

METAL COMPLEXES OF 2, 6-BIS(2-HYDROXYPHENYL)PIPERIDIN-4-ONE LIGAND AND THEIR ANTIMICROBIAL, ANTIOXIDANT ACTIVITIES

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ABSTRACT

We have been studied that the biological importance of Ruthenium(III), Cobalt(II) and Copper(II) metal complexes, we had approaches the synthesis 2, 6-bis(2-hydroxyphenyl)piperidin-4-one ligand(L) of having different binding atoms and also prepared novel metal complexes. The synthesized ligand and their respective metal complexes were characterized by elemental analysis, conductivity measurements, magnetic susceptibility, electronic spectral studies, infrared spectroscopy studies, ¹H NMR spectral studies and Mass spectral studies. This study highlights the biological activities such as antioxidant, antibacterial activity of metal complexes and also

compared with standard compounds.

KEYWORDS: Ruthenium(III), Cobalt(II), Copper(II) metal complexes, DPPH activity, antibacterial activity.

INTRODUCTION

Recently, Mishra and group were studied on anti-HIV Ru(II) complexes, which they suggested that flavones complexes possess as a anti-HIV agent. Eilatin-containing octahedral ruthenium complexes inhibit HIV-1 replication in CD⁴⁺ HeLa cells and in human peripheral blood monocytes with IC(50) values of approximately 1 μ M. Similar metal complexes that

lack eilatin display 15-100-fold lower anti-HIV activities. $[\text{Ru}(\text{bpy})(\text{pre-eilatin})]^{2+}$ a complex that contains a nonplanar analogue of eilatin, shows significantly lower nucleic acid binding and lower anti-HIV activity than eilatin complexes. Their interaction with aqueous buffered calf thymus DNA was measured [1a-1b]. These results prompted additional screening for anti-HIV (human immunodeficiency virus) activity against DNA replication in H₉ lymphocytes and cytotoxic activity against eight tumor cell lines.^[2]

From above literature survey of ruthenium(II), cobalt(II) and copper(II) metal complexes, we had approaches the synthesis of various ligands of having different organic moieties and their biological applications in various field. The synthesized ligands and their respective metal complexes were characterized by elemental analysis, conductivity measurements, magnetic susceptibility, electronic spectral studies, infrared spectroscopy studies, ¹H NMR spectral studies, mass spectral studies and thermogravimetric analysis.

