



Synthesis, Spectral Characterization, Electrochemical Behaviour, Invitro Antimicrobial and DPPH Radical Scavenging Activities of Iron (II), Cobalt (II) Complexes with imidazolyl terpyridine

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Abstract: Imidazole terpyridine (L) and its Fe (II), Co(II) metal complexes were synthesized. IR, UV-vis, Mass, elemental analysis, electrical conductance and magnetic moments is techniques were applied for characterization. Cyclic voltammetry studies of Fe (II) and Co(II) complexes in DMSO show that the complexes able to stabilize low oxidation states of Fe(I) and Co(I). The complexes were evaluated for antimicrobial and DPPH radical scavenging activities. They showed varying degree of activity with the values between 28.95-97.36 μm in DPPH radical scavenging activity. These synthesized metal complexes were found to be less active than the ligand and standard for DPPH radical scavenging activity. The antimicrobial activities of the metal complexes were found to be more active than the ligand and the antifungal activity of the ligand, Fe (II) complex are insensitive.

Keywords: itpy, DPPH, antimicrobial, CHN elemental analysis, cyclic voltammetry.

1. Introduction

Imidazole and its derivatives form complexes with a number of transition metal ions. Imidazole is biologically the most important since the imidazole nitrogens of histidyl residues coordinate to metal ions in many metalloproteins, Azathioprine, dacarbazine and metronidazole are some examples of drugs possessing imidazole moiety. Imidazolyl terpyridine metal complexes have shown wide range of DNA binding behaviours and cytotoxic effects. Iron is an essential element involved in an enormous range of functions. It is necessary for haemoglobin synthesis and oxidative processes of living tissues, as it exists at the active site of molecules responsible for oxygen transport and electron transport¹. Since the first reported studies into the biological activity of Co complexes² in 1952, diverse structurally characterized cobalt complexes showing antitumor³,⁴ antimicrobial^{5,6} antifungal^{7,8} antiviral^{9,10} and antioxidant¹¹ activities have been reported. We have synthesized 4'-(1H-imidazol-2-yl)-2,2':6',2''-terpyridine and its metal (bivalent Fe, Co) complexes and studied their biological activities.

2. Experimental

2.1 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 itpy and its metal complexes were 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 itpy and its metal complexes was recorded in Finnigan LCQ6000 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 were 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.

2.2 Materials and Methods

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

2.3 Synthesis and Characterization of 4'-(1H-Imidazol-2-yl)-2,2':6',2''-terpyridine

4'-(1H-imidazol-2-yl)-2, 2':6', 2''-terpyridine (itpy) was synthesized using a slight modification of known procedure available in literature^{12,13}. 2-Acetyl pyridine (1.12 ml, 10mmol) and 1H-imidazol-2-carbaldehyde (0.73g, 5 mmol) were intimately mixed by grinding the mixture in a mortar and pestle until the formation of an orange red powder. The powder was added to a suspension of ammonium acetate (2.5 g) in glacial acetic acid (10 ml) and heated to reflux for 3 hr. The crude product was precipitated by the addition of water (5 ml). The product was filtered, washed with water and then with cold ethanol. It was column chromatographed using silica gel and 1:1 methanol-dichloromethane solvent system. (Yield: 1.43 g, 82 %). Elemental analysis, found (calcd) for itpy: C, 71.93 (72.24); H, 4.22(4.35); N, 23.07(23.41); mass $m/z=299.0$.

2.4 Synthesis of Iron (II), Cobalt (II) Perchlorate Complexes with itpy

Complex 1: To the hot solution of hydrated $\text{Fe}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ (50mg, 0.196 mmol) in methanol, methanolic solution of itpy (117.49mg, 0.39 mmol) was added slowly and the mixture was stirred for 1h. The violet colored solid separated out upon slow evaporation of the solvent. It was filtered, washed with diethyl ether and then dried. Yield: 1.03 g, 81%. Elemental analysis, found (cal) for itpy: C, 49.95 (50.65); H, 2.94(3.05); N, 15.91(16.42); M, 6.40(6.55); mass, $m/z=853.0$.

Complex2: To the hot solution of $\text{Co}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ (100mg, 0.273 mmol) in methanol, methanolic solution of itpy (163.6 mg, 0.55 mmol) was added slowly and the mixture was stirred for 1h. A red brown solid crystallized on slow evaporation of the solvent. It was filtered, washed with diethyl ether and then dried. Yield: 1.0 g, 75 %. Elemental analysis, found (cal) for itpy: C, 49.26 (50.47); H, 2.90(3.04); N, 17.02(16.36); M, 6.89(6.88); mass, $m/z=855.9$.

