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Synthesis, Characterization and Application of 1, 10 Phenanthroline and 2, 6 Pyridine Dicarboxamide Ligands Supported Five Coordinated Cu (II) System for DNA Binding and Antibacterial Activity

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A B S T R A C T

The copper (II) complex [Cu(1,10-phen)(2,6-pyridinedicarboxamide)]$(NO_3)_2$ has been synthesized and characterized by Fourier transform infrared spectroscopy (FT-IR), Ultraviolet-visible spectroscopy (UV), Electron paramagnetic resonance spectroscopy (EPR) and Photoluminescence (PL) analysis. The copper complex was connected to two sides, 1,10-phenanthroline and 2,6 py-dicarboxamide ligands, providing a five-coordinate Cu (II) complex. The commercial heterocyclic base coordinated copper metal complex was found to have antimicrobial activity against Gram-positive and gram-negative bacteria. It is noted that the commercial ligand with a copper metal complex was found to be more biologically active, and the DNA-binding properties of the copper complex were investigated by UV, PL, and cyclic voltammetry measurements. The obtained results indicate that the Cu (II) complex binding in DNA through an intercalation mode and the binding constant (Kb) value of the complex as 1.9×10^4 M^{-1}, respectively.

Introduction

The study of copper metal complexes is of recent interest because of their probable use as metallo-drugs, and the insertion of heterocyclic ligand has been effective in the development of novel compounds. That have an extensive range of pharmacological properties such as antiviral, antibacterial, and cytotoxic (Rudrapal, 2014). In particular, antibacterial activity has devoted great awareness. A bacterial disease is a group of infections due to uncontrolled cell propagation (Caon, 2019). Still, some studies support the suggestion of complex control as an antibacterial approach (Brewer, 2001). Copper is a crucial trace metal and use for centuries as copper ions or copper complex to make germ-free liquids, solids, and human tissues (Daniel *et al.,* 1989). Biological

activities of metal complexes change with ligands or metal ions increase or decreased physical activities are reported in several metal complexes, (More *et al.*, 2001). The interaction of copper metal containing multidentate aromatic ligands, especially N-containing heterocyclic ligands they raise much awareness in recent year (Adelaid and James, 2013). The chelating agents such as 1,10-phenanthroline and 2, 6-pyridine dicarboxamide have played a vital role in bioinorganic chemistry (Gude *et al.*, 2005). 1, 10-phenanthroline is one of the most popular metal coordinating ligand in mononuclear complexes with remarkable structural and electronic properties (Agwara *et al.*, 2008). Mixed ligand complexes containing 2, 6-pyridine dicarboxamide as a secondary ligand were of significant substance. They act potential models for enzymemetal ion substrate complexes due to their stabilities (Ajay *et al.*, 2012).

The copper complexes bind with DNA using intercalation mode and N-donor aromatic ligands have been commonly used to develop nuclease binding activity (Khalafi-Nezhad *et al.*, 2005). In the extension of our work in this field (Santhakumar and Arumugham, 2012). we present in detail the synthesis and structural characterization of a new pentagonal coordinated Cu (II) complex such as [Cu(1,10-phen)(2,6-pyridinedicarboxamide)]$(NO_3)_2$ from 1,10-phenanthroline and 2,6-pyridinedicarboxamide. The emission properties, DNA binding studies, and antibacterial activity of the mixed-ligand Cu (II) complex were furthermore studied.

Experimental section

Materials and Instrumentation

In commercial reagents such as ethanol, $Cu(NO_3)_2.3H_2O$, 1,10-phenanthroline monohydrate and 2,6-pyridinedicarboxamide are all analytical grade. With Plasmid pBR322 DNA was purchased from Bangalore Genie, India. Disodium salt of calf thymus DNA (CT-DNA), ethidium bromide and Tris-(hydroxymethyl) aminomethane (Tris) were received from Sigma Aldrich. The IR spectra were analyzed by Perkin Elmer spectrometer with using KBr as pellets, and the range is 4000–400 cm^{-1}.

