



ANTIOXIDATIVE, ANTITUMOUR AND ANTIMICROBIAL PROPERTIES OF Co (II), Ni(II) and Cd(II) COMPLEXES WITH BITPY

G. Krishnaveni^{1*}, M. Seenii Mubarak², R. Elayaperumal¹ and M. Kiruthika³

¹Assistant Professor of Chemistry, J. J. College of Engineering and Technology, Tiruchirappalli-620 009, India.

²Associate Professor, Department of Chemistry, Jamal Mohamed College, Tiruchirappalli-620 020, India.

³Assistant Professor of Chemistry, Arinar Anna Government Arts College, Musiri, India.

***Corresponding Author: Dr. G. Krishnaveni**

Assistant Professor of Chemistry, J. J. College of Engineering and Technology, Tiruchirappalli-620 009, India.

Article Received on 01/02/2018

Article Revised on 22/02/2018

Article Accepted on 12/03/2018

ABSTRACT

Three transition metal (II) complexes with 4'-(1H-benzimidazol-2-yl)-2,2':6',2''-terpyridine (bitpy) ML₂ (M = Co, Ni and Cd, L = bitpy) have synthesized and characterized by using elemental analysis, UV-visible, NMR, Mass, electrical conductance, magnetic moment and IR spectroscopic techniques. The antioxidant activity of the complexes was evaluated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method. In this work, we have observed that the newly synthesized Cd (II) complex was found to be much more effective free radical scavengers than the ligand and other complexes. The antitumour activities of bitpy and its metal complexes were tested by MTT assay against human breast cancer (MCF-7) cell lines. The Cd(II) complex exhibited an potential effect against MCF-7 cell lines. Antimicrobial assay of the above ligand and complexes against two gram positive (*S.aureus*, *B.subtilis*), two gram negative (*K.aerogenes*, *E.coli*) and two fungi (*A.niger*, *C.albicans*) showed that the ligand exhibited significant activity than the other complexes.

KEYWORDS: bitpy, metal (II) complex, antioxidantant, anticancer, antimicrobial.

INTRODUCTION

The rapid spread of cancer has attracted many authors to search for new structures which may be used in designing novel potent, selective, and less toxic anticancer reagents. Organo-metallic chemistry is one of the growing areas which are widely used in the design of novel potential chemotherapeutic agents.^[1-3] For the last 30 years increased research attention has been focused on the synthesis, spectroscopic characterization, electrochemistry and biological properties of polypyridyl based metal ion complexes. The terpyridine ligands are more rigid than the bidentate ligands such as bipyridine and phenanthroline. The physicochemical and biological properties of the terpyridine metal complexes can be tuned favourably by putting a suitable substituent on the 4' position of the terpyridine. A few researchers have investigated with benzimidazolyl terpyridine metal complexes.^[4-6] In view of this an attempt has been made to synthesize and characterize the ligand L1 and its metal ion complexes with Co^{II}, Ni^{II} and Cd^{II}. so as to evaluate their electrochemical properties and biological activities such as, antioxidants, anticancer and antimicrobial activities.

Physical Parameters

C, H and N were estimated by using Elemental Vario EL III CHNS/O elemental analyser. The FT-IR and UV-vis

spectra of bitpy(L1) and its metal complexes was recorded as KBR pellets in the range of 400-4000 cm⁻¹ region using a Shimadzu FT-IR 8000 spectrophotometer and Perkin Elmer lambda 35 Uv-vis spectrophotometer in the region of 200-800 nm respectively. The ESI-MS spectra of the bitpy and its metal complexes was recorded in Finnigan LCQ 6000 advantage max ion trap mass spectrometer equipped with an electron spray source. The magnetic moments were carried out at room temperature by using a Gouy magnetic balance. Molar conductance was measured by DMF solutions at room temperature using a digital conductivity bridge, systronics direct reading conductivity meter 304 with a dip type conductivity cell. Cyclic voltammetric studies of the complexes was carried out by using three electrode systems in a single compartment comprising of glassy-carbon working electrode and potentials were referenced to standard calomel electrode.

MATERIALS AND METHODS

2-acetyl pyridine, hydrated metal(II) perchlorate are procured from Sigma Aldrich, USA and used as received. Other materials like sodium hydroxide, ammonium acetate and solvents like methanol, acetonitrile, ethanol, diethyl ether, and glacial acetic acid were of reagent grade.

Synthesis and Characterization of 4'-(1H-benzimidazol-2-yl)-2,2':6',2''-terpyridine L1.

4'-(1H-benzimidazol-2-yl)-2,2':6',2''-terpyridine (L1) was prepared^[7,8] by 2-Acetylpyridine and 1H-benzimidazole-2-carbaldehyde were combined using a mortar and pestle. The grinding was continued until the formation of an orange red powder. The powder was added to a suspension of ammonium acetate in glacial acetic acid and heated to reflux for 3 hours. The crude product was precipitated out by the addition of water. The product was filtered, washed with water and cold ethanol and it was column chromatographed using 1:1 methanol dichloromethane system to get white powder.

