Synthesis, Characterization, Antibacterial and Antioxidant Activities of 2,5-Dihydroxy-benzaldehydethiosemicarbazone and their Cu(II) and Pd(II) Complexes

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Abstract- Complexes of Copper(II) and Palladium(II) with general composition of $M(L)_2Cl_2$, have been synthesized with the ligand containing 2,5-dihydroxybenzaldehydethiosemicarbazone (L). The ligand characterized by HRMS, NMR and FT-IR & Raman studies. All the complexes were subjected to characterization by elemental analyses, IR & Raman, electronic and EPR spectral studies. The FT-IR spectral data of ligand indicate the coordination of sulphur and azomethine nitrogen with the central metal ion. The electronic, FT-IR and EPR studies reveal on elongated tetragonal geometry for cu(II) and square planar geometry for Pd(II) complexes. The synthesized ligand and its Cu(II) and Pd(II) complexes were evaluated for their antimicrobial and antioxidant activity.

Key words- Antibacterial, Antioxidant activity, Benzaldehydethiosemicarbazone, Electronic, FT-IR, NMR and Raman spectra.

1 INTRODUCTION

The families of thiosemicarbazone compounds have been studied due to their wide range of potential in medical applications [1]. They form highly stable and intensely colored complexes and are used for spectrophotometric determination of metal ions in different media [2]-[4]. Thiosemicarbazones are five membered chelating ligands coordinating to the metal ions through the sulphur and one of the hydrazinic nitrogen atoms [5].

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The metal complexes, particularly the first row transition metal complexes, exhibit prounced biological activities such as, antibacterial [6], antifungal [7], antitumour [8], anti-oxidant [9], and other biological activities [10]-[12], as compared with free thiosemicarbazones. Literature is abound with antiproliferative potential of Pd-thiosemicarbazone complexes as demonstrated on human breast cancer, including tumor cell lines resistant to cisplatin [13]-[14].

Based on the significance of metal complexes, the present study is focused on the synthesis, spectral characterization, antimicrobial and scavenging activities of Cu(II) and Pd(II) complexes with a thiosemicarbazone (L) derived from 2,5-dihydroxybenzaldehyde.

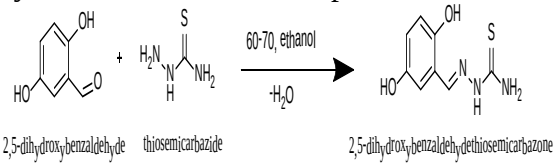
2 Experimental

2.1 Materials and methodologies

All the chemicals used were of analytical grade. 2,5-dihydroxybenzaldehyde and it was purchased from AVRA synthesis Pvt. Ltd. Thiosemicarbazide was purchased from Sd-Fine chemicals and palladium (II) chloride was purchased from Sigma Aldrich. Copper(II) chloride and other organic solvents were obtained from the commercial sources and were used after further purification.

2.2 Synthesis of ligand (DHBATSC)

The ligand (L) was synthesized by refluxing a solution of 2,5-dihydroxybenzaldehyde (10 mmol) ethanolic solution and thiosemicarbazide (10 mmol) ethanolic solution. The reaction mixture was refluxed for 4 hr. And then cooled to room temperature and its purity was checked by thin layer chromatography (TLC). White crystals were precipitate and filtered, washed with cold ether and dried under vaccum with anhydrous P_4O_{10} (yield 76%). The solid compound was recrystallized from acetonitrile solution with slow evaporation process. The schematic representation of synthesis is depicted below.



2.3 Syntheses of complexes

A mixture of hot methanolic solution of free ligand (0.002 mol) and hot methanol solution of (0.001 mol) of the metal chloride solutions ($CuCl_2.2H_2O/PdCl_2$) was refluxed for 4 hr. The obtained precipitate was filtered and washed with methanol and ether and dried under vacuum with anhydrous P_4O_{10}. The purity of the complexes is checked by TLC, elemental (%) and chemical analysis data are shown in Table 1.