The elemental analysis study was performed on a Perkin Elmer micro analyzer at 24 ^oC. The UV-Vis and Fluorescence emission spectrum of the complex was absorbed from the Shimadzu UV-2450 spectrophotometer and Jobin Young Fluorolog 3 spectrophotometer. The Molar conductivity measurements were evaluated using a Control Dynamics (India) conductivity meter.

Synthesis of [Cu (1, 10-phen)(2,6-pyridinedicarboxamide)] complex

2 mmol (330 mg) of 2,6-pyridinedicarboxamide was dissolved in a 5ml ethanol solution. Then, The ethanolic solution of $Cu(NO_3)_2$. $3H_2O$ (483.0 mg, 2 mmol) was added slowly into the same solution. Then the prepared solution was stirred few minutes, and 2 mmol of 1,10-phenanthroline monohydrate (396 mg) in ethanol solution was also added under magnetic stirred for 6 h at 60 °C. Afterwards, the mixture cooled down at ambient temperature, and the obtained blue solution evaporated. After a week, the blue colour copper complex was collected and A schematic evidence reaction is shown in Scheme 1.

The calculated yield is 84% for $C_{19}H_{13}CuN_2O_5$: C 56.09; H 3.22; N 17.21, O 7.86, Cu 15.62; Found (%): C 55.78; H 3.57; N 16.64, O 6.93, Cu 14.98;. IR (KBr, cm^{-1}): 3425 cm^{-1}, 1724 cm^{-1}, 1627 cm^{-1}, 1515cm^{-1}, 479 cm^{-1} (N-H, C=O,C=N,C=C,M-N) respectively. UV-Vis (H_2O), λ/nm (ε/M^{-1} cm^{-1}):271(5372 π–π*), 294(1645 n–π*), 674 (d→ d).Conductance ΛM (Ω^{-1} cm^2 mol^{-1}) in water at 27 °C: 96.

DNA binding studies

UV-Vis absorption analysis of copper (II) complex with DNA binding

The CT-DNA is dissolved in 50 mM NaCl/5 mM Tris-HCl buffer (pH 7.2) solution to have produced

a ratio of 1.8, then this shows that the DNA was adequately free of protein. The UV absorbance band displays at 271 and 294 nm to observe. By measuring UV absorption at 271 nm, which CT-DNA's molar absorption coefficient is 5372 M^{-1} cm^{-1} and the DNA content can be determined. In the 5 mM Tris-HCl/50 mM NaCl buffer, pH 7.2, electronic spectra of [Cu(1,10-phen)(2,6-pyridinedicarboxamide)]$(NO_3)_2$ (1 X 10^{-5} M) were obtained both before and after the addition of CT-DNA (r = 0.0, 0.5, 1.0, 1.5, 2.0, and 2.5), where r is the molar ratio of DNA and complex. The following equation was used to calculate the intrinsic binding constant Kb for the interaction of the investigated complex with CT-DNA using absorption spectral titration data.

$$[DNA]/(\varepsilon a-\varepsilon f) = [DNA]/(\varepsilon b-\varepsilon f) + 1/Kb(\varepsilon b-\varepsilon f)\ldots.(1)$$

Where ε_a, ε_f and ε_b correspond to $A_{obsd}/[Cu]$, the extinction coefficient for the free copper complex, and the extinction coefficient for the copper complex in the fully bound form, respectively. In the plot of [DNA] / (ε_a - ε_f) vs. [DNA], K_b is then given by the ratio of slope to intercept.