2.5. 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 medium¹⁴. 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 – 37 °C for 24 hours and the plates incubated with the fungi were incubated for 48 hours at the same temperature.

2.6 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 hydrazyl¹⁵⁻¹⁹. The reduction capability of DPPH induced by antioxidants has been determined by the decrease in its absorbance measured at

517nm .The sample solution in DMSO (5, 10, 15 or 20 μL) was mixed with 1mL 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 mixtures 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.

3. Result and Discussion

3.1 Elemental Analyses

Elemental analyses give satisfactory results for ligand and its metal complexes. CHN values are in close agreement with expected molecular formulae assigned to the ligand and its metal complexes. The mass and elemental analysis data are presented in the experimental section. The IR and mass spectral data confirm that the structure of the ligand and complexes.

3.2 Mass Spectra and Molar Conductance

Ligand 1 shows its molecular ion peak at $m/z = 299.0$ (Fig. 1) which confirms its molecular mass and molecular formula. The mass spectrum of Complex 1 (Fig.2) shows its molecular ion peak at $m/z = 853$. The complex measures molar conductance at $32.40 \text{ S cm}^2\text{mol}^{-1}$ in DMF solution indicating the non-electrolytic nature of the complex [14] due to the coordination of perchlorate anions to the metal centre. Complex 2, exhibits its molecular ion peak at $m/z = 855.9$ and measures a molar conductance in DMF solution at $34.36 \text{ S cm}^2\text{mol}^{-1}$ predicting that the perchlorate ions have coordinated to the cobalt (II) ion and that the complex is non-ionic in nature²⁰.

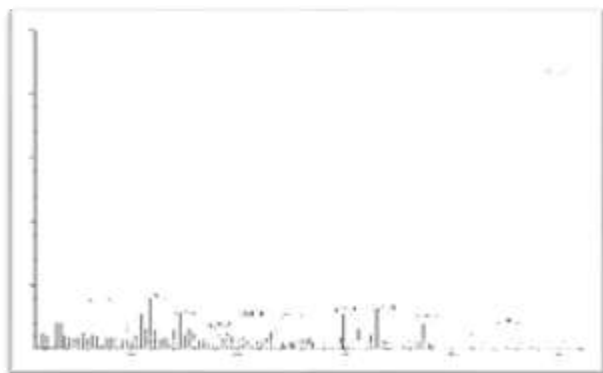


Fig.1 Mass Spectrum of Ligand 1

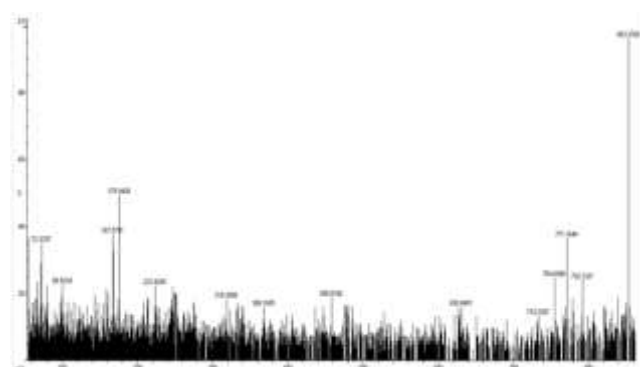


Fig.2 Mass Spectrum of Complex 1

3.3 Electronic Spectral and Magnetic Studies on itpy (L1) and its Metal Complexes

The L1 consists of two bands at 217 nm and 315 nm. These bands are assigned to $\pi-\pi^*$ and $n-\pi^*$ transition. The electronic spectra of the complex 1 showed two bands at 12850 cm^{-1} (single d-d transition) and 17376 cm^{-1} . These bands are assigned to ${}^5\text{T}_{2g} \rightarrow {}^5\text{E}_g$ ²¹⁻²⁴ and charge transfer band²⁵. The magnetic moment of complex 1 was found to be 5.3 BM^{24} . These electronic spectra and magnetic values proposed octahedral environment around the metal ion. On the other hand, the electronic spectra of the complex 2 showed three bands at 17331 cm^{-1} (ν_3), 20150 cm^{-1} (ν_2) and 30490 cm^{-1} . The first two bands are assigned to ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{1g}(\text{P})$, ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{A}_{2g}(\text{F})$. The third band is assigned to M-L charge transfer. The magnetic moment of the Complex 2 was found to be 4.6 BM^{21-24} . These results are typically characteristic for an octahedral configuration for the cobalt complex.