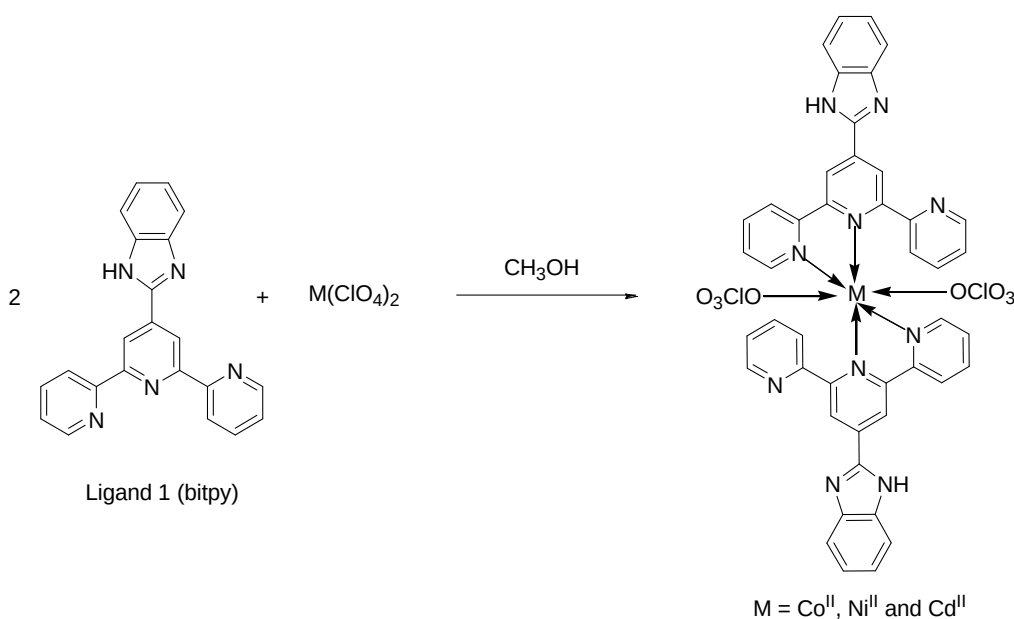
Synthesis of Cobalt (II), Nickel (II) and Cadmium (II) Perchlorato Complexes of L1

Complex 1: To a hot solution of $\text{Co}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ in methanol, bitpy dissolved in methanol was added slowly and the mixture was refluxed for 4 hour. The red

coloured solid that separated out upon slow evaporation of solvent was filtered, washed with diethyl ether and dried.

Complex 2: Hot solution of $\text{Ni}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ and bitpy in methanol were mixed and the mixture was stirred and refluxed for 4 hour. The pale green solid that separated out upon slow evaporation of the solvent was filtered, washed with diethyl ether and dried.

Complex 3: To a hot solution of $\text{Cd}(\text{ClO}_4)_2 \cdot \text{H}_2\text{O}$ in methanol, bitpy dissolved in methanol was added slowly and the mixture was stirred for 4h. The greenish yellow solid that separated upon slow evaporation of solvent was filtered, washed with diethyl ether and dried. The overall reaction between the metal perchlorate $\text{M}(\text{ClO}_4)_2$ and bitpy (L1) in methanol medium is shown in below.



Synthesis of Metal Complexes with bitpy

DPPH Radical Scavenging Assay

The free radical scavenging abilities of the terpyridyl ligand and its metal complexes have been determined by their interaction with the stable free radical 2, 2'-diphenyl-1-picryl hydrazyl.^[9-13] The reduction capability of DPPH induced by antioxidant has been determined by the decrease in its absorbance measured at 517 nm. The sample solution in DMSO (5, 10, 15 or 20 μL) was mixed with 1 mL of 1.5×10^{-5} M DPPH solution in ethanol so as to make the desired concentrations of 5-20 $\mu\text{g/mL}$ and the mixture have been kept in dark for 30 minutes. The quantity of DPPH remaining in each mixed solution has been determined by measuring the absorbance of mixed solution at 517 nm using a spectrophotometer. The decrease in the absorbance of DPPH in the mixed solution indicates the free radical scavenging activity of the test drug. L-ascorbic acid has

been used as standard and ethanol has been employed as control.

Invitro Anticancer Activity Screening- MTT Assay^[14]

3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme present in living cells namely succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple coloured formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells present in the sample. After 48 h of incubation, 15 μL of MTT (5 mg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at 37⁰ C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100 μL of DMSO and then the absorbance was measured at 570 nm using a

micro plate reader. Percentage cell growth inhibition was calculated using relative cell viability.

$$\text{Relative Cell Viability (\%)} = \frac{\text{Absorbance of test drug}}{\text{Absorbance of control}} \times 100$$

$$\% \text{ Cell Growth Inhibition} = 100 - \text{Relative Cell Viability}$$

Antimicrobial Assay

The *in vitro* antimicrobial assay of the test drugs were tested for certain bacteria and fungi by using the Disc Diffusion Method and Mueller Hinton agar medium.^[15] A loopful of strain in each was inoculated in 30 mL of the nutrient broth and incubated for 24 hours at 37°C to activate the strains. The dried surface of each agar-agar plate was swabbed with the respective suspension of the bacterial/fungal strain using a sterile cotton swab. The test drugs were dissolved in DMF to prepare stock solutions and concentrations of 100, 250 and 500 mg/mL were made by suitable dilutions. The sterilized filter paper discs were completely saturated with the test compounds and the impregnated dried discs were placed on the dried surface of inoculated agar plate. The agar plates inoculated with the bacterial organisms under test were incubated at 35°C to 37°C for 24 hours and the plates incubated with the fungi were incubated for 48 hours at the same temperature.