MATERIALS AND METHODS

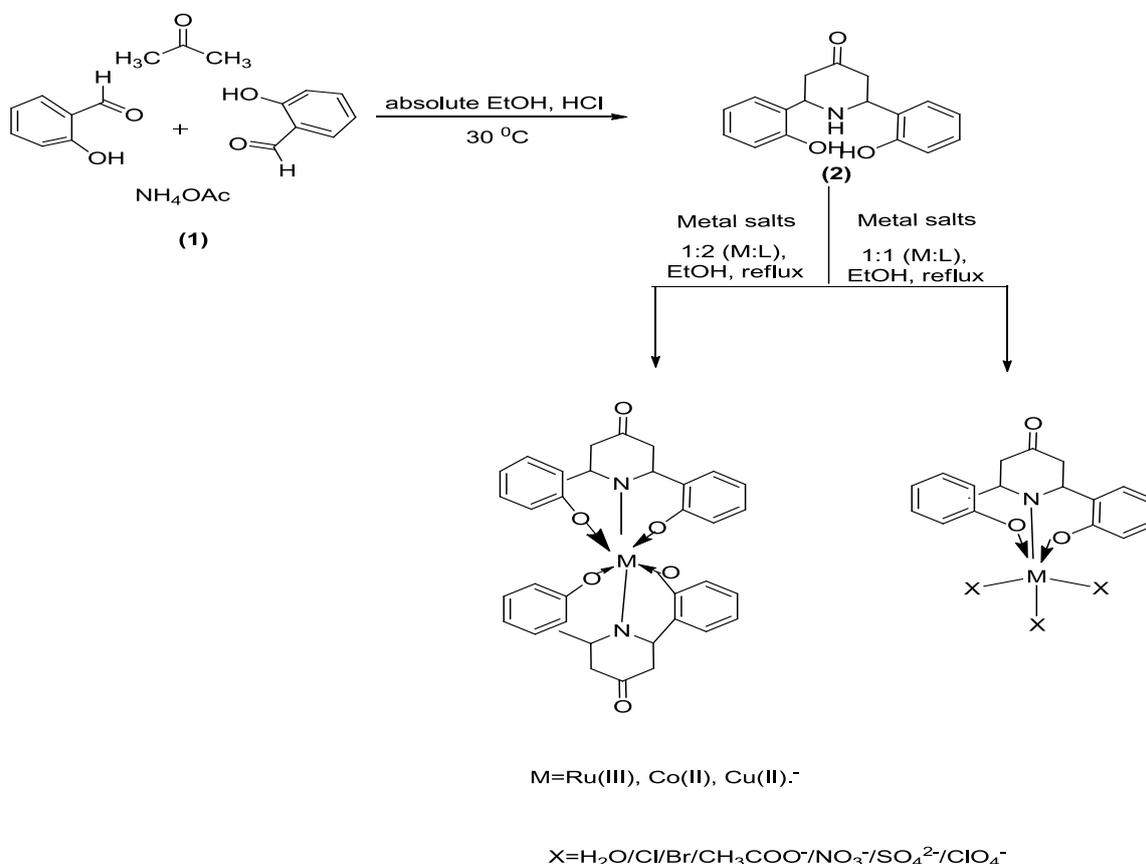
All reagents and solvents were purchased from Merck (Darmstadt, Germany) chemical AR grade and were used as provided. DPPH and BHA were purchased from Sigma-Aldrich chemical Co. (St. Louis, MO, USA). TLC analysis was performed on alumina sheets precoated with silica gel 60F-254 and SiO₂, 200-400 mesh (Merck) was used for column chromatography. ¹H (400 MHz), ¹³C (100.56 MHz) and ⁷⁷Se (76.29 MHz) NMR spectra were obtained on a Bruker 400 MHz NMR spectrometer. Chemical shifts are cited with respect to SiMe₄ as internal (¹H and ¹³C) and Me₂Se as external (⁷⁷Se) standards. Mass spectral studies were carried out on a Bruker Daltonics 6000 plus mass spectrometer with ESI-MS mode analysis.

General procedure of preparation of ligand(L)

Synthesis of 2, 6-bis(2-hydroxyphenyl)piperidin-4-one ligand(L) (C₁₇H₁₇NO₃) is similar to that of Niclova *et. al.*, 0.1 mol (7.7 g) of ammonium acetate was dissolved in 30 ml of ethanol and 0.2 mol (21.24 ml) of salicylaldehyde and 0.1 mol (7.35 ml) acetone were added to the solution. The mixture was heated to boiling for 30 min, and set aside for a day. The oily base obtained was converted into its hydrochlorides by the addition of conc. HCl (5 ml) and the separated solid was filtered, dried and recrystallised with liq. NH₃ which upon dilution with water, the free base separated was filtered and recrystallised from absolute ethanol.^[3]

Preparation of metal complexes (M₁-M₁₀)

Synthesis of metal complexes. An alcoholic solution of ligand (L) (2 mmol) was refluxed with (1 mmol) of metal chlorides in ethanol on water bath for 2-3 h. Then, to the reaction mixture (2 mmol) of sodium acetate was added and reflux was continued for 3h. The separated complex was filtered, washed thoroughly with water, ethanol and ether. Finally the complexes were dried in vacuum over fused CaCl₂.^[4]



Scheme-1: Synthesis of Ligand (L) and their metal complexes.

