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Cytotoxicity and Antibacterial Studies of Newly Synthesized Novel Heterocylcic Pyridine Derivative and its Metal Complexes

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ABSTRACT

A pyridine derivative 2-((*E*)-1-(2-hydrazinyl-4-methyl-6-phenyl-pyridine-3-carboyl)ethyl)pyridine-4-carbonitrile (CPHPC) ligand and its 3*d*-metal(II) complexes has been synthesized (where [M = Co(II), Ni(II) and Cu(II)]. The physico-chemical, analytical data, UV-Vis, FT-IR, ^{1}H NMR and ESR spectrum methods were used to characterize all of the synthesized complexes. Spectral investigations of metal(II) complexes revealed that the metal ion is surrounded by an octahedral geometry. Low conductance values indicated that the metal(II) complexes behave as non-electrolyte. The cytotoxic activity on lung cancer cell lines and hepatic cancer cell lines A549 and HepG2, respectively, with the ligand and their metal complexes were tested with MTT assay. The ligand and its metal complexes were tested for diverse harmful bacterial strains using the agar well diffusion method on Gram-negative bacteria such as *Pseudomonas desmolyticum*, *Escherichia coli* and *Klebsiella aerogenes*, as well as Gram-positive bacteria *Staphylococcus aureus*.

KEYWORDS

Pyridine derivatives, Metal(II) complexes, Cytotoxicity studies, Antibacterial activity.

INTRODUCTION

A vast number of heterocyclic compounds having pyridine ring have been linked to a variety of pharmacological effects, including anticonvulsant [1], antimicrobial [2,3], antiviral [4], anticancer [5] and anti-HlV [6], Pyridine derivatives have been produced and employed as intermediates with the parent molecule 2-chloropyridine-3-carboxylic acid and they display antifungal and anticancer properties. Recent synthetic methods to anticancer [7,8] medicines have shown that *N*-alkylated 2-pyridones are key intermediates in the production of polycyclic molecules of biological significance.

New pyridine derivatives have been synthesized, resulting in fascinating heterocyclic scaffolds that can be used to build various chemical libraries of drug-like compounds for biological screening. Recently, it is reported that terpyridine derivatives showed a promising cytotoxicity activities with A549, MCF7, A2780 cell lines [9]. Some of the pyridine derivatives (pyridine-2-carboxamidrazone), whose importance in terms of antimycobacterial activity of a number of derivatives are reported [10,11].

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Several nitrogen-containing heterocyclic systems exhibit a wide range of therapeutic activities, which is why a recent study on the synthesis of new heterocycles of 2-hydrazinyl-4-methyl-6-phenylpyridine-3-carbonitrile condensed with 2-acetylpyridine was undertaken. This can result in the development of new biologically active compounds. We designed and synthesized new pyridine derivative as well as its transition metal(II) complexes, namely Co(II), Ni(II) and Cu(II). These complexes were characterized using various techniques and also tested for cytotoxicity and antibacterial activity.

EXPERIMENTAL

The chemicals *viz*. benzoyl acetone, cyanoacetamide, sodium ethoxide, triethylamine, phosphoryl chloride, hydrazine monohydrochloride, 2-acetyl pyridine-4-carbonitrile, metal(II) chloride and cobalt(II) chloride were purchased from Sigma Aldrich. All the other chemicals and solvents were of analytical reagent grade and purchased from the commercial sources.

