min^{-1}. The UV-Vis spectra were recorded on a PG spectrophotometer model T+80 at 298 K. Magnetic susceptibility measurements of the metal complexes were done on a Gouy balance at room temperature using $Hg[Co(SCN)_4]$ as a calibrant. Molar conductance was measured on an Elico CM-180 conductometer using 1 mmol L^{-1} solutions in DMF. A HANNA 211 pH meter at 298 K equipped with a CL-51B combined electrode was used for pH measurements, calibrated against standard buffers (pH 4.02 and 9.18) before measurements. Quantum chemical calculations for 3-methoxysalicyaldehyde and tryptophan (MST) ligand structure were performed using MOPAC2000 [13] with

WinMOPAC 2.0 [14] as a graphic interface. At the beginning, structure the starting material was optimized with the eigenvector-following routine (EF) [15] using the semi-empirical PM3 method [16, 17]. To investigate the 3D structure and molecular stability for the Cu-MST complex accurate density functional theory (DFT) calculations were done using Gaussian 03 software package [18]. Calculations were carried out at DFT level of theory with pseudo potential functions, 6-311G (p,d) [19] basis set for ligand atoms, and LANL2DZ [20] basis set with effective core potential (ECP) for Cu ion. Antimicrobial screening was carried by using agar well diffusion. Viscosity measurements were performed by using viscometer immersed in a thermo stated water bath maintained at 25 ^{o}C. Gel electrophoresis was visualized under UV a transilluminator and photographed with a Panasonic DMC-LZ5 Lumix Digital Camera.

Synthesis of Schiff base amino acids

To a stirred solution of 3-methoxysalicylaldehyde or 4-diethylaminosalicylaldehyde (5 mmol, 0.76 g, 0.995 g respectively) in ethanol, 40 ml of the appropriate (L-Phenylalanine (P), DL-Tryptophan (T), L-Histidine (H)) (5 mmol, 0.825 g, 1.045 g, 1.02 g respectively) was added. The resultant mixture in each case was heated under reflux for 2h. The solid products were collected by filtration, washed with hot ethanol, dried at room temperature and finally stored in an air tight sample vial for further use [21].

Complexes preparation

Aqueous solutions of the amino acids were prepared by dissolving (5 mmol, 0.825 g, 1.045 g, 1.02 g respectively in 40 ml of aqueous-ethanol mixture) of each (P, T, H). Each of the solutions was mixed with 3-methoxysalicylaldehyde (5 mmol, 0.76 g, 50 ml hot ethanol) or 4-diethylaminosalicylaldehyde (5 mmol, 0.995 g, 50 ml hot ethanol). Then the mixture was stirred at 70 ^{o}C for 1 hour. Cu(II) acetate mono hydrate solution of (2.5 mmol, 0.5 g in 40 ml aqueous-ethanol mixture) was added to the previous mixtures. The color changed from yellow to (green for MSTCu, dark green for DSPCu and bright brown for DSHCu) after stirring at room temperature for 3 hour. The obtained products were evaporated over night. The obtained solid products were filtered, washed with water, and dried in vacuum over anhydrous $CaCl_2$ [2, 3, 22]. The structures of the investigated Schiff base amino acid ligands and their complexes are shown in Table 1.

Magnetic susceptibility measurements

The magnetic susceptibility measurement is one of the most useful methods available to the coordination chemists for studying the electronic structure of a transition metal complex. It provides fundamental information about the bonding and stereochemistry of the metal complexes [23]. The magnetic properties of the coordination compounds are based on the effect of the ligands on the spectroscopic terms of the metal ions. The susceptibility per gram atom of a paramagnetic metal ion in a particular compound is determined by measuring the molar susceptibility of the compounds and applying diamagnetic corrections for the other ions or molecules in the compounds. The diamagnetic corrections can be determined by various methods [24], however Pascal's constants are more often used to calculate the corrections. The magnetic susceptibility and moments can generally be calculated by using the following relationships [25].

$$\mu_{eff} = 2.83\sqrt{\overline{X}_M T}$$

Where μ_{eff}, the magnetic moment (in Bohr Magneton), B. M. where T = temperature (K).

$$\chi M = \bar{\chi} M - (\text{diamag. corr.})$$

χM molar magnetic susceptibility before correction, $\bar{\chi} M$ molar magnetic susceptibility after correction.

Kinetic data for thermogarvimetric analysis (TGA) of the prepared complexes

The kinetic parameters of decomposition processes of complexes namely activation energy (E*), enthalpy (H*), entropy (S*) and Gibbs free energy change of the decomposition (G*) were evaluated graphically by employing the Coats-Redfern relation [26].

$$\log\left[\frac{\log\{W_\infty/(W_\infty - W)\}}{T^2}\right] = \log\left[\frac{AR}{(\phi E^*)}\left(1-\frac{2RT}{E^*}\right)\right] - \frac{E^*}{2.303RT} \qquad (1)$$

Where W_∞ is the mass loss at the completion the decomposition reaction, W is the mass loss up to temperature T, R is the gas constant and ϕ is the heating rate. Since 1-2RT/E* = 1, the plot of the left hand side of equation (1) against 1/T would give straight line. E* was then determined from the slope and from the intercept, the Arrhenius constant, A, was obtained. The other kinetic parameters; the entropy of activation (S*), enthalpy of activation (H*) and the free energy change of activation (G*) were calculated using the following equation:

$$S^* = 2.303\ R \log \frac{Ah}{kT} \qquad (2)$$

$$H^* = E^* - RT \qquad (3)$$

$$G^* = H^* - TS^* \qquad (4)$$

Where, (k) and (h) are the Boltzmann's and Planck's constant, respectively.

Antibacterial bioassay

All the prepared Schiff base amino acid ligands and their complexes were screened for their antibacterial activity against two Gram-positive (*Bacillus subtilis* and *Micrococcus luteus*) and one Gram-negative (*Escherichia coli)* bacterial strains using the agar well diffusion method [2, 3, 27]. The solution of complexes and ligands were prepared by dissolving in DMSO at a concentration (15 and 30 mg/ml). Paper discs of Whatman No.1 were sterilized in an autoclave and saturated with solution of metal complexes, the test organisms were grown on nutrient agar medium in Petri plates. After that, holes were formed in the agar using a sterile crook borer and these holes were completely filled with the test solutions. The Petri dishes were incubated for 24 h at 37 ^{o}C. Ciprofloxacin was used as standards for antibacterial activity.

Antifungal screening

Antifungal activities of all the synthesized Schiff base amino acid ligands and their complexes were studies against three fungal cultures (*A. niger, C. glabrata and S. cerevisiae*) using disk diffusion method [2, 3, 28]. These fungal species were isolated from the infected parts of the host plants, potato dextrose agar. The fungus strains were directly mixed with potato dextrose agar and dispersed into the Petri dishes. The discs were soaked in DMSO in which the test complexes were dissolved (concentration of ligands and complexes 15 and 30 mg/ml). Filter paper discs of 6 mm in size were saturated with solution of complexes. The plates were inverted and incubated at 35 ^{o}C for 48 h. The

plates were then observed and the diameters of the inhibition zones were measured. Amphotricine B was used as standard for antifungal activity.

Antiviral activities of the prepared complexes

For Herps Simplex Virus (HSV): the Vero cell line was maintained in RPMI 1640 (Gibco, Tunisia) supplemented to fetal bovine serum (10 %, v/v), L-Glutamin (2 μM), penicillin (100 U/μl), and streptomycin (100 μg/ml). Cells were incubated at 37 °C in a 5% CO_2 humidified atmosphere. Coxsakievirus B_3 Nancy strain (kindly provided by Pr. Bruno Pozzetto, Laboratory of Bacteriology-Virology, Saint-Etienne, France) was propagated in Vero cells.

Confluent Vero cell cultures were treated with three non-cytotoxic concentrations of the submitted sample during and after virus infection in two sets of experiments as follows: (1) 5 x 10^4 TCID50 of the virus was exposed to essential oil for one hr at 37 °C. Then 100 μl of the mixture were added to the cells cultured fluently in 96-well flat-bottom microtiter plate (100 μl); (2) Cells were treated with sample (100 μl) for one hr at 37 °C. After one hour of incubation at 37 °C, 5 x 10^4 TCID50 of the virus (100 μl) were added.

All plates were incubated at CO_2-incubator for 48 hrs. The viability of the infected and non-infected cells was evaluated according to the absorbance values of formazan using the MTT inclusion assay, as described in cytotoxicity assay. The percentage of protection was calculated as follows:

$$\text{Percent protection} = [(\text{ODT}) - (\text{ODC})\ \text{V}] / [(\text{ODC})\ \text{M} - (\text{ODC})\ \text{V}] \times 100$$

Where (ODT), (ODC) V and (ODC) M are the absorbance of the test sample, the absorbance of the virus-infected control (no compound) and absorbance of the mock-infected control (no virus and no compound), respectively. The 50 % inhibition concentration (IC50) was calculated by regression curve analysis, which is defined as the concentration of the essential oil that inhibits the viral infection by 50 %.

For Tobacco Mosaic Virus (TMV): plants grown in Magenta® vessels were ready for mechanical inoculation with the virus within 2 weeks. Czech isolate of Turnip Tobacco mosaic virus (TMV) [29] maintained in the virus collection at the Institute of Plant Molecular Biology was used for this study. Singular virus infection was checked by sucrose density gradient during the virus purification, electron microscopy, preparation of antiserum and serology. The inoculum was prepared from infected B. pekinensis *cv*. Manoko plants with mosaic symptoms 3 weeks after inoculation. A leaf sample was homogenized in a mortar in a 1: 10 (w/v) ratio with 0.1 μ phosphate buffer, pH = 7.0, and filtered through 0.22 m sterile filters (Millipore). Mechanical inoculation of plants was conducted on the first 2 leaves in a flow box using a cotton pad soaked in inoculum mixed with carborundum powder.