RESULTS AND DISCUSSION

a) Stoichiometry

The stoichiometries of Ru(III), Co(II) and Cu(II) complexes were concluded from their elemental analysis. All the synthesized complexes are colored and are stable in air, non-hygroscopic, have high melting points and are insoluble in water, but soluble in coordinating solvents such as DMF, DMSO, THF as well as acetonitrile. The analytical data are depicted in Table-1. Suggest that the metal: ligand ratio is [1: 1] & [1: 2], they can be represented by the general formulae;

[M(L)₂X], [M(L)X], Where M = Ru(III), Co(II), Cu(II). L= Ligand, X = Cl.

b) Conductometric studies

All the complexes were anhydrous in nature and insoluble in methanol, ethanol, acetone and benzene. They are soluble in dimethyl sulphoxide and in dimethyl formamide. The general structures of the metal complexes were shown in Scheme-1. The 10^{-3} M solution of the complex in dimethyl sulfoxide (DMSO) was used to determine the molar conductance. The conductivity data reveals that the complexes are non-electrolytic in nature. The metal and anions are estimated using standard procedure (Vogel A. I., 1962). Elemental analysis data are in well agreement with the calculated value and the data are presented in Table-1.

c) Magnetic susceptibility measurements

The magnetic susceptibility measurements of the complexes were obtained at room temperature using Gouy's balance. Pure $\text{Hg}[\text{Co}(\text{SCN})_4]$ was synthesized is used as calibration standard. The effective magnetic moment values of the complexes are in Table-2. The transition element of ruthenium having electronic configuration $[\text{Kr}] 4d^6 5s^2$ and Ru(III) ion being a d^5 system has five unpaired electron in 4d shell and its complexes are expected to have magnetic moments close to the spin only value of about 5.91 BM, irrespective of the bond type involved. The magnetic moment of mononuclear Ru(III) complexes usually lies in the range 5.12-5.95 BM have no major interaction between the unpaired electron on different manganese ions and essentially temperature independent.

And in the copper complexes having electronic configuration $[\text{Ar}] 3d^{10} 4s^1$ and Cu(II) ion being a d^9 system has one unpaired electron in 3d shell and its complexes are expected to have magnetic moments close to the spin only value of about 1.91 BM, irrespective of the bond type involved. The magnetic moment of Cu(II) complexes usually lies in the range 1.75-1.91 BM have no major interaction between the unpaired electron on different manganese ions and essentially temperature independent.^[5]

d) Electronic spectral measurements

The Electronic Absorption Spectral Studies of ligands (L) and some of metal complexes had carried out in the region 250-1100 nm was measured by spectrophotometer. Weighed samples were dissolved in appropriate DMF of HPLC grade at a concentration of 10^{-6} and 10^{-7} M. were measured and carried out at room temperature. The electronic absorption spectrum of the Ligands is depicted in Table-2. and Figure-1. Exhibited two absorption bands around 340 nm and 420 nm respectively. The first band around 340 nm corresponds to $\Pi \rightarrow \Pi^*$ of the nitrogen contain group and second band at 420 nm corresponds to $n \rightarrow \Pi^*$ transitions. The

new ruthenium(III) complexes are paramagnetic, indicating the presence of ruthenium in the +3 oxidation state. The electronic spectra of all the complexes in DMSO show a band in the 270–285 nm region. This band has been assigned to the charge-transfer transition arising from the excitation of an electron from the metal t^2_g level to the unfilled molecular orbitals derived from the p^* level of the ligands, in accordance with the assignments made for other similar octahedral ruthenium(III) complexes.^[6-8]

The spectrum of the Ru(III) complexes are represented in spectrum of Figure-2 respectively, the electronic spectra's shows that the dominated by one strong MLCT $[\text{Ru}(\text{L})_2\text{X}]$ transition at 20000 cm^{-1} due to the HOMO–LUMO excitation. There are two weak transitions in the $20000\text{--}22500\text{ cm}^{-1}$ region due to HOMO-LUMO and HOMO-LUMO excitations of Ru(III). ruthenium(III) ion which has five d electrons and The ground term arising from the $t^3_{2g} e^2_g$ configuration in an octahedral field is $^4T_{1g}$ and having three spin allowed d-d transitions, $\nu_1 = ^4T_{1g}(\text{F}) \rightarrow ^4T_{2g}(\text{F})$, $\nu_2 = ^4T_{1g} \rightarrow ^4A_{2g}(\text{F})$, $\nu_3 = ^4T_{1g}(\text{F}) \rightarrow ^4T_{1g}(\text{P})$.