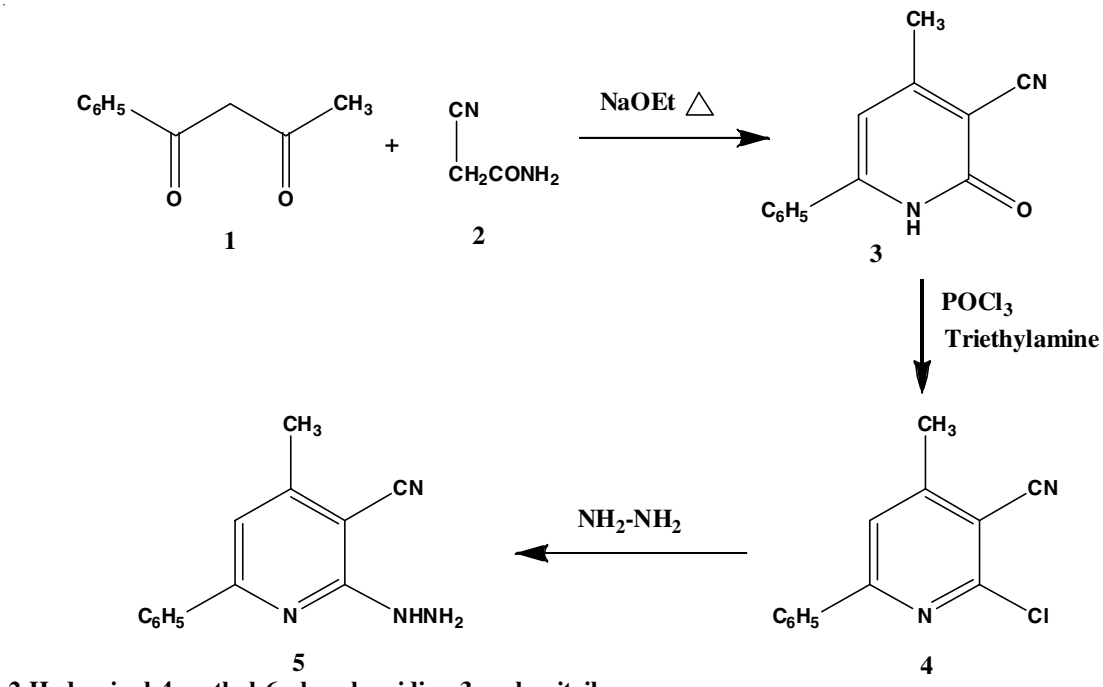
Physical measurements: The elemental analysis (C, H and N) was performed on a Perkin-Elmer 2400 CHN analyzer. Melting points were determined in open capillaries using a G LAB melting apparatus and are reported uncorrected. UV-visible spectra of DMF solutions were recorded on a UV-210 ELICO spectrometer in the spectral window of 200-800 nm. Infrared (FTIR) spectra were recorded using a Bruker FT/IR vector 22 spectrometer in the form of KBr pellets. ^{1}H NMR were recorded in solution state with AVANCE III 500 NMR spectrometer.

Synthesis of ligand: 2-Hydrazinyl-4-methyl-6-phenylpyridine-3-carbonitrile was synthesized according to the reported method [12]. In brief, hydrazine hydrochloride (2.4 g, 0.01 mmol) dissolved in 20 mL methanol was added to 2-acetylpyridine 4-carbonitrile (1 mL, 0.01 mmol) in hot 10 mL methanolic solution with the addition of few drops of conc. HCl. The reaction mixture was placed in a round bottom flask and allowed to reflux for 2 h. A yellow solid precipitate generated when the reaction mixture was cooled to room temperature. The product was collected by filtration, washed with MeOH and dried under vacuum (**Schemes I** and **II**). Yield 72%; m.p. 162-164 ºC; IR (KBr, cm^{-1}): 3320 (NH), 2235 (C≡N), 1625 (C=N), 1025 (arom. C-H); ^{1}H NMR; δ 2.34 (s, 3H CH_3), 2.43 (s, 3H, CH_3), 6.71 (s, 1H Py-H), 7.33-7.80 (m, 5H.Ar-H), 8.32 (d, 1H, Py-H), 8.52 (s, 1H, Py-H), 9.28 (d, 1H, Py-H); MS ($C_{21}H_{16}N_6$) *m/z* (%): 352.68.

Synthesis of metal(II) complexes: A hot methanolic solution (20 mL) of ligand mixture (3.52 g 0.1 mol) was added to a solution of metal(II) chlorides (M = Co^{2+}, Ni^{2+} and Cu^{2+}) (0.05 mol) in H_2O (50 mL) with addition of 2 mL of 1 M NaOH solution. The resulting colourless solution was refluxed for 2 h and then allowed to gently concentrate by evaporation at room temperature for a period of time, resulting in a solid substance which was washed with a minimum amount of MeOH and dried in air.

