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Synthesis and characterization of copper (II) complex with Pyrrolidine dithiocarbamate: interaction with bovine serum albumin, DFT computation and antibacterial activity

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Abstract
A four coordinated square planar copper (II) complex formulated as [Cu $(PDTC)_2$] (1) (PDTC = Pyrrolidine dithiocarbamate) was synthesized and characterized by elemental, physico-chemical and spectroscopic methods. The electronic structure and spectral properties of the PDTC and complex 1 has been explained by DFT and TDDFT calculations. The lower HOMO-LUMO gap of PDTC indicating molecule requires small excitation energy. The interactions of copper (II) complex towards bovine serum albumin (BSA) were examined with the help of absorption and fluorescence spectroscopic tools. An antibacterial activity of the complex was studied by agar disc diffusion method and minimum inhibitory concentration (MIC) strain against *Escherichia coli, Vibrio cholerae, Streptococcus pneumonia and Bacillus cereus.* The complex exhibited significant activities against one gram-positive (*S. pneumoniae*) and one gram-negetive bacteria (*E. coli*).

Keywords: Copper complex; DFT study; BSA binding; Optical properties; antibacterial activity

1. Introduction
Copper(II) complexes have been described as a plastic metal ion because the chemistry of its complexes exhibit different coordination numbers with many kinds of irregular coordination geometries, such as tetrahedral or square planar four-coordinate, octahedral six-coordinate, and square pyramidal or trigonal–bipyramidal five-coordinate [1] while Cu(I) demands, in general, tetrahedral geometry [2]. The redox change Cu(II)/Cu(I) or vice versa is associated with structural change which requires large reorganization energy. Copper is a redox-active nutrient that is indispensable at unusually high bodily levels for usual brain function. Owing to the large oxygen capability and oxidative metabolism of brain tissue, neurons and glia alike require copper for basic respiratory and antioxidant enzymes cytochrome c oxidase and Cu/Zn superoxide dismutase (SOD), respectively. In addition, copper is a necessary cofactor for many brain-specific enzymes that control the homeostasis of neurotransmitters, neuropeptides, and dietary amines.
Pyrrolidine dithiocarbamate (PDTC) is a synthetic organosulfur compound, which is largely used in cell biology and molecular biology [3]. PDTC belongs to a group of dithiocarbamate, which is known to exert antioxidant and radical scavenger functions. PDTC may inhibit or induce apoptosis; its effect on apoptosis depends on the experimental context, density, cell types, and the presence of Cu and Zn. PDTC may also block ionizing radiation-induced apoptosis in freshly-isolated normal mouse spleen cells. Because of its apoptosis- inducing capability, PDTC has been proposed as an anticancer agent [4].
Bovine serum albumin (BSA) has been one of the most widely studied groups of proteins, particularly of its structural similarity with human serum albumin. BSA consists of three homologous domains (I, II, III) and each domain contain of two sub-domains [5]. Since a drug binding with serum albumin may have an important in pharmacokinetics and the determination of the dosage form of the drug, the changes in fluorescence intensities of BSA-drug complex could give considerable information regarding the binding characteristics and the therapeutic effectiveness of drugs. Consequently, the binding of drugs to serum albumin considered as a model in protein chemistry to study the binding behaviour of proteins, research field in chemistry, and clinical medicine [6].
In this work we have synthesized thioether containing square Planar Cu(II) complex having the general formula [Cu$(PDTC)_2$]. The electron transfer mechanism of copper (II) complex is investigated by cyclic voltammetry. Electronic structure and electrochemical properties of the complex has been supported by DFT calculation. TDDFT calculation has been used to simulate the experimental electronic spectra of the complex. The BSA protein binding study of the copper (II) complex has been performed spectroscopically. In vitro antibacterial activities of synthesized compound against some specific microbes were also studied.