e) FT-IR spectral studies

The structure of ligand confirmed by using IR spectrum. The imported infrared spectral data of prepared Ligands and their metal complexes are represented in Table-3. And spectrums are presented in supplementary data. The IR discussions of ligand and metal complexes are as follows, *Ligand (L)*; In Ligand (L) showed a broad band at $3000\text{--}2500\text{ cm}^{-1}$ indicated the presence of the -O-H group in the ligand which is disappear after the complex formation reveals that the M-O bond formation, this band appears near far-infrared region i.e., $500\text{--}450\text{ cm}^{-1}$. A medium band at $1360\text{--}1250\text{ cm}^{-1}$, which was assigned to the formation of the C-O group and also a medium band of the primary amines groups appears at about $1750\text{--}1650\text{ cm}^{-1}$ this band frequency slightly shifted to $15\text{--}25\text{ cm}^{-1}$ due the formation of complexes of M-N bond this band appears near far-infrared region i.e., $450\text{--}410\text{ cm}^{-1}$.^[9]

f) ^1H NMR spectral studies

The ^1H NMR data the ligands and metal complexes are presented in Table-4. The ^1H -NMR spectra were recorded in DMSO- d_6 in a multiplet from $\delta(7.89\text{--}8.01)$ ppm for aromatic protons (Ar-H) in ligand (L). In ligand (L) the signals within the range of $\delta(1.31\text{--}1.55)$ ppm are attributable to aromatic ring protons and the signal range in between $\delta(6.98\text{--}6.04)$ ppm for N-H proton, The O-H proton shows peak between $\delta(7.68\text{--}6.64)$ ppm and at $\delta(2.78\text{--}2.89)$ signal

attributed for the –CH proton. The spectrum of ligand(L) are presented in supplementary data.^[10]

Biological evaluation

Antioxidant activity

The synthesized ligand and their respective metal complexes exhibited effective antioxidant scavenging activities. From all the synthesized complexes of ligand (L) are having electron withdrawing nitrogen moiety promotes scavenging of Free radical activity and also the scavenging of Free radical increases after complexation with above said ligand. this changes is due to because the metal moiety was in electron deficient state this property make the complexes to scavenge the free radical very effectively than the free ligand as well as standard drug. Finally the complexes $[\text{Co}(\text{C}_{17}\text{H}_{17}\text{NO}_3)(\text{Cl})]$, $[\text{Ru}(\text{C}_{17}\text{H}_{17}\text{NO}_3)_2]$ shows potent antioxidant activity^[11] and complexes remain complexes shows moderate activity, all the tested ligand and metal complexes responds against the free radical scavenging strain significantly these data are reproduced in Table-5. and graphical representation in Graph-1.

Antimicrobial activity

On the basis of observed zones of inhibition, it was found that, in general, all the prepared ligands and their metal complexes responded against all the tested bacterial and fungal strains significantly.^[12,13] The investigation of antibacterial screening data revealed that the tested compounds showed moderate to good antibacterial and antifungal activities against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Aspergillus flavus*, *Chrysosporium keratinophilum* and *Candida albicans* respectively.

Table-1: Elemental analysis, melting point, molecular weight and molar conductance data of ligand and their metal Complexes.

Sl. No.	Compounds	Yield (%)	Mel.point (°C)	Molecular Weight	Elemental analysis Calc. (found) (%)						Molar conduct. (Ohm ⁻¹ cm ⁻² mol ⁻¹)
					M	C	H	N	O	anion	
1	L (C ₁₇ H ₁₇ NO ₃)	67	191-193	283	--	72.07 (72.30)	6.05 (6.61)	4.94 (5.01)	16.94 (17.05)	-	10.58
2	[Ru(C ₁₇ H ₁₇ NO ₃) ₂]	72	221-222	665	9.77 (9.81)	72.51 (72.55)	6.67 (6.70)	5.00 (5.04)	17.01 (17.10)	-	16.21
3	[Cu(C ₁₇ H ₁₇ NO ₃) ₂]	78	205-206	639	8.41 (8.45)	72.81 (72.86)	7.17 (7.18)	5.40 (5.41)	17.13 (17.14)	-	15.47
4	[Co(C ₁₇ H ₁₇ NO ₃) ₂]	73	201-203	629	8.30 (8.35)	72.81 (72.83)	8.62 (8.64)	4.51 (4.53)	17.41 (17.42)	-	13.54
5	[Ru(C ₁₇ H ₁₇ NO ₃)(Cl)]	67	224-225	417	12.51 (12.53)	70.62 (70.70)	7.21 (7.22)	4.87 (4.89)	17.70 (17.72)	12.51 (12.55)	12.47
6	[Cu(C ₁₇ H ₁₇ NO ₃)(Cl)]	68	210-211	391	8.30 (8.35)	73.21 (73.24)	7.52 (7.54)	4.28 (4.28)	16.72 (16.74)	8.30 (8.32)	11.64
7	[Co(C ₁₇ H ₁₇ NO ₃)(Cl)]	75	184-185	381	8.30 (8.35)	71.26 (71.30)	5.58 (5.61)	5.03 (5.05)	17.71 (17.78)	8.30 (8.35)	16.41