2.4 Physical measurement

NMR spectrum was recorded with a model Bruker Biospin AG-400 MHz (IIT Madras) using DMSO-d_6 as a solvent and TMS as an internal standard. Mass spectrum of the ligand was recorded in ESI mode using Bruker HRMS. Elemental analyses (CHN) were performed by using FLASH 1112 series. FT-IR spectra (KBr pellet) were recorded in the region 4000-400 cm^{-1} on a FT-IR spectrum Thermo scientific nicolet-380 spectrophotometer. Raman spectra were recorded in the range 1800 to 400 cm^{-1} using a confocal Raman microscope equipped with a 432 nm He-Ne Laser source. The electronic spectra were recorded in DMF solution on a UV Shimadzu 3600 spectrometer. The EPR spectrum was recorded as polycrystalline samples at 298 K on a Bruker-ER073 instrument equipped with an EMX microX source.

2.5 Biological studies

2.5.1 Antibacterial activity

Invitro antibacterial activity of the ligand and their Cu(II) and Pd(II) complexes were evaluated against Gram-positive (Bacillus cereus and Staphylococcus aureus) and Gram-negative (Escherichia coli, Klebsiella pneumoniae and Salmonella typhi) bacteria by Kirby-Bauer disk diffusion method on Mueller-Hinton agar. Streptomycin (30 µg/ml) was used as standard and antibiotic discs of 6 mm were placed on Mueller-Hinton agar medium. The metal complexes were dissolved in DMSO and 20 µl of compound in different concentrations ranging from 25 to 200 µg/ml were transferred on to the sterile discs and incubated at 37 °C for 24 hr. Control measurements were carried out with DMSO. The zone of growth inhibition was determined by measuring the diameter of clear zone (including the disc) on the agar medium around the disc in mm. All the experiments were carried out in triplicates and mean zones of inhibition were recorded.

2.5.2 Antioxidant activity

Free radical scavenging property of ligand and their Cu(II) and Pd(II) complexes against DPPH (2, 2-Diphenyl-1-picrylhydra-zyl) was determined according to method described previously with slight modifications [15]. An antioxidant reacts with DPPH by donating hydrogen and reduces DPPH. About 1 ml of 0.2 mM. DPPH solution in methanol was mixed with the different concentrations (10-100 µg/ml) of the ligand and their complexes of Cu(II) and Pd(II). Ascorbic acid (25-100 µg/ml) was taken as a reference control. The reaction mixture was shaken well and kept in dark for 15 min at room temperature. The absorbances of the sample and control solutions were measured at 517 nm.

The percent of radical scavenging activity was calculated from the following equation.

% of Inhibition = (A control – A sample / A control) × 100

The IC_{50} values were calculated by plotting I % values as a function of complex concentrations.

3. Results and discussion

3.1 NMR and Mass spectra

Characteristic NMR signals (DMSO-d_6, TMS) ^{1}H NMR Fig. (1) and ^{13}C NMR (400 MHz, δ ppm) exhibit following signals: δ 11.34 (1H, s, NH), δ 9.21 (1H, s, OH), δ 8.82 (1H, s, OH), δ 8.30 (1H, s, −HC=N−), δ 8.07 (1H, br s, NH), δ 7.79 (1H, br s, NH_2) δ 6.68-7.22 (m, 3H, aromatic). ^{13}C NMR: δ 177 (C=S); δ 149 (C=N); δ 111-140 (aromatic) [16]. The electronic impact mass spectrum of ligand Fig. (2), owed a molecular ion peak at m/z = 212 (M+1) amu corresponding to species ($C_8H_9N_3O_2S$) which confirms the proposed structure of the formulae.