Fluorescence spectral analysis

The fluorescence spectra were recorded with excitation at 480 nm and emission at 614 nm at normal temperature. In the experiment, [Cu(1,10-phen) (2,6-pyridinedicarboxamide)] $(NO_3)_2$ was titrated into samples containing 1 X 10^{-4} M of DNA and 8 X 10^{-5} M ethidium bromide in a 5 mM Tris-HCl/50 mM NaCl buffer. Using the above equation, Stern-Volmer quenching constants were calculated.

$$Io/I = 1 + Ksv\ r\ldots(2)$$

Where I_0 and I are the fluorescence intensities in the absence and presence of complex, respectively, Ksv is a linear Stern – Volmer, and r is the total complex concentration to that of DNA. In the Plot of I_0/ I vs [complex] / [DNA], Ksv is given by the ratio of slop to intercept.

Cyclic voltammetric

Cyclic voltammetry (CV) analysis was performed on a CHI 602D type electrochemical workstation by standard 3-electrode system. The three-electrode system consisted of glassy carbon (GC) electrode as the working electrode, silver chloride (Ag/AgCl) as the reference electrode and Pt wire as the counter electrode.

The 0.1 g of lithium perchlorate was used as supporting electrolyte. Before each experiment, the solution was served by purging pure N_2 for 15 min, and nitrogen atmosphere was kept over the solution during the experiments.

Antimicrobial activity

The disc dispersal method tested in vitro antimicrobial screening of the Cu (II) complex for its effect on certain human pathogenic bacteria. The complex was stored at room temperature and dissolved in water. Gram-positive (*Bacillus cereus* and *Staphylococcus aureus*) and Gram-negative (*Enterobacter cloacae* and *Staphylococcus haemolyticus*) bacteria were grown in nutrient agar medium and incubated at 37 ºC for 24 h followed by a frequent subculture to fresh medium and were used as test bacteria.

Then, the agar loaded Petri plates were inoculated with a loop full of bacterial culture and spread throughout the Petri plates uniformly with a sterile glass spreader. To each disc, the test complex (10 ppm) and reference ciprofloxacin (1 µg/disc for bacteria) were added with a sterile micropipette. The plates were then incubated at 35 ± 2 ºC for 24–48 h and 27 ± 1 ºC.

Plates with a disc containing respective solvents served as control. Inhibition was recorded by measuring the diameter of the inhibitory zone after the incubation period. The entire experiments were employed continuously and the total values were provided (Kanagavalli *et al.*, 2019).

Results and Discussion

FT-IR analysis

The functional group of the copper complex was confirmed using the FT-IR spectrum in the range of 4000–400 cm-1(Ezhilarasan and Arumugham, 2020). The tentative course of the FT-IR spectral peaks, like the ligands and copper (II) ion, were useful aids for confirmation of coordination activity. The C=N peaks appeared at 1627cm^{-1} vibration mode. It is clearly identified that the M-N band intensity frequency appeared at 479cm-1 (Baskaran *et al.,* 2020). For the Cu(II) complex, the stretching vibrations of the carbonyl group and C=C are assigned at 1724 cm^{-1} and 1515 cm^{-1}, respectively, and the two peaks represent the chalcone derivatives. Furthermore, the sharp intensity peak was obtained in the vibration range at 3425cm^{-1} for the N-H band of the complex compound. Therefore, all the above spectral data confirmed the Cu (II) complex formation shown in Fig 1.

The electronic spectra of Cu (II) complex

UV-Vis spectroscopy in DMSO solution was used to examine the electronic properties of the metal complex. A strong absorption band at 271 and 294 nm is attributed to ligand-centred charge transfer (LLCT) in conjunction with $\pi \rightarrow \pi*$ and $n \rightarrow \pi*$ transitions of the 1, 10-phenanthroline and 2,6-pyridinedicarboxamide ligands (Afzal Hussain *et al.,* 2019). In addition to introducing a new absorption band at 674 nm, which can be attributed to d-d transitions of a five coordinate copper (II) complex to be promoted with chromophore and may be confirmed to $^{2}E_{g} \rightarrow {}^{2}T_{2g}$ possible metal-ligand transition Fig. 2 depicts the prepared spectral data.