Table-2: Electronic spectral and magnetic susceptibility data of metal complexes.

Sl.No.	Complexes	Electronic spectra (cm ⁻¹)				Magnetic moment[BM]
1	L (C ₁₇ H ₁₇ NO ₃)	14796	16354	25986	34567	--
2	[Ru(C ₁₇ H ₁₇ NO ₃) ₂]	20120	22287	28906	35562	5.83
3	[Cu(C ₁₇ H ₁₇ NO ₃) ₂]	15886	18375	27674	35580	1.85
4	[Co(C ₁₇ H ₁₇ NO ₃) ₂]	14842	16342	25815	34642	3.84
5	[Ru(C ₁₇ H ₁₇ NO ₃)(Cl)]	20864	22313	28834	35809	5.92
6	[Cu(C ₁₇ H ₁₇ NO ₃)(Cl)]	15931	17345	27761	35705	1.90
7	[Co(C ₁₇ H ₁₇ NO ₃)(Cl)]	14806	16324	25254	34726	3.81

Table-3: Important IR spectral data of Ligand and their metal complexes.

Sl.No.	Compounds	IR Frequencies (cm ⁻¹)					
		O-H	N-H	C=O	M-N	M-O	M-X
1	L (C ₁₇ H ₁₇ NO ₃)	3046-3010	3129-3150	1730-1607	-	-	-
2	[Ru(C ₁₇ H ₁₇ NO ₃) ₂]	-	-	1725-1650	645-600	450-465	-
3	[Cu(C ₁₇ H ₁₇ NO ₃) ₂]	-	-	1720-1750	590-570	430-420	-
4	[Co(C ₁₇ H ₁₇ NO ₃) ₂]	-	-	1780-1790	630-590	460-455	-
5	[Ru(C ₁₇ H ₁₇ NO ₃)(Cl)]	-	-	1695-1600	650-620	455-450	-
6	[Cu(C ₁₇ H ₁₇ NO ₃)(Cl)]	-	-	1650-1620	550-500	460-455	-
7	[Co(C ₁₇ H ₁₇ NO ₃)(Cl)]	-	-	1670-1650	600-550	480-430	-

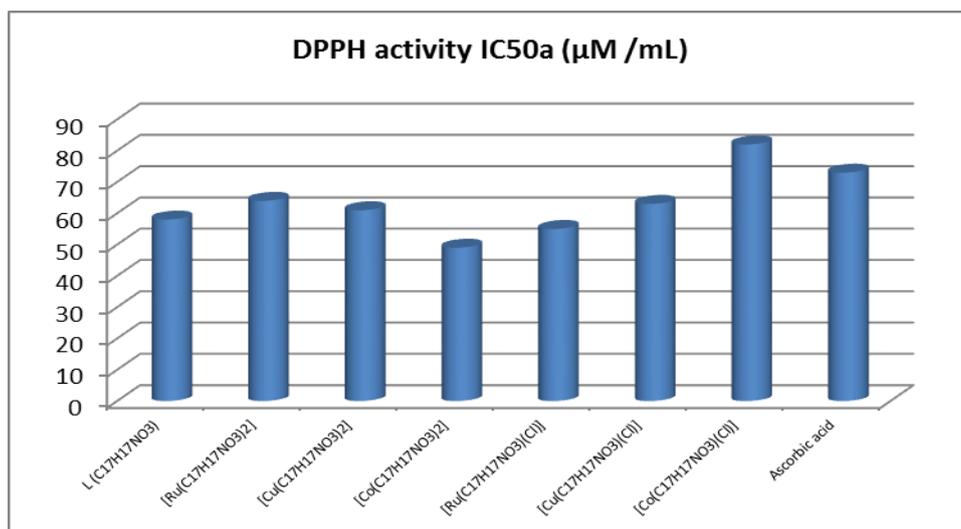
Table 4: Important ¹H NMR spectral data of ligand and their metal complexes.

