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Synthesis, spectral characterization, DNA binding ability and antibacterial screening of copper(II) complexes of symmetrical NOON tetradentate Schiff bases bearing different bridges

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ABSTRACT

A novel series of four copper(II) complexes were synthesized by thermal reaction of copper acetate salt with symmetrical tetradentate Schiff bases, N,N′bis(o-vanillin)4,5-dimethyl-l,2-phenylenediamine (H_2L^1), N,N′bis(salicylaldehyde)4,5-dimethyl-1,2-phenylenediamine (H_2L^2), N,N′bis(o-vanillin)4,5-dichloro-1,2-phenylenediamine (H_2L^3) and N,N′bis(salicylaldehyde)4,5-dichloro-1,2-phenylenediamine (H_2L^4), respectively. All the new synthesized complexes were characterized by using of microanalysis, FT-IR, UV–Vis, magnetic measurements, ESR, and conductance measurements, respectively. The data revealed that all the Schiff bases (H_2L^{1-4}) coordinate in their deprotonated forms and behave as tetradentate NOON coordinated ligands. Moreover, their copper(II) complexes have square planar geometry with general formula [CuL^{1-4}]. The binding of the complexes with calf thymus DNA (CT-DNA) was investigated by UV–Vis spectrophotometry, fluorescence quenching and viscosity measurements. The results indicated that the complexes bind to CT-DNA through an intercalative mode. From the biological activity view, the copper(II) complexes and their parent ligands were screened for their *in vitro* antibacterial activity against the bacterial species *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosai* by well diffusion method. The complexes showed an increased activity in comparison to some standard drugs.

1. Introduction

Deoxyribonucleic acid (DNA) plays a significant role in the life process. This implies carrying the inheritance information, the biological synthesis of proteins and enzymes through the replication and transcription of genetic information in living cells. Also DNA is considered the primary target molecule for most anticancer and antiviral therapies according to cell biologists. Since DNA is an important cellular receptor, many chemicals exert their antitumor effects through binding to DNA thereby changing the replication of DNA and inhibiting the growth of the tumor cell [1]. Thus new and more efficient antitumor drugs were designed [2,3].

The interaction between DNA and transition metal complexes is an important fundamental issue in life sciences [4]. Consequently the design and synthesis of new complexes that interact with DNA are of considerable interest nowadays [5–7].

It is known that copper is a bioessential element with relevant oxidation states [8]. A diverse variety of copper-containing metalloenzymes occur in nature. It is revealed that Azurins [9], plastocyanins [10] and laccases [11] are involved in electron transfer reactions. Moreover, Tyrosinases [12] and ascorbate oxidases [13] are used in oxygenation reactions. Also, Hemocyanins [14] are copper-containing oxygen transport metalloproteins found in arthropods and mollusks. The role played by copper ions in the active sites of a large number of metalloproteins droved many researchers to design and characterize copper complexes as models for a better understanding of biological systems [15–17].

The aim of the present work is to investigate the interaction of calf thymus DNA (CT-DNA) with novel copper(II) complexes synthesized via thermal reaction with symmetrical tetradentate N_2O_2 Schiff base ligands derived from condensation reactions of 4,5-dimethyl-1,2-phenylendiammine and 4,5-dichloro-1,2-phenylendiammine with o-vanillin and salicylaldehyde. The new synthesized complexes have been fully characterized by different spectroscopic techniques. Moreover, from biological activity view the *in vitro* antibacterial activity of the new copper(II) complexes were screened against the bacterial species *Staphylococcus aureus*,

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Staphylococcus epidermidis, *Escherichia coli* and *Pseudomonas aeruginosai* by well diffusion method.

2. Experimental

Microanalyses (C, H and N) of all the synthesized compounds were performed on a JEOL JMS-AX500 MASS SPECTROMETER. FT-IR spectra (4000–400 cm^{-1}, KBr discs) of the samples were recorded on a Unicam-Mattson 1000 FT-IR. The electronic spectra were recorded on Shimadzu UV 1800 Spectrophotometer, equipped with a PC, using UVProbe software, version 2. Fluorescence spectra were recorded on a Jenway 6270 Fluorimeter. The molar conductivities of the complexes in dimethyl sulfoxide (DMSO) solution (1×10^{-3} M) were measured at room temperature by using Jenway 4010 conductivity meter. Magnetic moments were determined on a Sherwood Scientific magnetic moment balance (Model N0: MK1) at room temperature (25 °C), using $Hg[Co(SCN)_4]$ as a calibrant. Corrections for diamagnetism of the constituents were made using Pascal constants [18]. Electron spin resonance (ESR) measurements of solid complexes were recorded at room temperature on Bruker EPR spectrometer at 9.706 GHz (X-band), the microwave power was (1.0 mW) with 4.0 G modulation amplitude, using 2,2-diphenylpyridylhydrazone (DPPH) as standard (g = 2.0037). Copper content of the complexes were estimated by atomic absorption spectroscopy after decomposing the complexes with concentrated HNO_3 using Buck Scientific 205 AAS [19].