EPR Spectra of copper (II) complex

The EPR spectra have explained the properties of unpaired electrons in the Para magnetism on copper (II) metal complex. In addition, the EPR spectra have given the most satisfactory information on pentagonal geometry with the bonding nature of metal ions. The X band EPR spectra of dehydrated fine substances of the Cu (II) complex d9-system are investigated in DMSO solution, and the revealing anisotropic signals obtained are shown in Fig. 4. The g values g 2.24 and g 2.04 (g > g > ge (2.0023) indicate axial symmetry, implying that an unpaired electron has a dx2-y2 orbital with a 2B1g ground state and complex pentagonal geometries. In the investigation, the EPR spectral g‖ value was recognised to be 2.24 for the copper (II) complex, and the atmosphere was covalent. The EPR spectral G value is calculated using the following formula: G = (g‖-2.0023/g-2.0023) and the found G value is 2.1113 (G < 4.0). In this case, g||> 2.4 and g||< 2.4 are characteristically followed by ionic and covalent behavior, respectively. The metal–ligand bonded EPR spectral result is displayed in Fig. 4.

DNA binding studies

UV-Vis absorption analysis was used to investigate the binding activities of the Cu (II) complex with double-stranded CT-DNA helix, followed by changes in absorbance and wavelength. Herein, the complex [Cu(1,10-phen)(2,6-pyridinedicarbox-amide)] $(NO_3)_2$ can be identified by the shape, and polarity nature of the side chains and heterocyclic ligand. This demonstrates the binding mode to DNA. The intensity of the complex is decreased (hypochromism) by the addition of CT-DNA, which is attributed to the interactions between DNA and the copper (II) complex, which can be noted in the absorption band. A complex with a ligand contribution -NH group exhibits similar hypochromism (Ali *et al.,* 2016). Resultant CT-DNA double helix possesses several hydrogen bonding units, involving a major groove interaction with the complex.

In this case, the NH group of the complex forms a hydrogen bond with CT-DNA, revealing in absorption spectra of the complex bound to DNA from side to side intercalation. The CT-DNA involves a strong stacking interaction using the planar heterocyclic aromatic rings of the ligand and the red-shift of the metal complex with DNA has

been confirmed. The binding constant Kb value was determined by plotting [DNA]/(a-f) versus [DNA] inset of Fig. 4.

Fluorescence spectroscopic studies

The relative binding of the complex to CT-DNA is investigated using the emission spectrum technique. Ethidium bromide (EB) emission intensity is utilised as a spectral probe. The fluorescence titration experiment, specifically the EB fluorescence displacing experiment, has been widely used to characterise the complex's interaction with DNA. DNA and EB have low intrinsic fluorescence intensities in Tris-HCl solution. However, EB's fluorescence intensity will increase with the addition of DNA due to its intercalation into the DNA.

EB can therefore be used to examine how a compound interacts with DNA. If the complex is able to intercalate into DNA, there will be fewer DNA binding sites accessible for EB, which will result in a quenching of EB's fluorescence. In our experiments, as shown in Fig. 5, the fluorescence intensity of EB at 614 nm displays a remarkably decreasing trend with an increase in complex concentration, suggesting that certain EB molecules have been released from EB-DNA after an exchange with the complex, which results in the fluorescence quenching of EB. This could be as a result of the metal complex displacing EB (whose fluorescence is increased upon DNA binding) from the DNA-binding sites, or it could be as a result of a much more straightforward quenching interaction with the DNA itself. We believe that the displacing of the DNA bound to EB by the copper (II) complex can be the cause of the rise in EB emission intensity with increasing complex concentration.

As a result, various Cu(II) complexes have been discovered to enhance the quenching properties of EB bound with DNA due to the interaction between the Cu(II) complex and DNA. The Cu (II) complex exhibited Kq values of $5.25 \times 10^{4} M^{-1}$.