Sl. No.	Compounds	Chemical shift(δ ppm)		
		ArC-H	O-H	N-H
1	L (C ₁₇ H ₁₇ NO ₃)	5.45-6.64	7.14-7.86	6.94-6.99
2	[Ru(C ₁₇ H ₁₇ NO ₃) ₂]	5.42-6.61	7.11-7.79	6.91-6.89
3	[Cu(C ₁₇ H ₁₇ NO ₃) ₂]	5.40-6.62	7.12-7.69	6.99-7.02
4	[Co(C ₁₇ H ₁₇ NO ₃) ₂]	5.43-6.66	7.15-7.70	6.88-7.03
5	[Ru(C ₁₇ H ₁₇ NO ₃)(Cl)]	5.49-6.67	7.12-7.81	6.91-7.00
6	[Cu(C ₁₇ H ₁₇ NO ₃)(Cl)]	5.39-6.60	7.10-7.66	6.92-7.04
7	[Co(C ₁₇ H ₁₇ NO ₃)(Cl)]	5.45-6.66	7.17-7.61	6.90-6.99

Table-5: Antioxidant activity (IC₅₀) of the ligand and their metal complexes.

Sl.No.	Compounds	DPPH activity IC ₅₀ ^a (μM /mL)
1	L (C ₁₇ H ₁₇ NO ₃)	58±0.10
2	[Ru(C ₁₇ H ₁₇ NO ₃) ₂]	64±0.13
3	[Cu(C ₁₇ H ₁₇ NO ₃) ₂]	61±0.09
4	[Co(C ₁₇ H ₁₇ NO ₃) ₂]	49±0.53
5	[Ru(C ₁₇ H ₁₇ NO ₃)(Cl)]	55±0.21
6	[Cu(C ₁₇ H ₁₇ NO ₃)(Cl)]	63±0.41
7	[Co(C ₁₇ H ₁₇ NO ₃)(Cl)]	82±0.11
Std.	Ascorbic acid	73±0.05

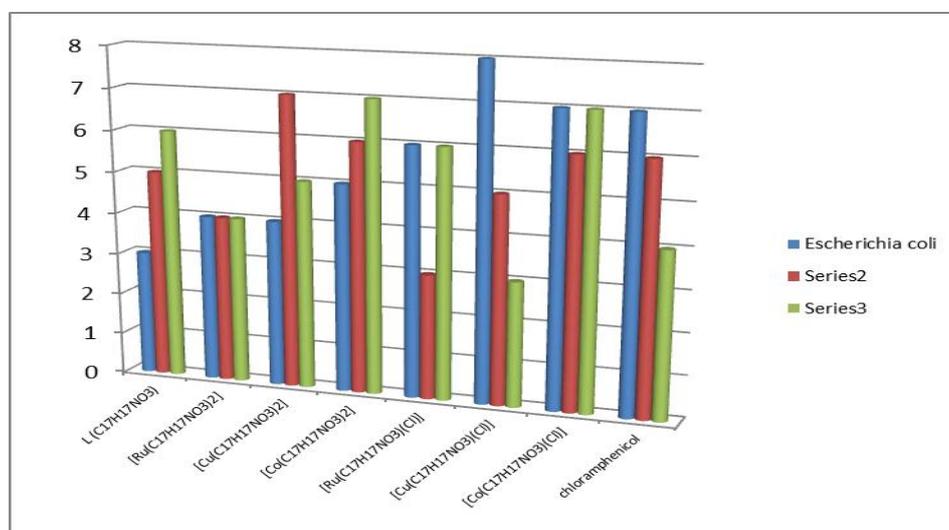
^a IC₅₀ = the concentration (μM/mL) exhibiting 50% inhibition of DPPH radical.