RESULTS AND DISCUSSION

Elemental Analyses, Mass Spectra and Molar Conductance

Elemental analyses are consistent with a 1:2 metal to ligand ratio for the complexes. Ligand 1 shows its molecular ion peak at $m/z = 349.0$ (Fig.1). The molecular ion peaks for Co, Ni and Cd complexes appeared at 954.9 (Fig.1), 955.7 and 1009.8 (Fig.2). The molecular ion peak indicates the presence of the two perchlorate groups inside the coordination sphere as well as the neutral character of the coordination complexes. The molar conductance value of the complexes was found to be 42.8, 38.5 and 42.0 $\text{S cm}^2 \text{mol}^{-1}$ in DMF solutions showing that all complexes are non electrolytic nature^[16] due to the coordination of perchlorate anions to the metal centre. The mass and elemental analysis data are confirm that the structure of the L1 and its metal complexes.

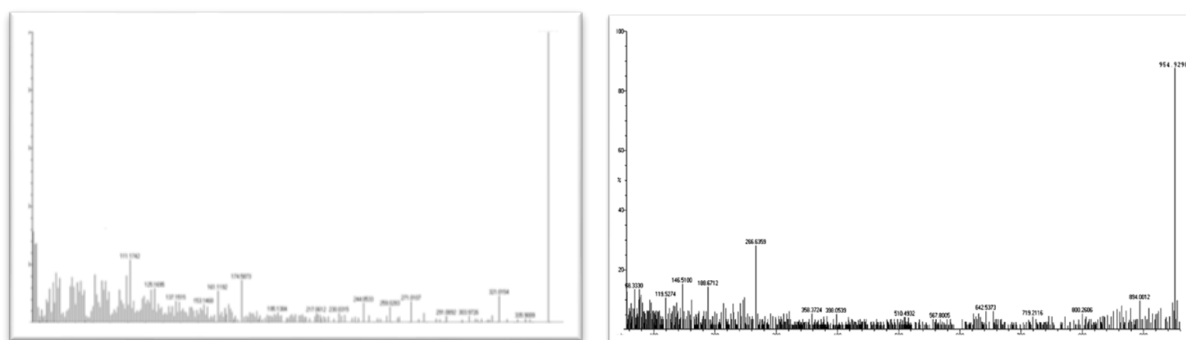


Fig.1 Mass spectra of L1 and its complex 1.

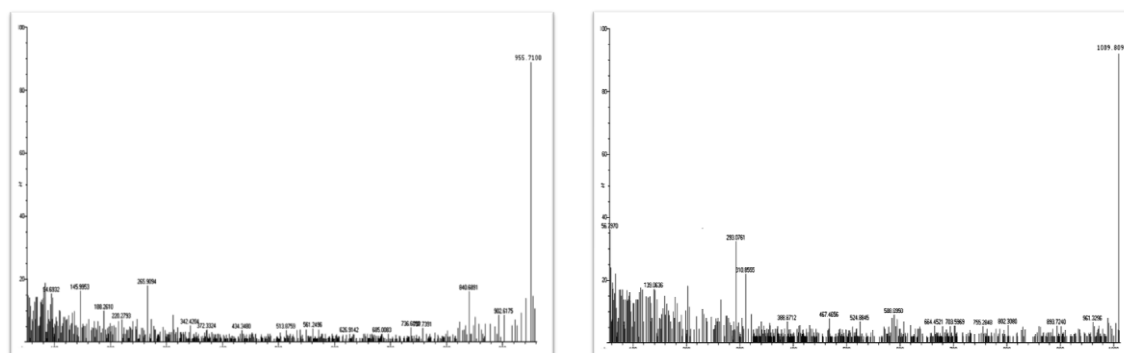


Fig. 2 Mass spectra of complex 2-3.

Electronic Spectral and Magnetic Studies on bitpy (L1) and its Metal Complexes 1-3

The L1 consist of three bands at 40486, 35336 and 31949 cm^{-1} . These bands are assigned to $\pi - \pi^*$ and $n - \pi^*$ transition (Fig.3). Generally compounds containing hetero atoms such as N, O, S etc., have nonbonding electrons (n) in addition to σ and π electrons. Thus the

terpyridine derivative shows both $\pi - \pi^*$ and $n - \pi^*$ transitions in its UV spectrum. Complex 1 showed three bands (Fig.3) at 20460, 17036 and 30488 cm^{-1} . These bands are assigned to ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{1g}(\text{P})$, ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{A}_{2g}(\text{F})$ and charge transfer band (Fig.2). Complex 2 showed three bands (Fig.3) at 28028, 17094 and 350450 corresponds to ${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{1g}(\text{P})$, ${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{1g}(\text{F})$

and charge transfer band (Fig.2).^[17-20] The magnetic moment of the Complex 1-3 was found to be 5.1-2.98 BM.^[20] These results are typically characteristic for an octahedral configuration for the metal complexes. The metal atom in the greenish yellow coloured Cd complex belongs to the $4d^{10}$ system and cannot exhibit d-d

electronic transition, but its colour may be due to charge transfer transition measured at 31847 cm^{-1} . However, based on results of elemental analysis, conductance, mass spectrum and IR spectrum the cadmium(II) complex is also proposed to have the octahedral geometry.