2.1. Materials

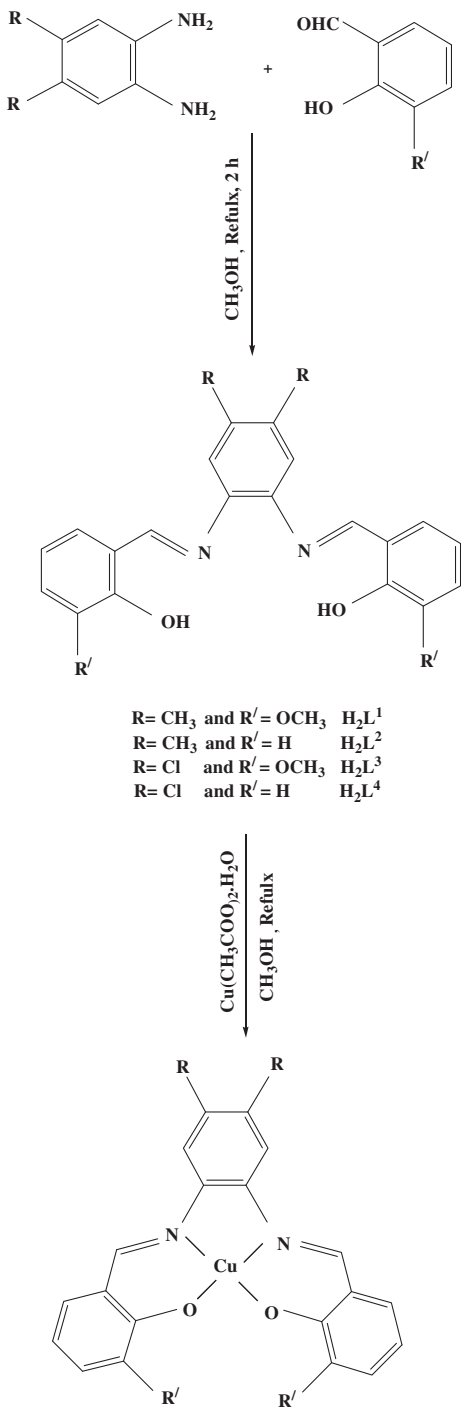
4,5-Dimethyl-1,2-phenylendiammine, 4,5-dichloro-1,2-phenylendiammine, o-vanillin, salicylaldehyde and $Cu(CH_3COO)_2{\cdot}H_2O$ were supplied from Aldrich. calf thymus DNA (CT-DNA) and ethidium bromide (EB) were purchased from Sigma Chemicals Co. (USA). All experiments of the interaction of the complexes with CT-DNA were carried out at room temperature in triply distilled water buffer containing 5 mM Tris–HCl/50 mM NaCl and adjusted to pH 7.2 with hydrochloric acid. The stock solution of CT-DNA (1 mM) was prepared in Tris–HCl/NaCl buffer, pH 7.2 (stored at 4 °C and used within 4 days). Solution of CT-DNA in the buffer gave a ratio of UV absorbance at 260 and 280 nm (A_{260}/A_{280}) of 1.87, indicating that the DNA was sufficiently pure and free of protein [20]. The CT-DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm [21]. All solvents were of analytical grade and were used without further purification.

2.2. Syntheses

Synthetic route of Schiff base ligands (H_2L^{1-4}) and their corresponding copper(II) complexes (1–4) is shown in Scheme 1.

2.2.1. Synthesis of the Schiff base ligand (H_2L^{1-4})

The ligands were synthesized as described in literature [22] by the following general procedure. 20 mmol of aldehyde was added drop wisely to a hot methanolic solution containing 10 mmol of the amine. The yellow solution obtained was refluxed for 2 h. After cooling, the precipitated product (Schiff base) was collected by filtration and recrystallized from methanol. The ligands were dried in a desiccator over anhydrous $CaCl_2$ under vacuum. The resultant product was washed with ethanol and the purity of the ligands was checked by TLC.



Scheme 1. Synthetic route of Schiff base ligands (H_2L^{1-4}) and their corresponding copper(II) complexes.

2.2.2. Synthesis of copper(II) complexes (1–4)

The copper(II) complexes were prepared by using the following general procedure. A methanolic solution (10 mL) of $Cu(OAc)_2{\cdot}H_2O$ (1 mmol) was added drop wisely to a hot methanolic solution (10 mL) of the corresponding Schiff bases. After complete addition, the mixture was refluxed at 80 °C for 3 h in case of H_2L^1 and H_2L^2 and 8 h for H_2L^3 and H_2L^4. Thereafter, the microcrystalline complex was separated on cooling and filtered under suction. The complex crystals were washed with hot petroleum ether 40–60 °C, recrystallized from chloroform and finally kept in a desiccator over anhydrous $CaCl_2$.

2.3. DNA-binding experiments

2.3.1. Absorption study

Absorption studies were performed with fixed complex concentrations (50 μM) while varying the CT-DNA concentration within 0–50 μM. Because of their low solubility in the buffer solution, the copper(II) complexes was dissolved in ethanol first to get a stock solution and then a small amount of the stock solution was added to the DNA solutions to get the desired concentration. While measuring the absorption spectra, equal increments of CT-DNA were added to both the complex solution and the reference solution to eliminate the absorbance of CT-DNA itself. From the absorption data, the intrinsic binding constant K_b was determined from a plot of [DNA]/($\varepsilon_a-\varepsilon_f$) versus [DNA] using the equation [23]:

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

where [DNA] is the concentration of DNA in base pairs, the apparent extinctions coefficient ε_a, ε_b and ε_f correspond to $A_{obs.}/[CuL^{1-4}]$, the extinction coefficient of the copper(II) complex in the free solution and the extinction coefficient of the copper(II) complex in the fully bound form, respectively. The intrinsic binding constant (K_b) is given by the ratio of the slope to the intercept.

2.3.2. Fluorescence study

The relative binding of the copper(II) complexes to CT-DNA was studied with an EB-bound CT-DNA solution in 5 mM Tris–HCl/NaCl buffer (pH 7.2) using fluorescence spectrophotometry. The fluorescence intensities of EB (10 μM) bound to CT-DNA (10 μM) were measured at 602 nm (524 nm excitation) after addition of different complex concentrations within range 0–50 μM. The relative binding of the copper(II) complexes to CT-DNA was determined by calculating the quenching constant (K) from the slopes of straight lines of Stern–Volmer equation [24]:

$$F_o/F=1+K\,r \quad (2)$$

where F_o and F are the fluorescence intensities in the absence and presence of the quencher, respectively and r = $[CuL^{1-4}]$/[DNA].

2.3.3. Viscosity measurements

Viscosities of CT-DNA at different complexes concentrations (0–60 μM) in buffer solution were measured using Ubbelodhe viscometer at the temperature of 25 ± 0.1 °C in a thermostatic water-bath. CT-DNA samples, averaging approximately 200 base pairs (bp) were prepared using sonication in order to minimize the complexities arising from DNA flexibility [25]. Different ratios of $[CuL^{1-4}]$/[DNA] were prepared before use, using [CT-DNA] = 100 μM. Average flow time was recorded after three time measurements for a sample with a digital stopwatch. Relative viscosities for CT-DNA in the presence and absence of complexes were calculated from the relation:

$$\eta=(t-t_o)/t_o \quad (3)$$

where t is the observed flow time of the CT-DNA-containing solution and t_o is the flow time of buffer alone [26]. The data were presented as $(\eta/\eta_o)^{1/3}$ vs mole ratio of $[CuL^{1-4}]$/[DNA] [27].