Graph-1: Antioxidant activity of the ligand and its metal complexes.

Table-6: Antibacterial activity of the ligand and its metal complexes. Inhibitory zone (diameter in mm) of the synthesized compounds against tested bacterial strains by well plate method.

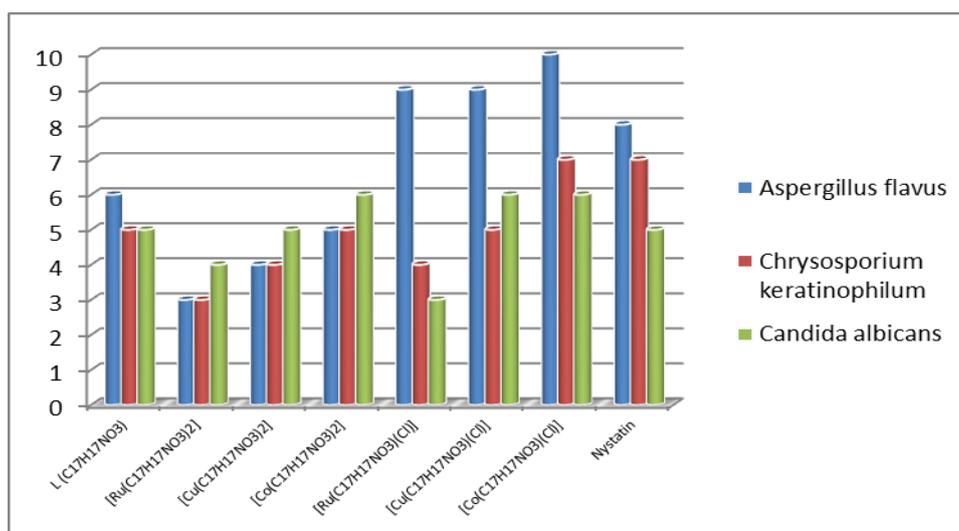
Sl. No.	Compounds	Escherichia coli	Staphylococcus aureus	Pseudomonas aeruginosa
1	L (C ₁₇ H ₁₇ NO ₃)	03 ± 0.12	05±0.01	06±0.08
2	[Ru(C ₁₇ H ₁₇ NO ₃) ₂]	04± 0.15	04±0.13	04±0.16
3	[Cu(C ₁₇ H ₁₇ NO ₃) ₂]	04± 0.11	07±0.02	05±0.04
4	[Co(C ₁₇ H ₁₇ NO ₃) ₂]	05±0.16	06±0.01	07±0.13
5	[Ru(C ₁₇ H ₁₇ NO ₃)(Cl)]	06±0.05	03±0.20	06±0.21
6	[Cu(C ₁₇ H ₁₇ NO ₃)(Cl)]	08±0.12	05±0.17	03±0.04
7	[Co(C ₁₇ H ₁₇ NO ₃)(Cl)]	07±0.19	06±0.21	07±0.12
Std.	chloramphenicol	07.0± 0.09	06.0± 0.14	04.0± 0.21



Graph-2: Antibacterial activity of the ligand and its metal complexes.

Table-7: Antifungal activity of the ligand and its metal complexes. Inhibitory zone (diameter in mm) of the synthesized compounds against tested bacterial strains by well plate method.

Sl.No.	Compounds	Aspergillus flavus	Chrysosporium keratinophilum	Candida albicans
1	L ($C_{17}H_{17}NO_3$)	06±0.17	05±0.04	05±0.01
2	$[Ru(C_{17}H_{17}NO_3)_2]$	03±0.11	03±0.15	04±0.05
3	$[Cu(C_{17}H_{17}NO_3)_2]$	04±0.19	04±0.22	05±0.01
4	$[Co(C_{17}H_{17}NO_3)_2]$	05±0.06	05±0.01	06±0.11
5	$[Ru(C_{17}H_{17}NO_3)(Cl)]$	09±0.14	04±0.21	03±0.14
6	$[Cu(C_{17}H_{17}NO_3)(Cl)]$	09±0.18	05±0.18	06±0.04
7	$[Co(C_{17}H_{17}NO_3)(Cl)]$	10±0.12	07±0.09	06±0.19
Std.	Nystatin	8±0.13	7±0.11	5±0.21



Graph-3: Antifungal activity of ligand and Metal complexes.