3.4 IR spectroscopy

The IR spectra of itpy ligand and its metal complexes exhibit several diagnostic vibrational frequencies of respective functional groups. The characteristic stretching details are summarized in Table 1. IR spectrum of L1 displays bands at 3300, 3013-2772, 1555-1437, 621 and 430 cm^{-1} assigned to $\nu(\text{NH})$, $\nu(\text{CH})$, pyridine ring

skeletal vibrations ($\nu_{C=C}$ & $\nu_{C=N}$), in plane and out-of- plane deformations respectively. The Complex 1, $2\nu_{C=C}$ & $\nu_{C=N}$ bands have shifted to higher position ($1616-1449\text{ cm}^{-1}$) and ($1618-1460\text{ cm}^{-1}$) due to the coordination of the pyridyl nitrogen atoms to the metal ion²¹. The pyridine ring in-plane deformation and out-of-plane deformation confirming the coordination of two nitrogen atoms at 1' and 1'' positions^{26, 27, 28}. The two new bands at $550, 474$ and $521, 450\text{ cm}^{-1}$ were also observed in the complexes and were not found in the free ligand. These bands attributed to M-N and M-O bonds in complexes 1-2²⁹(Table1) respectively. Based on these data, Complex 1, 2 proposed to have octahedral structure.

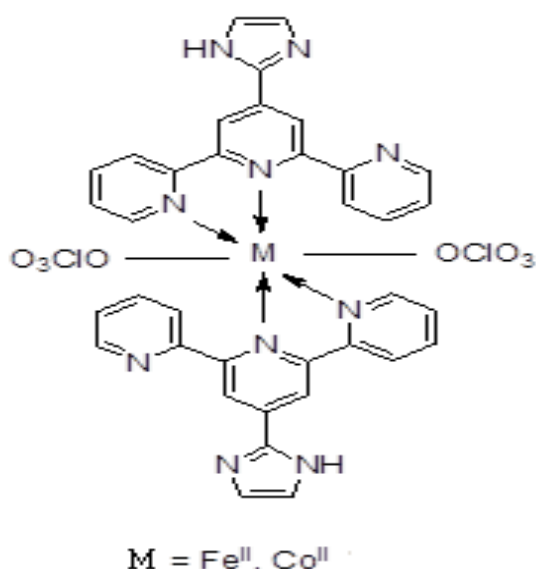


Table 1 Selected IR Absorptions for itpy (L1) and its Metal Complexes

Sl. No	Compound	ν_{NH}	ν_{CH}	pyridine ring skeletal vibration	pyridine ring In plane deformation	pyridine ring out-of-plane deformation	ν_{ClO}	ν_{MO} & ν_{MN}
1	itpy (L)	3300	3013 - 2772	1555 - 1437	621	430	-	-
2	[Fe(itpy) ₂ (ClO ₄) ₂] Complex 1	3400	3074	1616 - 1449	627	460	1244 1088	550 521
3	[Co(itpy) ₂ (ClO ₄) ₂] Complex 2	-	3069	1618 - 1460	627	450	1246 1107	474 450

