Synthesis of novel 2-chloro-6-{[(*E*)-{2-hydroxy-3-methoxy-5-[(*E*)-1,3-thiazol-2-yldiazenyl]phenyl}methylidene]amino}-4-nitrophenol and its Co(II) Cu(II) complexes : biological studies and Detection of Hg^{2+} by $[Co(CEN)_2]$/GCE

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Abstract

In this study, the novel azo-dye 2-chloro-6-{[(*E*)-{2-hydroxy-3-methoxy-5-[(*E*)-1,3- thiazol-2-yldiazenyl]phenyl}methylidene]amino}-4-nitrophenol (CEN/$C_{17}H_{12}ClN_{10}O_{10}S$) and Co (II), Cu (II) complexes were synthesized. The structure of synthesized metal complexes and CEN were characterized by physico-chemical technique by FT-IR, elemental analysis, UV-Vis, mass spectra, ^{1}HNMR spectra and thermogravimetric analysis; all spectral data suggests trident ligand and prepared complexes shows octahedral geometry with PXRD exhibit crystanality nature of complexes. Ligand and its [Co(CEN)2], [Cu(CEN)2] were tested for their in vitro antimicrobial and antioxidant properties. The [Co(CEN)2] act as a good antioxidant agent. In this connection, we designed a new sensor for electrochemical determination of Hg^{2+} by CV. The GCE was modified with [Co(CEN)2] to enhanced, because the electron transfer behavior of the electrode. The modified [Co(CEN)2]/GCE has long linear range of 10-70 µM/L, sensitivity of 4.059 $\mu A \mu M^{-1} cm^{-2}$ and LOD (limit of detection) is 3.333 µM/L respectively in PBS pH 7.0. The modified biosensors is monitoring the mercury in aqueous water and recovery result obtained. Prepared sensor $[Co(CEN)_2]$/GCE has more stability and showed less leaching property.

Keyword: Azo-dye, Metal complexes, Detection of mercury, Antimicrobial, Antioxidant.

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1. INTRODUCTION

The azo schiff bases ligand are applicable in lots of diverse areas have a good fastness, high dyeing strength properties, absorption characteristics, antimicrobial activity, molecular structure, and nonlinear optic properties [1]. In previous years, the synthesis heterocyclic azo schiff base ligand and its complexes were found to have significantly biopharmaceutical activity like antioxidant, anticancer, antimicrobial, antidiabitic, anti-tuberculosis and DNA cleavage studies e.t.., [2-6].

Electrochemical detection of Hg^{2+}, mercury is a heavy and toxic element. Is used in batteries, thermometers, barometers and many industries. Mercury and its derivatives compounds have been parts of widespread pollutant of aquatic environment and it is dangerous in both natural ecosystem and human species. Mercury is very hazards known to be highly toxic element, then its harmful effects to aquatic plants and animals like as fishes, moreover human living system. It causes Minamata disease, irritation to the eyes, skin and stomach pain; difficulty to breathing cough chest pain headache etc., mercury exposure to human body its effect on neurological and behavioral disorders arising in living system [7]. Thus, the determination of Hg^{2+} is very important. Literature showed that cyclic voltammogram is a potential, sensitive, rapid and reliable method for the detection of Hg^{2+},. This method is used to investigate the electrochemical feature of a substance in solution.

In our lab focused on synthesis of transition metal complexes, evolution of on drug resistance properties and the electrochemical detection of bioactive molecules using modified electrodes. In continuation of our research work here-in we report the synthesis and characterization of 2-chloro-6-{[(*E*)-{2-hydroxy-3-methoxy-5-[(*E*)-1,3-thiazol-2-yldiazenyl]phenyl}methylidene]amino}-4-nitrophenol (CEN/$C_{17}H_{12}ClN_{10}O_{10}S$) and its [Co(CEN)2] , [Cu(CEN)2] complexes screening by anti-microbial and antioxidant activities. The modified [Co(CEN)2]is used to detect mercury bioactive (Hg^{2+}) in various concentrations using CV techniques with good anodic peak current have been evaluated

2. MATERIALS AND MEASUREMENTS

2.1 Source and instrumentations

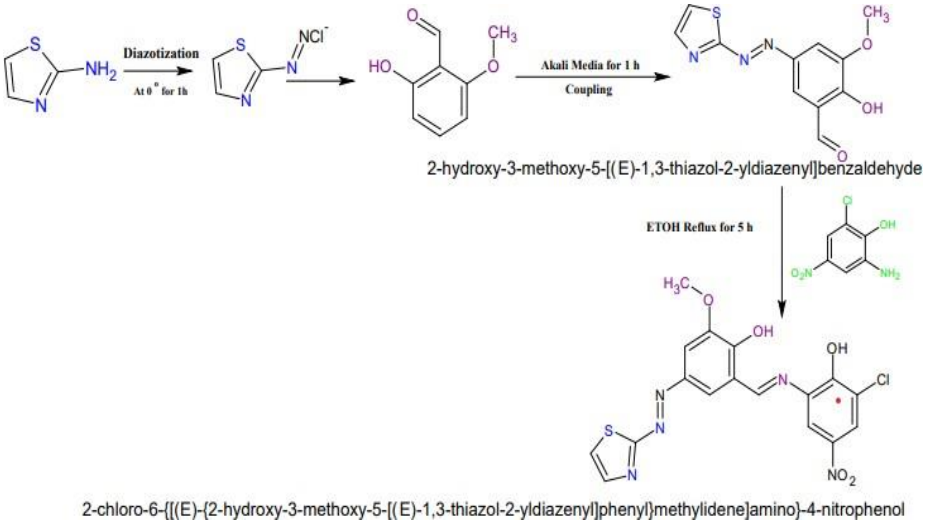
All the chemicals used were of analytical grade and purchased from Himedia chemical company which were used without further purification. The melting point is determined by the digital melting point apparatus, electrothermal IA9100. The synthesized compounds were characterized by UV-Vis spectrophotometer in the range 200-800 nm using systronics 119 model in DMF solvent. The elemental analysis was performed on a CHN analyser carlo erba 1108 analyser. The IR spectra were recorded using a KBr pellet in a Shimadzu FT-IR spectrophotometer in the range 4000–400 cm^{-1}. ^1H NMR spectrum from JEOL JNM-ECZ400S/L spectrometer of the ligand was recorded with an internal standard tetramethylsilane. The electro-chemical analysis was done by CHI 620E analyzer Inc. made in the U.S.A. Mass spectra of the compounds were determined on LC-MS water AQUITY-2777C mass spectrometer. The molar conductance measurement was conducted using an ELICO-CM82 conductivity meter. The magnetic moment of the complexes was noted at 28 °C by Gouy balance version 7550 using Hg[Co(NCS)4] as a calibrant. The TGA of complexes was carried out on SII Exstar TG/DTA 6300 instrument from the laboratory temperature to 1000 °C with a scan rate of 10 °C/ min.