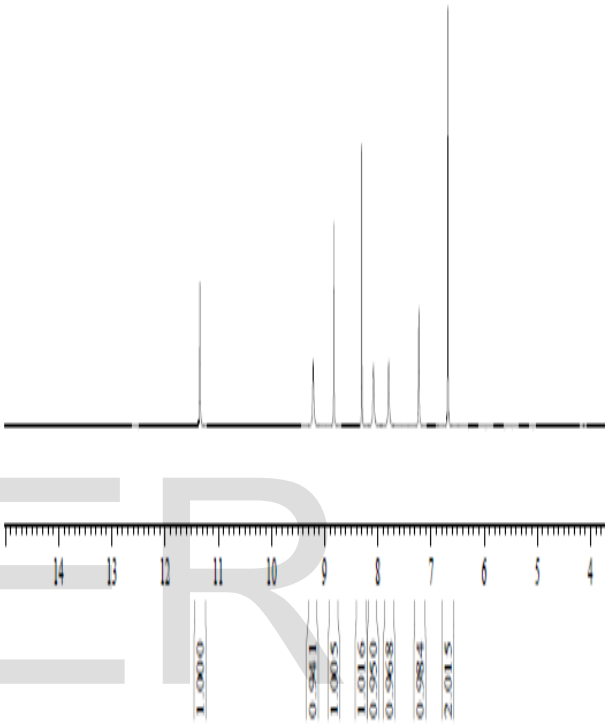


Figure 1: ^{1}H NMR spectrum of the ligand

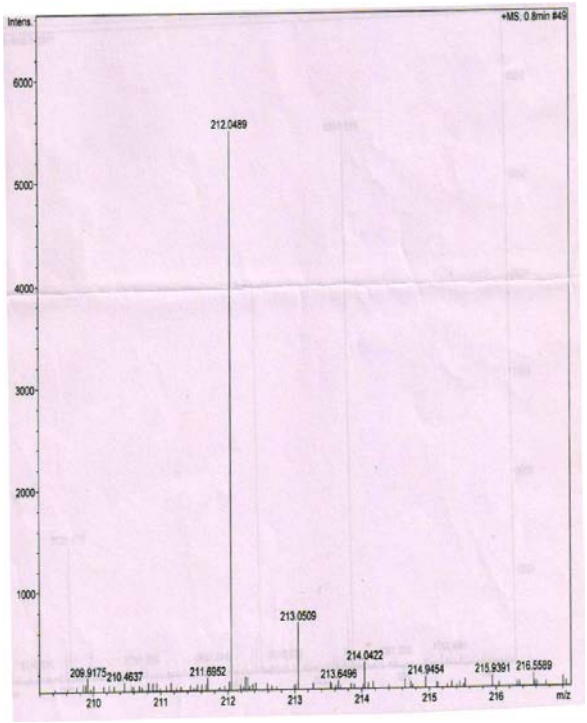
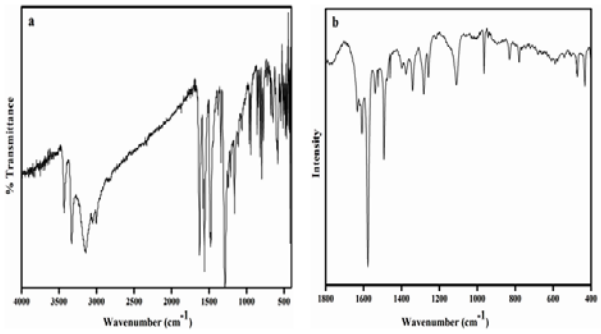


Figure 2: Mass spectrum of the ligand.

3.2 FT-IR and Raman spectra

The FT-IR and Raman spectra of different band assignments of the ligand DHBATSC and its metal complexes are listed in Table 2. DHBATSC has the characteristic thioamide moiety (-HN-C(S) NH), which is present in thione form. The spectra of DHBATSC show the absence of bands in 2500-2600 cm^{-1} region indicating the presence of the free DHBATSC in thione form [17], and it shows a strong IR and Raman bands at 1628 and 1612 cm^{-1} Fig. (3). Which is corresponding to the azomethine, υ(HC=N), group. In the spectra of the complexes, the shift of this band to lower frequency is observed, suggesting the participation of azomethine nitrogen in the coordination to metal ions [18]-[19]. This feature is further supported by the shift of υ(N-N) band to higher frequency upon the complexation in the free ligand at 958 and 951 cm^{-1}, in IR and Raman respectively. On the other hand, the participation of the deprotonated thiol sulfur in coordination was indicated by the shift of IR band at 820 cm^{-1} in the free ligand, 800, 798 cm^{-1} for IR in Cu(II) and Pd(II) complexes respectively [20]. The υ(NH) band shifting is supported to the thione sulfur participation in coordination. DHBATSC act as a neutral bidentate ligand through thione sulfur as well as azomethine nitrogen atoms. The spectra of the complexes show that new bands are observed in the IR near 470 cm^{-1} Cu(II) and



472 cm^{-1} for Pd(II) complexes.