2. Experimental
2.1 Materials and Methods
Pyrrolidine dithiocarbamate (PDTC) was purchased from Aldrich and other chemicals were obtained from commercial sources and used as received, unless otherwise stated. The elemental (C, H, N) analyses were performed on a Perkin Elmer model 2400 elemental analyzer. Copper analysis was carried out by Varian atomic absorption spectrophotometer (AAS) model-AA55B, GTA using graphite furnace. Electronic absorption spectra were recorded on a SHIMADZU UV-1800 spectrophotometer. The fluorescence spectra were obtained in the Fluorimeter (Hitachi-2000). IR spectra (KBr discs, 4000–400 cm^{-1}) were recorded using a Perkin-Elmer FTIR model RX1 spectrometer. The room temperature magnetic susceptibility measurements were performed by using a vibrating sample magnetometer PAR

155 model. Molar conductances ($_M$) were measured in a systronics conductivity meter 304 model using ~10^{-3} mol L^{-1} solutions in appropriate organic solvents. Scans were collected on dry samples in the range of 10–80^0. Electrochemical measurements were performed using computer-controlled CH-Instruments (Model No- CHI620D), All measurements were carried out under nitrogen environment at 298 K with reference to SCE electrode in dimethyl sulphoxide using [n-Bu_4N]ClO_4 as supporting electrolyte. Powdered XRD was recorded in a 'Rigaku Miniflex-II' X-ray diffractometer using CuKα radiation (λ=0.154056nm).

2.2 Preparation of [Cu(PDTC)$_2$] (1)

To a 15 mL methanolic solution of PDTC (0.082 g), 10 mL methanolic solution of $Cu(OAC)_2 \cdot 2H_2O$ (0.05 g) was added drop wise under stirring condition. The stirring was continued for 1 h and then refluxed for 4 h. The product was collected by filtration and washing with cold methanol and water then dried in vacuo. The pure crystallized product was obtained from methanol.

[Cu(PDTC)$_2$]: Yield 80-85%; $C_{10}H_{16}N_2S_4Cu$ (**1**)

Complex **1**: $C_{10}H_{16}N_2S_4$ Cu: Anal.Found; C,33.80; H, 4.50; N, 7.88; Cu, 17.86; Calc: C, 33.74; H, 4.38; N, 7.56; Cu, 17.64. IR (cm^{-1}): ν_{C-N}, 1322; $\nu_{C=S}$, 936, ν_{C-S}, 826.; ν_{Cu-S}, 436, m.p. 242 ± 1^{o}C. Magnetic moment (μ, B.M.) : 1.76. Conductivity (Λo, $ohm^{-1}\,cm^2\,mol^{-1}$) in acetonitrile: 146.

2.3 Computational methods

All calculations were carried out using the GAUSSIAN 09 program package with the aid of the GaussView visualization program [7]. The molecular calculations were performed in the gas phase using Density Functional Theory (DFT) using the B3LYP (Becke three-parameter Lee–Yang–Parr) exchange correlation functional. The 6–31G(d) basis set for C, H, N and O atoms and 6–311G(d) basis set for S atoms were used [8]. The vibrational frequency calculations were performed to ensure that the optimized geometries represent the local minima of potential energy surface and there are only positive eigen–values. The lowest vertical electronic excitations based on B3LYP optimized geometries were computed using the time-dependent density functional theory (TD-DFT) in acetonitrile using the same B3LYP level and basis sets.

2.4 BSA Protein binding experiments

The interaction between copper (II) complex and BSA was performed by fluorimetric titration. A 3.0 mL portion of aqueous solution of protein was titrated by addition of the appropriate concentration of copper (II) complex solution (to give a final concentration of 3.2×10^{-6} mol L^{-1}). For every addition, the mixture solution was shaken and allowed to stand for 10 minute and then the fluorescence intensities were measured with an excitation wavelength of 280 nm.