2.2.1 Synthesis of the ligand [CEN]

The azo Schiff base 2-chloro-6-{[(*E*)-{2-hydroxy-3-methoxy-5-[(*E*)-1,3-thiazol-2-yldiazenyl] phenyl}methylidene]amino}-4-nitrophenol (CEN/$C_{17}H_{12}ClN_{10}O_{10}S$) was synthesized as following literature [1, 2 and 8]. The 1st step 1,3-thiazol-2-amine (5 mM of 0.5g) was dissolved in a mixture of (8.5 mL strong hydrochloric acid and 4.5 mL water) the condition of the reaction was maintained at 0-5 °C and 5 mM/0.34g of sodium nitrite in 3 mL of water was added to above hydrochloric mixture at 0–5 °C for 18 min. Then, it was maintained for 2 h to complete diazotization. After, 2 h diazoniumsalt obtained. Further 2nd step the 1,3-thiazol-2-amine diazoniumsalt solution was added drop

wise to a coupling solution of (5 mM/0.76g) of 2-hydroxy-3-methoxybenzaldehyde) which was dissolved KOH alkali media and maintained pH at 4-6. After the complete the reaction maroon solid precipitate (2-hydroxy-3-methoxy-5-[(*E*)-1,3-thiazol-2-yldiazenyl]benzaldehyde (HMB) was obtained and separated, dried for next continued step.

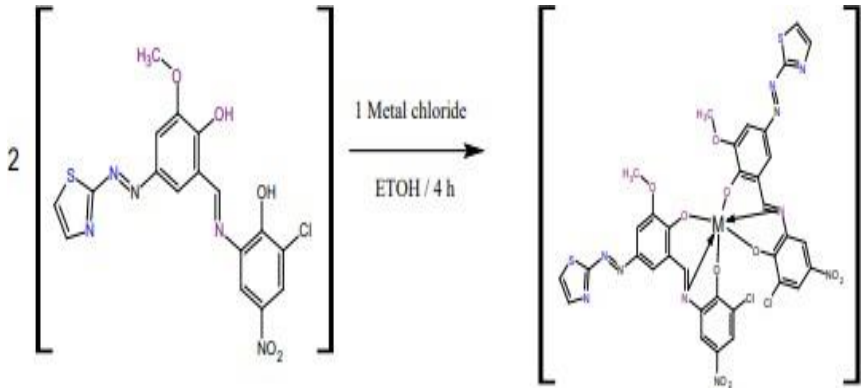
Furthermore, the 3rd step preparation of CEN followed by (1:1) ratio of 2 mM/0.58g HMB and 2 mM/0.4g of 2-amino-6-chloro-4-nitrophenol in 18 mL ethanol taken in a round bottom flask. To which 5 drop of acetic acid was added as a catalyst, and reflux for 4 h at 60-70 ˚C. The progresses of the reaction were confirmed by TLC using pet ether and chloroform in (1:2) ratios. Moreover reaction mixture was quenched with ice cold water CEN ligand was form and collected by filtration; filtered CEN was rinsed with ethanol and recrystallization from methanol. The obtained CEN was dried under oven at 50 ˚C for 1h after then cooled in lab temperature and kept in desiccator the yield is 73 %. [8,9] The route of scheme 1 is represented in below.



Scheme1: 2-chloro-6-{[(*E*)-{2-hydroxy-3-methoxy-5-[(*E*)-1,3-thiazol-2- yldiazenyl] phenyl} methylidene]amino}-4-nitrophenol (CEN/$C_{17}H_{12}ClN_{10}O_{10}S$)

2.2.2 Preparation of Co(II) and Cu(II)

The Co(II) and Cu(II) complexes have been prepared according to the following literature in the form of 1:2 stoichiometric ratio like as [Co(II) (2 mM/0.5g) : CEN (4 mM)/1.8g] was dissolved in hot 23 mL of ETOH under reflux on water bath at 61 °C for 3 h. Moreover after Complete the reaction the color precipitate collected via filtration and washed with ethanol, the precipitate dried over in a desiccator [8, 9]. The similar synthetic route was followed for preparation of Cu(II) complexes is depicted in scheme 2 the yield obtained is 75–80%.



Scheme 2 Synthesis of [Co(CEN)$_2$] and [Cu(CEN)$_2$]

2.3 Electrochemistry

Before modification, the GCE surface was polished with alumina slurries followed by sequential sonication in 1:1 (ethanol, HNO_3 and deionized water) for 5 min to obtain a clean surface. Then 0.2 mg of $[Co(CEN)_2]$ was dissolved with DMF and homogenized by sonication for 20 min. Afterwards, 5 µL of $[Co(CEN)_2]$ in DMF drop coated on the electrode surface area. The modified $[Co(CEN)_2]$/GCE was used to detect mercury in phosphate buffer (pH7) solution [10, 11].

2.4 Biological studies

2.4.1 Antimicrobial activities

Antimicrobial activity of CEN and its $[Co(CEN)_2]$, and $[Cu(CEN)_2]$ complexes analysed using agar well difusion method against the bacterial cultures of *escherichia coli* (gram negative bacteria) and *bacillus subtilis* (gram positive bacteria). *Ciprofoxine* as a consider standard for screening of antibacterial agent (control). The synthesised compounds are assessed against in vitro antifungal cultures are *aspergillius favus* and *candida albicans* with standard *fluconazole*. The test compounds had been dissolved DMSO to get a concentration of 0.5 and 1 mg/cm^3. Sample poured petri plates were incoculated with microorganism and incubated at 37 °C for 24 h [12, 13]. The zone inhibition value of the compounds was determined by (mm) in mean method.