Figure 3: (a) FT-IR (b) Raman spectrum of the ligand.

3.3 Electronic absorption spectra

The electronic absorption spectrum of ligand exhibited two intraligand absorption maxima at 37878 and 32051 cm^{-1}. These two bands are assigned to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions respectively. In addition to this, the spectrum exhibits a band at 27700 cm^{-1}. This band is assigned to $n \rightarrow \pi^*$ transition of the azomethine group and is found to shift to longer wavelength on coordination through azomethine nitrogen [21]. The absorption spectrum of the ligand and Cu(II) complex is shown Fig. (4). The spectrum exhibit bands at 14925 and 23980 cm^{-1} which are assigned to d-d transitions $^2B_{1g} \rightarrow {}^2B_{2g}(d_{x^2-y^2} \rightarrow d_{xy})$ and $^2B_{1g} \rightarrow {}^2E_g(d_{x^2-y^2} \rightarrow d_{xz}, d_{yz})$ respectively [22]. The spectral data suggests that the symmetry is elongated tetragonal geometry [23]. In addition two bands are observed at 27173 cm^{-1}

and as 29411 cm^{-1} these two bands are attributed to intraligand transitions. A careful comparison electronic absorption spectrum of ligand with the spectrum of Pd(II) complex showed that the absorption maxima shifts to longer wavelength, 35335 and 23640 cm^{-1}. This shift is due to extended conjugation. The optical absorption band at 23474 cm^{-1} in Pd(II) complex is due to d-d transition which showed square planar symmetry in the complex [24].

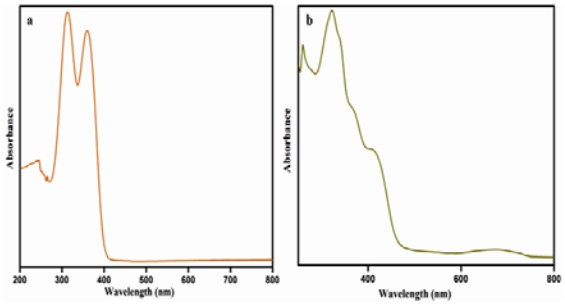


Figure 4: Electronic absorption spectra of ligand (a) and Cu(II) complex (b).

3.4 EPR studies

EPR spectrum of polycrystalline copper complex was recorded at room temperature on X-band ESR spectrometer with microwave frequency 9.760 GHz and 100 KHz field modulation. The spectrum is shown in Fig. (5). The spectrum exhibit three hyperfine lines corresponding to $g_{\parallel}$. For Cu(II) ion S = 1/2 and I = 3/2, one would expect four hyperfine lines. From these hyperfine lines, the effective $g_{\parallel}$ value is calculated and is found to be $g_{\parallel}$ = 2.19. The hyperfine splitting constant $A_{\parallel}$ is found to be 167 G. The resonance signals corresponding to $g_{\perp}$ exhibit three hyperfine lines only. From this, the effective $g_{\perp}$ value is calculated, and is found to be $g_{\perp}$ = 2.06 and the hyperfine splitting constant $A_{\perp}$ is found to be 52 G. The calculated g values provide valuable information on the electronic ground state of the ion. For g values, $g_{\parallel} > g_{\perp} > g_e$, the ground state of the ion is $d_{x^2-y^2}$ which suggests an elongated tetragonal symmetry [25]. The exchange coupling interaction between two copper ions is obtained from the expression given by Hathaway.

$$G = (g_{\parallel} - 2.0023) / (g_{\perp} - 2.0023)$$

If the value of G > 4, it indicates that the exchange interaction is negligible, while < 4, it indicates the considerable exchange interaction between copper ions. In the present work, the obtained G value (3.25) is less than 4.0 which suggests that there is appreciable exchange interaction between copper ions.

4. Biological studies

4.1 Antibacterial activity

The antibacterial activity of the ligand and their complexes were evaluated by the presence of zone of inhibition (Table 3). The Cu(II) and Pd(II) complexes showed moderate to good inhibition zones, when compared to standard antibiotic streptomycin. The zone of inhibition increased. With the increase in concentration, however, the ligand did not show any activity against the test bacteria except for Escherichia coli. The Cu(II) complexes showed significant activity against all organisms on comparison with Pd(II) complex.