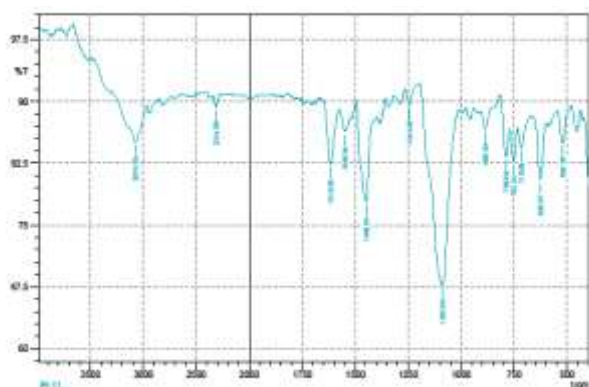


Fig3 FT-IR Spectrum of Complex 1

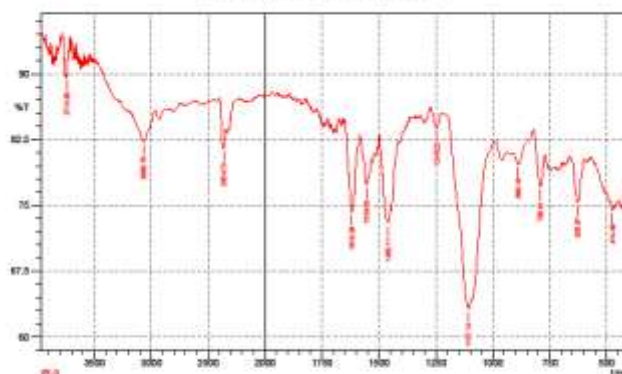


Fig 4 FT-IR Spectrum of Complex 2

3.5 Cyclic Voltammetry

To investigate the redox properties of the complexes, the cyclic voltammograms were recorded for the complexes in DMSO using the same cell set up. The cyclic voltammograms of the complexes have been measured at 50, 100 and 200 mVs^{-1} scan rates (Fig.5, 6). Complex 1, the E_{pa} represents the energy required for oxidation of the central metal ion and the E_{pc} represents energy needed for reduction of the metal ion at a particular scan rate. The E_{pc} values of 1.015, 0.992 and 1.584 V feature the reduction of Fe^{I} to Fe^{II} species. Reoxidation of Fe^{I} to Fe^{II} species has happened at E_{pa} values of 1.607, 1.709 and 2.062 $\text{V}^{30,31}$. The ΔE_{p} between peak potentials are higher than 200 mV indicating an irreversible one electron redox couple of $\text{Fe}^{\text{II}}/\text{Fe}^{\text{I}}$. The $E_{1/2}$ indicate that the Fe^{II} species cannot be reduced easily due to stronger σ - donating ability of ligand. The peak current ratios is less than unity suggesting that electron transfer is followed by a chemical reaction.

Complex2 undergoes reduction to Co^{I} species at cathodic peak potentials of 1.033, 1.487 and 1.441 V. But the reduced form of Co^{I} species reoxidizes to Co^{II} species at the respective anodic peak potentials of 1.501, 1.688 and 1.809 V. The ΔE_{p} values higher than 200 mV which reveal that an irreversible one electron redox couple of $\text{Co}^{\text{II}}/\text{Co}^{\text{I}}$ exists 30,31 . The peak current ratios less than unity suggesting that the electron transfer is followed by a chemical reaction. The I_{pa} values have increased with increase of $v_{1/2}$ to suggest that the redox process is diffusion controlled. The $E_{1/2}$ values, are all positive to show that the complex2 may not undergo easy reduction as it is stabilized by the higher σ - donating ability of the ligand.

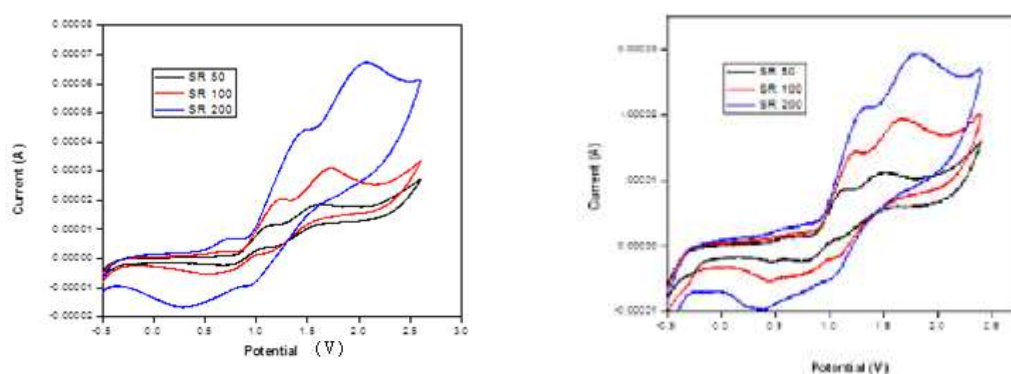


Fig. 5, 6 Cyclic Voltammograms of Complex 1, 2 at 50,100 and 200 mVs^{-1}

4. Biological Screening

4.1. Antimicrobial Activity

The results of antibacterial, antifungal activities of itpy (L1) and its metal complexes in comparison with that of the standard drug (ciprofloxacin, nystatin) are furnished in Table2,3 and Plates 1, 2. From the result we can see that itpy showed very little antimicrobial activity against *E.coli*, and insensitive against *S.aureus*, *B.subtilis*, *K.aerogenes*, *A.niger* and *C.albicans*. Complex 1 was insensitive *S.aureus*, *E.coli*, *A.niger* and *C.albicans* and little active against *B.subtilis*, *K.aerogenes*. The cobalt complex 2 exhibits a significant increase activity against *S.aureus*, *B.subtilis*, *K.aerogenes* and moderate activity against *E.coli*, *A.niger* and *C.albicans*.