2.4.2 Antioxidant studies

The scavenging ability of (DPPH) 2, 2-diphenyl-1-picrylhydrazyl radical is used extensively for the evaluation of synthesised CEN ligand and its complexes by spectrophotometrically. The solution of 24 mg of DPPH with 100 mL of methanol and storing it at 20 °C until needed. The working solution was made by our research group diluting the 2 mL of DPPH solution added to all synthesized substances were produced in DMSO at 25, 50, 100 and 200 µg/mL concentrations and the solution achieved by stirring. The result was measuring the absorbance at 517 nm with a spectrophotometer following literature [12, 13] and kept at lab temperature for 35 min in the dark. The percentage of inhibition was determined using the formula below. The IC_{50} value of compounds and IC_{50} value was determined by standard method.

% Inhibition of DPPH activity = [Control Absorbance - Sample Absorbance /Control Absorbance]
× 100

3. RESULT AND DISCUSSION

3.1. Characterization

The synthesis of CEN ligand coordinate with Co(II) and Cu(II) metals are produces the complexes formula $[Co(CEN)_2]$ and $[Cu(CEN)_2]$. The prepared ligand and its complexes were spectral characterized by different spectroscopic techniques. The complexes are soluble in DMF and DMSO solvents. The molar conductance measurements of the complexes used in DMF (1×10^{-3} M) solution reported 12-16 Ω^{-1} cm^2 mol^{-1} indicate non-electrolytic behavior [14, 15]. The experimental analytical data are reported in Table 1.

Table 1 Physical properties and analytical data of CEN and its complexes

Compounds	Colour	Mol.Wt	M.P °c	Elemental analysis (%) (Cal)				λm ($\Omega^{-1}cm^{2-1}$)
				C	H	N	M	
CEN	Maroon	433.82	138-139	47.07 (44.23)	2.79 (2.21)	16.14 (15.17)	-	-
$[Co(CEN)_2]$	Red	922.55	166-168	44.26 (44.23)	2.19 (2.21)	15.18 (15.17)	6.39 (6.40)	17.5
$[Cu(CEN)_2]$	Scarlet	927.16	140-143	44.04 (44.08)	2.17 (2.15)	15.11 (15.12)	6.85 (6.87)	13.4

3.2 ^{1}H NMR Spectral Data

The newly synthesized CEN has been characterized via ^{1}H NMR in DMSO-d_6 solvent at laboratory temperature and depict in

Figure 1. The Ar-OH group of two proton assigned as a singletat 13.554 and 11.845 ppm (s, 2H, Ar-OH) and the azomethine proton resonance showed singlet with respect to 9.808 ppm (s, 1H, CH=N). The signals by view of aromatic protons appeared between 8.094-7.54 ppm (m, 6H, Ar-H). The methoxy group protons reveal a singlet on 3.36 ppm(s, 3H, Ar-OCH_3) [14, 15].

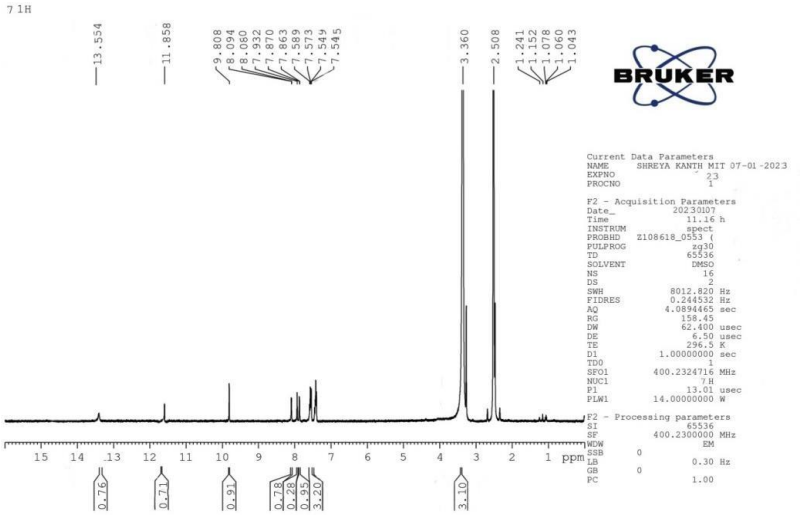


Figure 1 ^{1}H NMR spectrum of CEN

3.3 IR spectral studies

Infrared spectroscopy is very essential tool to find the functional groups in synthesized CEN and its metal complexes. The IR spectral data are provided in the Table 2. The CEN showed a band due to its phenolic ν(OH) group at 3407 cm^{-1} while its [$Co(CEN)_2$] and [$Cu(CEN)_2$] appeared at 3445 and 3483 cm^{-1} respectively. A azometonine group peak appeared for CEN at 1634 cm^{-1}, for [$Co(CEN)_2$] at 1595 cm^{-1} and [$Cu(CEN)_2$] showed a peak at 1601 cm^{-1}. The peaks are shifted different wavelength region indicates Involvement in the complexes formation. The (-N=N-) functional group of synthesized CEN shows 1411 cm^{-1} but its complexes show different wavelength region between 1461-1449 cm^{-1} is attributed. These wavelength regions indicate formation of coordinated bond for complexes. In addition, the spectral peaks appeared at 519-531 cm^{-1} is due to (M-N) and (M-O) stretching at 609- 610 cm^{-1} confirms the respectively, for bond formation. The spectra are shown in Supplementary Figure S1 [14, 15].

Table 2 FT-IR spectral data of azo dye CEN ligand and its metal complexes

Compound	ν(–OH)	ν(C=N)	ν(N=N)	ν(M-N)	ν(M-O)
CEN	3407	1634	1411	-	-
[$Co(CEN)_2$]	3445	1595	1461	531	610
[$Cu(CEN)_2$]	3483	1601	1449	519	609

<Figure S1>

3.4 UV studies

The prepared CEN and its coordinated complexes were investigated by electronic spectral studies between 200-800 nm at the concentration 10^{-6} M in DMF solvent. The CEN revealed three distinct absorption bands exhibits at 30120, 20,202 and 15,220 cm^{-1} attributed to the corresponding π→π* and n→π* transitions assigned and this indicates due to the interaction of azo-aromatic

chromosphere intermolecular charge transfer. In $[Co(CEN)_2]$ exhibited $^6A1g \rightarrow ^4T1g$, $^1A2g \rightarrow ^2T1g(F)$ and $^4A2g(F) \rightarrow ^3T2g(F)$ transition assignment due to at 29,940, 23,041 and 19,801 cm^{-1} respectively and along with its magnetic moment is 3.83 BM shows distorted octahedral geometry [14, 15]. The $[Cu(CEN)_2]$ complex is attributed two absorption peak at 24,752 and 20,618 its transition assignments is $^2B1g \rightarrow ^2A2g$ and $^4T1g(F) \rightarrow ^4A2g(F)$ its magnetic moment is 1.81 BM, it is revealed octahedral geometry. The wavelength is shifted various regions in complexes compared to ligand due to charge transfer from ligand to metal, while all transition appeared in bathochromic shift. The spectral results of the electronic spectral studies were presented in Table 3 and Figure 2 [14- 18].