2.5 Antimicrobial Screening

The antibacterial activities of the ligand (PDTC) and its copper (II) complex have been studied by agar disc diffusion method [9]. The ligand and Copper complex are soluble in DMF solvent by using two gram negative bacteria (*Escherichia coli, Vibrio cholerae)* and two gram positive bacteria *(Staphylococcus aureus, Bacillus cereus).* Chloramphenicol was used as reference antibacterial drug. The solution of ligand and its copper (II) complex were added to the agar plates. The DMF solvent was used as a negative control. Incubation of the plates was done at 37°C for 24 hours, inhibition of the organisms was measured and used to calculate mean of inhibition zones in millimetres. The antimicrobial activities of the title complex (average of three measurements) estimated by minimum inhibitory concentrations (MIC; μg/mL) are listed in (Table 2).

3. Results and Discussion

3.1 Synthesis and characterization

The square planar copper (II) complex [Cu(PDTC)$_2$]was obtained in good yield by the reaction of copper(II) acetate with Pyrrolidine dithiocarbamate (PDTC) in methanol at ambient temperature. The complex is soluble in organic solvents including ethanol, methanol, acetonitrile, DMF, and DMSO. The conductivity in acetonitrile (Λo, $ohm^{-1}\,cm^2\,mol^{-1}$) is 146 at 300 K, suggesting that the complex is a non-electrolyte in solution. At room temperature, the magnetic moment of complex is 1.76 B.M. corresponds to one unpaired electron, which indicates complex is a distorted square planar geometry.

3.2 Infrared and Electronic spectral studies

Infrared spectral data of the ligand, pyrrolidine dithiocarbamate (PDTC) shows several bands at 1323, 939, 827 and 2968 cm^{-1} due to C-N, C=S, C- S and C-H bond stretching vibrations in the solid state respectively. These bands are shifted to lower frequency on complexation with $Cu(OAc)_2$, with 1:2 stoichiometry ratio. New vibrations at 426 cm^{-1} indicates Cu-S bond, which are not present in the free ligand. The appearance of these vibrations confirmed the involvement of only sulphur atom in chelation with Copper ion.

The electronic spectra of pyrrolidine dithiocarbamate (PDTC) and its copper complex were recorded in acetonitrile at room temperature. The spectra of the pyrrolidine dithiocarbamate exhibit main peaks: at 354 nm, which was attributed to $\pi \rightarrow \pi^*$ or $n \rightarrow \pi^*$ intra ligand transitions. The copper (II) complex absorption band observed at about 287 nm is associated with $\pi \rightarrow \pi^*$ and a weak band observed at the 684 and 532 nm is well in agreement with the d–d transition for copper (II) in the square planar geometry [10]. To simulate the experimental electronic spectra of the complexes TDDFT calculations have been performed in acetonitrile. The contour plots of selected molecular orbitals of PDTC (Figure 1) and complex-1 are shown in **(**Figure 2**)**.The calculated transitions at 692 and 594 nm have mixed ILCT, MLCT and LMCT character, along with a minor contribution of d–d transitions (Table 1). The other transitions at 443 and 296 nm correspond to mixed LMCT and ILCT characte.