Cyclic voltammetry study

The use of cyclic voltammetry to study the interaction between complexes and DNA is a useful addition to the previously used UV-Vis investigation methods. A typical cyclic voltammogram of a 0.01 mM complex solution without and with DNA was performed in DMF at a glassy carbon (GC) working electrode (Fig. 6). A single cathodic peak was observed in the forward scan, corresponding to the reduction of complex. The absence of an anodic peak in the reverse scan indicates that the process is irreversible. When CT-DNA was added to a complex solution, there was a significant decrease in peak current height and a shift in peak potential to more -ve values. The addition of a very large excess of DNA seemed to have no effect on the cyclic voltammetric behaviour, indicating that the decrease in peak current of the complex after the addition of DNA was caused by the binding of [Cu(1,10-phen)(2,6-pyridinedicarboxamide)] $(NO_3)_2$ complex to the DNA. When the concentration of DNA increases, the changes in peak current and potential gradual increases. This demonstrates that the complex interacted with CT-DNA.

Table.1 Antibacterial zone inhibition of Cu(II) complex

Zone of Inhibition in mm				
S.No	**Test Organism**	**100µg/ml**	**50µg/ml**	**Control (Ciproflaxcin)**
1	*Enterobacter cloacae*	14 mm	12 mm	16 mm
2	*Staphylococus Haemolytics*	13 mm	12 mm	16 nm
4	*Bacillus cereus*	13 mm	11 mm	15 mm
5	*Staphylococcus aureus*	14 mm	11 mm	15 mm

Scheme.1 A schematic representation of the synthesis process for Cu (II) complex.

Fig.1 FT-IR spectrum of Cu (II) metal complex

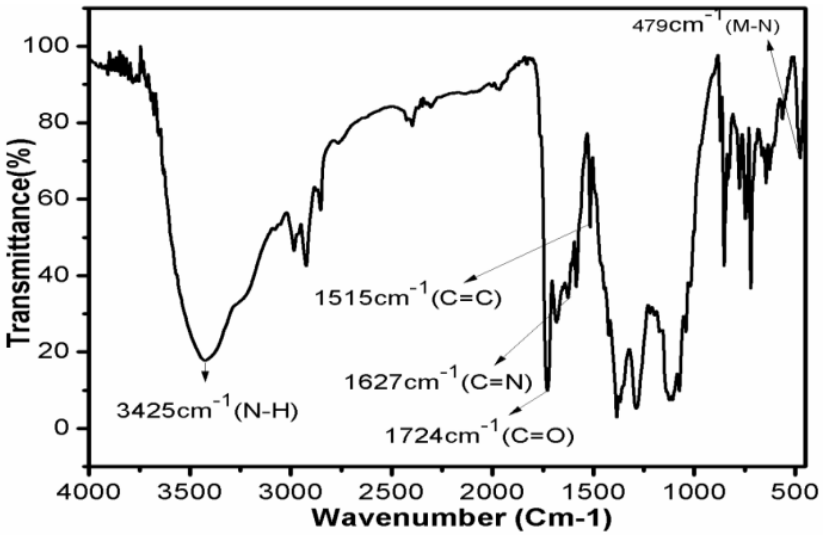


Fig.2 The UV–Vis spectrum of complex Cu(II) complex with inset image of d-d transition