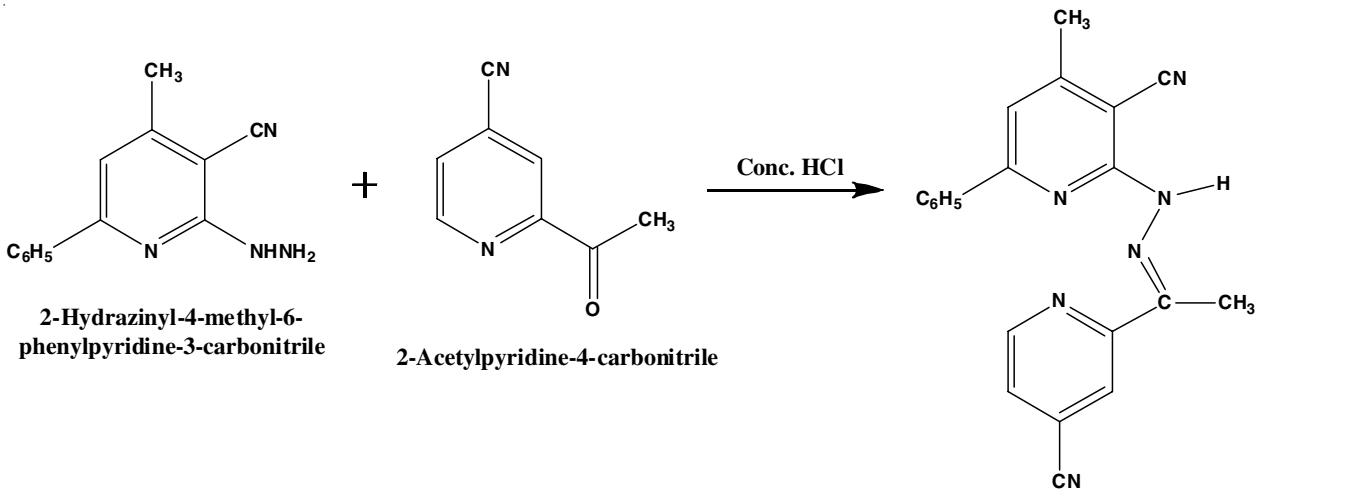
ESR spectrometry: The electronic spectrum of copper complex was determined using a JES-FA200 ESR spectrometer (ESR-JEOL, Japan) with a standard frequency (X band) of 8.75-9.65 GHz, sensitivity of 7 × 10^9 spin/0.1 mT, resolution of -2.35 μT and temperature range of +200 ºC to liquid nitrogen temperature.

Determination of cytotoxicity: Ligand and its metal(II) complexes cytotoxicity in human lung cancer (A549) and human hepatocarcinoma (HepG2) cell lines were examined in MTT assay (National Centre for Cell Science, Pune, India). Cell lines were grown in the presence of Dulbeccos Modified Eagles Medium (DMEM) with supplemented foetal bovine serum and antibiotics for 24 h at 37 ºC in a humidified atmosphere of 5% CO_2. The cells were planted at a density of 25 × 10^3 cells per well in 96-well plates. The cytotoxicity of varied



2-Hydrazinyl-4-methyl-6-phenylpyridine-3-carbonitrile

Scheme-I: Synthesis of 2-hydrazinyl-4-methyl-6-phenylpyridine-3-carbonitrile



Scheme-II: Synthesis of 2-((*E*)-1-(2-hydrazinyl-4-methyl-6-phenylpyridine-3-carboyl)ethyl)pyridine-4-carbonitrile [CPHPC]

TABLE-1
ANALYTICAL AND PHYSICAL CHARACTERIZATION DATA FOR LIGAND AND ITS METAL COMPLEXES

Compound	m.w. Found (calcd.)	m.p. (°C)	Colour	Yield (%)	Elemental analysis (%): Found (calcd.)			Molar conductivity (Ω^{-1} cm^2 mol^{-1})
					C	H	N	
CPHPC	352.68 (352.39)	162-164	Yellow	72.48	71.50 (71.57)	4.57 (4.57)	23.82 (23.85)	–
[Co(CPHPC)$_2$]	763.25 (763.71)	> 290	Dark green	69.22	66.08 (66.04)	4.22 (4.22)	22.02 (22.00)	26.36
[Ni(CPHPC)$_2$]	763.61 (763.47)	> 290	Brown	60.00	66.05 (66.06)	4.22 (4.22)	22.01 (22.01)	30.58
[Cu(CPHPC)$_2$]	767.92 (768.32)	> 290	Black	61.93	65.68 (65.64)	4.19 (4.19)	21.88 (21.87)	22.14