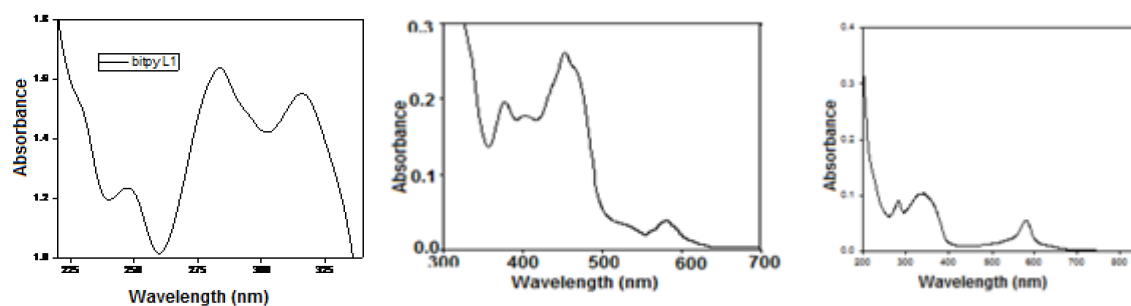


Fig. 3 UV - visible Spectrum of bitpy (L1) and complex 1-2.

IR spectroscopy

The IR spectra of bitpy (L) and its metal complexes exhibit several diagnostic vibrational frequencies of respective functional groups (Table 1). IR spectrum of L1 displays bands (Fig 4) at 3399 , 3057 - 2922 , 1523 - 1431 , 624 and 434 cm^{-1} assigned to $\nu(\text{NH})$, $\nu(\text{CH})$, pyridine ring skeletal vibrations ($\nu\text{C}=\text{C}$ and $\nu\text{C}=\text{N}$), in-plane and out-of-plane deformations respectively. The Complex 1-3 (Fig 4,5) $\nu\text{C}=\text{C}$ and $\nu\text{C}=\text{N}$ bands have

shifted to higher position (Table 1) due to the coordination of the pyridyl nitrogen atoms to the metalion.^[21] The pyridine ring in-plane deformation and out-of-plane deformation confirming the coordination of two nitrogen atoms at 1' and 1' positions.^[22-24] The new band at 500 - 520 cm^{-1} was also being observed in the complexes and was not found in the free ligand. These bands attributed to M-N and M-O bonds in complexes 1-3^[25] respectively.

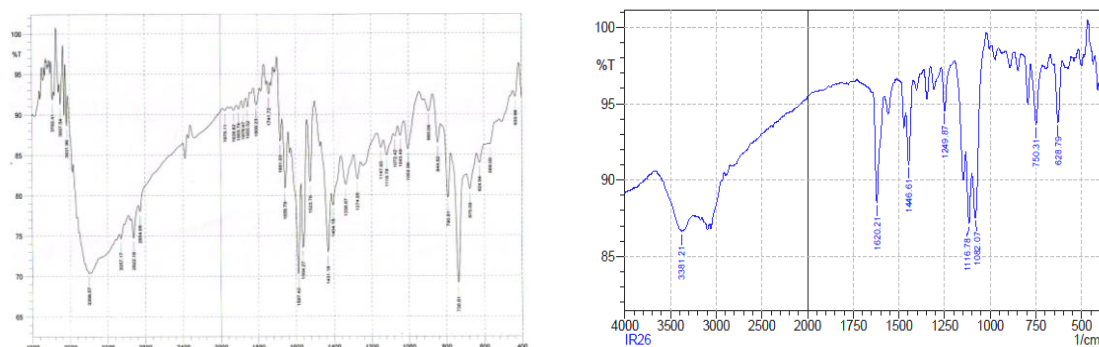


Fig. 4 IR Spectrum of L1 and its Complex 1.

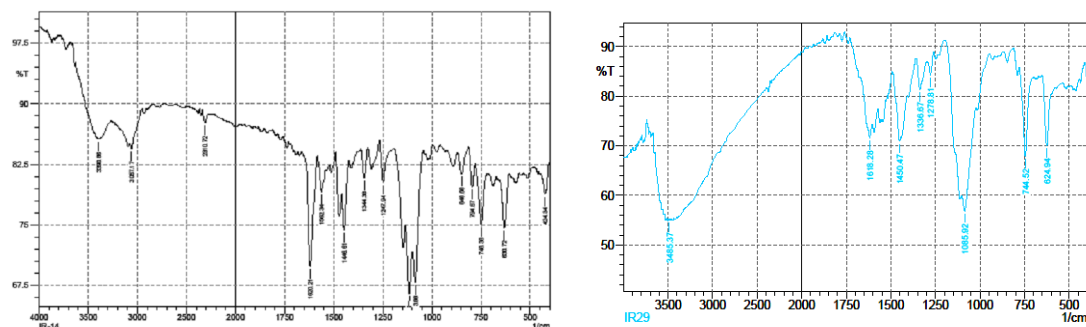


Fig. 5 IR Spectrum of Complex 2-3.

Table 1: IR Absorption Frequencies (cm⁻¹) for bitpy (L1) and its Metal Complexes (1-3).

S. No	Compound	ν_{NH}	ν_{CH}	$\nu_{\text{C=N}} \ \& \ \nu_{\text{C=C}}$ of Terpyridine	In plane pyridine ring deformation	Out-of-plane pyridine ring deformation	ν_{ClO}	$\nu_{\text{MO}} \ \& \ \nu_{\text{MN}}$
1	bitpy(L1)	3399	3057 - 2922	1523 - 1431	624	434	-	-
2	[Co(bitpy) ₂ (ClO ₄) ₂] Complex 1	3381	3094	1516 - 1447	629	500	1117 1082	500
3	[Ni(bitpy) ₂ (ClO ₄) ₂] Complex 2	3391	3057	1562 - 1447	632	516	1115 1084	516
4	[Cd(bitpy) ₂ (ClO ₄) ₂] Complex 3	3485 - 3300	-	1550 - 1450	625	520	1115 1086	520