2.4. Anti bacterial screening

The *in vitro* biological activity of the investigated Schiff bases and their copper(II) complexes was tested against the bacteria strains: (i) *S. aureus*, (ii) *S. epidermidis* (iii) *E. coli*, and (iv) *P. aeruginosai* by well diffusion method using nutrient agar as medium. The stock solution was prepared by dissolving the compounds in DMSO. In a typical procedure, a well was made on agar medium inoculated with microorganism. The well was filled with the test solution using a micropipette and the plate was incubated for 24 h at 37 °C. During this period, the test solution diffused and the growth of the inoculated microorganism was affected. The inhibition zone was developed, at which the concentration was noted. Preliminary screening was performed using two concentrations, 100 μg/1.0 mL and 200 μg/1.0 mL of the tested compounds. DMSO was used as a control under the same conditions for each organism and no activity was found. Finally the activity results are calculated as a mean of triplicates. The standard drugs streptomycin and amoxycillin were also tested for their antibacterial activity at the same concentration under similar conditions to that of the test compounds.

3. Results and discussion

The physical properties of the ligands and the complexes are grouped in Table 1. The elemental analysis data (Table 1) was found to agree well with the proposed formulae of the complexes and confirmed the $[CuL^{1-4}]$ composition with 1:1 [M:L] ratio. Generally all the complexes were colored, quite stable towards air, insoluble in water and soluble in acetone, ethanol, CH_2Cl_2, DMF CH_3CN and DMSO, respectively. The Molar conductance values (Λ_m) of the complexes in DMSO (1×10^{-3} M) solution at 25 °C were found in the range 8.45–15.7 Ω^{-1} mol^{-1} cm^2. These low values indicated that all of the complexes have non-electrolytic nature [28].

3.1. Characterization of the complexes

3.1.1. Infrared spectra

In order to study the binding of Schiff bases ligands (H_2L^{1-4}) to Cu(II) ion, a comparison between the IR spectra of the complexes and the free ligands were made. The selected IR spectra of the ligands and their copper(II) complexes along with their tentative assignments are presented in Table 2. The IR spectra of $[CuL^1]$ and $[CuL^2]$ complexes as a representative example are shown in Fig. 1. It is illustrated that all the IR spectra of the complexes are

Table 1
Yield, molecular weight, micro analysis and conductivity data of the Schiff bases ligands (H_2L^{1-4}) and their copper(II) complexes.

Complexes	Yield (%)	M (Wt)	Color	(Calculated) Found (%)					Λ_m (Ω^{-1} cm^2 mol^{-1})
				C	H	N	Cl	Cu	
H_2L^1 $C_{24}H_{24}N_2O_4$	89	404.46	Orange	(71.27) 71.01	(5.98) 5.77	(6.92) 6.78	–	–	–
$[CuL^1]$ (1) $(C_{24}H_{22}N_2O_4)Cu$	85	465.99	Brown	61.86 (61.79)	4.75 (4.68)	6.01 (5.86)	–	(13.63) 13.56	14.9
H_2L^2 $C_{22}H_{20}N_2O_2$	63	344.41	Yellow	(76.72) 76.21	(5.85) 5.72	(8.13) 7.98	–	–	–
$[CuL^2]$ (2) $(C_{22}H_{18}N_2O_2)Cu$	79	405.94	Dark green	65.09 (64.98)	4.46 (4.37)	6.90 (6.88)	–	15.65 (15.61)	15.70
H_2L^3 $C_{22}H_{18}N_2O_4Cl_2$	88	445.30	Yellow	(59.34) 59.21	(4.07) 3.92	(6.29) 6.11	(15.92) 15.75	–	–
$[CuL^3]$ (3) $(C_{22}H_{16}N_2O_4Cl_2)Cu$	75	506.83	Brown	(52.13) 52.03	(3.18) 3.13	(5.52) 5.46	(13.99) 13.87	(12.53) 12.49	9.74
H_2L^4 $C_{20}H_{14}N_2O_2Cl_2$	70	385.25	Yellow	(62.35) 62.29	(3.66) 3.60	(7.27) 7.25	(18.40) 18.38	–	–
$[CuL^4]$ (4) $(C_{20}H_{12}N_2O_2Cl_2)Cu$	63	466.78	Brown	(51.46) 51.38	(2.59) 2.53	(6.00) 5.93	(15.19) 15.02	(13.61) 13.58	8.45

Table 2
The infrared (cm^{-1}), UV–Vis spectral data of the Schiff bases ligands (H_2L^{1-4}) and their copper(II) complexes.

Compound	(OH)	ν(C═N)	ν(C—O)	ν(Cu—O)	ν(Cu—N)	UV–Vis λ_{max} (nm) (Ethanol)
H_2L^1	3389(br.)	1610(s)*	1276(m)	–	–	224[a], 308[b]
[CuL^1] (1)	–	1612(s)	1334(m)	488(w)	436(w)	228[a], 311[b], 400[c]
H_2L^2	3428(br.)	1617(s)	1278(m)	–	–	223[a], 301[b]
[CuL^2] (2)	–	1615(s)	1329(m)	497(w)	441(w)	224[a], 305[b], 397[c]
H_2L^3	3394(br.)	1608(s)	1274(m)	–	–	216[a], 297[b]
[CuL^3] (3)	–	1606(s)	1299(m)	466(w)	435(w)	219[a], 299[b], 388[c]
H_2L^4	3444(br.)	1616(s)	1279(m)	–	–	214[a], 295[b]
CuL^4] (4)	–	1608(s)	1332(m)	554(w)	475(w)	218[a], 288[b], 386[c]

*IR peak intensity; s, strong; m, medium; w, weak; br., broad.
[a] π–π^*.
[b] n–π^*.
[c] MLCT.