Interaction of the prepared complexes with Calf Thymus DNA (CT-DNA)

- **Absorption spectral studies**

Electronic absorption spectrum of the complex was recorded before and after addition of CT-DNA in the presence of Tris-HCl buffer (pH 7.2). Different concentration of metal complexes was titrated with incremental amounts of CT-DNA over the range (3 –30 μM). The equilibrium binding constant (K_b) values for the interaction of the complex with CT-DNA were obtained from absorption spectral titration data using the following equation [2, 3, 30].

$$[\text{DNA}]/ (\varepsilon_a - \varepsilon_f) = [\text{DNA}]/ (\varepsilon_b - \varepsilon_f) + 1/K_b\ (\varepsilon_b - \varepsilon_f)$$

Where ε_a, ε_f and ε_f are the molar extinction coefficient observed for the charge transfer absorption at a given DNA concentration, the extinction coefficient at the complex free in solution and the extinction coefficient of the complex when fully bound to DNA respectively. K_b and [DNA] are the equilibrium binding constant and the concentration in nucleotides respectively. A plot of [DNA]/ (ε_a - ε_f) versus [DNA] gives K_b as the ratio of the slope to the intercept. The standard Gibb´s free energy for DNA binding was calculated from the following relation.

$$\Delta G^o_b = -RT \ln K_b$$

Where R, T and K_b are the general gas constant, the absolute temperature and the binding constant respectively [2, 3]

- **viscosity measurements**

Various concentrations of complex (10–250 μM) were added into the viscometer to give a specific mole-ratio r (r = [complex] / [DNA]) while keeping the DNA concentration constant at (250 μM). The control sample was carried out on EB by using the same method. After thermal equilibration, the time of the solution's flowing through the capillary was determined by a digital stopwatch. The data were founded as $(\eta/\eta_0)^{1/3}$ versus the mole-ratio values, where η and η_0 are the viscosity of DNA in the presence and absence of complex, respectively. Viscosity values were calculated from the observed flow time of DNA containing solutions (t) and corrected for buffer solution (t_0), $\eta = (t - t_0)/t_0$ [2, 3, 22, 31].

- **Agarose gel electrophoresis**

Solutions of DNA were freshly prepared before each experiment using doubly distilled water containing 0.1 M Tris - HCl buffer, pH = 7.2. The concentration of DNA solutions was determined by UV-Vis spectrophotometer at 260 nm using a value of 6600 $M^{-1}cm^{-1}$ for the absorption coefficient. The purity (freedom from bound protein) was assessed from the ratio of the absorbencies at 260 nm and 280 nm. In general, the commercial DNA preparation was found to be free of protein ($A_{260\ nm}/A_{280\ nm}$= 1.9) according to this criterion and no further purification was attempted. Cleavage reactions were run between the metal complexes and DNA, and the prepared solutions were subjected to electrophoresis on 1 % agarose gel prepared in TBE buffer (45 μM Tris, 45 μM boric acid and 1 μM EDTA, pH 7.3). Then (20 micron) of ethidium bromide was added to the above solution and mixed well. The gel was mounted into electrophoretic tank; enough electrophoretic buffers were added to cover the gel. DNA sample (10 micron) and metal complex (20 micron) were mixed with loading dye (bromophenol blue) and incubated for 30 min at 25 ^oC, then loaded into the well of the submerged gel using a micropipette. The electrophoresis was performed at a constant voltage (100 V) for about 1-2 h (until bromophenol blue had passed through 50% of the gel) in TBE buffer. The gel was visualized under UV a transilluminator and photographed with a Panasonic DMC-LZ5 Lumix Digital Camera [2, 3, 32].

Results and discussion

Characterization of the prepared Schiff base amino acid ligands

^{1}H NMR and ^{13}CNMR spectra of the prepared Schiff base amino acid ligands:

The ^{1}HNMR spectrum (DMSO-d_6, ppm) of MST ligand show singlet signal at 13.6 for COOH proton respectively, singlet signal at 8.2 for CH=N proton respectively, multiple signals at 7.4 – 6.6 for nine aromatic protons respectively. Also they show singlet signal at 4.3 for OH proton respectively, singlet signal at 3.7 for CH aliphatic proton respectively and singlet signal at 3.8 for three OCH_3 protons respectively. Furthermore, they show doublet

signals at 3.0 for three CH_2 protons respectively and the ^{1}HNMR spectrum of MST ligand showed singlet signal at 10.9 for NH proton respectively.

The ^{13}CNMR spectrum of MST display the signals corresponding the different non-equivalent carbon atoms at different values of δ as follows: at δ 171 ppm (COOH) due to carboxylic acid group in the amino acid, at δ 168 ppm (CH=N) due to azomethine, at δ 165-70 ppm (14CH – Ar) due to carbon atoms of aromatic rings, at δ 58 ppm (OCH_3) due to carbon atom of methoxy group, at δ 52 ppm (CH) due to aliphatic carbon atom, at δ 29 ppm (CH_2) due to carbon atom of methyl group connected with aromatic ring

The ^{1}HNMR spectrum (DMSO-d_6, ppm) of DSP and DSH ligands show singlet signal at 11.0 and 11.5 for COOH proton respectively, singlet signal at 8.5 and 8.3 for CH=N proton respectively, multiple signals at 7.4 – 6.1 and 7.5– 6.0 for five and eight aromatic protons respectively. Also they show singlet signal at 5.0 and 4.3 for OH proton respectively, singlet signal at 3.4 and 3.5 for CH aliphatic proton respectively and quartet signals at 3.4 and 3.3 for four $2CH_2$- CH_3 protons respectively. Furthermore, they show triplet signals at 1.1 and 0.9-1.2 for six $2CH_3$-CH_2 protons respectively and the ^{1}HNMR spectrum of DSH ligand showed singlet signals at 9.5 for NH proton respectively.

The ^{13}CNMR spectrum of DSP and DSH display the signals corresponding the different non-equivalent carbon atoms at different values of δ as follows : at δ 191 and 197 ppm (COOH) due to carboxylic acid group in the amino acid, at δ 165 and 163 ppm (CH=N) due to azomethine, at δ 163- 99 and 158 −100 ppm (12CH – Ar) and (9CH – Ar) due to aromatic carbon atoms, at δ 50 and 52 ppm (CH) due to aliphatic carbon atom, at δ 40 and 38 ppm (CH_2-N) corresponding to carbon atom in diethyl group, at δ 39 and 35 ppm (CH_2-Ar) corresponding to carbon atom connected with aromatic group, at δ 17 and 13 ppm (CH_3) due to carbon atom in diethyl group.

Characterization of the prepared Schiff base amino acid ligands and their complexes

Microanalysis measurements

Results of empirical formula, Molecular weight, melting points (for ligands), decomposition points (for complexes), elemental analysis and magnetic moment measurements are given in Table 2. The conductivity values with different concentrations in the range from (1.4×10^{-3} to 18.8×10^{-3}) Mol dm^{-3} are shown in Table 2. The molar conductivity measurements were carried out in dimethylformamide (DMF). All the prepared Schiff base amino acid ligands and their complexes were relatively low, indicating that these compounds have the non-electrolytic nature [2, 3, 22, 33]. The microanalysis results suggest that 3-methoxysalicylaldehyde or 4-diethylaminosalicylaldehyde amino acid Schiff bases behave as monobasic tridentate ONO ligands and coordinate to Cu(II) in octahedral geometry according to the general formula$[Cu(HL)_2].nH_2O$. Magnetic susceptibility measurements values of the prepared Schiff base amino acid ligands are diamagnetic and for their complexes are (1.8−2.2 B. M.) which suggests octahedral geometry of the complexes, Table 2 [2, 3, 22, 34].

IR and Electronic spectra for the investigated compounds

Results of IR spectra are showed in Table 3. The wavelengths at maximum absorption band (λ_{max}) and the molar absorptivity (ε) of the different bands in the recorded spectra of the complexes (Fig. 1) are given in Table 4.

The IR spectra provide valuable information regarding the nature of functional group attached to the metal atom. The IR spectrum of the free ligand is compared with the spectra of the complexes. The IR spectrum of the free Schiff base amino acid ligands exhibit a broad band around 3441-3389 cm^{-1}, which is attributed to the stretching

frequency of the aromatic hydroxyl substituent group, perturbed by intramolecular hydrogen bonding [O—H---N] between phenolic hydrogen and azomethine nitrogen atoms [35, 36, 37]. Further, the appearance of the OH band around 3465-3395 cm^{-1} in the spectra of the complexes with an increase in intensity indicates that the hydroxyl oxygen is coordinated to the M(II) ion without proton displacement [38]. The ligands exhibit the characteristic (C=N) band in the 1651– 1632 cm^{-1} region, while the complexes the (C=N) were observed in the 1628-1610 cm^{-1} region. The (C=N) stretching frequency is shifted to a lower frequency, indicating that decrease in the (C=N) bond order due to the coordinate bond of the metal with the imine nitrogen lone pair [39]. The ν(C - O phenolic) vibration of ligands are observed around 1293-1235 cm^{-1} , which get shifting to lower or higher frequency region in the complexes indicating coordination of phenolic oxygen [40]. The ligands exhibit other two intense bands at (1430-1362), (1588-1572) cm^{-1} corresponding to symmetric stretching and asymmetric stretching frequencies of (COOH) group, respectively of the organic ligand. On complexation symmetric and asymmetric bands were shifted to a higher frequency or remained unaltered in the position of the ligands [41]. The difference between symmetry and asymmetry stretching vibration of COO^- group showed that amino acid Schiff bases coordinated through COO^- group [42]. At 695-688 cm^{-1} and 542-569 cm^{-1} to ν(Cu-N) and ν(Cu-O) stretching, respectively [43, 44].