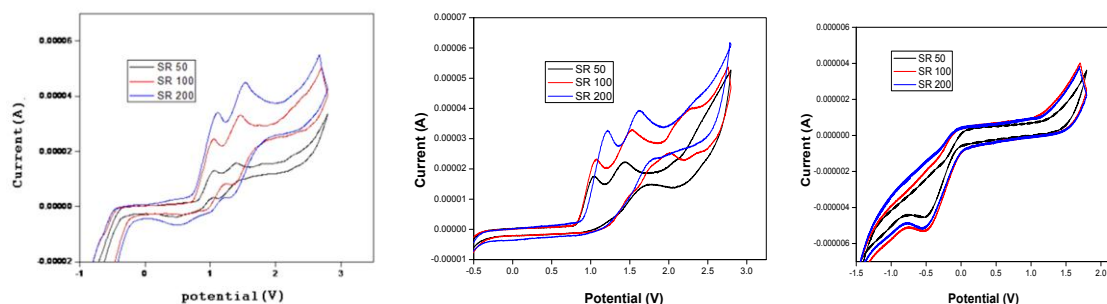
Cyclic Voltammetry

Electrochemical studies have been carried out on complexes 1-3 (Fig 6). The cyclic voltammograms have been recorded at three different scan rates viz. 50, 100 and 200 mVs⁻¹. Complex 1-3, the values (Table 2) of E_{pa} indicate the energy required for oxidation and the values of E_{pc} indicate the energy required for reduction of the metal ion.^[26-27] The ΔE_{p} values are higher than 200 mV in complex 1-2, indicates an irreversible redox couple.

But complex 3 showed less than 200 mV but higher than 60 mV suggest a quasi-reversible redox process. The peak current ratio for complex 1,3 are less than unity suggesting that the electron transfer is followed by chemical reaction. i.e. EC mechanism is followed. But the complex 2, these ratios are higher than unity and indicate that the electron transfer is not followed by a chemical reaction. The positive values of $E_{1/2}$ show that the metal complexes cannot undergo easy reduction.

Table 2: Electrochemical Parameters Measured for Co, Ni and Cd Complexes with bitpy.

Compound	Scan Rate (mVs ⁻¹)	E_{pa}^1 (V)	E_{pa}^2 (V)	E_{pc} (V)	ΔE_{p} (mV)	$E_{1/2}$ (V)	$I_{\text{pa}}^1 \times 10^{-6}$ (A)	$I_{\text{pa}}^2 \times 10^{-6}$ (A)	$I_{\text{pc}} \times 10^{-6}$ (A)	$I_{\text{pa}}/I_{\text{pc}}$
[Co(bitpy) ₂ (ClO ₄) ₂] Complex 1	50	1.0488	1.3794	1.0026	376.8	1.191	1.3147	1.5672	3.0819	0.5085
	100	1.151	1.5488	1.099	449.8	1.3239	2.4273	3.2841	7.8633	0.4176
	200	1.2373	1.6489	1.3606	288.3	1.5047	3.3803	4.4524	2.3131	1.9248
[Ni(bitpy) ₂ (ClO ₄) ₂] Complex 2	50	1.0257	1.4238	1.7436	-319.8	1.5837	1.7388	2.2552	1.471	1.5331
	100	1.1112	1.551	1.9716	-420.6	1.7613	2.3278	3.2721	2.518	1.2994
	200	1.1963	1.6068	1.7667	-159.9	1.6867	3.2229	3.9267	2.2699	1.7299
[Cd(bitpy) ₂ (ClO ₄) ₂] Complex 3	50	-	0.0676	0.0056	62	0.0366	--	2.8688	-6.0655	-0.4729
	100	-	0.0476	-0.0706	118.2	0.0591	--	2.7759	-8.5464	-0.3248
	200	-	0.4469	0.3850	62	0.416	--	0.6398	0.5453	1.1733

**Fig.6 Cyclic Voltammograms of Complex 1-3 at 50, 100 and 200 mVs⁻¹****Antioxidant activity screening**

The bitpy and its metal complexes were evaluated for DPPH radical scavenging activity.^[28, 29] The antioxidant activity of each test drug increases with increase in its concentration. The determined results (Table 3) show that the bitpy (L1), complex 2,3 exhibits the highest activity at 94.74% while the complex 1 displays the

lowest activity at 92.1% at the concentration of 20 µg/mL.

Table 3: *Invitro* Antioxidant Activities of bitpy (L1) and its Metal Complexes (1-3).

Sl. No	Test Drug	Concentration (µg/mL)	Absorbance	% DPPH Scavenging Activity
1.	bitpy(L1)	05	0.19	50.00
		10	0.10	73.68
		15	0.05	86.84
		20	0.02	94.74
2.	[Co(bitpy) ₂ (ClO ₄) ₂] Complex 1	05	0.19	50.00
		10	0.10	73.68
		15	0.07	81.58
		20	0.03	92.11
3.	[Ni(bitpy) ₂ (ClO ₄) ₂] Complex 2	05	0.17	55.26
		10	0.09	76.32
		15	0.04	89.47
		20	0.02	94.74
4.	[Cd(bitpy) ₂ (ClO ₄) ₂] Complex 3	05	0.17	55.26
		10	0.09	76.32
		15	0.04	89.47
		20	0.02	94.74
5.	Standard (Vitamin C)	2.0	0.15	60.52
		2.5	0.10	73.68
		3.0	0.05	86.84
		3.5	0.01	97.34
6.	Control (Ethanol)	-	0.38	-

Antiproliferative Effect of L1 (bitpy) and its Metal Complex

The present study examined the efficacy of Ligand - 1 and its metal complexes 1-3 to inhibit the human breast cancer cell lines (MCF-7) at 24 h as determined by MTT assay. The cells were treated with five different concentrations (Table 4-7) of test drugs ranging from 0.25 to 100 µM. The free ligand as well as the complexes suppressed the growth of breast cancer cell lines in a dose dependent manner.