Table 3 The electronic spectral data of CEN and its metal complexes

Compounds	bsorptions in (cm^{-1})	Transitions assignment	Magnetic moment μeff(BM)
CEN	31,746 27,774 15,220	n→π *n→π *	
$[Co(CEN)_2]$	29,940 23,041 19,801	$^6A1g \rightarrow ^4T1g$ $^1A2g \rightarrow ^2T1g(F)$ $^4A2g(F) \rightarrow ^3T2g(F)$	3.83
$[Cu(CEN)_2]$	24,752 20,618	$^2B1g \rightarrow ^2A2g$ $^4T1g(F) \rightarrow ^4A2g(F)$	1.81

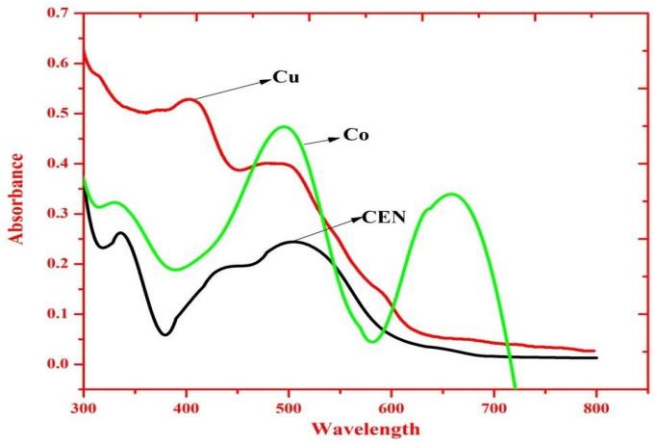


Figure 2 Electronic spectra of CEN, $[Co(CEN)_2]$ and $[Cu(CEN)_2]$

3.5 XRD studies

The crystallinity of synthesized metal complexes was determined by PXRD pattern in this order to scan with continuous speed at 10°/min in the region between 5–90° at constant a wave length 1.54 Å and inter-planar distance (d). The inter-planar space was determined using Bragg's formulae $n\lambda = 2d\sin\theta$ recorded data are given in Table 4 and the diffraction patterns are showed in Figure 4. The diffractogram depict the peak for $[Co(CEN)_2]$ shows 4 its relative intensity 2θ reflections and it's $h^2+k^2+l^2$ values are good agreements with 1, 2, 3 and 6 hence it represents cubic nature. The $[Cu(CEN)_2]$ also $h^2+k^2+l^2$ values noted as 1, 3, 3 and 11 diffractions, these diffractions indicate cubic structure of complex [15-18]. Further, the lattice parameter of Co and Cu complexes provide a=b=c= 4.53 and 3.41 Å respectively. All synthesized metal complexes data relived the molecules are arranged in crystalline nature as shown in Figure 3.

Table 4 XRD data of CEN ligand and its metal (II) complexes

Compound	Point	2θ	Sinθ	$Sin^2\theta$	$Sin^2\theta \times 1000$	$h^2+k^2+l^2$	h k l	D		a inA°
								Obs	Cal	

[Co(CEN)2]	1	25.29	0.218	0.047	47.524	1	1 0 0	3.53	3.51	3.53
	2	37.53	0.321	0.103	103.04	2	1 1 0	2.39	2.42	3.39
	3	45.74	0.388	0.150	150.54	3	1 1 1	1.98	1.88	3.43
	4	66.9	0.551	0.303	303.60	6	2 1 1	1.39	1.33	3.42
[Cu(CEN)$_2$]	1	19.05	0.165	0.027	27.221	1	1 0 0	4.66	4.65	4.66
	2	32.11	0.276	0.076	76.176	3	1 1 1	2.78	2.76	4.83
	3	33.88	0.291	0.084	84.682	3	1 1 1	2.64	2.65	4.58
	4	45.88	0.389	0.151	151.32	6	2 1 1	1.97	1.92	4.84
	5	66.9	0.551	0.303	303.60	11	3 1 1	1.39	1.33	4.63

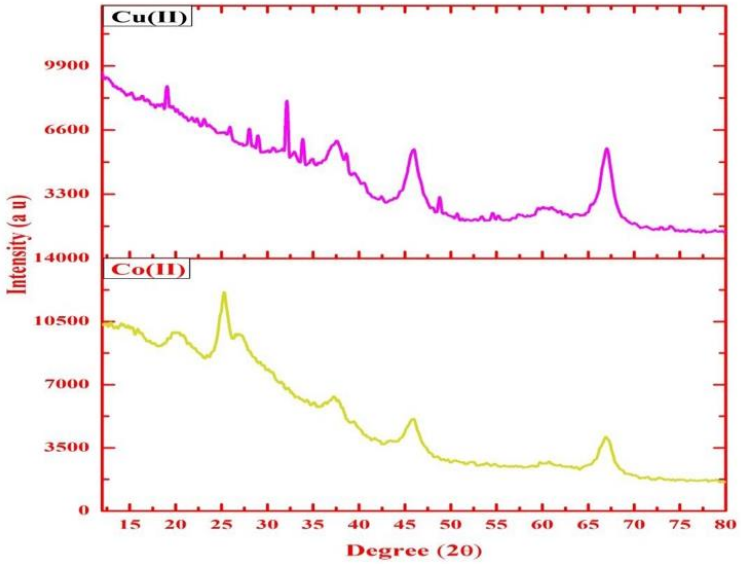


Figure 3 XRD patterns of [Co(CEN)$_2$] and [Cu(CEN)$_2$]

3.6 Mass spectra of syntheses compounds

The mass spectra of the novel CEN ligand peak attributable to the given molecular ions peak m/z 433.09 (Cal: 433.89) represents in Figure 4 and its [Co(CEN)$_2$] , [Cu(CEN)$_2$] complexes m/z ion peak showed at 922.5(Cal: 922.93) and 927.1 (Cal: 927.56) respectively, as data shown in elemental analysis Table 1