very similar. This finding would indicate that, the four complexes have the same IR coordination structures. Generally, the position and/or the intensities of IR peaks are expected to change upon chelation. Moreover, the appearance of new peaks are also guiding for chelation. The discussion is confined to the most important vibrations of the 4000–400 cm^{-1} region in relation to structure. It can be clearly seen that the absence of the characteristic aldehydic carbonyl stretching bands, and the appearance of the azomethine —C═N— bands at 1608–1617 cm^{-1}. This behavior gives an evidence for formation of the Schiff bases. Also, the broad band of medium intensity which appeared in the region 3389–3444 cm^{-1} was assigned to the intra-molecular hydrogen-bonded O—H group [29]. As the hydrogen bond becomes stronger, the bandwidth increases, and this band sometimes cannot be detected [30]. The strong band attributed to (C═N) stretching vibrations of imine nitrogen, present in the free ligands was found to be shifted to lower wave numbers in the range 1606–1615 cm^{-1} in accordance with the coordination of the azomethine to the metal ion for all the complexes [31]. Coordination of the Schiff base to the metal through the nitrogen atom is expected to reduce electron density in the azomethine as a result of the withdrawal of electron density from the nitrogen atom and lower the C═N absorption frequency. On the other hand, the involvement of the deprotonated phenolic-OH group in chelation is confirmed by clarifying the effect of chelation on the ν(C—O). The medium intensity band in the region 1274–1279 cm^{-1} was ascribed to the phenolic C—O stretching vibration in the case of salicylideneanilines [32]. On complexation this band was shifted to higher frequency in the range 1299–1334 cm^{-1}. The co-ordination of the azomethine nitrogen and phenolic oxygen were further supported by the appearance of two non-ligand bands at 488–554 cm^{-1} and 435–475 cm^{-1} due to ν(Cu—O) and ν(Cu—N) [33,34], respectively. From the above arguments together with the elemental analyses, it was concluded that Schiff base ligands behave as a dianionic tetradentate Schiff base ligand with NOON donor sites.

3.1.2. Electronic spectra and magnetic susceptibility

Magnetic measurements and electronic spectra were conducted in order to obtain information about the geometry of the complexes. The values of magnetic susceptibility for the copper(II) complexes are given in Table 3. The magnetic susceptibility values (μ_{eff}) of all these complexes were in 1.74–1.81 BM range, which was consistent with presence of a single unpaired electron [35]. This behavior suggest square-planar geometry for the copper(II) complexes [36]. On the other hand, the electronic spectra of the free ligands and the complexes were recorded in ethanol (1×10^{-5} M). The absorption region and the assignment of the absorption bands are listed in Table 2. Evidently the electronic spectra of the free ligands exhibit two absorption bands at the range 214–224 nm and 295–308 nm which were assigned to π–$\pi^*$and $n \rightarrow \pi^*$ transitions within the Schiff base ligands. These absorptions also present in the spectra of copper(II) complexes but they undergone bathochromic shift. This shift in the spectra of the complexes supported the coordination of the ligands to Cu(II) ion. Furthermore, the UV–Vis spectra of the complexes showed an intense band in the region 386–400 nm which can be considered as ligand to metal charge transfer (LMCT) transitions as those were absent in the spectra of the respective free ligands.

3.1.3. Electron spin resonance spectra

The ESR spectra of copper(II) complexes provide valuable information in studying the Cu(II) ion environment. ESR spectra of the polycrystalline copper (II) complexes exhibited an isotropic signal, without any hyperfine splitting. Representative ESR spectra of [CuL^1] and CuL^2complexes are given in Fig. 2. The g tensor values of the copper (II) complexes could be used to obtain the ground state [37]. For ionic environment $g_{||}$ is normally 2.3 or larger, but for covalent environment $g_{||}$ is less than 2.3 [38]. The trend in the observed "g" values of copper (II) complexes at room temperature (Table 3), was $g_{||} > g_{\perp} > g_e$ (2.0023). This trend provide an evidence of localization of the unpaired electron in d_{x2-y2} orbital [39,40]. The 'g_{aver}' values of the complexes were found to be in the range 2.09–2.12. Moreover, for the reported copper(II) complexes, $g_{||} < 2.3$ value, indicating the covalent character of the metal-ligand bond [40]. This behavior is further supported by calculating the axial symmetry parameter (G) using Kneubuhl's method $G = (g_{||} - 2.0023)/(g_{\perp} - 2.0023)$ [41]. According to Hathaway [42], if the G value >4, the exchange interaction is negligible, while a value of <4 give an indication for considerable exchange interaction in the complex. The axial symmetry parameter (G) of the reported copper(II) complexes were <4 suggesting exchange interaction in the solid state.

3.2. DNA binding studies

DNA binding is the critical step for DNA cleavage in most cases. Many workers [43,44] suggested two major binding modes of interaction of substrate with DNA. The first suggestion is that the metal complexes squeeze in between the double helix through hydrogen bonding. The second suggestion based on covalent binding, where two major sites are available for the metal ion to interact with the DNA. In the later case, one being the electron donor groups of the bases, more preferably at the guanine N-7 and the other is the phosphate moieties of the ribose-phosphate backbone.

3.2.1. UV–Vis spectroscopic studies

The application of electronic absorption spectroscopy in DNA-binding studies is one of the useful techniques. The binding of copper(II) complexes with DNA helix have been characterized through absorption spectral studies by following changes in absorbance