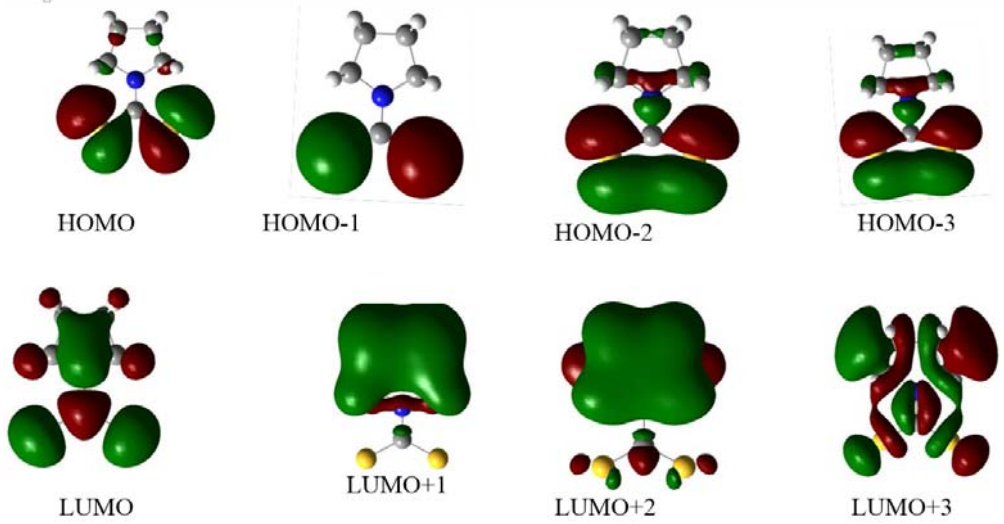

Fig 1: Contour plots of some selected molecular orbitals of PDTC

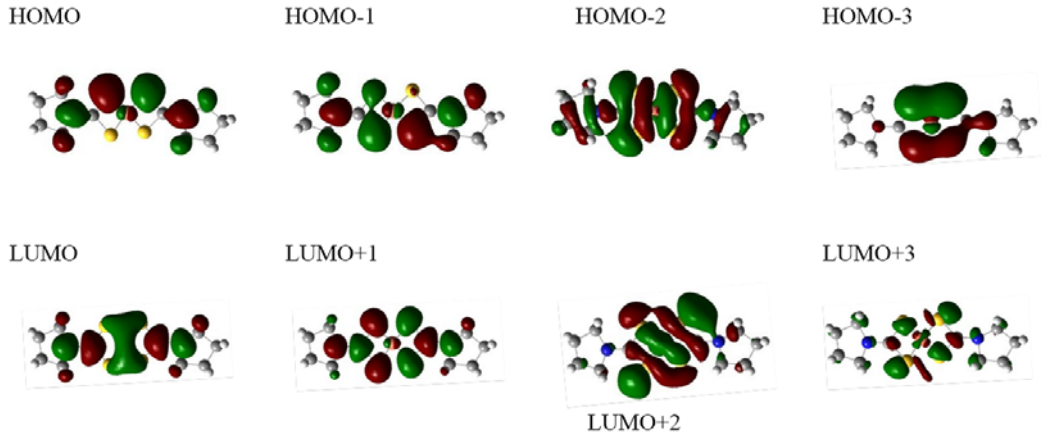

Fig 2: Contour plots of some selected molecular orbitals of $[Cu(PDTC)_2]$

Table 1: Vertical electronic excitations calculated by the TDDFT/B3LYP/CPCM method and experimental absorption bands of $[Cu(PDTC)_2]$

$E_{excitation}$ (eV)	$\lambda_{excitation}$(nm)	Osc. strength *f*	Transition	Character	$\lambda_{expt.}$($M^{-1}cm^{-1}$)
1.4923	692.80	0.2260	HOMO-1 →LUMO+1	π→π*	684
2.0465	605.83	0.0000	HOMO → LUMO+3	n/π→π*	
2.0857	594.45	0.0028	HOMO→ LUMO+2	n/π→π*	592
3.0730	443.46	0.0001	HOMO→ LUMO+4	π→π*	432
3.4926	354.99	0.0002	HOMO →LUMO+5	π→π*	
3.9452	314.27	0.2351	HOMO-1 → LUMO	π→π*	
4.1752	296.96	0.1998	HOMO-3 → LUMO	n→π*	287
4.4962	275.75	0.0001	HOMO-1 → LUMO+1	π→π*	
4.7140	263.01	0.0007	HOMO-2 → LUMO+2	π→π*	

Table 2: Antimicrobial activities of the title compounds evaluated by the minimum inhibitory concentration (MIC in μg/mL).