Table 2 Antibacterial Activities of itpy (L1) and its Metal Complexes

Sl. No	Test Drug	Zone of Inhibition (mm)											
		<i>S. aureus</i>			<i>B. subtilis</i>			<i>K. aerogens</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	itpy(L1)	-	-	-	-	-	-	-	-	-	10	10	16
2	[Fe(itpy) ₂ (ClO ₄) ₂] Complex 1	-	-	-	8	10	15	12	15	18	-	-	-
3	[Co(itpy) ₂ (ClO ₄) ₂] Complex 2	15	16	20	16	18	22	15	19	22	12	15	16
4	Ciprofloxacin Standard	-	-	35	-	-	40	-	-	30	-	-	38

Table 3 Antifungal Activities of itpy and its Metal Complexes

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	itpy(L1)	-	-	-	-	-	-
2	[Fe(itpy) ₂ (ClO ₄) ₂] Complex 1	-	-	-	-	-	-
3	[Co(itpy) ₂ (ClO ₄) ₂] Complex 2	15	16	18	14	14	18
4	Nystatin (Standard)	-	-	30	-	-	25



Escherichia coli

Bacillus subtilis

Klebsiella aerogenes

Plate.1 Antibacterial and Antifungal Activities of itpy (L1) and Complex 1

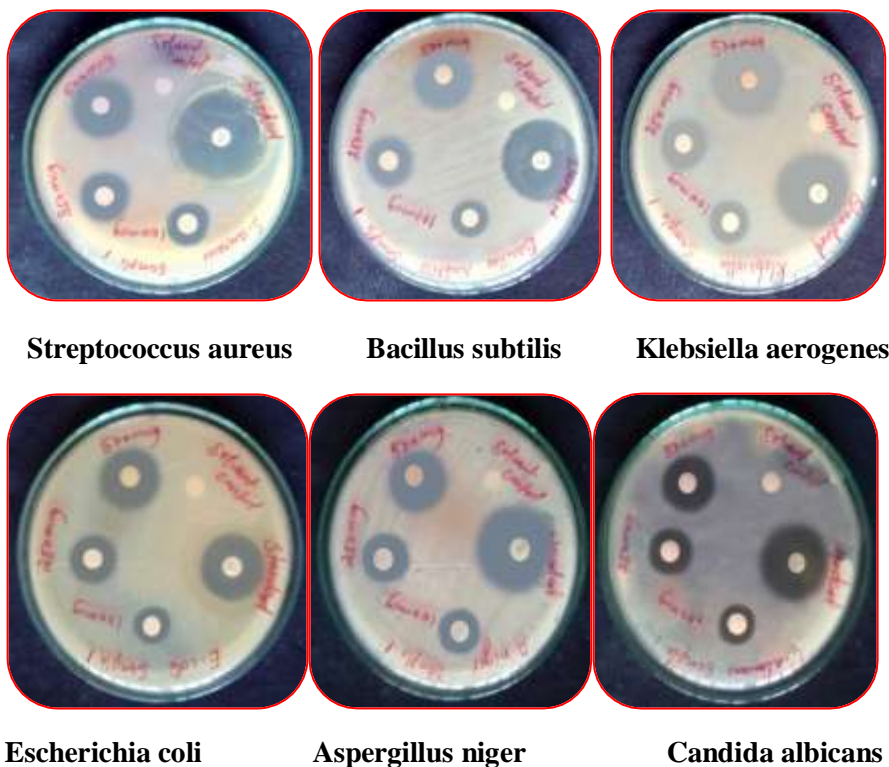


Plate 2. Antibacterial and Antifungal Activities of Complex 2

4.2. Antioxidant Activity Screening

The itpy and its metal complexes were evaluated for DPPH radical scavenging activity^{32, 33}. The antioxidant activity of each test drug increases with increase in its concentration. The determined results show that the itpy (L1) exhibits the highest activity at 97.36 % while the Fe^{II} complex displays the lowest activity at 73.68 % at the concentration of 20 µg/mL. The decreasing order of overall antioxidant activities is itpy > Complex 2 > Complex 1

5. Conclusion

In this study, we have reported the synthesis of itpy ligand and its Fe(II), Co(II) complexes. The structural characterizations of the synthesized compounds were made by spectroscopic methods, elemental analysis, electrical conductance, magnetic studies and cyclic voltammetry. From the spectroscopic characterization, it is concluded that itpy act as bidentate ligands, coordinating through its two 1, 1' positions N atoms to the metal is exhibited in fig. the redox properties of these complexes are able to stabilize the +1 oxidation state of iron and Cobalt. The biological activity screening showed that cobalt complex has more activity than iron complex and itpy ligand against the tested bacteria and fungi. Finally, this research emphasizes the interesting potential role of cobalt complex as antioxidants.

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