The IC₅₀ values for free ligand(L1), Co^{II}, Ni^{II} and Cd^{II} complexes treated MCF-7 cells were obtained at 51.16, 34.06, 13.7 and 1.36 µM or at 17.85, 32.52, 13.09 and 1.37 µg/mL respectively. Overall, IC₅₀ value for Cd^{II} complex was found to be less than 2 µg/mL, suggesting that Cd^{II} could exert very strong anti-proliferative effect when compared to the free ligand and other complexes tested with breast cancer cell lines at low doses. Also the Ni^{II} complex measuring IC₅₀ value of 13.09 µg/mL may be considered to exert moderate anti-cancer activity.^[30-31]

Table 4: Percentage Cell Growth Inhibitions and IC₅₀ Values Determined for bitpy (L1)

Concentration of Test Drug (µM)	Mean Absorbance	Relative Cell Viability	Percentage Cell Growth Inhibition	IC ₅₀	R ²
0.25	0.387	96.52	3.48	51.16 µM or 17.85 µg/mL	0.9834
2.50	0.374	93.2	6.80		
25.00	0.296	73.86	26.14		
50.00	0.190	47.39	52.61		
100.00	0.127	31.79	68.21		
Control	0.401	-	-		

Table 5: Percentage Cell Growth Inhibitions and IC₅₀ Values Determined for Co(II) Complex.

Concentration of Test Drug (µM)	Mean Absorbance	Relative Cell Viability	Percentage Cell Growth Inhibition	IC ₅₀	R ²
0.25	0.369	97.97	2.03	34.06 µM or 32.52 µg/mL	0.9853
2.50	0.356	94.35	5.65		
25.00	0.252	66.88	33.12		
50.00	0.105	27.83	72.17		
100.00	0.062	16.44	83.56		
Control	0.377	-	-		

Table 6: Percentage Cell Growth Inhibitions and IC₅₀ Values Determined for Ni(II) Complex.

Concentration of Test Drug (μM)	Mean Absorbance	Relative Cell Viability	Percentage Cell Growth Inhibition	IC ₅₀	R ²
0.25	0.352	93.38	6.62	13.7 μM or 13.09 μg/mL	0.9919
2.50	0.319	84.55	15.45		
25.00	0.136	36.22	63.78		
50.00	0.083	23.68	76.32		
100.00	0.029	7.87	92.13		
Control	0.377	-	-		

Table 7: Percentage Cell Growth Inhibitions and IC₅₀ Values Determined for Cd(II) Complex.

Concentration of Test Drug (μM)	Mean Absorbance	Relative Cell Viability	Percentage Cell Growth Inhibition	IC ₅₀	R ²
0.25	0.312	77.76	22.24	1.36 μM or 1.37 μg/mL	0.983
2.50	0.167	41.58	58.42		
25.00	0.009	2.25	97.75		
50.00	0.000	0.00	100.00		
100.00	0.000	0.00	100.00		
Control	0.401				

Antimicrobial activity

Bacterial species such as *S.aureus*, *B.subtilis*, *K.aerogenes* and *E.coli* and fungal species such as *A.niger* and *C.albicans* were used in the study. Ciprofloxacin, Nystatin was the standard antibiotics used in the antibacterial and fungal screening. The inhibition activities of the test compound increase with increase of concentrations of test compound (Table 5, 6, Plate1-4). A comparison of the activities of the standard, bitpy (L1) and its metal complexes (1-3) against *S.aureus* shows the order : ciprofloxacin > bitpy > Co > Cd. Comparing the antibacterial activities of test drugs against *B.subtilis* shows the order : ciprofloxacin > bitpy ~ Co > Ni. Here the Cd^{II} complex shows nil activity against *B.subtilis*. In the case of *K.aerogenes* the activities decrease in the

order: ciprofloxacin > Co > bitpy. When the test drugs are assayed against *E.Coli*, it is observed that the Ni^{II} and Cd^{II} complexes do not display any activity and the order of activity shown by other compounds is ciprofloxacin > bitpy. Thus it is seen that a few compounds tested are not sensitive to the bacteria used.

The antifungal activity results indicate that the test drugs show enhanced activity against the test fungi (*A.niger* and *C.albicans*) when the concentrations of drugs are increased.. The activities of test drugs against *A.niger* decrease in the order : nystatin > bitpy ~ Ni > Co > Cd. The sensitivities of test drugs to *C.albicans* are found to decrease in the order : nystatin > Ni > bitpy > Co.

Table 8: Antibacterial Activities of bitpy and its Metal Complexes (1-3).

Sl. No	Test Drug	Zone of Inhibition (mm)											
		<i>S. aureus</i>			<i>B. subtilis</i>			<i>K. aerogenes</i>			<i>E. coli</i>		
		100 mg/L	250 mg/L	500 mg/L	100 mg/L	250 mg/L	500 mg/L	100 mg/L	250 mg/L	500 mg/L	100 mg/L	250 mg/L	500 mg/L
1	bitpy(L1)	17	20	24	12	16	18	8	8	10	15	16	20
2	[Co(bitpy) ₂ (ClO ₄) ₂] Complex 1	14	16	20	14	15	18	18	20	24	13	16	18
3	[Ni(bitpy) ₂ (ClO ₄) ₂] Complex 2	-	-	-	8	10	15	-	-	-	-	-	-
4	[Cd(bitpy) ₂ (ClO ₄) ₂] Complex 3	10	15	18	-	-	-	-	-	-	-	-	-
5	Ciprofloxacin Standard	-	-	35	-	-	40	-	-	30	-	-	38

Table 9: Antifungal Activities of bitpy and its Metal Complexes (1-3).