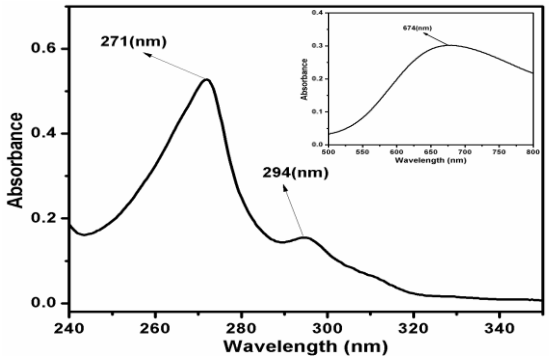


Fig.3 The EPR spectrum of Cu (II) complex

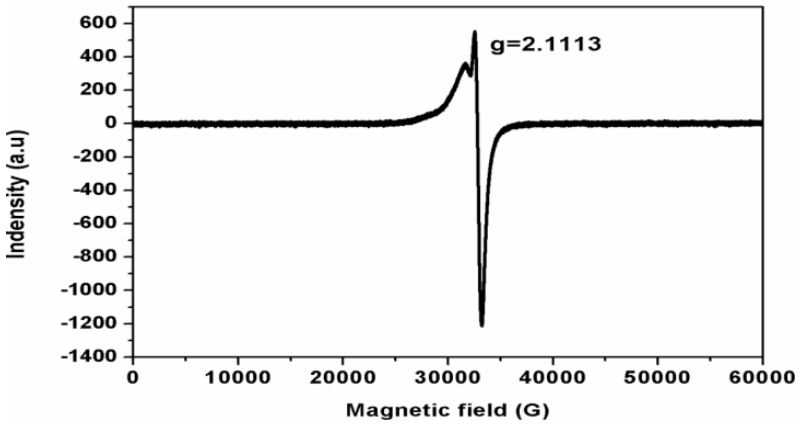


Fig.4 Absorption spectral traces on addition of CT DNA to [Cu(1,10-phen)(2,6-pyridinedicarboxamide)] $(NO_3)_2.2H_2O$ (show by arrow). Inset plot of [DNA]/(εa-εf) Vs [DNA] for absorption titration of CT DNA with complex.

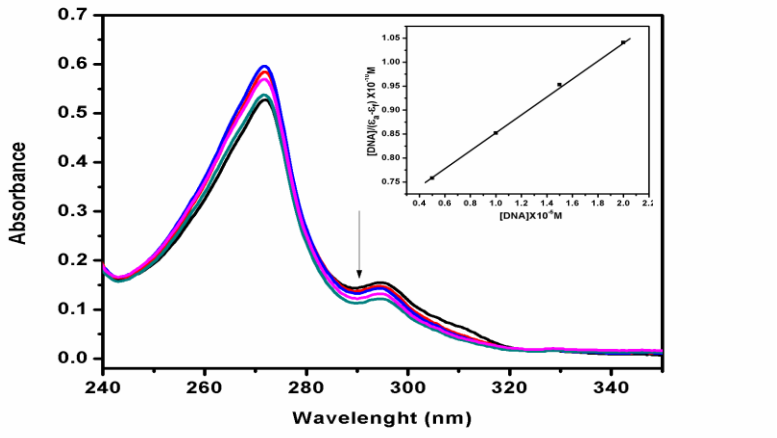


Fig.5 Fluorescence Emission spectra (excited at 480 nm) of the CT DNA-EB system($4X10^{-5}$M) EB, $4X10^{-5}$M CT DNA) in the absence (block line) and presence (color line) of 0.8-4.0 $X10^{-5}$ M [Cu(1,10-phen)(2,6-pyridinedicarboxamide)] $(NO_3)_2.2H_2O$

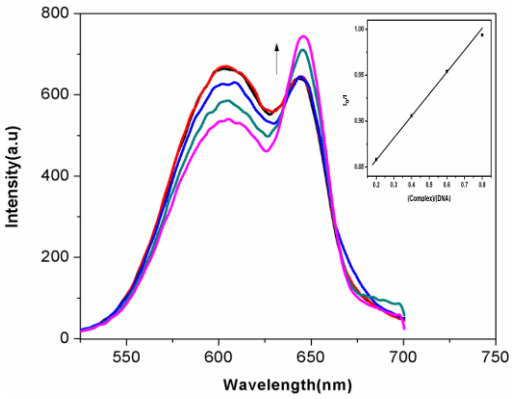


Fig.6 Cyclic voltammograms of [Cu(1,10-phen)(2,6-pyridinedicarboxamide)] $(NO_3)_2.2H_2O$ in DMF in absence(block line) and presence (red line) of DNA.