The electronic absorption spectra of the Schiff bases recorded in DMF is composed of three bands in the 200 - 800 nm region. The first band A appearing below 300 nm region can be assigned to the π-π* transition of the aromatic rings. The second band B is observed within the wavelength range 315-431 nm is due to transition between the π-orbital localized on the central azomethine (-CH = N-) bond [45] while the third band C located within the 363-379 nm region can be ascribed to charge transfer within the entire Schiff base molecule. This band is observed in o-hydroxyl Schiff bases and is based on strong intramolecular hydrogen bonding between the hydroxyl group of the salicylidene and the azomethine nitrogen [46]. The charge transfer bands being more sensitive to solvent changes than bands resulting from local transitions. A broad band from 507 to 630 nm, which indicating that band could be mainly attributed to d → d transition in an octahedral structure of the prepared complexes [47, 48]

Thermogravimetric analysis (TGA)

In the present investigation, heating rates were suitably controlled at 10 $^oC\ min^{-1}$ under nitrogen atmosphere, and the weight loss was measured from the ambient temperature up to 750 ^oC. The experimental results are given in Table 5. Thermogravimetric analyses of the Schiff base amino acid complexes were used to: (i) get information about the thermal stability of these new complexes, (ii) decide the water molecules (if present) are inside or outside the inner coordination sphere of the central metal ion, and (iii) suggest a general scheme for thermal decomposition of these complexes [49]. Thermal analyses providing important information regarding thermal stability of substances and thus indirectly provide clues to the structure and composition of the substances. In thermogravimetry the change in mass of the sample is recorded as a function of temperature. It provides the analyst with quantitative measurements of change in weight associated with any transition. TG can directly record the loss in weight with time or temperature due to dehydration or decomposition. Routine measurements can be made at temperature range from ambient to 750 ^oC with inert atmosphere [50]. Thermogravimetric analyses of synthesized complexes are observed in 3 steps, (i) a small weight loss which is assigned to loss of lattice water molecules, except DSPCu complex is stable up to 113.1 ^oC, indicating that absence of lattice water molecules, (ii) maximum weight loss which is assigned to the loss of coordinated water molecules, but in this paper all complexes don't contain coordinated water molecules, (iii) gradual weight loss can be assigned to complete decomposition of ligand moiety around the metal ion. Finally complexes are converted into their metal ion.

Kinetic Data for TGA of the prepared complexes

The data is tabulated in Table 6. The activation energy of decomposition was in the range 14.4-111.9 KJ /mol. The high values of the activation energies reflect the thermal stability of the Complexes. The entropy of activation had negative values in all the complexes, which indicating that the decomposition reactions proceed with a lower rate than the normal one.

Determination of stoichiometry of the investigated complexes

The stoichiometry of the various complexes formed in solutions via the reaction of Cu(II) with the studied ligands was determined by applying the spectrophotomertic molar ratio [51] and continuous variation methods [52] as shown in Figs 2, 3. Maximum in the curve at X_{ligand} =0.62-0.73 implicates a 1: 2 (metal ion to ligand) molecular association.

The formation constants of the investigated complexes

Stability constant is equilibrium constant for the formation of a complex in solution. Stability constant is a measure of the strength of the interaction between the reagents that come together to form the complex. There are two main kinds of complexes, i) compounds formed by the interaction of a metal ion with a ligand and ii) supramolecular complexes. There are many areas of application in chemistry, biology and medicine. The apparent formation constants (K_f) of the studied Cu(II) Schiff base amino acid complexes formed in solution were determined from the spectrophotometeric measurements using the continuous variation method [2, 3, 22] according to the following relation:

$$K_f = \frac{(A/A_m)}{4C^2\,(1-A/A_m)^3}$$

Where A_m, (A) and C are the absorbance of the maximum formation of the complex, the arbitrary chosen absorbance values on either sides of the absorbance pass and the initial concentration of the metal respectively. The obtained K_f values are indicating that high stability of the prepared complexes. The values of K_f of the prepared complexes increase in the following order: DSPCu > DSHCu > MSTCu. The values of the stability constant and Gibbs free energy of the investigated Cu(II) Schiff base amino acid complexes are cited in Table 7. The negative values of Gibbs free energy mean that the reaction is spontaneous and favorable.

Stability range of the investigated complexes

The pH profile (absorbance vs. pH) presented in Fig. 4 showed typical dissociation curves and a wide stability pH range [(4-11for DSPCu and DSHCu) and (6-9 for MSTCu)] of the studied complexes. This means the formation of the complex greatly stabilizes the Schiff base amino acid ligands. This pH range means the prepared complexes are more favorable to different physiological reactions.

Molecular modeling

Figs. 5, 6 show the optimized structures and electronic configuration of MST and its Cu complex. MST has numerous donor groups; however three of them are oriented toward each other. Those groups are the carboxylate (COO^-), hydroxyl (OH) and imine group (-N=C).Optimized [$Cu(MST)_2$] complex structure has local octahedral geometry. The stability of this complex is due to the aromatic rings formed between the metal and donor atoms. To

relative stability of the complex was evaluated by calculating the metal-ligand interaction energy ($E_{complex} - E_{reactants}$). According to the current DFT approximation this energy was found to be -39 kcal/ mol. Moreover quantum chemical descriptors were also calculated to estimate the chemical reactivity of the complex. These descriptors include, the highest occupied molecular orbital (HOMO), the lowest unoccupied molecular orbital (LUMO), the energy gap ($\Delta E = E_{LUMO}$-E_{HOMO}), chemical hardness (η), electronic chemical potential (μ) and electrophilicity index (ϵ) of the prepared Schiff base amino acid (MST) and its complex. Chemical Hardness (η) is associated with the reactivity of a chemical system. The harder and less reactive the molecule corresponds to the larger η. Electronic chemical potential (μ) is a measure of electronegativity of the molecule. The greater η, the less stable or more reactive is the complex. Electrophilicity Index (ϵ), measures the capacity of a species to accept electrons [16]. Table 8 summarizes the calculated descriptors, and reflects the high electrophilic capacity of the complex compared with the ligand itself. The small value of η explains the intense color of the complex.

To further compare the theoretical calculations the experimental results, frequencies single point calculations at same level of DFT approximation. From these calculations, IR vibrational spectrum for ligand and complex models was generated (cf Table 3). The following table stats the calculated vibrations for the main function groups in ligand and complex models. The difference between the calculated and experimental values (~100 cm^{-1}) may be attributed to the approximation method used.

Application of the prepared Schiff base amino acid ligands and their complexes

Antimicrobial studies

The measured zones of inhibition against the growth of various microorganisms are summarized in Table 9. The antimicrobial screening results exhibited marked enhancement in activity on coordination with the Cu(II) ion against more testing bacterial and fungal strains Figs. 7, 8. Furthermore, the activities of the prepared Schiff base amino acid ligands and their complexes were reported in Table 10.

The morphology of the cell wall is key factor that influences the activity of antimicrobial agents. The increase in biological activity of the metal chelates may be due to the effect of the metal ion on the normal cell process [53]. Chelation considerably reduce the polarity of the metal ion because of partial sharing of their positive charge with the donor group and possible p-electron delocalization within the whole chelate ring system that is formed during coordination. Chelation could enhance the lipophilic character of the central metal atom and hence increasing the hydrophobic character and liposolubility of the complex favoring its permeation through the lipid layers of the cell membrane. The antimicrobial activity of the three complexes can be referred to the increase of their lipophilic character which in turn deactivates enzymes responsible for respiration processes and other cellular enzymes, which is playing a vital role in various metabolic pathways of the tested microorganisms. Activities of the synthesized complexes were confirmed by calculating the activity index according to the following relation:

$$\textbf{Activity index (A)} = \frac{\textbf{Inhibition Zone of compound (mm)}}{\textbf{Inhibition Zone of standard drug (mm)}} \textbf{ x 100}$$

Complexes have more activity against Gram-positive bacteria than against Gram negative pathogens. The results show that Gram-positive bacteria were inhibited more strongly than Gram-negative bacteria and that can be explained by considering the structural features of both bacterial types. Gram-negative bacteria possessing an extra outer layer on top of the peptidoglycan and this has been found to be highly impermeable. However, Gram-positive bacteria have polysaccharides in their cell wall called teichoic acid, which is negatively charged and have facilitated the passage of

the positive metal ions [54]. An increase in lipophilicity of a metal complex enhances bacterial cell membrane penetration and blocking of metal binding sites on enzymes.

Antiviral activities of the prepared complexes

In principle, a molecule can act as an anti-viral drug if it inhibits some stage of the virus replication cycle, without being too toxic to the body's cells. The possible modes of action of anti-viral agents would include being able to:

1. Inactivate extracellular virus particles.
2. Prevent viral attachment and/or entry.
3. Prevent replication of the viral genome.
4. Prevent synthesis of specific viral protein(s).
5. Prevent assembly or release of new infectious virions.

Antiviral activities of the prepared complexes were investigated by determination of minimum cytotoxic and minimum inhibitory concentration which is tabulated in Table 11. The activities of the prepared complexes were higher in the sequence: DSPCu > MSTCu > DSHCu. The results show that the DSHCu complex has the highest concentration that inhibit the replication of a virus after overnight incubation and the highest concentration that toxic the virus.

Methodology for DNA binding analysis using absorption spectral studies

The application of electronic absorption spectroscopy in CT-DNA binding studies is one of the most important techniques [55]. The DNA binding studies were characterized by absorbance maximum at 382 for MSTCu, 380 for DSPCu and 368 for DSHCu. The addition of increasing higher concentration of DNA led to hypochromic in their visible absorption spectra as a result of the formation of more stable complexes. The interaction of complexes with DNA resulted in the decrease of absorption intensity accompanied by a shift towards lower wavelengths (2 to 14 nm), which corresponds to 25.9 % to 38.5 % reduction (Hypochromism). The spectral changes were used to evaluate the intrinsic binding constant (K_b), it observed 0.8 x 10^5, 1.8 x 10^5 and 9.4 x 10^5 M^{-1} for MSTCu, DSPCu and DSHCu, which are intercalated into DNA base pairs [56].The complexes may bind with DNA through intercalative interaction, which is brought by the π-π interaction between the complex which possesses an aromatic ring moiety in the ligands and the aromatic heterocyclic bases of DNA. The electronic absorption spectra of the prepared complexes in the absence and presence of different concentration of buffered CT-DNA are given in Table 12 and Fig. 9.