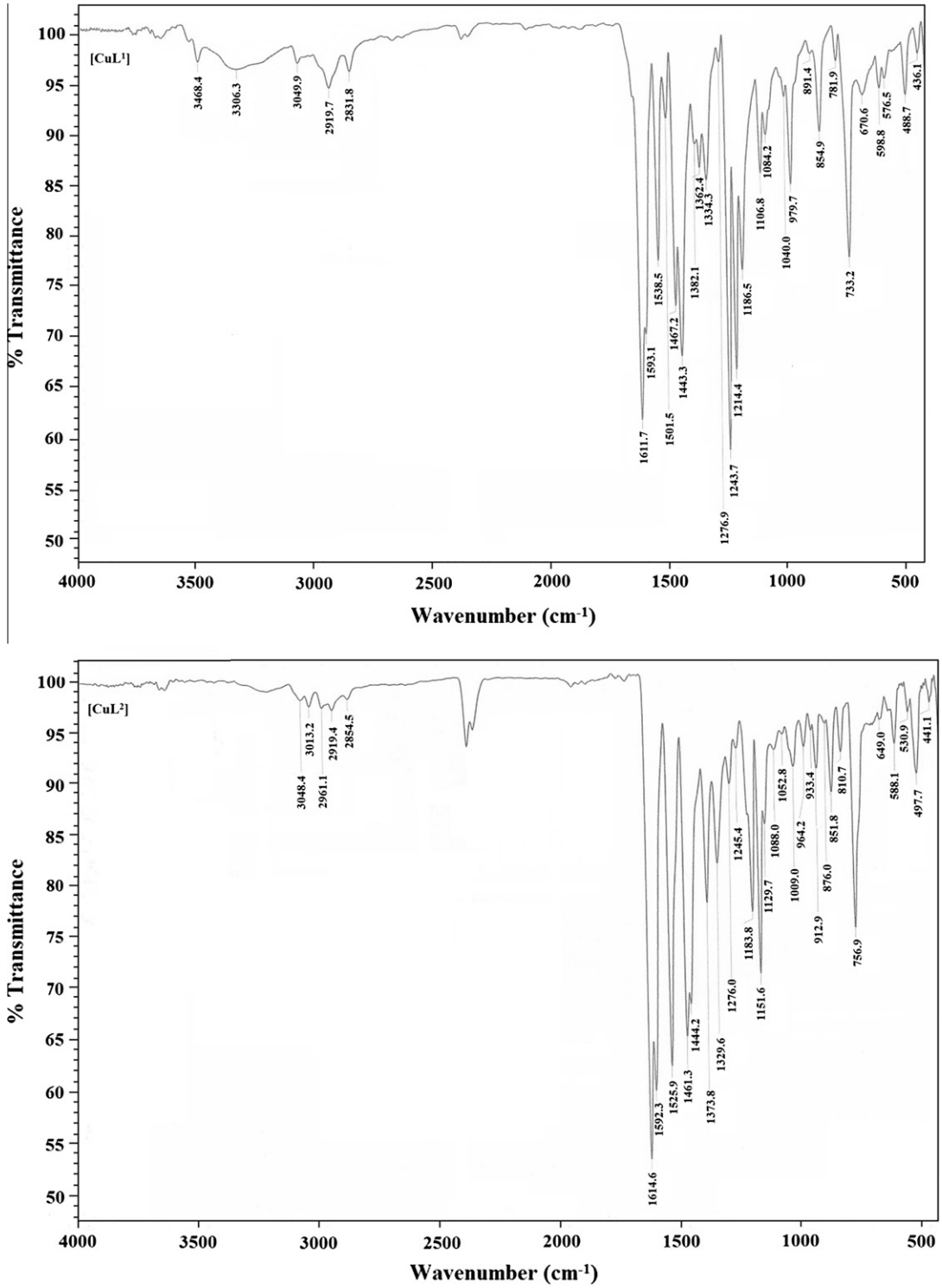


Fig. 1. FT-IR spectra of [CuL^1] and [CuL^2] complexes.

Table 3
The μ_{eff} and g parameter values of the reported copper(II) complexes (1–4).

Complex	$\mu_{eff.}$ (BM)	$g_{\|\|}$	$g_{\perp}$	$^*g_{aver.}$	G
[CuL^1] (1)	1.78	2.15	2.06	2.12	2.56
[CuL^2] (2)	1.80	2.14	2.07	2.11	2.03
[CuL^3] (3)	1.74	2.11	2.05	2.09	2.26
[CuL^4] (4)	1.81	2.11	2.04	2.08	2.85

* = $1/3(g_{\perp} + 2\, g_{\|})$; $G = (g_{\|}\text{-}2.0023)/(g_{\perp}\text{-}2.0023)$.

and shift in wavelength. In general, the absorption spectra of metal complex bound to DNA through intercalation exhibit significant hypochromism and bathochromism shift due to the strong π–π* stacking interaction between the aromatic chromophore ligand of metal complexes and the base pairs of DNA [44]. The absorption spectra of copper(II) complexes in the absence and presence of DNA, using a constant concentration of the copper(II) complex are given in Fig. 3. The results showed that as the DNA concentration increased, the transition bands of the complexes exhibited

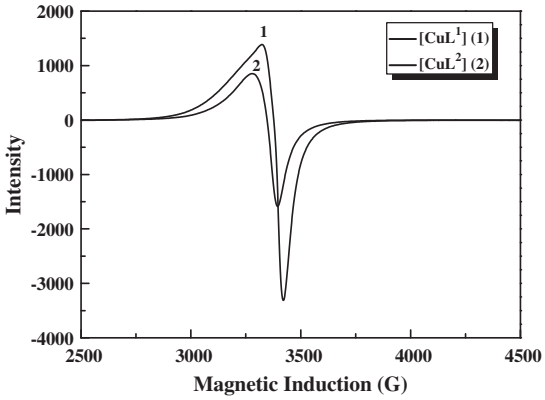


Fig. 2. ESR spectra of [CuL^1] and [CuL^2] complexes at 298 K.

hypochromism with a red shift of 6 nm, 4 nm, 2 nm and 1 nm for complexes [CuL$^{1-4}$] (1–4) respectively. This behavior gives an indication for binding of the four complexes to DNA. Moreover, it is observed that the extent of hypochromism was 45.60% for complex 1, 32.31% for complex 2, 21.67% for complex 3 and 17.67% for complex 4, respectively. The extent of hypochromism is consistent with intercalative interaction [45]. Generally, the extent of the hypochromism is commonly consistent with the strength of intercalative interaction [46].

The affinity of the complexes toward DNA was determined quantitatively by calculating the intrinsic binding constant (K_b) from the observed spectroscopic changes by regression analysis using Eq. (1). The K_b values were $4.754 \times 10^4\ M^{-1}$ for complex 1, $4.257 \times 10^4\ M^{-1}$ for complex 2, $3.637 \times 10^4\ M^{-1}$ for complex 3, and $2.144 \times 10^4\ M^{-1}$ for complex 4, respectively revealing that 1 > 2 > 3 > 4 in binding to CT-DNA.

3.2.2. Fluorescence quenching studies

Fluorescence measurements were carried out to get further proof for the binding of the copper(II) complexes to DNA. The binding of the copper(II) complexes to CT-DNA was evaluated by measuring the fluorescence emission intensity of EB bound to CT-DNA as a probe. EB (3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide) is a large, flat basic conjugate planar molecule that resembles a DNA base pair. EB shows weak reduced fluorescence intensity in buffer due to quenching by solvent molecules. When EB bound to DNA, EB fluorescence is enhanced due to its intercalation into the helix and again it is quenched by the addition of another molecule that displaces EB from DNA [47]. The fluorescence measurement for the copper(II) complexes showed that no emission band was observed either with or without CT-DNA at ambient temperature. The fluorescence intensity of EB (10 μM) bound to DNA (10 μM) at 602 nm in the absence and the presence of copper(II) complexes (1–4), using variable complex concentrations are shown in Fig. 4. In case of the addition of complexes showed a significant decrease in the fluorescence intensity of the EB-DNA