concentrations of metal complexes (25, 50, 100, 200 and 400 µg/mL) against human cancer cell lines were examined in MTT assay. The IC_{50} value was determined after a statistical analysis of the cytotoxicity of metal complexes against cancer lines in the presence of MTT.

Antibacterial activity: The antibacterial activity of metal (II) complexes against different pathogenic bacterial strains both Gram-negative bacteria *Pseudomonas desmolyticum* (NCIM-2028), *Escherichia coli* (NCIM-5051) and *Klebsiella aerogenes* (NCIM-2098) and Gram-positive *Staphylococcus aureus* (NCIM-5022) strains (purchased from National Centre for Cell Science, Pune, India) by agar-well diffusion method. Nutrient agar plates were prepared by dissolving 37.0 g of nutrient agar media in 1000 mL of distilled water and sterilizing them in an autoclave at (121 ºC) 15 lbs pressure for 15-20 min. Following sterilization, nutrient agar medium was poured into sterile petri dishes and allowed to solidify, followed by 100 mL of 24 h mature broth culture of individual pathogenic bacterial strains in nutrient broth while spreading all over the surface of agar plates with a sterilized L-shaped glass rod. Following that under aseptic conditions, 6 mm wells were made into each petri plate using a sterile steel cork borer. Then after, different concentration of metal complexes (100, 200, 300 and 400 µg/mL) was dispersed in 10% DMSO solution and while standard antibiotic ciprofloxacin used as a positive control and control in to the wells. The plates were incubated for 24 h at 37 ºC. Following the incubation period, the zone of inhibition around the wells was measured in millimetres. The antibacterial activity was determined in triplicate, preceded by the bactericidal activity of metal(II) complexes.

RESULTS AND DISCUSSION

A pyridine derivative 2-((*E*)-1-(2-hydrazinyl-4-methyl-6-phenyl-pyridine-3-carboyl)ethyl)pyridine-4-carbonitrile (CPHPC) ligand and its 3*d*-metal(II) complexes were found to be very stable at room temperature. All the metal complexes are soluble in DMF. Elemental analysis values were in good agreement for complexes with a ratio of 1:2. All of them were neutral and non-conducting compounds (Λ_m = 22.1-30.5 Ω^{-1} cm^2 mol^{-1}) [13]. The physico-chemical and analytical data of ligand and its metal(II) complexes are given in Table-1.

Electronic spectral bands: The electronic UV-visible spectra obtained in DMF solutions exhibit one very broad absorption band in the 760-601 nm region it is due to *d–d* transitions, a medium intense band at 318 nm region for charge transition and a strong sharp band at 298-280 nm region for π→π* transition [14-17]. The electronic spectral data of the metal(II) complexes are shown in Table-2. Electronic spectrum of [Co(CPHPC)$_2$] in high concentration (visible region) is shown in Fig. 1.

TABLE-2
UV-VISIBLE DATA OF METAL COMPLEXES

Complex	Wavelength (nm)	Frequency (cm^{-1})	Assignment
[Co(CPHPC)$_2$]	298	33,557	π→π* transition
	601	16,638	*d-d* transition
	667	14,992	*d-d* transition
[Ni (CPHPC)$_2$]	280	35,714	π→π* transition
	620	16,129	*d-d* transition
[Cu(CPHPC)$_2$]	318	31,446	CT transition
	760	13,157	*d-d* transition