Methodology for DNA binding analysis using viscosity measurements

Viscosity measurements were also carried out to provide clues for a binding model between complexes and DNA. In the presence of lower concentrations of complexes, no significant changes were observed in the relative viscosity of DNA. However, at higher concentrations of complexes, the relative specific viscosity of DNA increased, but the increase is less than that observed for the typical intercalator EB, indicating that intercalative as shown in Fig. 10. A classical intercalation model results in lengthening of the DNA helix as base pairs to an increase of DNA viscosity [57, 58]. This observation suggests that the mode of DNA binding by complexes involved base pair intercalation.

Agarose gel electrophoresis of CT-DNA interaction with the investigated complexes

DNA binding studies are important for the rational design and construction of new and more efficient drugs targeted to DNA. The molecules interact reversibly with double stranded DNA, primarily through three modes: (i)

electrostatic interactions with negatively charged nucleic sugar–phosphate structure, (ii) binding interaction with two groves of DNA double helix and (iii) intercalation between the stacked base pairs of native DNA [59].

The cleavage efficiency of complexes was compared to that of the control is due to their efficient DNA binding ability control experiment using DNA alone does not show any significant cleavage of CT-DNA even on longer exposure time. The variation in DNA–cleavage efficiency of ligands/transition metal complexes was due to their difference in binding ability of ligands /complexes to DNA. The intensity of lanes Fig. 11 were higher in the sequence DSHCu > DSPCu > MSTCu. It was clear concluded that as the complexes was observed to cleave the DNA, therefore inhibits the growth of the pathogenic organism by cleaving the genome.

Conclusion

In this paper, coordination chemistry of Schiff base amino acid ligands, obtained from the reaction of 3–methoxysalicylaldehyde (MS) or 4-diethylaminosalicylaldehyde (DS) with α-amino acids (L-phenylalanine (P), L-histidine (H), DL-tryptophan (T)). The resulted Schiff base amino acid ligands are mono anionic tridentate ligands. Cu(II) complexes have been synthesized by using the above Schiff base amino acid ligands. Results of physical measurements showed that Cu(II) ion is coordinated by two phenolic oxygen atoms, two azomethine N atoms and two carboxylate O atom to form octahedral complexes with general formula $[Cu(HL)_2].nH_2O$. The prepared Schiff base amino acid ligands and their complexes have non-electrolytic nature. Moreover, the obtained K_f values increased in the following order DSPCu > DSHCu > MSTCu. The prepared complexes bind to DNA via an intercalative mode according to the spectral, viscosities measurements and gel electrophoresis. Furthermore, antimicrobial and antiviral for the prepared complexes were screened. These finding clearly indicated that transition metal based complexes have many potential applications including new therapeutic reagents for diseases. In addition, these complexes showed better therapeutic drug for antimicrobial treatment.

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List of Tables:

Table 1: The 2D structure of Schiff base amino acid ligands derived from 3-Methoxysalicylaldehyde (MS) or 4-Diethylaminosalicylaldehyde (DS) and their complexes

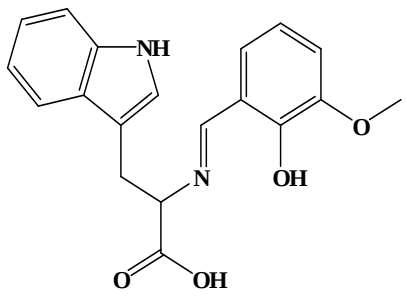
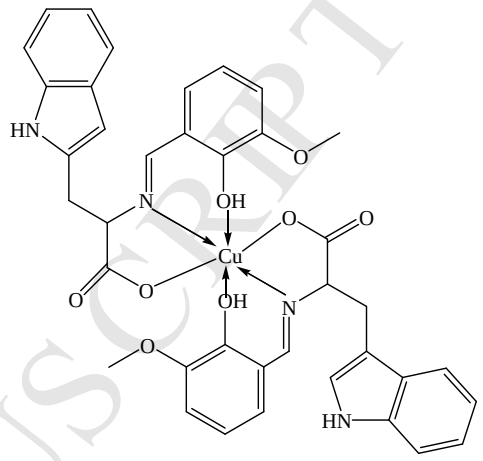
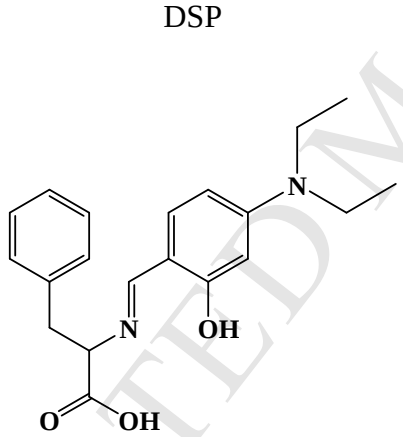
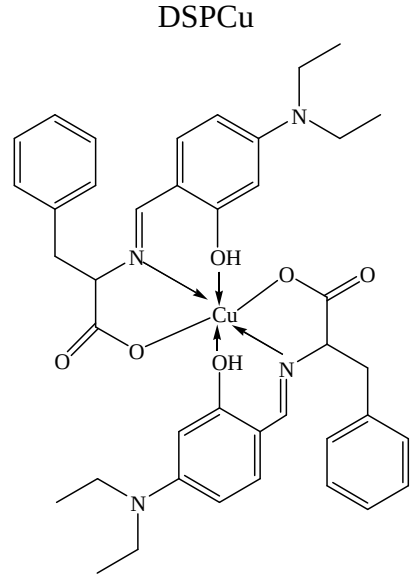
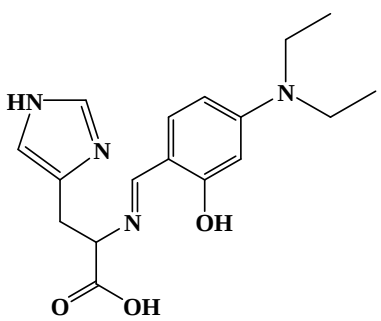
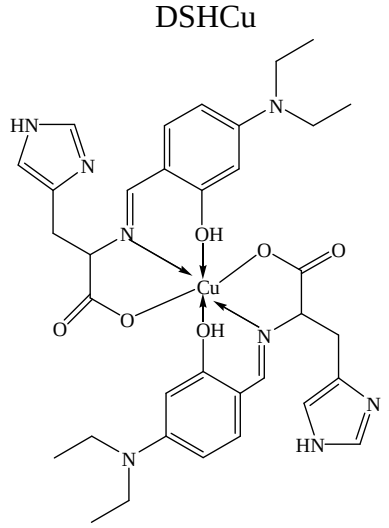
Amino acids	Aldehyde	
	3- MethoxySalicylaldehyde (MS)	
Tryptophan (T)	MST 	MSTCu 
	4-DiethylaminoSalicylaldehyde (DS)	
Phenylalanine (P)	DSP 	DSPCu 
Histidine (H)	DSH 	DSHCu 

Table 2: Analytical and physical data of Schiff base amino acid ligands and their complexes

Comp.	Molecular formula (M. wt.)	μ_{eff} (B. M.)	Λ_m, Ohm^{-1} cm^2 mol^{-1}	M. p. and Decomp. (^oC)	Elemental Analysis found (calc.) %		
					C	H	N
MST	$C_{19}H_{18}N_2O_4$	Diamagnetic	20.5	240	67.28	5.10	8.05
	(338.0)				(67.31)	(5.22)	(8.18)
MSTCu	$C_{38}H_{37}O_{9.5}N_4Cu$	1.8	7.7	280	59.49	4.68	7.21
	(764.5)				(59.54)	(4.74)	(7.33)
DSP	$C_{20}H_{24}N_2O_3$	Diamagnetic	8.2	160	70.40	6.98	8.11
	(340.0)				(70.52)	(7.06)	(8.23)
DSPCu	$C_{40}H_{46}O_6N_4Cu$	1.8	4.9	>300	64.73	6.20	7.55
	(741.5)				(64.81)	(6.29)	(7.59)
DSH	$C_{17}H_{24}N_4O_3$	Diamagnetic	19.4	275	61.82	6.67	16.97
	(330)				(61.91)	(6.72)	(17.02)
DSHCu	$C_{34}H_{44}O_7N_8Cu$	2.2	8.7	>300	55.17	5.95	15.14
	(739.5)				(55.22)	(6.01)	(15.21)

Table 3: IR spectral data of the investigated Schiff base amino acid ligands and their complexes.

Comp.	υ(OH)/H_2O	υ_{St} (-C=N)	υ_S(COO)	υ_A(COO)	υ_{ph}(C-O)	υ (Cu-N)	υ (Cu-O)
MST (found)	3389 (s)	1651 (s)	1362 (m)	1588 (w)	1293 (m)	-	-
MST (calc.)	3535	1761	1605	1655	1265	-	-
MSTCu (found)	3395 (s)	1628 (s)	1352 (m)	1566 (m)	1224 (m)	695 (m)	563 (m)
MSTCu (calc.)	3485	1701	1595	1535	1225	650	425
DSP	3441 (s)	1632 (s)	1405 (s)	1573 (s)	1235 (s)	-	-
DSPCu	3465 (s)	1618 (s)	1380 (m)	1554 (w)	1183 (w)	689 (m)	542 (m)
DSH	3438 (s)	1640 (s)	1430 (m)	1572 (w)	1280 (m)	-	-
DSHCu	3457 (s)	1630 (s)	1415 (m)	1567 (m)	1237 (m)	688 (w)	569 (w)

S = strong, m = medium, w = weak, ph = phenolic

Table 4: Molecular electronic spectra, λ_{max} (nm) and ε_{max} ($dm^3mol^{-1}cm^{-1}$) of the prepared Schiff base amino acid ligands and their complexes in DMF at 298 K against DMF as a blank.