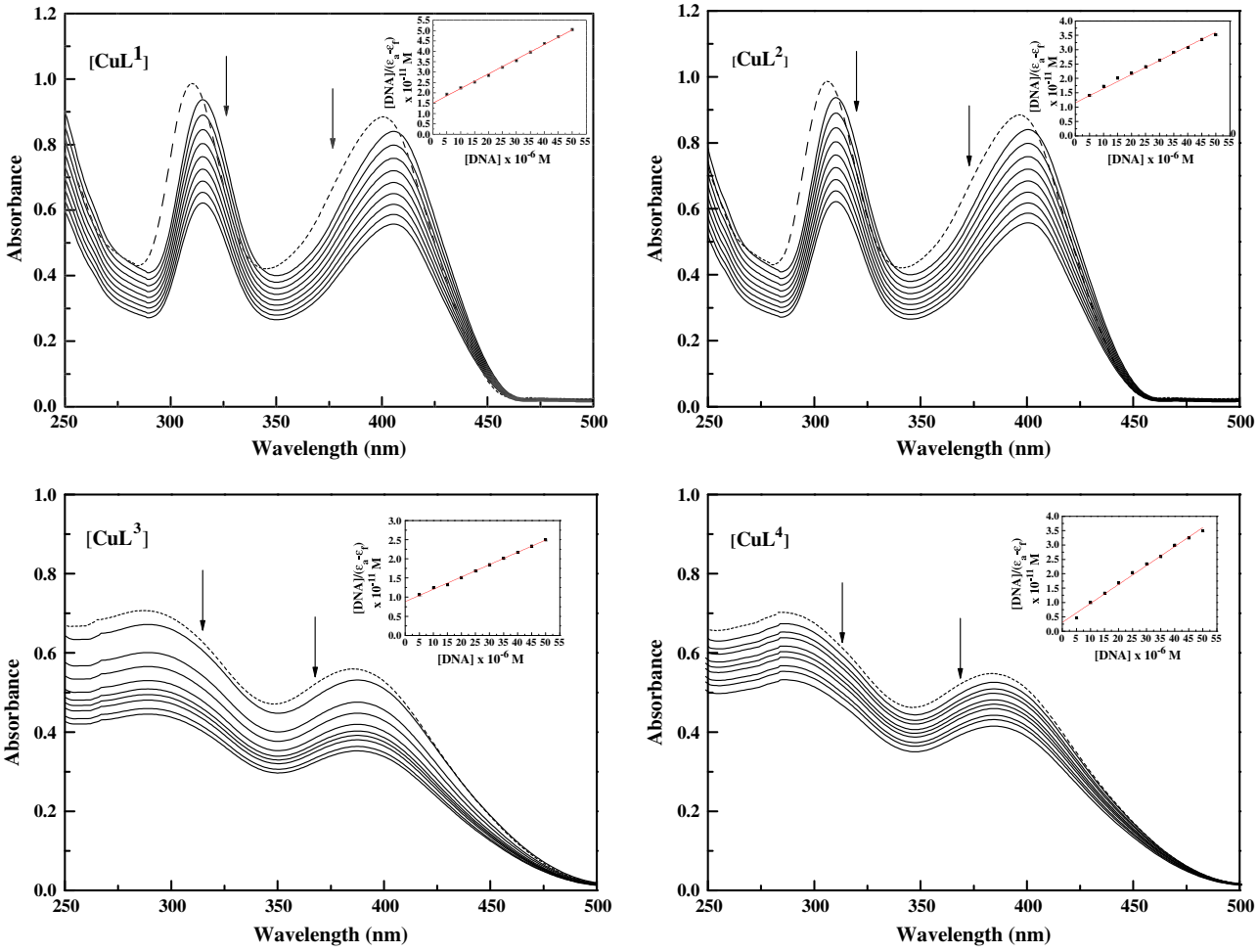


Fig. 3. Absorption spectra of copper(II) complexes (50 μM) in the absence (dashed line) and presence of CT-DNA (5,10, 15, 20, 25, 30, 35, 40 and 50 μM) at 25 ± 0.1 °C. Arrows show the changes in absorbance with respect to an increase of DNA concentration (Inset: plot of [DNA] vs [DNA]/($\varepsilon_a - \varepsilon_f$)).

system. The decrease in fluorescence intensity give an indication of the binding of the copper(II) complexes to DNA. The extent of the quenching gives a measure of the DNA binding propensity of the complexes. These results indicate that the copper(II) complexes could partially displace EB from the EB-DNA system, as often observed in intercalative complex-DNA modes [48].

The fluorescence quenching of EB bound to DNA by the copper(II) complexes (1–4) is plotted against r values (r = [complex]/[DNA]) and it was found to be in agreement with the Stern–Volmer equation (Fig. 4 inset). The Stern–Volmer constant (K) value give an indication about the degree of interaction the copper(II) complexes to CT-DNA. The K values corresponding to copper(II) complexes (1–4) were obtained from the slope of F_o/F–r plot using Eq. (2), were found to be 1.95, 1.57, 1.05, and 0.96 respectively. These values give an indication for the binding ability of the copper(II) complexes with CT-DNA follow the order 1 > 2 > 3 > 4 which in consistent with absorption study.

3.2.3. Viscosity studies

The binding of the copper(II) complexes (1–4) with CT-DNA was further elucidated by measuring the relative specific viscosity of DNA after the addition of varying concentration of complexes. Viscosity studies give valuable information regarding mode of binding metal complexes with DNA in the absence of crystallographic structural data [49]. Also, viscosity measurements are proved to be least ambiguous to support a complex-DNA binding model [50] as these measurements are very much sensitive to length change. When a small molecule intercalate between the DNA base pairs, it unwinds the DNA helix and hence lengthen it, resulting in significant increase in the viscosity of DNA solution. However, a partial and/or non-classical intercalation of ligand may bend (or kink) the DNA helix, resulting in the decrease of its effective length and concomitantly its viscosity [49]. The effect of the investigated copper(II) complexes on the viscosity of CT-DNA solution was studied in order to assess the binding mode and strength of these complexes with CT-DNA. The effects of copper(II) complexes (1–4) on the viscosity of rod-like CT-DNA at 25 ± 0.1 °C are shown in Fig. 5. Viscosity experimental results clearly showed that the relative viscosity of CT-DNA increased steadily on addition of increasing concentration of the copper (II) complexes in the following order 1 > 2 > 3 > 4. Several factors which include the shape and hydrophobicity of the complex as well as extension of planarity and the presence of additional donor functionalities on the ligand have been cited to be an importance in rendering these complexes to be strong intercalative binding agents. The complexes 1 and 2 intercalate more strongly and deeply than the complexes 3 and 4, leading to the greater increase in viscosity of the CT-DNA with an increasing concentration of complex. The viscosity increase of CT-DNA is ascribed to the intercalative binding mode of the copper(II) complexes which cause the effective length of the DNA to increase [51].