Sl. No	Test Drug	Zone of Inhibition (mm)					
		<i>Aspergillus niger</i>			<i>Candida albicans</i>		
		100 mg/L	250 mg/L	500 mg/L	100 mg/L	250 mg/L	500 mg/L
1	bitpy(L1)	16	16	20	15	18	19
2	[Co(bitpy) ₂ (ClO ₄) ₂] Complex 1	15	16	19	10	14	16
3	[Ni(bitpy) ₂ (ClO ₄) ₂] Complex 2	15	18	20	10	16	21
4	[Cd(bitpy) ₂ (ClO ₄) ₂] Complex 3	08	09	15	-	-	-
5	Nystatin Standard	-	-	30	-	-	25

Note: Zone size less than 15 mm - Least active; 16 – 20 mm - moderately active; Above 20 mm – highly active

*Streptococcus aureus**Bacillus subtilis**Klebsiella aerogenes**Escherichia coli**Aspergillus niger**Candida albicans***Plate 1. Antibacterial and Antifungal Activities of Ligand 1.***Streptococcus aureus**Bacillus subtilis**Klebsiella aerogenes**Escherichia coli**Aspergillus niger**Candida albicans***Plate 2. Antibacterial and Antifungal Activities of Complex 1.**

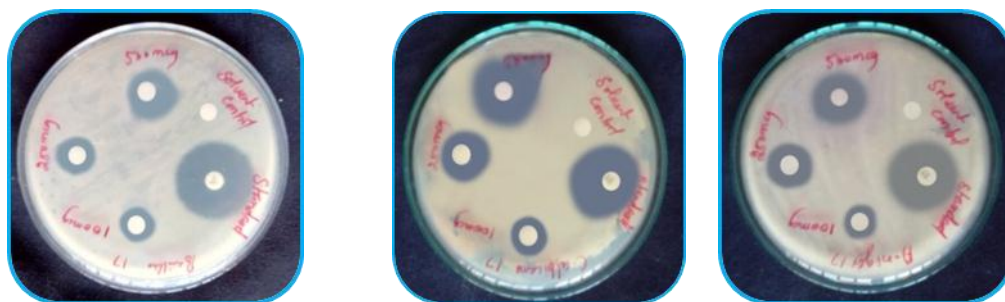
*Bacillus subtilis**Aspergillus niger**Candida albicans*

Plate 3. Antibacterial and Antifungal Activities of Complex 2.

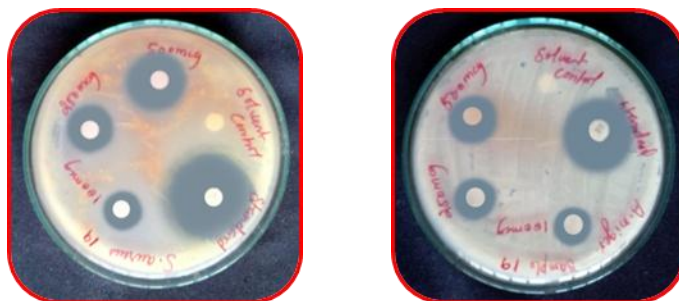
*Streptococcus aureus**Aspergillus niger*

Plate 4. Antibacterial and Antifungal Activities of Complex 3.

CONCLUSION

In this work, we have reported the synthesis of bitpy (L1) and its metal complexes. The structural characterizations of the synthesized compounds were made by spectroscopic methods (FT-IR, Electronic, ESI-MS), elemental analysis, electrical conductance, magnetic studies and cyclic voltammetry. From the spectroscopic characterization, it is concluded that bitpy act as bidentate ligands, coordinating through its two 1, 1' positions to the metal. The biological activity screening showed that the complexes selectively inhibited the bacterial (*S.aureus*, *B.subtilis*, *K.aerogenes* and *E.coli*) and fungal (*A.niger*, *C.albicans*) species, the ligand has more activity than other complexes. The antioxidant properties of ligand and complexes have been tested using DPPH radical scavenging method in which ligand and its metal complexes exhibited potential antioxidant activity. Complex 3 shows strong antiproliferative effect on the breast cancer cell lines. However, further studies on the mechanisms of antioxidant and invivo study of anticancer are required.

ACKNOWLEDGEMENTS

The authors are thankful to SAIF-IIT Chennai, SMS-EUMIC lab, Periyar Maniyammai Pharmaceutical College –Tiruchirappalli, India.