Comp.	λ_{max}(nm)	ε_{max} ($dm^3mol^{-1}cm^{-1}$)	Assignment
MST	431	100	n→π*
	315	31	n→π*
	247	37	π→π*
MSTCu	614	104	d-d band
	379	438	LMCT band
	246	650	Intraligand band
DSP	389	75	n→π*
	250	136	π→π*
DSPCu	630	100	d-d band
	375	1192	LMCT band
	244	1664	Intraligand band
DSH	397	76	n→π*
	249	101	π→π*
DSHCu	507	46	d-d band
	363	220	LMCT band
	242	315	Intraligand band

Table 5: Thermal analysis of the prepared complexes

Complex	Temperature ^oC	Fragment loss %		Weight loss %	
		Molecular formula	Molecular weight	Found	Calc.
MSTCu	20.7-240.2	$1.5H_2O$	27.0	3.75	3.81
	241.5-289.7	$C_{12}H_{10}N_2O_2$	214.0	26.99	27.06
	290.9-340.5	$C_8H_8O_5$	184.0	23.75	24.07
	381.8-527.5	$C_{18}H_{16}N_2O$	276.0	36.97	36.39
Residu	>750	Cu	63.5	8.23	8.31
DSPCu	113.1-209.3	C_8H_8N	118.0	15.14	15.23
	209.3-339.3	$C_{12}H_{15}NO_3$	221.0	28.67	28.80
	339.3-456.8	$C_{20}H_{23}N_2O_3$	334.0	45.90	45.95
Residu	>750	Cu	63.5	8.49	8.53
DSHCu	15.3-157.9	H_2O	18.0	2.35	2.41
	157.9-396.1	$C_7H_6N_3O_4$	196.0	26.47	26.50
	396.1-445.3	$C_{11}H_{16}NO_2$	194.0	26.19	26.24
	445.3-530.9	$C_{16}H_{20}N_4$	268.0	36.17	36.24
Residu	>750	Cu	63.5	8.49	8.52

Table 6: The kinetic and the thermodynamic data

Complex	Temp. (^oC)	E* KJ/mol	A S^{-1}	Thermodynamic Parameters		
				S* (J/mol)	H* (KJ/mol)	G* (KJ/mol)
MSTCu	218.9	1.0	1.3	-240.3	-1.8	50.8
	272.6			-242.19	-2.3	63.7
	329.3			-243.7	-2.7	77.5
	469.5			-246.6	-3.9	111.9
DSPCu	143.6	0.5	0.6	-242.1	-1.2	33.6
	280.0			-247.61	-2.3	67.0
	411.7			-250.8	-3.4	99.8
DSHCu	64.8	1.0	1.2	-230.7	-0.5	14.4
	313.0			-241.8	-2.0	57.8
	455.8			-246.9	-3.8	108.8

Table 7: The formation constant (K_f), stability constant (pK) and Gibbs free energy ($\Delta G^{\neq}$) values of the prepared complexes in aqueous - ethanol at 298 K.

Complex	Type of complex	10^9 K_f	pK	ΔG^* kJ mol^{-1}
MSTCu	1: 2	1.1	20.8	-51.6
DSPCu	1: 2	2.5	21.6	-53.6
DSHCu	1: 2	1.8	21.3	-52.8

Table 8: The calculated HOMO, LUMO, energy gap (ΔE), chemical hardness (μ), chemical reactivity (η) and electrophilicity index (ϵ)

Comp.	HOMO (ev)	LUMO (ev)	ΔE (ev)	η (ev)	μ (ev)	ϵ (ev)
MST	-4.20	-1.18	3.05	1.53	-2.69	2.36
[Cu-MST]	-5.13	-3.25	1.88	0.94	-4.19	9.34

Table 9: Results of antimicrobial bioassay of the prepared Schiff base amino acid ligands and their complexes in DMSO

Comp.	Inhibition Zone (mm)											
	Fungi						Bacteria					
	A. niger		*C. glabrata*		*S. cerevisiae*		*B. subtilis*		*E. coli*		M. *luteus*	
Conc.(mg/ml)	15	30	15	30	15	30	15	30	15	30	15	30
MST	5	10	3	7	5	8	4	9	3	8	6	11
MSTCu	12	24	8	13	11	17	15	24	9	20	16	32
DSP	4	8	3	7	5	8	4	9	3	8	6	11
DSPCu	13	24	9	14	11	19	13	23	9	18	14	30
DSH	5	9	3	8	5	8	5	9	4	8	6	10
DSHCu	10	23	9	13	11	19	12	19	8	18	12	28
Ciprofloxacin	-	-	-	-	-	-	19	33	13	28	24	42
Amphotricine B	16	29	11	17	14	23	-	-	-	-	-	-

A. niger: ***Asperagillus niger*****, C. glabarta:** ***Candida Glabarta*****, S. cerevisiae:** ***Saccharomyces cerevisiae***

B. subtilis: ***Bacillus subtilis*****, E. coli:** ***Escherichia coli*****, M. luteus:** ***Micrococcus luteus***

Table 10: Results of activity index (%) for antimicrobial assay of the prepared Schiff base amino acid ligands and their complexes

Comp.	Activity index (%)					
	Bacteria			Fungi		
	B. subtilis	*E. coli*	M. *luteus*	*A. niger*	*C. glabrata*	*S. cerevisiae*
MST	27.3	28.6	26.2	34.5	41.2	34.8
MSTCu	72.7	71.4	76.2	82.8	76.5	73.9
DSP	27.3	28.6	26.2	27.6	41.2	34.8
DSPCu	69.7	64.3	71.4	82.2	82.4	82.6
DSH	27.3	28.6	23.8	31.0	47.1	34.8
DSHCu	57.6	64.3	66.7	82.8	76.5	78.3

Table 11: Antiviral activities of the prepared complexes

Compoud	Minimum cytotoxic concentration (µg/ml)	Minimum inhibitory concentration			
		Herpes simplex virus-1 (KOS)	Herpes simplex virus2- (G)	Herpes simplex virus-1 TK- VMW1837	Tobacco Mosaic Virus
MSTCu	43	102.7	86.2	38.9	16.60
DSHCu	84	> 400	> 400	> 400	98.30
DSPCu	64	1.2	1.3	10.2	1.45
BVDU	> 400	0.0256	> 400	0.64	-
ACG	> 400	0.0768	0.0768	0.64	-
DHPG	> 100	0.0038	0.0064	0.32	-

Table 12: Spectral parameters for DNA interaction with the prepared complexes

Complex	λ_{max} free (nm)	λ_{max} bound (nm)	Δn (nm)	Chromism (%)[a]	Type of Chromism	$10^5 K_b$ $mol^{-1}dm^3$	ΔG^* kJ mol^{-1}
MSTCu	620	628	8	31.4	Hypo	0.8	-28.2
	382	380	2	38.5	Hypo		
DSPCu	634	648	14	53.8	Hyper	1.8	-30.2
	380	367	13	30.4	Hypo		
DSHCu	498	485	13	73.9	Hyper	9.4	-22.9
	368	374	6	25.9	Hypo		

[a]**Chromism (%) = ($A_{free} - A_{bound}$) / A_{free}**

List of Figures:

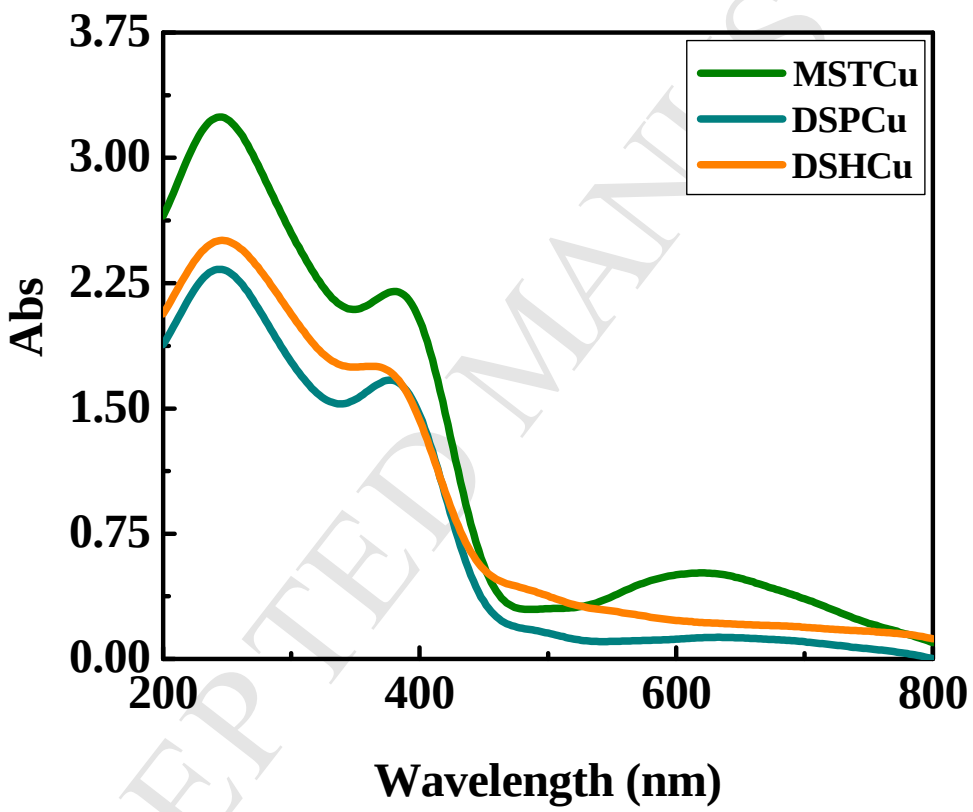


Figure 1: Molecular electronic spectra of (1) [DSHCu] = 8.0 x 10^{-3} mol dm^{-3}, (2) [DSPCu] = 1.4 x 10^{-3} mol dm^{-3}, (3) [MSTCu] = 5.0 x 10^{-3} mol dm^{-3}.