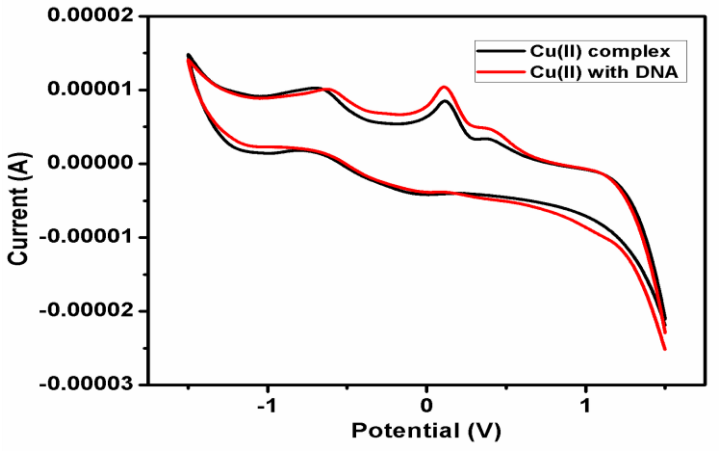
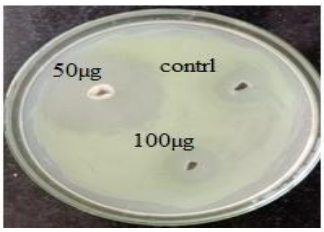


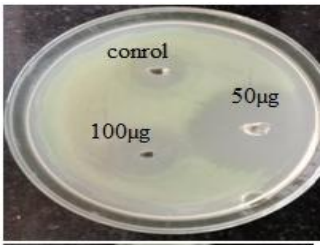
Fig.7 Represents the antibacterial activity of [Cu(1,10-phen)(2,6-pyridinedicarboxamide)]$(NO_3)_2$ complex against selected bacterial pathogenic strains (Enterobacter cloacae, Staphylococcus haemolytics, Bacillus cereus, and Staphylococcus aureus).

Staphylococcus haemolyticus *Enterobacter cloacae*

Staphylococcus aureus



Bacillus cereus



Antibacterial activity of [Cu (phan) (2, 6-pyridinedicarboxamide)]

The agar well diffusion assay was used to test the antibacterial activity of [Cu(1,10-phen)(2,6-pyridinedicarboxamide)]$(NO_3)_2$ against pathogenic bacteria such as *Enterobacter cloacae, haemolyticus, Bacillus cereus* and *Staphylococcus aureus.* The inhibition zones (mm) around each well containing [Cu(1,10-phen)(2,6-pyridinedicarbox-amide)]$(NO_3)_2$ solution were measured after 24 hours of incubation. *Bacillus cereus* and *Staphylococcus haemolytics* exhibited a minimum inhibition zone of 13 mm)at 100 µg/ml. [Cu(1,10-phen)(2,6-pyridinedicarboxamide)](NO3)2 exhibits maximum antibacterial activity against *Enterobacter cloacae* and *Staphylococcus aureus* with 14 mm as the zone of inhibition. All of the tested bacterial strains showed susceptibility to [Cu(1,10-phen)(2,6-pyridinedicarboxamide)].In contrast, positive control ciprofloxacin (10µg/ml) exhibits a maximum zone of inhibition against *Staphylococcus haemolyticus* (Baskaran *et al.*, 2005). Conversely, Ciprofloxacin showed sensitivity towards all the tested bacterial strains except Staphylococcus haemolyticus

In the present study, one novel aromatic ligands 1,10-phenanthroline and 2,6-pyridinedicarboxamide and their metal complex are synthesized and characterized by the various physicochemical and spectral methods. The electronic data have confirmed the five coordinated geometry for the complex. The interaction of metal complex with CT-DNA has been successfully explored by UV-vis, electronic absorption spectroscopy and CV measurements. The resultant recommended that the complex is capable to bind with CT-DNA using an intercalative mode. We established that metal complex having more anti-bacterial activity than their free ligands, prominence potential applications in the field of bioinorganic chemistry. These investigations will be helpful in developing innovative metal chelates based drugs for the dealing of bacterial infections.

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