REFERENCES

- Alardyce C S, Dorcier A, Scolaro C, Dyson P J.(Development of Organometallic (Organo-transition Metal) Pharmaceuticals). Applied organometallic chem, 2005; 19: 1-10.
- Van Riji S H, Sadler P J. (Current Application and future potential for bioinorganic chemistry in the development of anticancer drugs). Drug Discovery Today, 2009; 14(23-24): 1089-1097.
- Li P Z, Liu Z Q. (ferrocenyl substituted curcumin:can it influence antioxidant ability to protect DNA) Eur. J. Med. Chem, 2011; 46(5): 1821-1827.
- Rajalakshmi S, Weyhermuller T and Unni Nair B. (Cu (II) complexes of terpyridine derivatives: A Footstep towards development of antiproliferative agent for breast cancer). J. Inorg. Biochem, 2012; 117: 48-59.
- Elayaperumal R and Dharmalingam P. (Metallonuclease activity of copper(II) complexes containing benzimidazolyl terpyridine). Der Chemica Sinica, 2013; 4: 170- 176.
- Kiruthika M, Elayaperumal R and Vennila T. (Synthesis, Characterisation And DNA Interactions Of A Mixed Ligand Ruthenium(II) Complex). Int. J.Chem Tech. Res, 2013; 5(4): 1839-1846.
- Cargill Thompson AMW. (The Synthesis of 2,2';6'2''-tepy ligands-Versatile building blocks for Sup Mole Chem). Coord Chem Rev, 1997; 160: 1-52.
- Cave GWV, Raston CL. (Effi Synthesis of Pyridine via a Sequential solvent less aldol Condensation and Michael Addition). J Chem Soc Perkin Trans, 2001; 1: 3258-3264.
- Ancerewicz J, Migliavacca E, Carrupt PA, Testa B, Dree FJ, et al. (Structure Prop relationships of trimetazidine derivatives and model compounds as potential antioxidants). Free Radical Biol Med, 1998; 25: 113-120.
- Karki SB, Treemanekam V, Kaufman MJ. (Oxidation of HMG-CoA reductase Inhib. By Tert-butoxyl and DPPH radicals; model reactions for

- predicting oxidatively sensitive compound during preformulation). *J Pharm Sci*, 2000; 89: 1518-1524.
11. Moridani MY, Pourahmad J, Bui H, Siraki A, O'Brien PJ. (Dietary flavonoid Fe complexes as cytoprotective superoxide radical scavengers). *Free Radical Biol Med*, 2003; 34: 243-253.
 12. Oktay M, Gulcin I, Kufrevioglu OI, (Determination of invitro antioxidant activity of Fennel seed extracts). *Liebensm Wiss U Technol*, 2003; 36: 263-271.
 13. Saito S, Kawabata J. (Effect of electron-withdrawing substance on DPPH Radi scavenging rea of protocatechuic acid its analogues in alcoholic solvents). *Tetrahedron*, 2005; 61: 8101-8108.
 14. Alley M.C, Scudiero D A, Monkes A, Hursey M L, Czerwinski M J, Fine D L, Abbott B J, Mayo J G, Shoemaker R H and Boyd M R. (Tetrazolium-based Assays for Cellular Viability: A Critical Examination of Selected Parameters Affecting Formazan Production). *J. Cancer Res*, 1988; 48:589.
 15. Verma RS, Nobles WL. (Antiviral, antibacterial and antifungal activities of isatin-N- Mannich bases). *J. Pharm Sci*, 1975; 64: 881-882.
 16. Geary WJ. (The use of conductivity measurements in organic solvents for the characterisation of coordination compounds). *Coord Chem Rev*, 1971; 7: 81-122.
 17. Nakamoto K, *Infrared and Raman Spectra of Inorganic and Coordination Compounds*: New York; Wiley Interscience, 1978.
 18. Lever ABP. *Inorganic Electronic Spectroscopy*: Elsevier; Amsterdam, 2000.
 19. Drago RS, *Physical Methods in Inorganic Chemistry*: New Delhi; East West Press, 1978.
 20. Figgis BN, *Introduction to Ligand Fields*: New Delhi; Wiley Eastern Ltd, 1976.
 21. Cotton FA, Wilkinson G: *Advanced Inorganic Chemistry*: New York; Wiley Interscience, 1972.
 22. Mallet C, Thummel RP, Hery C. (The conformational Proproperty of Eu complexes of polymethylene bridged derivatives of tepy). *Inorg Chim Acta*, 19932; 10: 223-231.
 23. Constable EC, Cargill Thompson AMW, (Novel didentate tepy Complexes of Ru). *Inorg Chim Acta*, 1994; 223: 177-179.
 24. Aimeloglou N, Michael PKB, Drew GB, Glaeser B, Holland F, et al. (2,2';6',2''-Tepy. Complexes of Mo and W). *J Organomet Chem*, 2000; 604: 191-196.
 25. Ferraro JR: *Low Frequency Vibration of Inorganic and Coordination Compounds*: New York; Plenum Press, 1971.
 26. Bard AJ, Isata LR: *Electrochemical Methods: Fundamentals and Applications*. New York; Wiley, 2001.
 27. Bard AJ, Faulkner LR: *Electrochemical Methods: Fundamentals and Applications*: John Wiley and Sons, USA, 1980; 193-197.
 28. Wu HC, Chen HM, Shiau CY., (Free Amino acids and peptides as related to antioxidant prop in protein hydrolysates of mackerel). *Food Res Int*, 2003; 36: 949-957.
 29. Ancerewicz J, Migliavacca E, Carrupt PA, Testa B, Dree F, et al. (Structure Prop Relation of trimetazidine derivatives and model compound as potential antioxidants). *Free Radical Biol Med*, 1998; 25: 113-120.
 30. Shier W T; "A Laboratory Manual of Low Cost Methods": Los Banos, *University of Pillippines*, 1991.
 31. Tofazzal M, Tarafder H, Asmadi A, Talib M S, Ali A M and Crouse K A. (Studies on coordination chemistry of a N-S donor ligand with ighter and heavier metal ions and biological activities of its complexes). *J. Trans. Met. Chem*, 2001; 26: 170-174.