In conclusion, it is suggested that, the copper(II) complexes (1–4) may bind with DNA through intercalative mode. The binding molecular mode for copper(II) complexes via an intercalative mode is represented in Scheme 2.

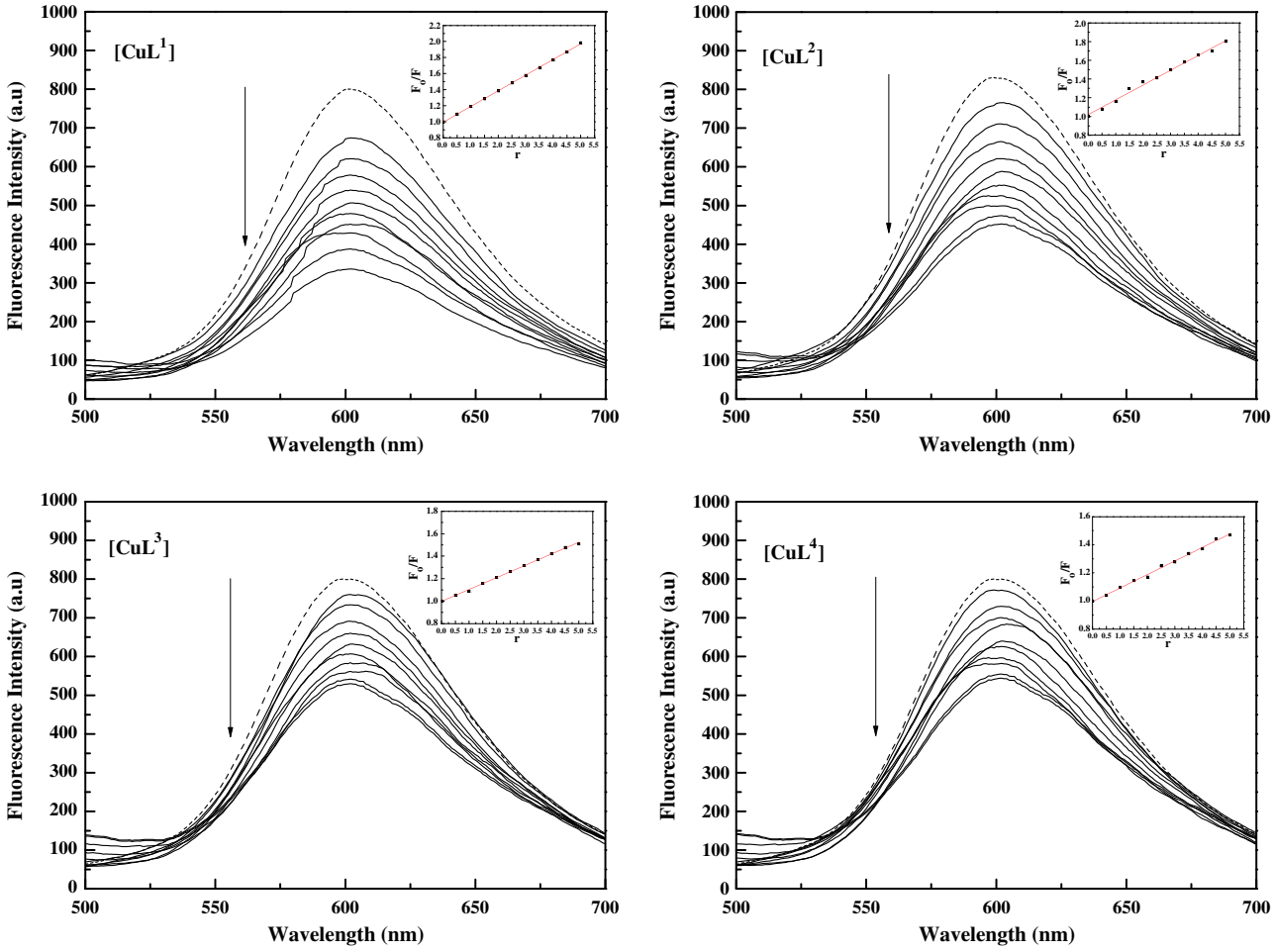


Fig. 4. Effect of increasing amount of copper(II) complexes (0–50 μM) on emission spectra EB-DNA (10 μM) at 25 ± 0.1 °C (dashed line in absence of Cu(II) complex). Arrows show the changes in emission intensity with respect to an increase of complex concentration (Inset: plot of Stern–Volmer plot of F_o/F vs [CuL]/[DNA]).

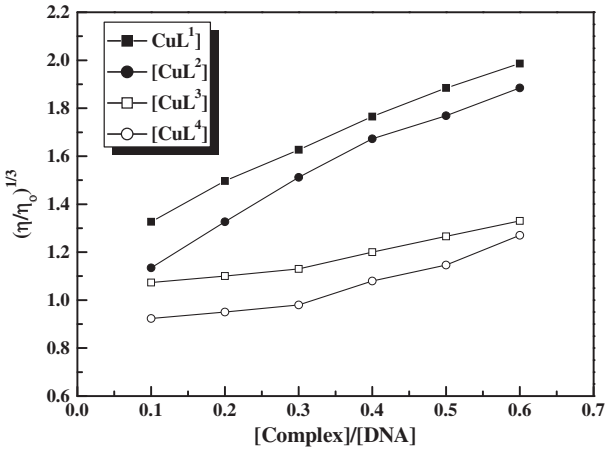
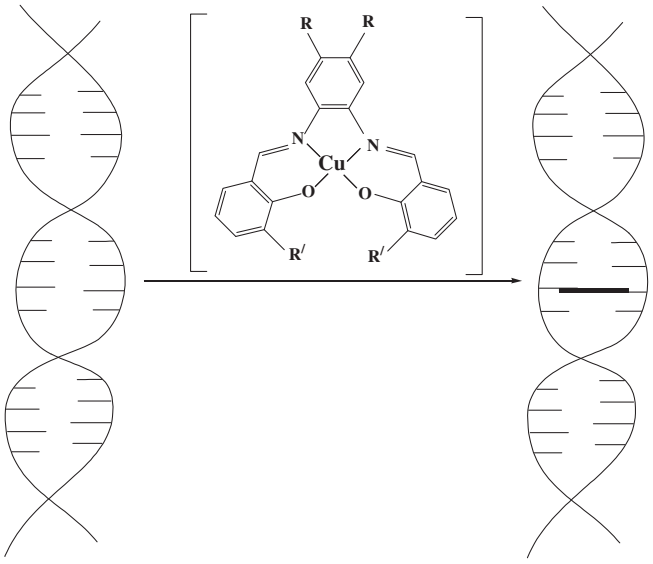


Fig. 5. Effect of increasing amount of copper(II) complexes on the relative viscosity of CT-DNA at 25 ± 0.1 °C. [DNA] = 100 μM and [complex] = 0–60 μM.