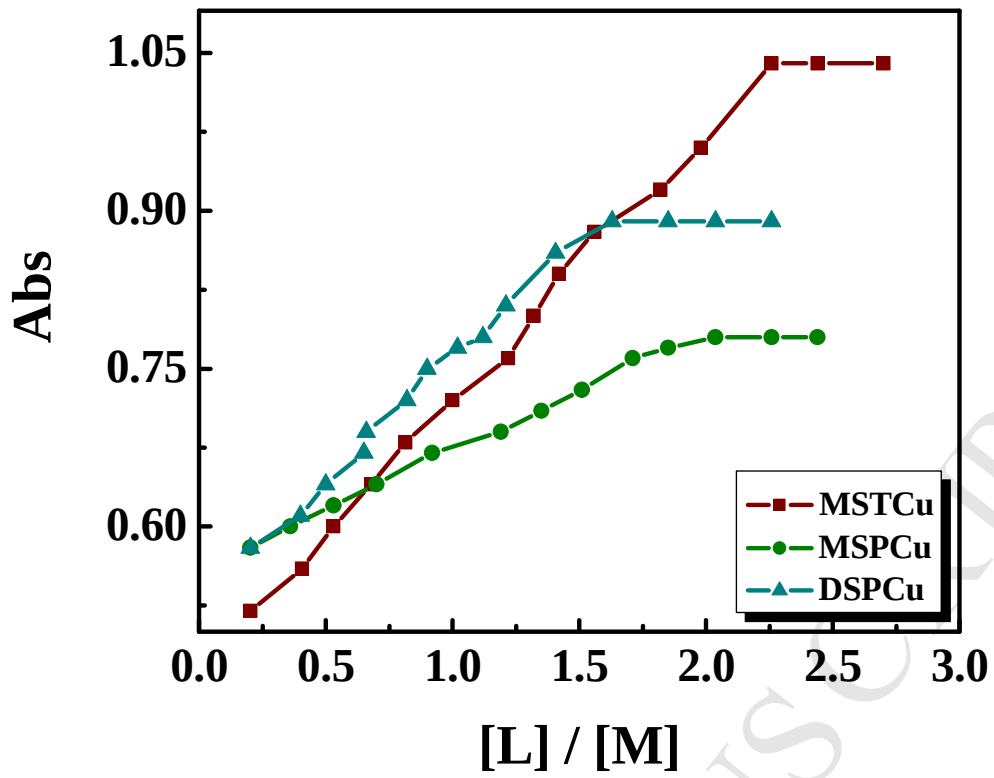


Figure 2: Molar ratio plot for the prepared complexes in aqueous-ethanol mixture at $[Cu^{2+}]$ = [MST] = 5.0 x 10^{-2} mol dm^{-3}, $[Cu^{2+}]$ = [DSP] = 2.8 x 10^{-2} mol dm^{-3} and $[Cu^{2+}]$ = [DSP] = 2.2 x 10^{-2} mol dm^{-3} and 298 K

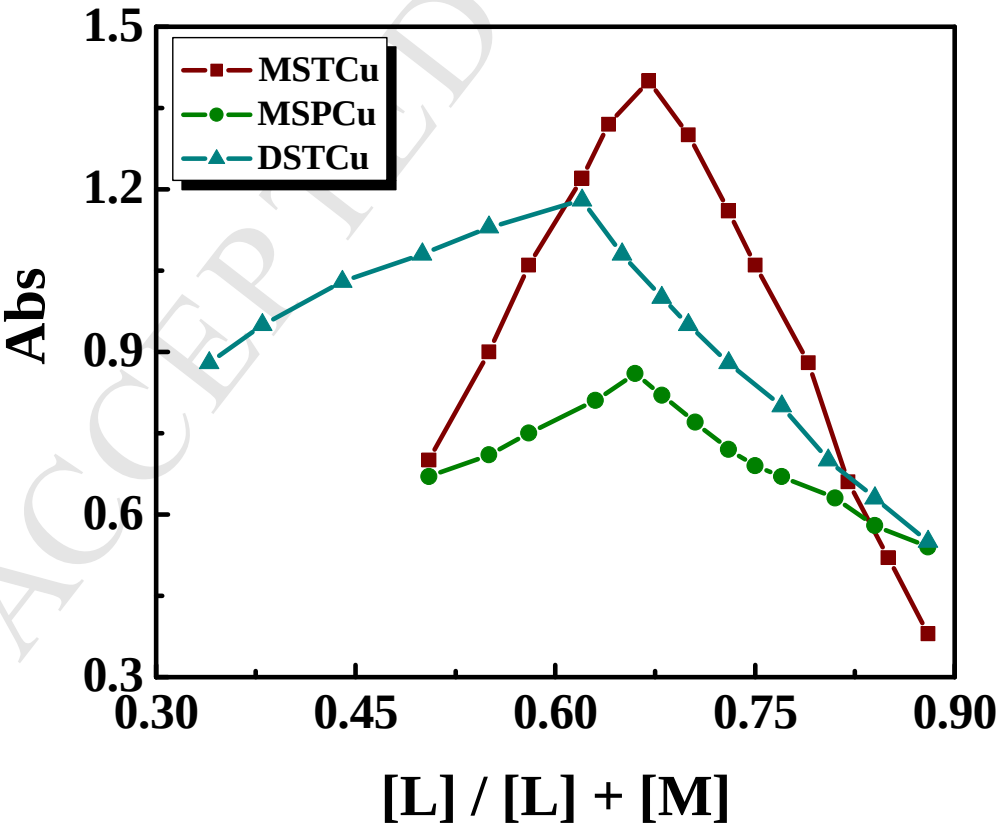


Figure 3: Continuous variation plot for the prepared complexes in aqueous-ethanol mixture at $[Cu^{2+}]$ = [DSH] = 2.5 x 10^{-2} mol dm^{-3}, $[Cu^{2+}]$ = [DSP] = 1.7 x 10^{-2} mol dm^{-3} and $[Cu^{2+}]$ = [MST] = 5.0 x 10^{-2} mol dm^{-3} and 298 K.

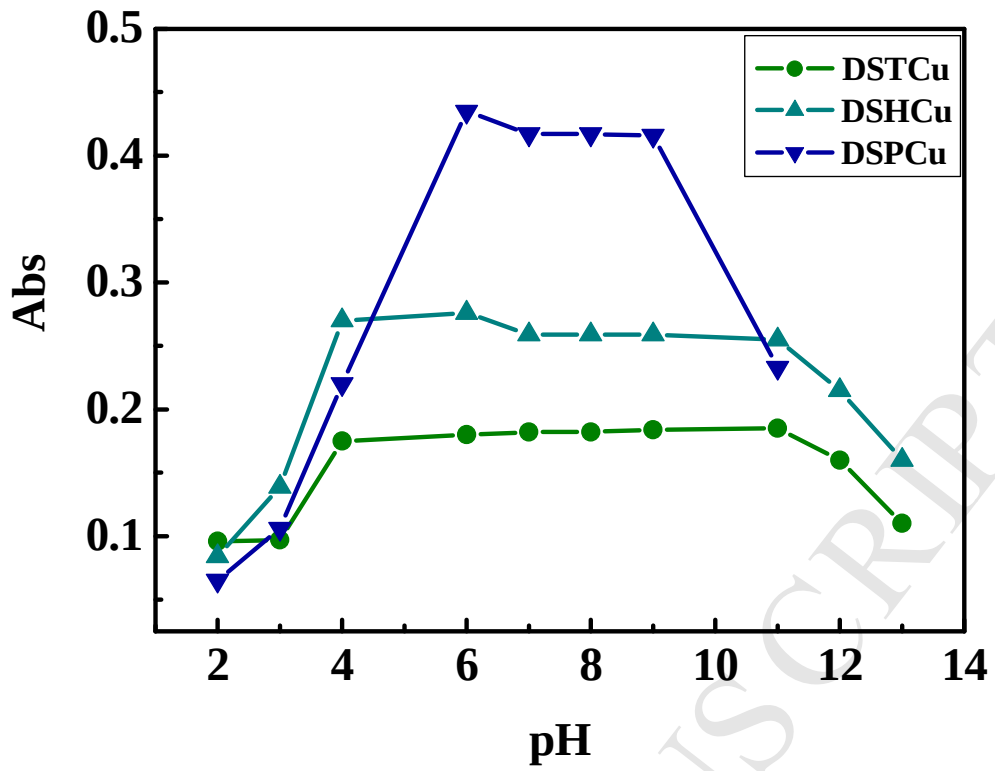


Figure 4: pH profile of the prepared complexes at [complex] = 1.0 x 10^{-3} mol dm^{-3}(except [DSHCu] = 5.4 x 10^{-3} mol dm^{-3}) and 298 K.