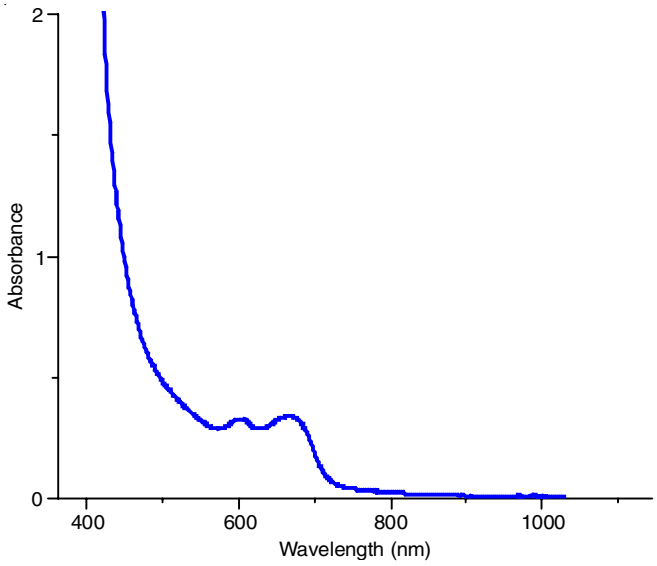


Fig. 1. Electronic spectrum of $[Co(CPHPC)_2]$ in visible region

IR spectral studies: The IR spectral data of the ligand [CPHPC] and its metal(II) complexes are given in Table-3. A medium intensity band around 3320 cm^{-1} due to ν(N-H). These observations suggest the non-involvement of atom N-H in coordination. The IR spectrum of the ligand display at 1625 cm^{-1}, which may be assigned due to the ν(C=N) azomethine. When compared to complex this band is shift lower frequency (1601 cm^{-1} to 1585 cm^{-1}) indicates the coordination of nitrogen in the complex formation. The participation of nitrogen in the coordination with the metal(II) ion is further supported by the new band appearance of ν(M-N) at 560-524 cm^{-1} in the infrared region [18-22].

ESR analysis of copper complexes: The ESR spectrum of copper (II) complex are recorded in DMF at 300 and 77 K (Fig. 2) and the spin Hamiltonian parameters of the metal-complexes are listed in Table-4. The observed spectral parameters reveals that $g_{\parallel} > g_{\perp}$ characteristic of an axially elongated octahedral geometry. The g avg value is less than 2.28 indicating the covalent character of the metal-ligand bond. Further, it is supported from the fact that the unpaired electrons lies predominantly in the $d_{x^2-y^2}$ orbital. The observed value of G for copper complex is 2.44, characteristic of mononuclear configuration, which also suggests that the exchange coupling is present and misalignment is appreciable. The α^2 value (0.72) points out appreciable in-plane covalency. The calculated value of $g_{\parallel}/A_{\parallel}$ for the copper(II) complex reveals slightly distorted structure. The orbital reduction factors such as $K_{\parallel}$ and $K_{\perp}$ are estimated from the equation. The $K_{\parallel}$ (0.307) > $K_{\perp}$ (0.234)

TABLE-3
IR SPECTRAL DATA (cm^{-1}) OF THE LIGAND AND ITS METAL(II) COMPLEXES

CPHPC	$[Co(CPHPC)_2]$	$[Ni(CPHPC)_2]$	$[Cu(CPHPC)_2]$	Assignment
3320	3295	3275	3288	N-H
2235	2210	2220	2215	CN
1625	1585	1595	1601	C==N
1025	985	987	992	Aromatic CH
	524	527	560	M-N