Compound	*E. coli*	*Vibrio cholerae*	*S. pneumoniae*	*Bacillus cereus*
PDTC	320	280	300	290
1	80	40	70	50
Chloramphenicol	10	14	20	18

3.3. Electrochemistry

The electrochemical studies of the copper (II) complex was examined by cyclic voltammetry using a Pt-disk working electrode and a Pt-wire auxiliary electrode in dimethylformamide using $[n\text{-}Bu_4N]ClO_4$ (0.1 M) as the supporting electrolyte. The complex exhibit a reduction peak at E_{pc} = -0.1057 V with a corresponding oxidation peak at E_{pa} = 0.5337 V at a scan rate interval 50–400 mV s^{-1} indicating quasi-reversible one-electron transfer process. The ratio of cathodic to anodic peak height was less than one. However,

the peak current increases with the increase of the square root of scan rates.

3.4. Electronic Structure

Full geometry optimization of PDTC and copper (II) complex **-1** were carried out using density functional theory (DFT) at the B3LYP level in their ground state shown in (Figure 3). From DFT study, HOMO of PDTC, the electrons are localised on C-S group but in complex electrons are largely localised in one S-atom of two pyrrolidine group. In LUMO of PDTC, electrons are largely localized on pyrrole ring and S-group but in complex electrons are localised between copper and S-group. The HOMO-LUMO energy gap of PDTC is 0.16574 eV and complex being 0.18196 eV. The lower HOMO-LUMO energy gap of PDTC indicates requires small excitation energy and more reactive.

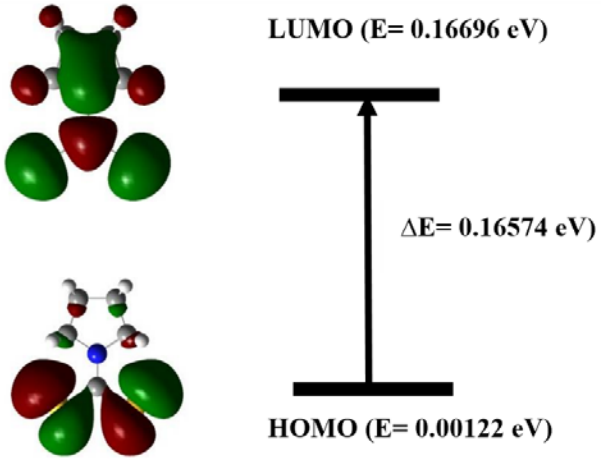

Fig 3: The HOMO-LUMO energy gap of PDTC

3.5. Powder X-ray diffraction study

The XRD pattern of Cu(II) complex show well defined crystalline peaks indicating that the samples are crystalline in nature [11]. An X-ray powder diffraction pattern of copper complex has been given in (Figure 4). The XRD of different scale particles are well coincident with each other and it means that different forms of complexes have the same structure. No peaks characteristics of impurities are detected in the XRD patterns.

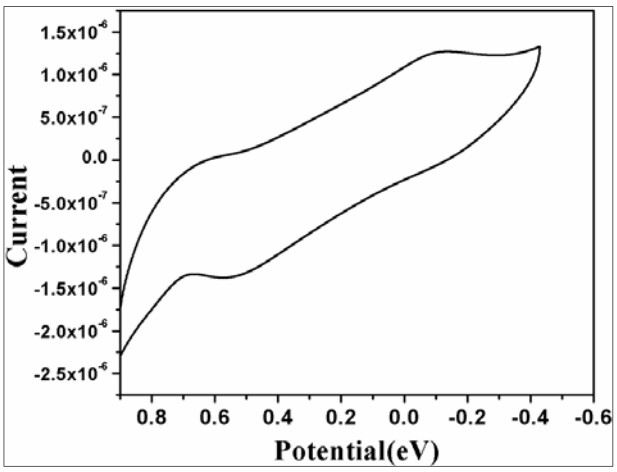

Fig 4: The cyclic voltammograms of complex-1

3.6. BSA Protein binding experiments

3.6.1. Absorption characteristics of BSA–Cu(II) complex

The absorption spectra of BSA in the absence and presence of copper (II) complex was studied at different concentrations in phosphate buffer, pH 7.4 given in (Figure 5). We observed that absorption of BSA increases frequently upon increasing the concentration of the complex may be due to the adsorption of BSA on the surface of the complex. From these data the apparent association constant (K_{app}) determined of the complexes with BSA has been determined using the Benesi-Hildebrand equation [12]:

$$1/(A_{obs} - A_0) = 1/(A_c - A_0) + 1/ K_{app}(A_c - A_0)[comp]$$

Where, A_{obs} is the observed absorbance of the solution containing different concentrations of the complex at 280 nm, A_0 and Ac are the absorbances of BSA and the complex at 280 nm, respectively, with a concentration of complex and K_{app} represents the apparent association constant. The enhancement of absorbance at 280 nm was due to adsorption of the surface complex, based on the linear relationship between $1/(A_{obs} - A_0)$ *vs* reciprocal concentration of the complex with a slope equal to $1/K_{app}(A_c - A_0)$ and an intercept equal to $1/(A_c - A_0)$.The value of the apparent association constant (K_{app}) of BSA determined from this plot and the value is 3.48×10^4 M^{-1}(R = 0.9968). This result indicated that copper complex strongly bind to BSA protein.

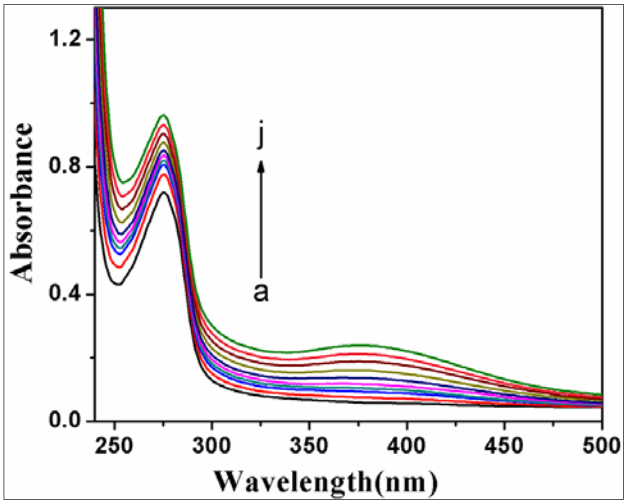

Fig 5: Electronic spectral titration of copper complex with BSA at 280 nm in phosphate buffer. Arrow indicates the direction of change upon the increase concentration of BSA.

3.6.2. Fluorescence quenching of BSA by the copper complex

The emission spectra of BSA in presence of different concentrations of complex were recorded in the wavelength range 300-550 nm by exciting the protein at 280 nm represented in (Figure 6). As seen, with increasing the concentration of the copper complex the fluorescence intensities of the proteins are regularly decreased. Fluorescence quenching is described by the Stern–Volmer relation [13]. In case of fluorescence quenching of BSA a linear plot between I_0/I against [complex] was obtained and from the slope we calculated the K_{SV} value is 4.26×10^5 M^{-1}(R = 0.99483).

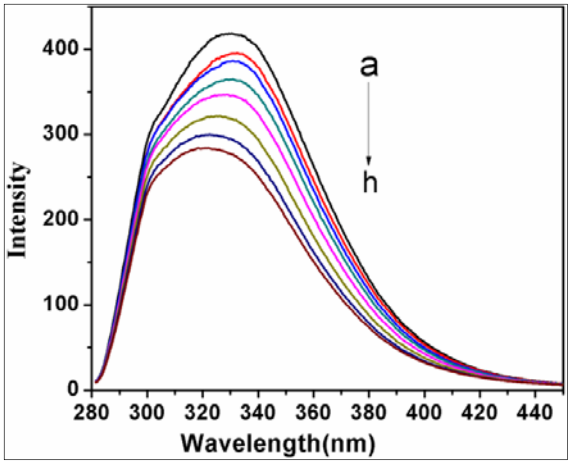

Fig 6: Change in fluorescence spectra of BSA through their titration with copper complex in phosphate buffer (pH 7.4). The concentration of complex varied from 0.0 to 3.2×10^{-6} M L^{-1}; λ_{ex} = 280 nm.