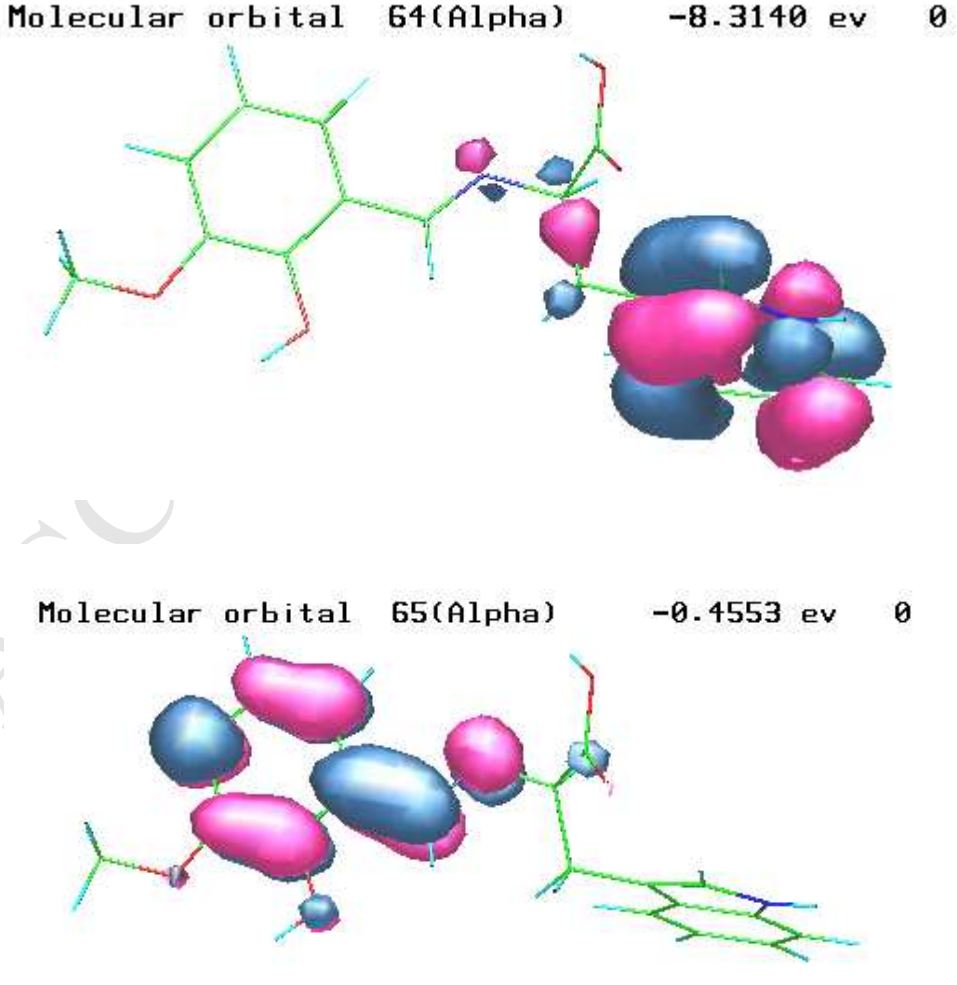


Figure 5: Optimized structures of MST ligand, HOMO-LUMO molecular orbitals with the calculated energy gap

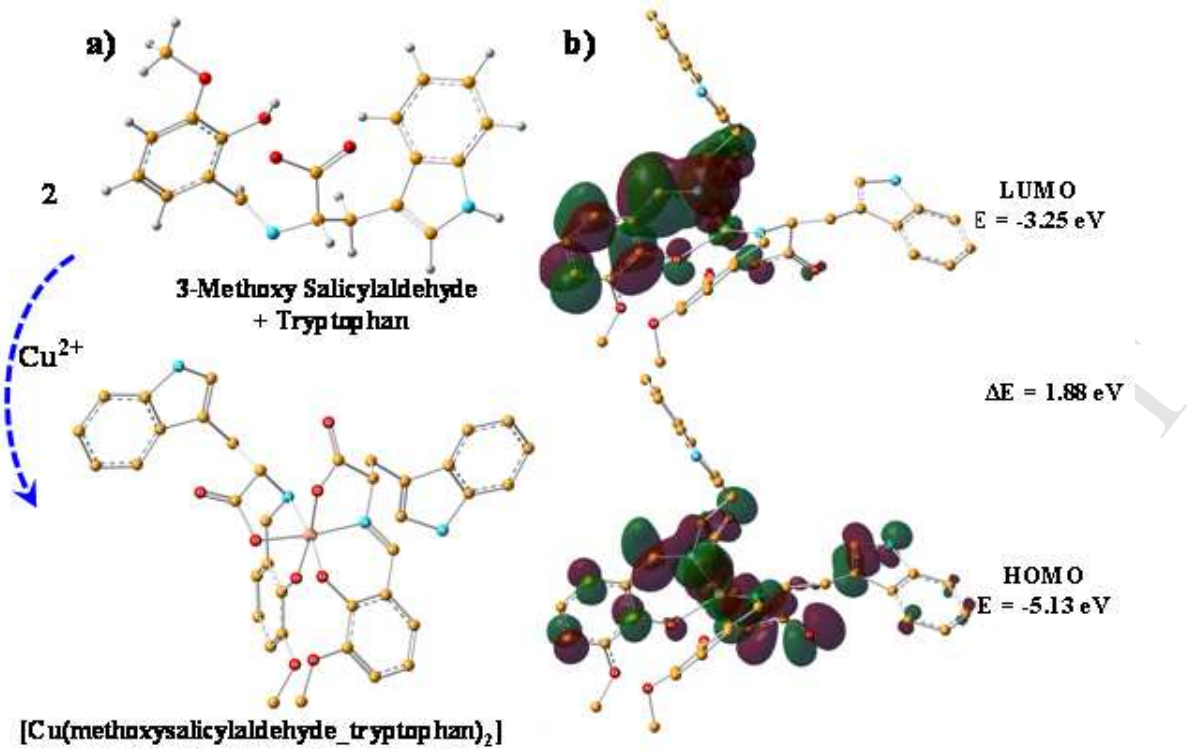


Figure 6 Optimized structures of a) MST ligand and the octahedral Cu complex. b) HOMO-LUMO molecular orbitals with the calculated energy gap.

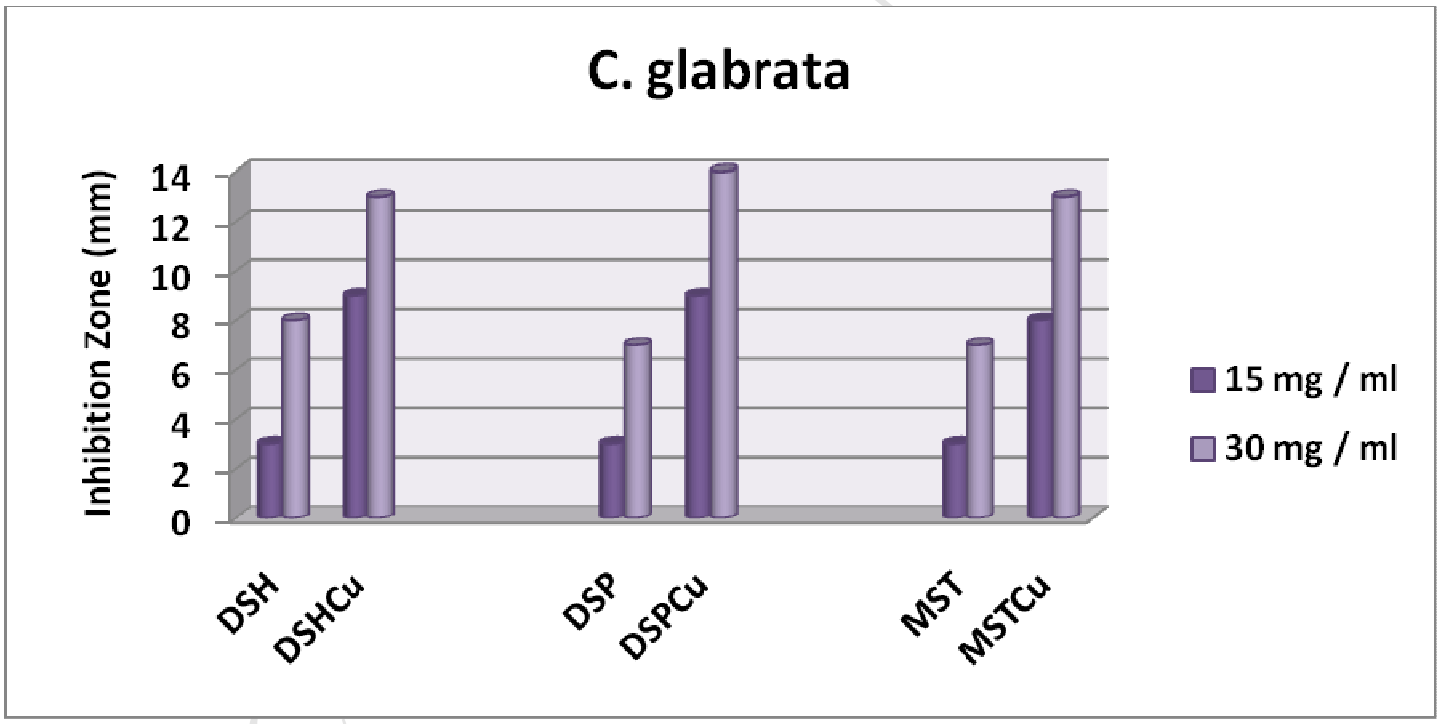


Figure 7: Antifungal evaluation of the investigated Schiff base amino acids and their complexes against *C. glabrata* fungi.