Scheme 2. Molecular mode for copper(II) complexes interaction with CT-DNA.

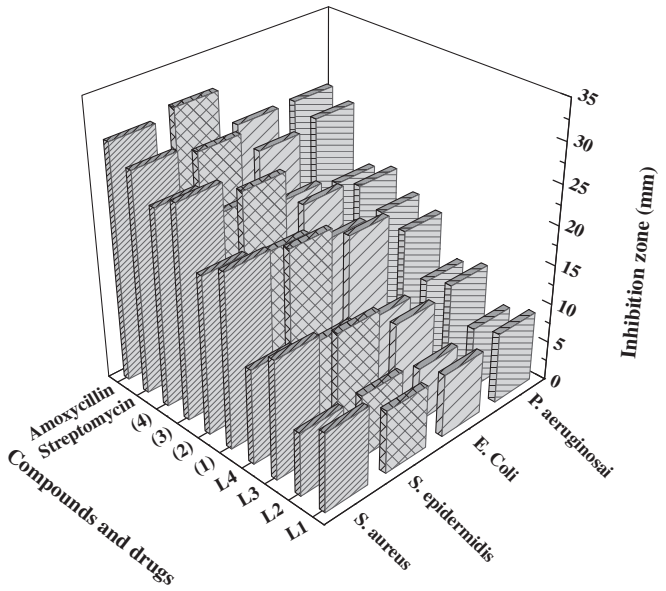


Fig. 6. Zone of inhibitions of reported compounds (200 μg/mL) and antibiotics against gram (+) and gram (−) bacteria strains.

3.3. Antibacterial screening

The Schiff base ligands H_2L^{1-4} and their copper(II) complexes (1–4) were tested for their inhibitory effects on the growth of bacteria strains: *S. aureus*, *S. epidermidis*, *E. coli*, and *P. aeruginosai*. The antibacterial activities of the prepared compounds are listed in Table 4. The results indicated that the tested complexes displayed more activity against the same microorganisms compared to the parent ligands under identical experimental conditions. The antibacterial activity of the reported compounds (200 μg/mL) is represented graphically in Fig. 6. The data showed that, the activity increase with increasing the concentration of the test solution. Such increase in the activity of the complexes compared to that of ligands can be explained on the basis of Overtone's concept [52] and Tweedy's Chelation theory [53]. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only the lipid-soluble materials which makes liposolubility as an important factor that controls the antibacterial activity. On chelation, the polarity of cation will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with

Table 4
Antibacterial activities of the Schiff bases ligands (H_2L^{1-4}) and their copper(II) complexes.

Compound	Diameter of inhibition zone (mm)							
	(Gram +ve)				(Gram −ve)			
	S. aureus		*S. epidermidis*		*E. coli*		*P. aeruginosai*	
	100 (ppm)	200 (ppm)	100 (ppm)	200 (ppm)	100 (ppm)	200 (ppm)	100 (ppm)	200 (ppm)
H_2L^1	7	10	6	9	6	8	6	9
H_2L^2	6	8	5	8	5	7	5	8
H_2L^3	10	15	10	14	9	11	10	12
H_2L^4	9	12	9	11	8	10	9	11
[CuL^1] (1)	19	22	17	21	16	19	15	16
[CuL^2] (2)	17	20	15	19	14	17	12	15
[CuL^3] (3)	23	27	23	25	17	20	19	19
[CuL^4] (4)	20	25	19	21	15	19	15	17
Streptomycin	25	28	21	27	21	24	22	25
Amoxycillin	28	30	27	31	23	26	24	26
DMSO	0	0	0	0	0	0	0	0

donor groups. Furthermore, the chelation increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity of the complexes. This increase in lipophilicity enhances the penetration of the complexes into lipid membranes, and blocks the metal binding sites of the enzymes of the microorganism. Metal complexes also disturb the respiration process of the cell and thus block the synthesis of the proteins that restricts further growth of the organism. Generally, the electronic nature (electron withdrawing and electron releasing) and position of substituents of the phenyl ring dictates the antimicrobial activities. The inhibitory action gets enhanced with the introduction of electron-withdrawing chloro groups in the phenyl ring, whereas, electron-releasing substituents such as methyl groups are less active compared to un-substituted phenyl ring [54].

4. Conclusion

Four mononuclear copper(II) complexes have been synthesized by direct thermal reaction between $Cu(OAc)_2{\cdot}H_2O$ and symmetrical neutral N_2O_2 donor tetradentate Schiff bases namely: N,N′bis(o-vanillin)4,5-dimethy-l,2-phenylenediamine (H_2L^1), N,N′bis(salicylaldehyde)4,5-dimethyl-1,2-phenylenediamine (H_2L^2), N,N′bis(o-vanillin)4,5-dichloro-1,2-phenylenediamine (H_2L^3) and N,N′bis(salicylaldehyde)4,5-dichloro-1,2-phenylenediamine (H_2L^4), respectively. The new complexes were fully characterized by physicochemical and spectroscopic methods. Characterizations of the new complexes have shown that, Cu(II) formed square planar complexes with 1:1 (metal:ligand) stoichiometry. The study of the interaction of complexes with CT-DNA was investigated by absorption spectrophotometry, fluorescence quenching and viscosity measurements. From the binding studies, it was evident that $[CuL^1] > [CuL^2] > [CuL^3] > [CuL^4]$ according to CT-DNA binding ability. Furthermore the novel complexes exhibited good antibacterial activity against the bacteria strains *S. aureus*, *S. epidermidis*, *E. coli*, and *P. aeruginosai* in compression with the standard drugs streptomycin and amoxycillin.

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