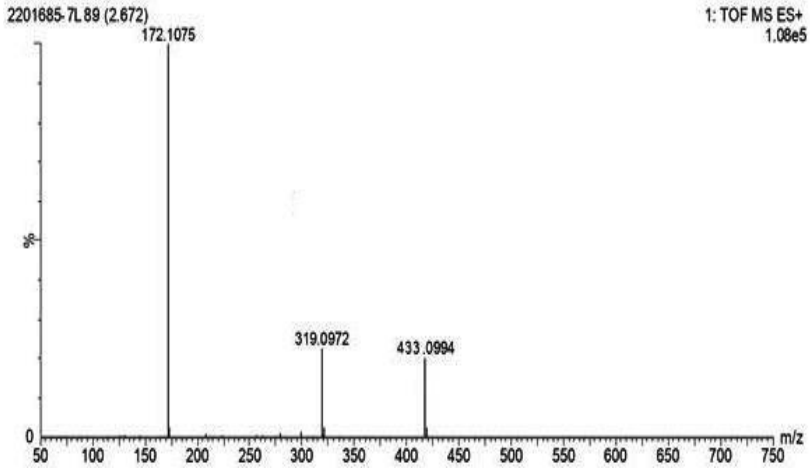


Figure 4 Mass of the CEN ligand

3.7 Thermal studies of compounds

The metal complexes were examined by thermo gravimetric analysis in the temperature range 25 to 1000 ℃ at a heating rate 10 ℃/min under nitrogen atmosphere. Thermograms of [Co(CEN)$_2$] exhibited three steps of degradations. The first step being at 22.5 to 255 ℃, which corresponds to the

mass loss of 58.5 % due to one pair of methoxy, chloride, nitro, hydroxyl and 2-diazenyl-1,3-thiazole molecules. In the second step 13.1 % weight loss between 255 to 368 °C is due to the prop-2-en-1-amine and (1E)-N-methylideneprop-en-1-amine removal molecules. Thethird step degradation denotes 20.1 % is because of the removal benzene and 2-methylphenol molecules in the temperature range 368-766 °C and at the end Co oxide residue formed. $[Cu(CEN)_2]$ exhibits two degradation steps, in the first step 26.8 % of mass loss is due to the dissociation of HMB molecules at temperature between 20.1-125°C. The second degradation step has occurred from 125-884°C showed the weight loss of 64.6 % CEN and 2-amino-6-chloro-4-nitrophenol molecules and at the end left over residue is CuO [25-29] as shown Table 5 and Figure5 [14-18].

Table 5 Thermal degradation data of complexes

Complex	Step	ecomposition Temp (°C)	Assignment Moiety left	Lossof mass(%)	Residue
$[Co(CEN)_2]$	1	22.5-255	$[(C_{14}Cl_2N_8O_8S_2)$/ one pair of methoxy, chloride, nitro, hydroxyl and 2-diazenyl-1,3-thiazole]	58.6	CoO
	2	255-368	$[(C_7H_8N_2)$/ prop-2-en-1-amine and (1E)-N-methylideneprop-en-1-amine]	13.1	
	3	368-766	$[(C_{13}H_{11}O)$/ benzene and 2-methylphenol]	20.2	
$[Cu(CEN)_2]$	1	20.1-125	$[(C_{11}H_{11}N_3O_2S)$/HMB]	26.8	CuO
	2	125-884	$[(C_{23}H_{17}Cl_2N_7O_7S)$/ CEN and 2-amino-6-chloro-4-nitrophenol]	64.6	

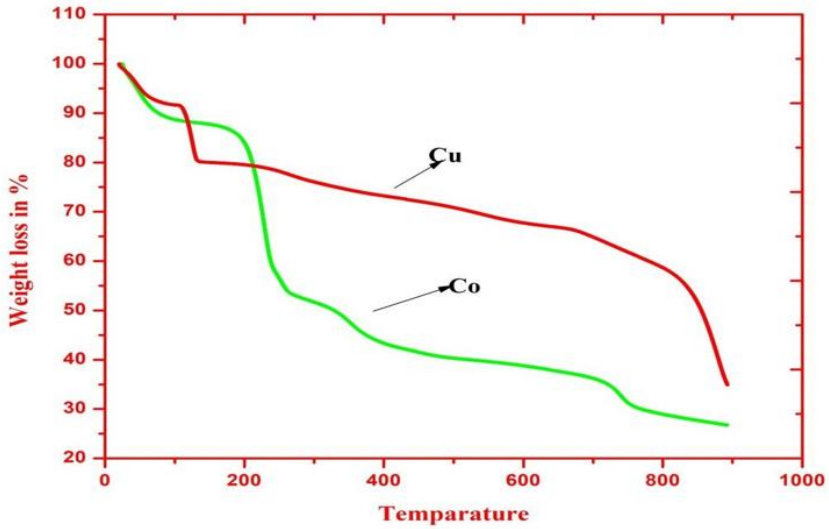


Figure 5 TGA curves for [Co(CEN)2] and [Cu(CEN)2]

3.8 Electrochemistry studies

3.8.1 Electrochemical study of different electrode

The cyclic voltammogram were performed to investigate the electrochemical properties of the synthesized $[Co(CEN)_2]$ molecule. The 0.2 mg $[Co(CEN)_2]$ in DMF were carried out with the pristine GCE as the working electrode scan at 50 mVs^{-1} in an inert nitrogen. Further $[Co(CEN)_2]$/GCE electrode was dried and washed with deionized water and rinsed in DMF and employed for electro catalytic sensor studies. The bare electrode showed redox peaks with very small peak currents, the $[Co(CEN)_2]$/GCE peaks are in agreement with the solution CV peaks

enhanced, the peaks indicating the successful modification of the electrode surface with $[Co(CEN)_2]$. The voltammogram of the $[Co(CEN)_2]$/GCE monstrate redox peaks signifying that the designed compound is redox active. The redox peak noted between 0.197–0.31 V can be ascribed in Figure 6 [19, 20].