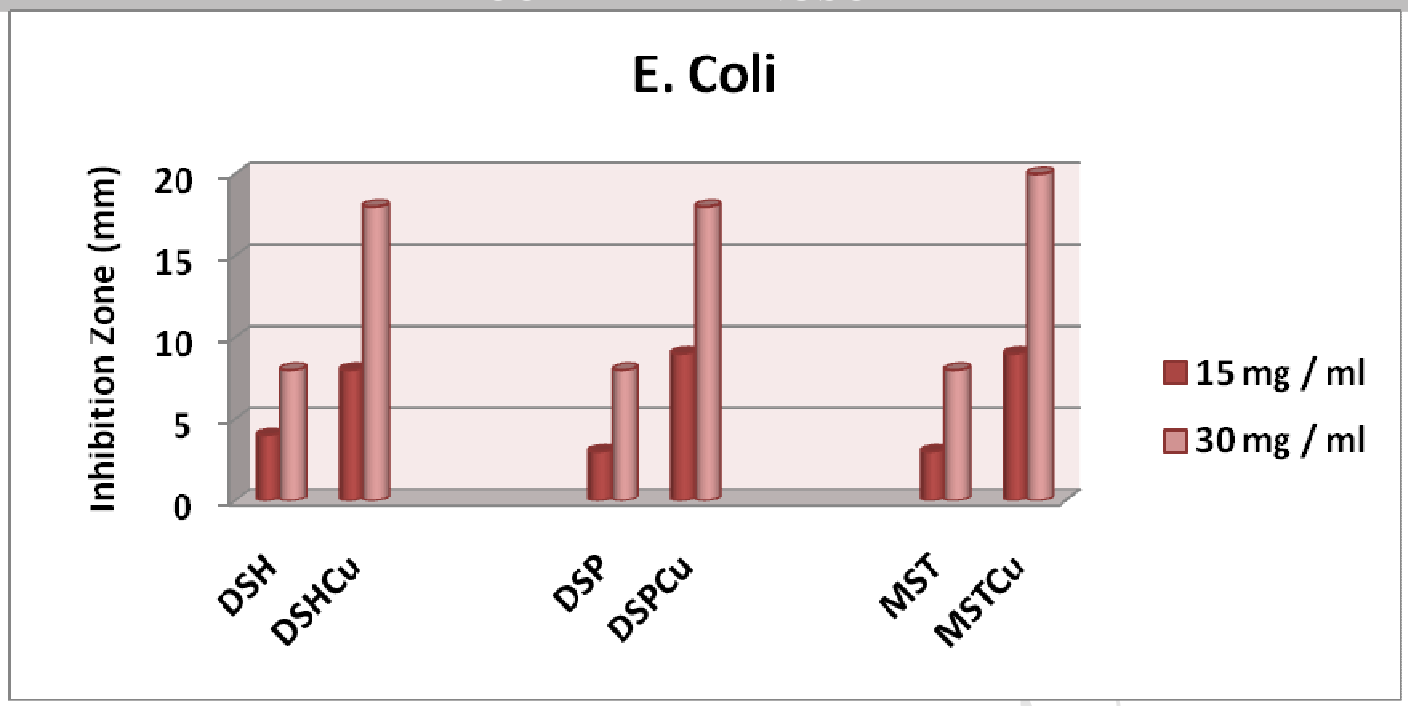


Figure 8: Antibacterial evaluation of the investigated Schiff base amino acids and their complexes against *E. coli* bacteria.

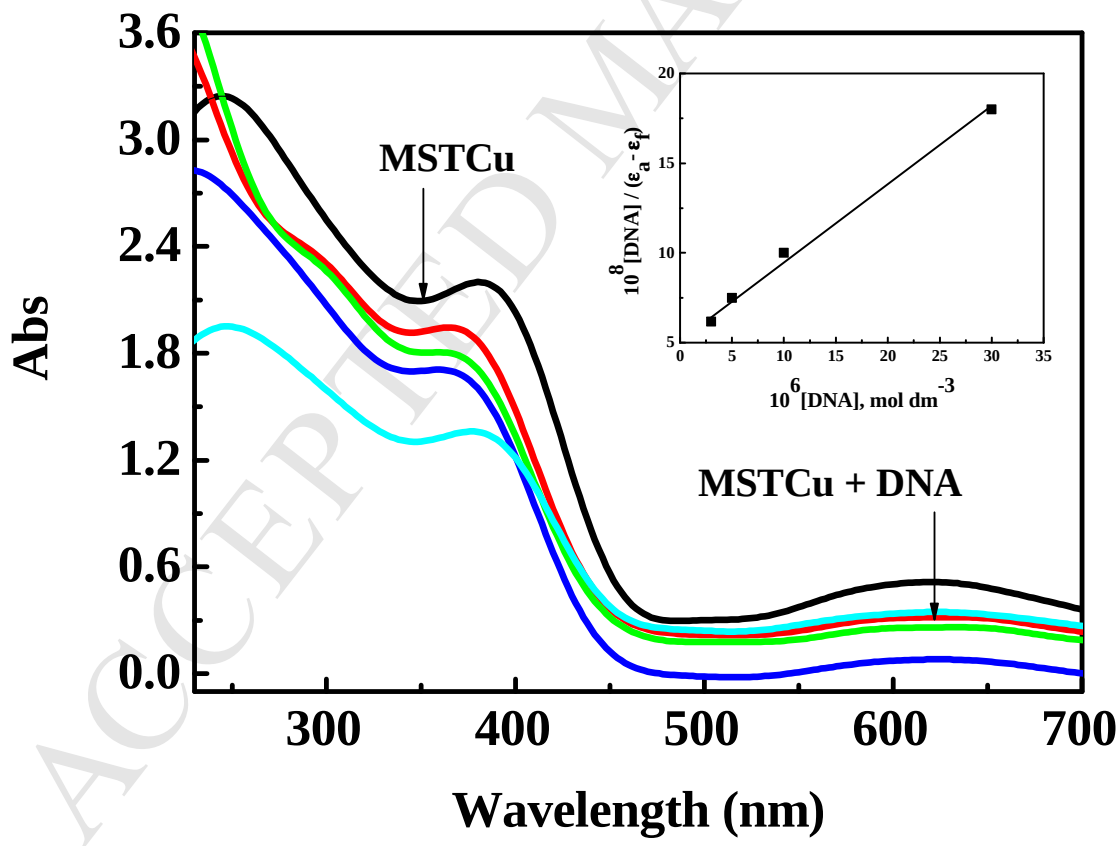


Figure 9: Spectral scans of the interaction of MSTCu complex (5.0 x 10^{-3} mol dm^{-3}) in 0.01 mol dm^{-3} Tris buffer (pH 7.2, 298 K) with CT – DNA (from 3 - 30 μm DNA). Plot of [DNA] / (ε_a - ε_f) versus [DNA] for the titration of DNA with MSTCu complex.

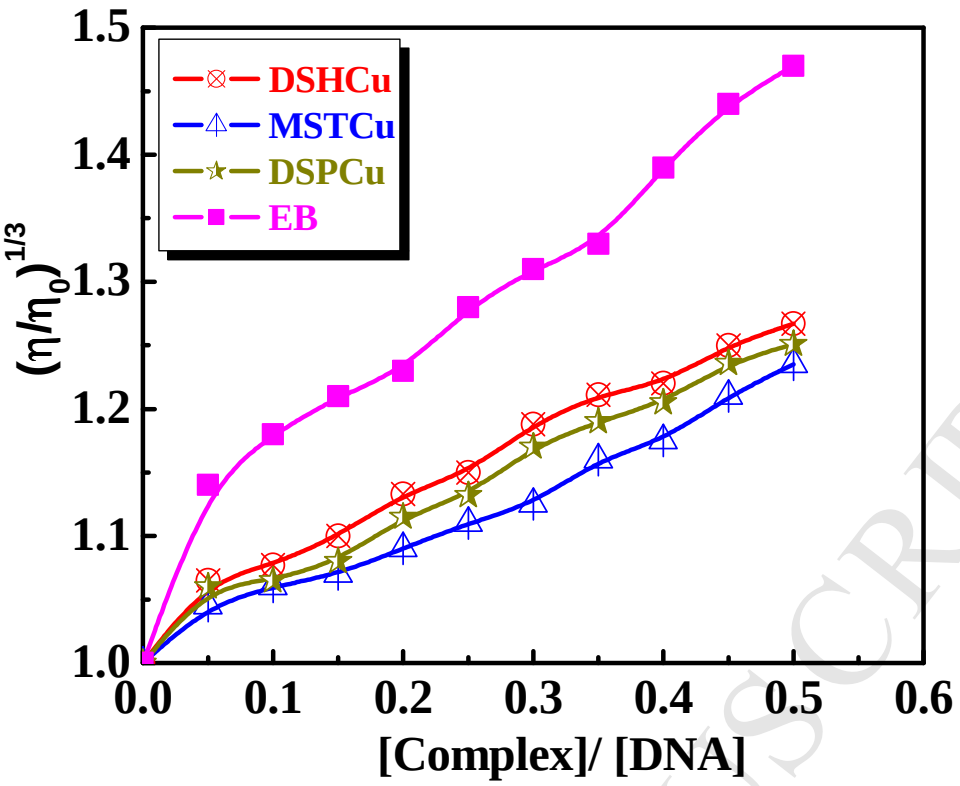


Figure 10: The effect of increasing concentration of the synthesized complexes on the relative viscosities of DNA at [DNA] = 0.5 mM , [complex] and [EB] = 25 -250 μM and 298K.

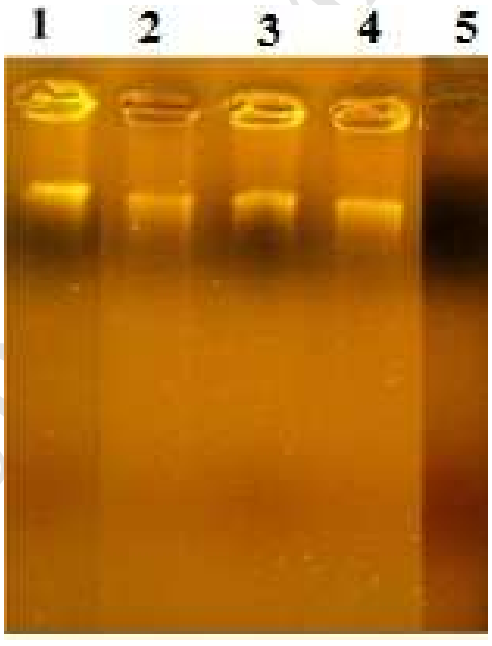


Figure 11: DNA binding results of Schiff base amino acid Complexes based on gel electrophoresis. Lane 1: CT – DNA, Lane 2: CT – DNA+DSHCu, Lane 3: CT – DNA+DSPCu, Lane 4: CT – DNA+MSTCu, Lane 5: MSTCu.

Highlights for review

1- **New Cu(II) Amino acid Schiff base chelates were synthesized and characterized**
2- **The prepared chelates were tested for their antimicrobial and antiviral activities.**
3- **DNA interaction of the prepared chelates was investigated**