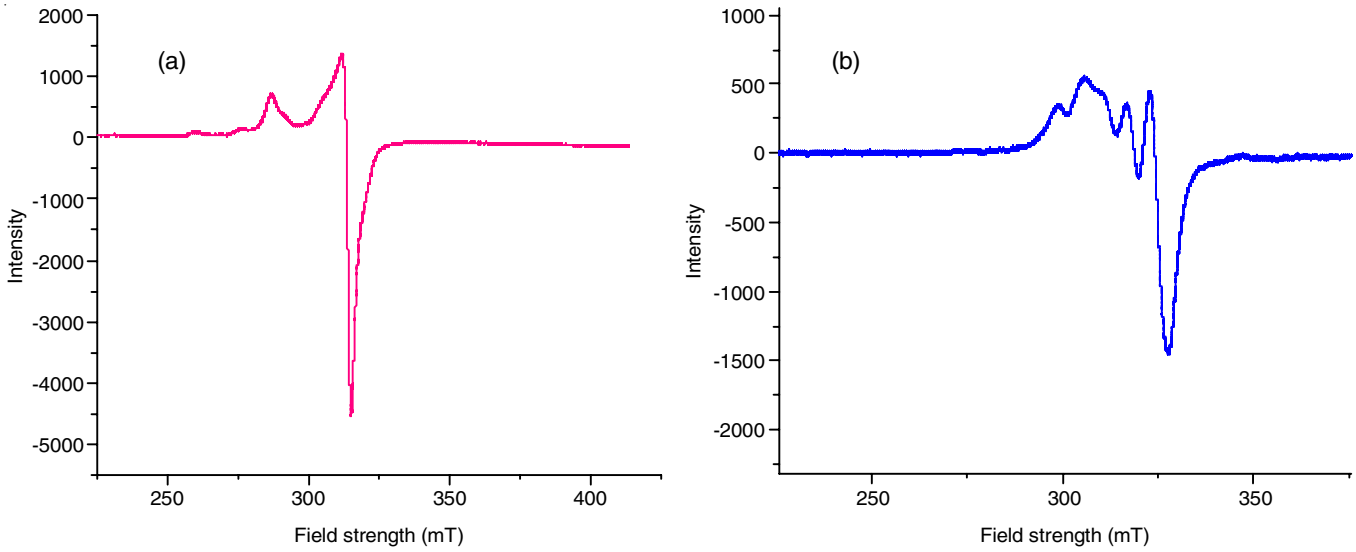


Fig. 2. ESR spectra of copper complex recorded at LNT (a) and RT (b)

TABLE-4
ESR SPECTRAL DATA OF COPPER COMPLEXES AT RT AND LNT

Complex	$g_{\parallel}$	$g_{\perp}$	g_{avg}	G	$A_{\parallel} \times 10^{-5}$	$A_{\perp} \times 10^{-5}$	$K_{\parallel}$	$K_{\perp}$	λ	α
$[Cu(CPHPC)_2]$ (RT)	2.07	2.03	2.04	2.44	–	–	–	–	–	–
$[Cu(CPHPC)_2]$ (LNT)	2.05	2.03	2.03	1.72	0.0298	0.0299	0.3077	0.234	760	0.7282

*RT = Room temperature; LNT = Liquid nitrogen temperature

indicates poor in-plane π bonding [23-27]. It has octahedral geometry, which is shown in Fig. 3.

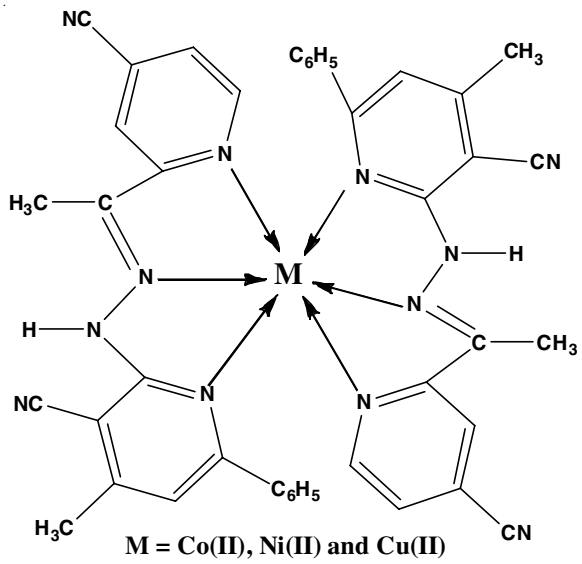


Fig. 3. Proposed structure of metal complexes

Cytotoxicity: On the basis of *in vitro* analysis, the cytotoxicity of synthesized ligand and its metal(II) complexes against A549 and HepG2 cell line cancer cells was assessed. The ligand and their metal(II) complexes were tested against cell lines with cisplatin as the positive control. The viability assay of cytotoxicity of ligand and metal(II) complexes against A549 and HepG2 cancer cell lines are given in Table-5. The decrease in cell viability with increasing metal(II) complex concentrations has shown significant cytotoxicity to accumulate the internal cells and higher stress, eventually leading to apoptosis [28-30].