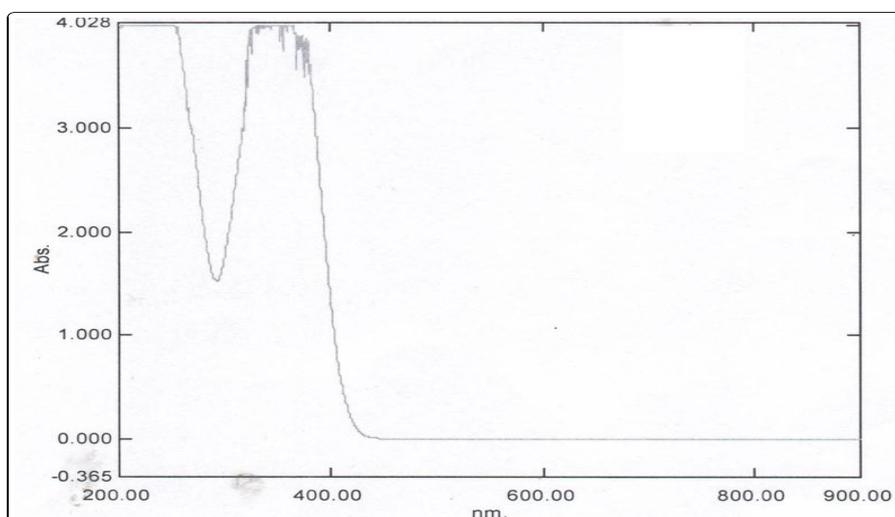


Fig-1: Electronic spectra of Ligand (L).

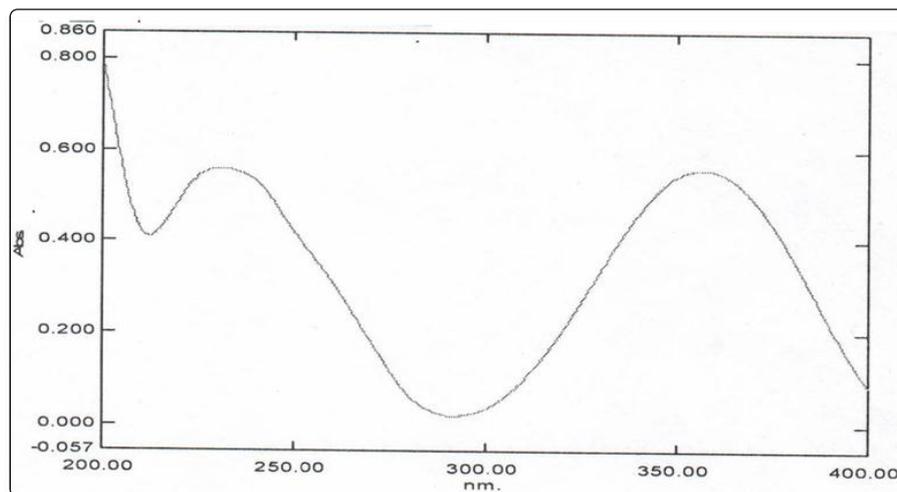


Fig-2: Electronic spectra of complex (2).

CONCLUSION

Work described here is involved the synthesis and spectroscopic characterization of ruthenium(III), cobalt(II) and copper(II) complexes with a new 2, 6-bis(2-hydroxyphenyl)piperidin-4-one ligand. These complexes were characterized by using different physicochemical techniques. These complexes are all neutral and found to have an octahedral geometry with the six donor atoms. The synthesized complexes have *in vitro* antimicrobial screening effects evaluated against three bacterial strains and fungal strains by disc diffusion method using nutrient agar medium for antibacterial studies and potato dextrose agar medium for antifungal studies And also these synthesized complexes reveals effective antioxidant activity by DPPH assay.

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