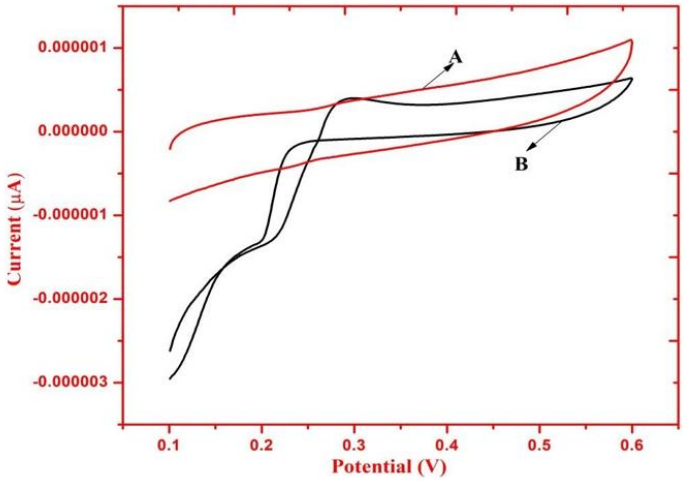


Figure 6 Cyclic votammogram of different electrodes a) bare GCE and b) $[Co(CEN_2)]$/GCE witt analyte thescan rate:50 mVs^{-1}

3.8.2 Electrocatalytic sensing of Mercury

Figure 7 exhibits the electrocatalytic redox peak of mercury from developed $[Co(CEN)_2]$/GCE in PBS pH 7 at scan rate 50 mVs^{-1}. The $[Co(CEN)_2]$/GCE exhibits its catalytic peak observed at 0.31 V is due tothe redox peak was noticed from the mercury analyte. The peak current at bare GCE was very minimal and redox peak appeared at higher positive over potential. The bare/GCE contrast to that of $[Co(CEN)_2]$, moreover the $[Co(CEN)_2]$/GCE enhanced peak current due to the fact that the resistive in nature for the charge transfer at the electrode as well as increases corresponding concentration of mercury in electrolyte. It may be accountable for the efficient electrocatalytic redox peak current of mercury. The long linearity curve in the 10-70 µM/L with LOD value of 3.333 µM/L and sensitivity of 4.059 $\mu A \mu M^{-1} cm^{-2}$. The value reveals an outstanding behaviour parameters of the developed $[Co(CEN)_2]$/GCE sensor and act as a good bioactive sensor compared with the previously literature and presented in the Table 6 [19-21].

Table 6 Comparison of the present work with different literature for mercury determination

Compound for Mercury	**Linearrange µM/L**	**Technique**	**LOD µM/L**	**sensitivity (µA μM^{-1} cm^{2})**	**Reference**
Fe1Co1/GCE	0.1-1.1	DPV	7.82	41.5	7
CoPc /GCE	0-0.03 mM	CV	81.94 nM	866.23 µA/mM	11
GCE/poly(CoTABImPc)	10-300	CV	4		19
[Co(CEN)2]/GCE	10-70	CV	3.333	4.05929	**This work**

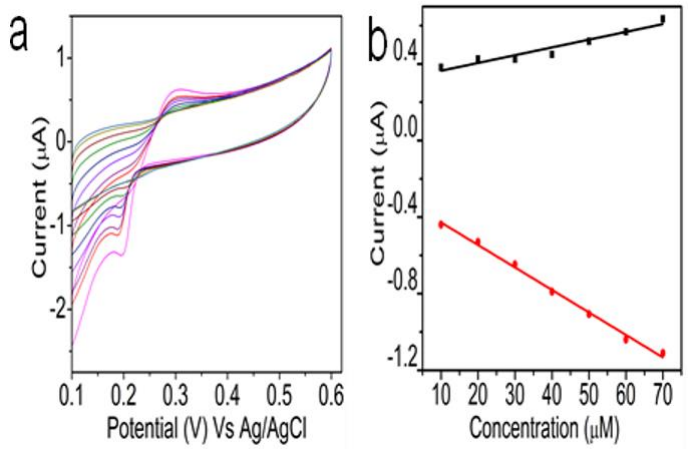


Figure 7 (a) Cyclic voltammogram of $[Co(CEN)_2]$/GCE concentration range from 10-70 μML^{-1} (b) Inset: Calibration plot of current vs concentration of mercury in the scan rate 50 mV/S at PBS pH 7.

3.8.3 Effect of scan rate

The influence of scan rate on $[Co(CEN)_2]$/GCE the redox behavior of the mercury was investigated at 0.01-0.05 mVs^{-1} scan rates in pH 7 PBS. The anodic peak intensity increases continuously with the increase of scan rate. A good linear relationship between the peak current and the square root of the scan rate, which the redox behavior of mercury indicates that electron transfer between PBS solution and the $[Co(CEN)_2]$/GCE based on diffusion controlled mechanism [20]. The diffusion coefficient of the redox reaction of mercury for $[Co(CEN)_2]$/GCE was calculated with the help of Randles-Sevcik equation [21]. The proposed scan peak current of mercury for modified electrodes can be represented as shown in Figure 8.

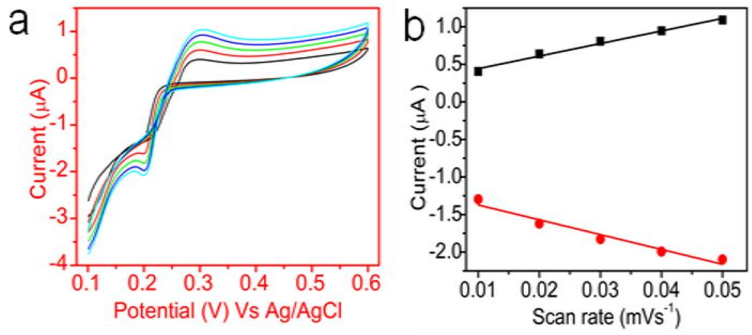


Figure 8 Cyclic voltammogram of mercury at [Co(CEN)2]/GCE at the scan rateranging from (0.01-0.05 mVs^{-1}). b) Plot of current (I) vs Scan rate

3.8.3 Application of the designed $[Co(CEN)_2]$electrode in real sample analysis

The sensor for commercial and practical purpose was assessed by the analysis of real sample. The designed $[Co(CEN)_2]$/GCE was employed sensing mercury in river water for the analysis. The 1.0 mL of the river water injected into 10 mL of PBS for mercury was determined by CV on the $[Co(CEN)_2]$/GCE. Generally, the standard addition methodology was applied to understand the matrix effects while detecting mercury in water samples. The recovery values were reported in Table 7 [20, 21]

Table 7 Determination of bio-active elements in River water

Sample of Mercury	Added (µmolL^{-1})	Detected (µmolL^{-1})	Recovery (%)
River water	5	4.58	91.6
	10	11.12	111.2
	20	18.71	93.55

3.9 Biological studies

3.9.1 Antimicrobial studies

The antimicrobial activity of the synthesized CEN and their metal complexes were carried out against *antifungal and antibacterial* cultures by agar well diffusion technique using *fluconazole and ciprofloxacin* as standard drug. The zone of inhibition values are illustrated in Tables 8 and graphical data are given in Figures 9 and 10. From the data, it is observed that all the isolated metal complexes significantly enhanced positive results against elected microbial strains compared to the free CEN ligand. The Co(II) complex showed grater activity against both the microbial activity because in complex enhancing the lipophilic feature can easily penetrate into the bacterial cell membrane by blocking the metal binding site inside the enzyme of microorganism [22,23]. Moreover, the efficiency for such results can be explained through Tweedy's chelation theory discussed in study [24].