Even though the concentrations were increased, the ligand alone had little effect. The activity increased dramatically as the quantities of metal(II) complexes increased. Copper(II) complex had the most activity, whereas nickel(II) and cobalt(II) complexes had identical activity with the A549 cell lines. For HepG2 cell lines, the activity show a slight different for metal(II) complexes, although the copper(II) complex has the highest activity, nickel(II) complex has a similar activity with copper(II) complex at 200 µg/mL. After that there is a drastic difference in activity as seen in the graphical representations (Figs. 4 and 5).

Antibacterial activity: The antibacterial activity of the ligand CPHPC and its metal(II) complexes was investigated. The zone of inhibition was measured in millimetres and the values of the substances studied are listed in Table-6. It is obvious

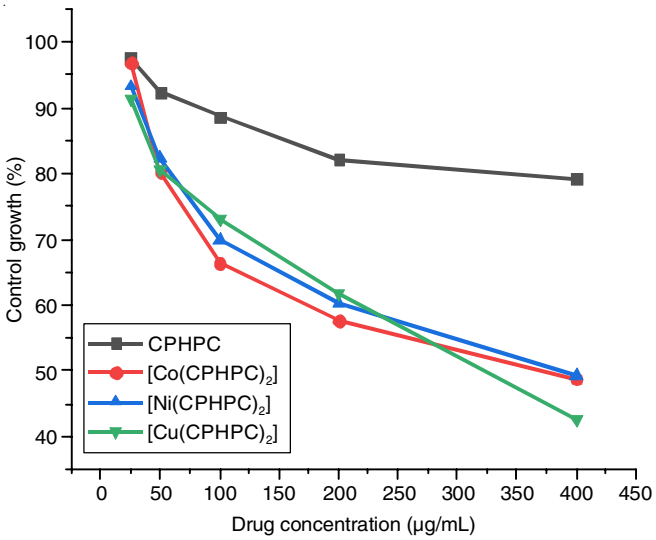


Fig. 4. Metal complexes with cytotoxicity effect on A549 cell lines

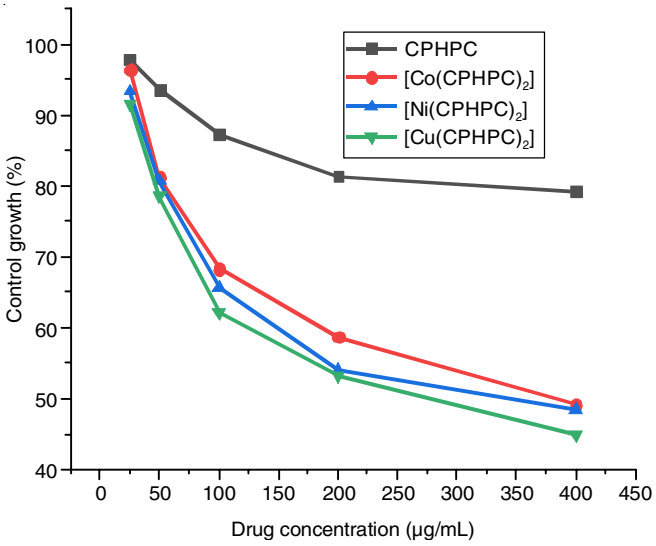


Fig. 5. Metal complexes with cytotoxicity effect on HepG2 cell lines

from the results that the metal(II) complexes have greater antibacterial action than the free ligand CPHPC. This is most likely owing to the complexes' increased lipophilicity. The increased activity of metal(II) complexes can be explained using Overton's concept and chelation theory [31-34]. One possible explanation for the observed increased activity after chelation is that the positive charge of the metal in the chelated complex is partially shared with the ligand donor atoms, resulting in electron delocalization throughout the chelate ring. This, in turn, increases the lipophilicity of the metal chelate and facilitates its permeation through the lipoid layers of bacterial membranes. Antibacterial