3.6.3. Analysis of binding Sites

Number of binding sites can be calculated from fluorescence titration data using the following equation [14]

$$\log [(I_0 - I)/I] = \log K_b + n\log[Q]$$

According to the experimental results, the linear fitting plots of log $[(I_0 - I)/I]$ versus log[Q] can be observed. The number of binding sites (n) evaluated from the intercepts of the linear plots respectively, as seen, the value of n is nearly 1 for binding of both complexes to the proteins used, which indicates that, in the binding reactions the molar ratio of protein to drug is 1:1.

3.7. Antibacterial activity

Antibacterial activity of the PDTC and its copper (II) complex are tabulated in (Figure 7). From this study it is incidental that, the complex exhibited significant activities against one gram-positive (*S. pneumoniae*) and one gram-negetive bacteria (*E. coli*). The complex also possessed moderate activities against two organisms (*Vibrio cholera*, *Bacillus cereus*). A comparative study of the ligand and complex shows that the complex is more effective than the uncoordinated PDTC. The increased activity of the metal chelates can be explained by overtone concept and the Tweedy chelation theory [15]. The delocalization of π-electrons in complex is more than ligand and indicating the complex is more lipophilic, which helps the penetration of the bacterial cell membranes [16]. The biological activity of the complex could be endorsed to tendency to further ligand replacement with the biological ligands such as DNA and Proteins.

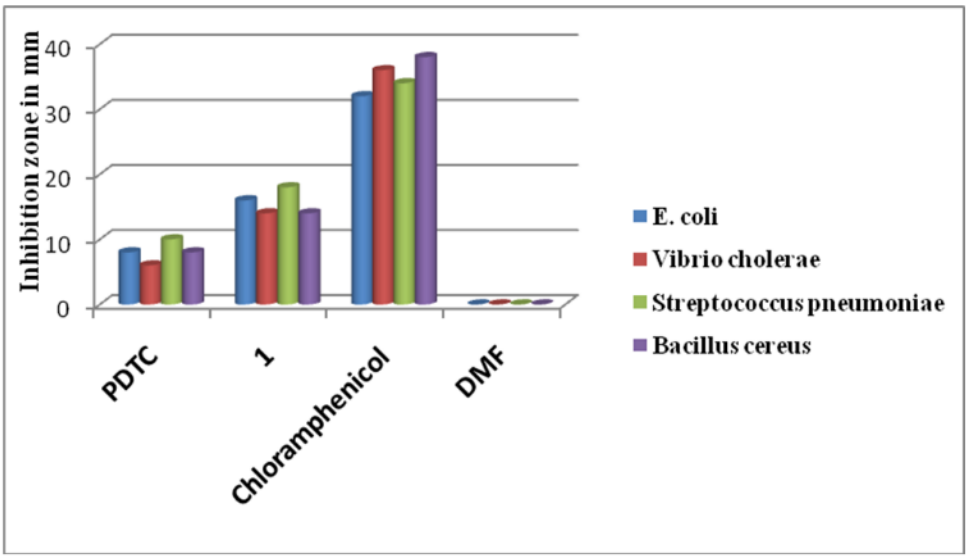

Fig 7: Comparison antibacterial studies of the investigated compounds (inhibition zone in mm) of PDTC and complex-1 with standard antibiotics.

4. Conclusion

Synthesis and characterization of mononuclear square planar copper (II) complex has been performed. The electrochemical study of these complexes showed a quasi-reversible one-electron transfer process. BSA-binding properties of the copper (II) complex investigated by absorption and fluorescence spectroscopic tools. All the result indicated that complex is outstanding binding with BSA protein. The antibacterial screening of ligand and its copper (II) complex displayed promising antibacterial activity compared to known antibiotic drug. The title complex exhibits significant biological activity showing the potential for its use as antibacterial agent.

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