Table 8 Antimicrobial activity of the CEN and its metal complexes

Bacterialcultures	Conc. mg/mL	Growth inhibition in mm			
		CEN	[Co(CEN)$_2$]	[Cu(CEN)2]	STD
Bacillussubtilis	25	1.16 ± 0.15	2.8 ± 0.35	2.16 ± 0.28	2.1 ± 0
	50	1.33 ± 0.28	3.26 ± 0.37	2.3 ± 0.55	2.2 ± 0
E. coli	25	1.2 ± 0.26	1.5 ± 0.26	1.3 ± 2.28	2.8 ± 0
	50	1.4 ± 0.1	2.3 ± 1.47	1.9 ± 1.53	1.9 ± 0
Fungal cultures	**Conc. mg/mL**	**Growth inhibition in mm**			
		CEN	**[Co(CEN)2]**	**[Cu(CEN)2]**	**Fluconazole**
A. favus	50	21	41.3	40.2	43
	100	51.5	58.5	57.1	76
Candidaalbicans	50	13	49.3	50.4	39
	100	53	67.1	66.9	74

*Each value is displayed average ± SD (standard division) of three replicates for the zone of inhibition *Std : Ciprofoxacin and Fluconazole M ± SD

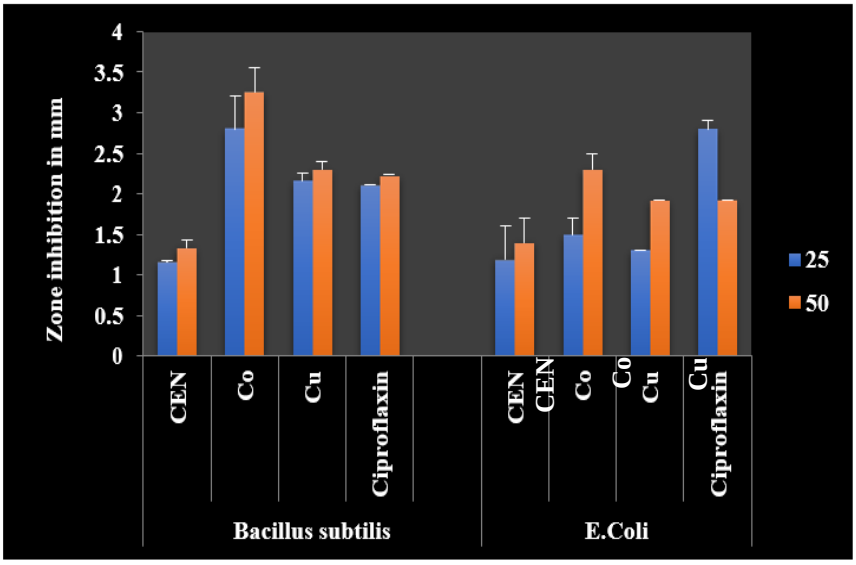


Figure 9 Graphical representation of antibacterial activity of CEN and its metal complexes**.**

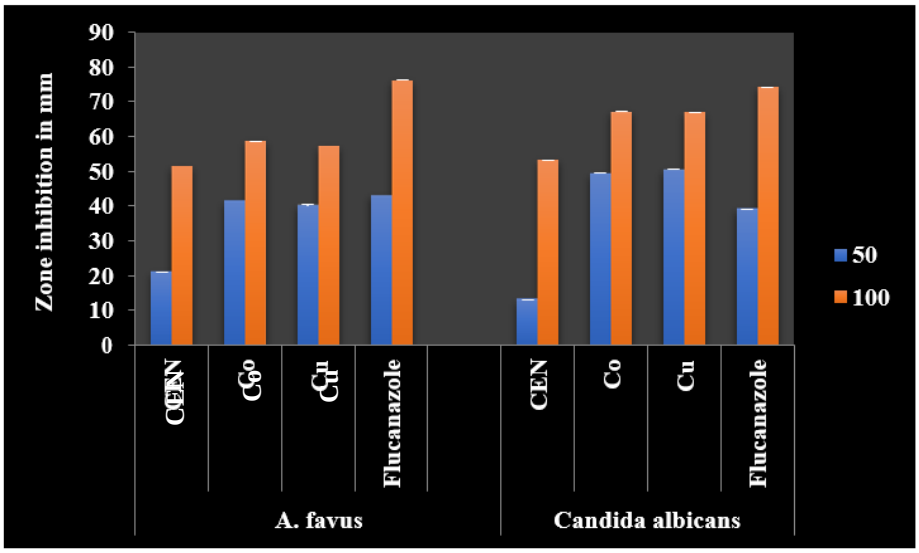


Figure 10 Graphical representation of antifungal activity of CEN and its metal complexes.

3.9.2 Antioxidant studies

The synthesized CEN and its metal (II) complexes are evaluated by DPPH scavenging activity in several concentrations. The metal (II) complexes shows promising results towards DPPH free radical which illustrate in Figure 11, whereas the $[Co(CEN)_2]$ complex explore more potential activity result compered by other complexes. Briefly at 200 µg/mL, the $[Co(CEN)_2]$ and$[Cu(CEN)_2]$ percentage inhibited DPPH free radical till 72.93 and 51.43% respectively results are entered in Table 9. IC_{50} value of compounds was determined and the result is denoted in the Table9 and Figure 12. The $[Co(CEN)_2]$ showed the minimum IC_{50} value of 57.67 µg/mL which can effectively best inhibits 50% of DPPH free radical [23-24].