TABLE-5
CYTOTOXICITY OF LIGAND AND METAL COMPLEXES AGAINST A549 AND HEPG2 CANCER CELL LINES

	Concentrations (µg/mL)											
	A549						HEPG2					
	25	50	100	200	400	IC_{50}	25	50	100	200	400	IC_{50}
CPHPC	97.54	92.36	88.62	82.14	79.23	–	97.84	93.56	87.24	81.36	80.34	–
$[Co(CPHPC)_2]$	96.84	80.29	66.32	57.69	48.78	374	96.38	81.29	68.34	58.75	49.23	381
$[Ni(CPHPC)_2]$	93.25	82.31	69.95	60.26	49.23	383	93.25	80.66	65.72	54.03	48.35	343
$[Cu(CPHPC)_2]$	91.43	80.52	72.96	61.62	42.64	321	92.34	78.62	62.09	53.26	44.89	282
Standard (cisplatin)	49.09	–	–	–	–	–	54.48	–	–	–	–	–

TABLE-6
ZONE OF INHIBITION VALUES (mm) OF LIGAND AND ITS COMPLEXES AGAINST DIFFERENT PATHOGENIC STRAINS

Sample	Concentration (µg/mL)	*S. aureus*	*E. coli*	*P. desmolyticum*	*K. aerogenes*
Ciprofloxacin	10	14.10	11.45	11.52	12.06
CPHPC ligand	100	1.23	0.89	0.93	1.04
$[Co(CPHPC)_2]$	100	2.24	2.06	2.04	2.12
	200	3.34	3.12	3.16	3.23
	300	4.82	4.63	4.48	4.53
	400	5.23	5.04	4.96	4.88
$[Ni(CPHPC)_2]$	100	2.40	2.24	2.68	2.44
	200	3.92	3.86	3.84	3.76
	300	4.77	4.56	4.28	4.22
	400	5.12	5.06	4.90	5.12
$[Cu(CPHPC)_2]$	100	3.11	2.46	2.52	2.56
	200	4.26	4.02	4.14	4.05
	300	6.44	5.92	6.04	6.23
	400	9.25	8.96	9.02	9.18

activity at various concentrations of the ligand and its metal(II) complexes with different pathogenic strains are shown in Fig. 6. Copper(II) complex, when compared to other metal(II) complexes has the highest antibacterial activity.

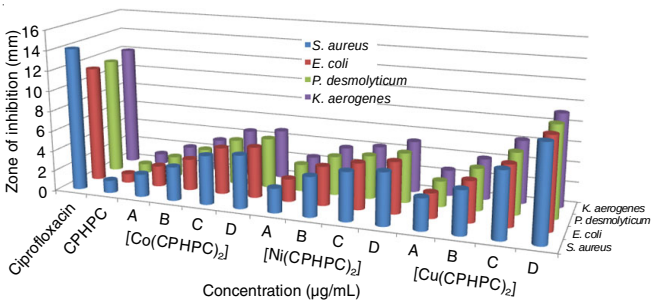


Fig. 6. Antibacterial activity at various concentrations of the ligand and their complexes with different pathogenic strains

Conclusion

A new pyridine derivative *i.e.* 2-((*E*)-1-(2-hydrazinyl-4-methyl-6-phenyl-pyridine-3-carboyl)ethyl)pyridine-4-carbonitrile ligand (CPHPC) as well as its transition metal(II) complexes (M = Cu(II), Co(II) and Ni(II) have been synthesized and characterized. The elemental analysis, conductivity and mass spectrometry revealed the complexes' stoichiometry and composition. The bonding properties of the synthesized ligand and metal(II) complexes were confirmed by FT-IR, UV-Vis, ^{1}H NMR and ESR spectral data. Based on the findings of copper(II) complex, ESR parameters suggest that the complex has an octahedral geometry. The decrease in cell viability associated with increasing metal(II) complex concentrations has resulted in considerable cytotoxicity, resulting in the accumulation of internal cells and increased stress, finally leading to apoptosis. Copper(II) complex had the highest activity, while nickel(II) and cobalt(II) complexes had the same activity for A549 and HepG2 cell lines. The antibacterial activity of the synthesized ligand (CPHPC) and its metal(II) complexes *in vitro* suggests that the complexes are more effective than the free ligand. When compared to other metal(II) complexes, copper(II) complex has the most antibacterial action.

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