Tabale 9 Antioxidant activity and IC_{50} value of CEN and its metal (II) complexes

Compounds	% Scavenging activity (Concentration in µg/mL)				IC_{50} value in(µg/mL)
	25	50	100	200	
CEN	34.55	37.96	39.45	40.54	78.68
$[Co(CEN)_2]$	58.98	63.78	68.23	72.93	57.67
[Cu(CEN)2]	39.18	46.04	48.12	51.43	66.67
Ascorbic acid	81.23	85.97	92.32	93.89	-

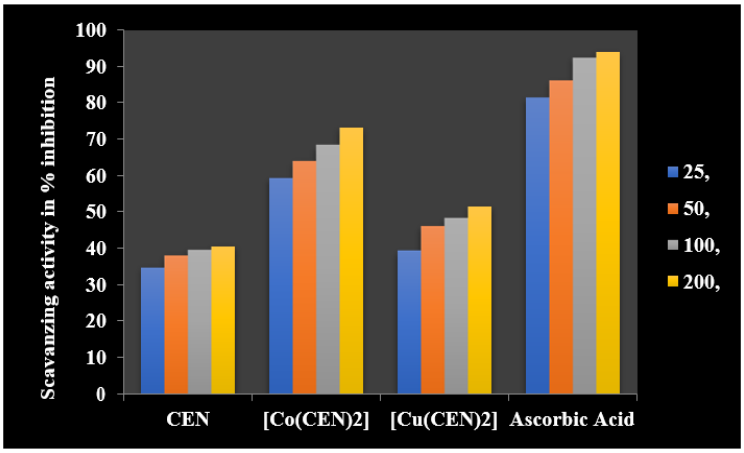


Figure 11 DPPH free radical scavenging activity of CEN and their complexes

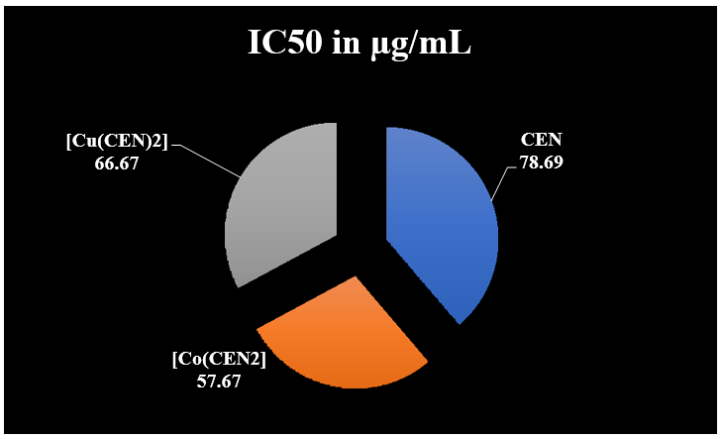


Figure 12 IC_{50} values of synthesized compounds for DPPH free radical scavenging activity

4. CONCLUSION

The novel 2-chloro-6-{[(*E*)-{2-hydroxy-3-methoxy-5-[(*E*)-1,3-thiazol-2-yldiazenyl] phenyl}methy-lidene]amino}-4-nitrophenol (CEN) and its $[Co(CEN)_2]$, $[Cu(CEN)_2]$ synthesized and the structure of the prepared compounds was elucidated by various spectro-chemical studies. The non-electrolytic nature of $[Co(CEN)_2]$ and $[Cu(CEN)_2]$ was

confirmed by the molar conductivity method. Infrared spectral data suggested the mode of bonding between the metal ion and CEN. Antimicrobial studies of synthesized metal complexes have shown that they are more significant inhibition than the CEN. The $[Co(CEN)_2]$ shows best antioxidant agent. The electrochemical study by modified electrode $[Co(CEN)_2]$/GCE showed well-established redox behavior and exhibited good results in the investigation of mercury by CV. In addition, The biocompatibility and synergistic effect of $[Co(CEN)_2]$/GC electrode showed an enhanced sensitivity is 4.059 ($\mu A\ \mu M^{-1}\ cm^{-2}$), LOD is 3.333 µmol/L and electrode exhibited good sensitivity, selectivity and stability. The modified biosensors essential for monitoring mercury in soil, water, food, aquatic animal, clinical samples and human living system. In this regard, spectrochemically, pharmaceutically and electrochemical results suggest that the synthesized compound act as good bio-catalytic molecule.

5. ACKNOWLEDGMENT

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Supplymentry Figures

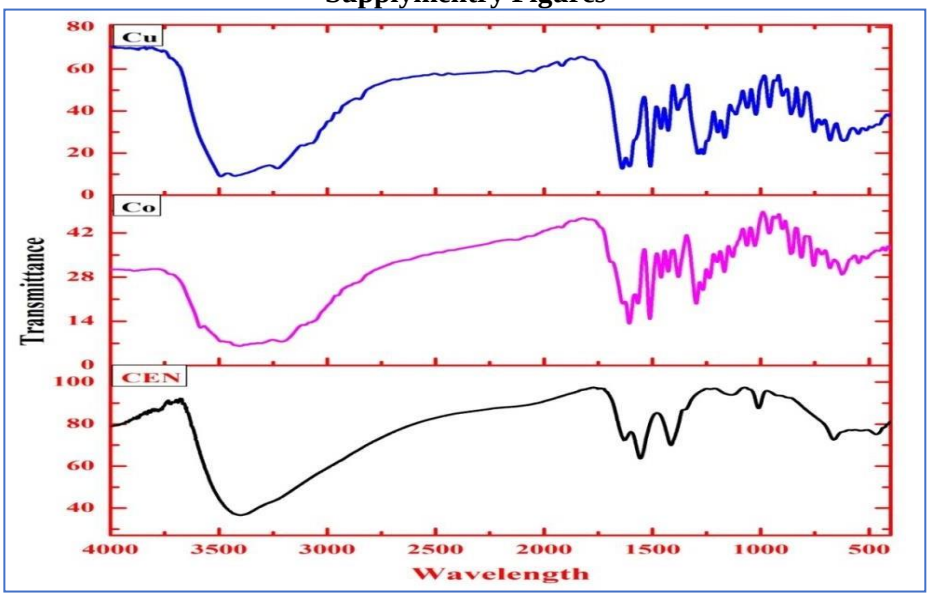

Figure S1 FT-IR spectral of CEN and their metal complexes

Graphical Abstract