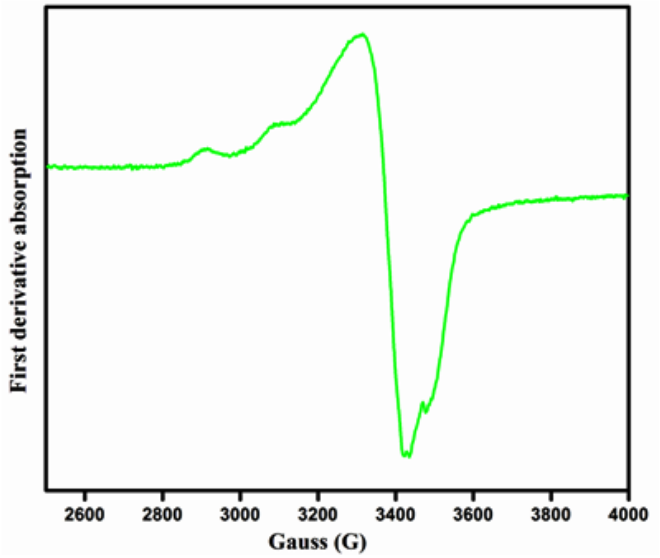


Figure 5. EPR spectrum of the Cu(II) complex.

2 Antioxidant activity

DPPH assay, the widely used invitro antioxidant assay, produce stable free radicals. It shows a strong absorption peak at 517 nm in visible spectrum due to the presence of an unpaired electron, in the presence of a free radical scavenger the electron becomes paired off and the absorption decreases with the respect to the number of electrons taken up. As shown in the Fig. (6). Support that the synthesized complexes exhibit good antioxidant property when compared with the ligand. The free radical scavenging activity of the ligand and their complexes increased with increasing concentration. The complex Cu(II) showed good bleaching of DPPH followed by Pd(II) and Ligand.

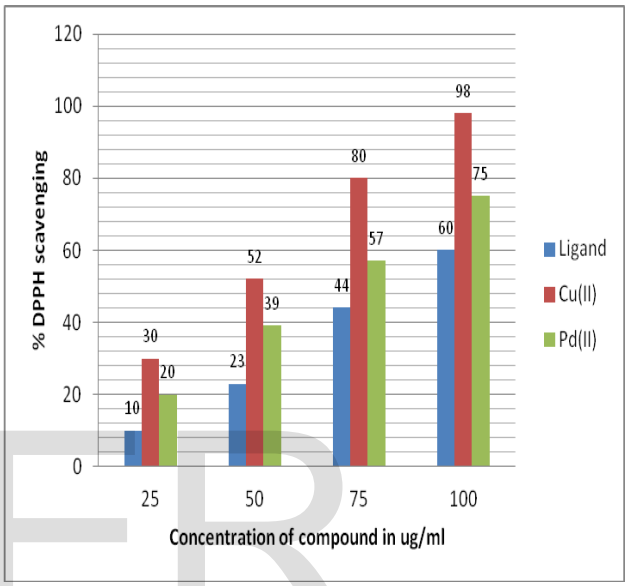


Figure 6. DPPH Scavenging activity of Ligand and their Cu(II) and Pd(II) complex.

Table 1: Analytical data for the ligand and its Cu(II) and Pd(II) complexes.

S.No	Compounds	Color	M.Wt.	M.P(°C)	Yield (%)	Elemental analyses data (%) (calculated)		
						C	H	N
1	Ligand	white	212.0489	265	76	45.32(45.49)	4.36(4.29)	19.76(19.89)
2	Cu(II)	Light green	767.1222	>300	66	35.89(35.70)	3.62(3.70)	14.76(14.69)
3	Pd(II)	Orange	809.9962	>300	65	33.17(33.21)	3.51(3.44)	13.76(13.67)

Table 2: Assignments of the FT-IR (cm^{-1}) and Raman (cm^{-1}) spectral bands of the ligand and their Cu(II) and Pd(II) complexes.

Ligand		Cu(II)	Pd(II)	
FT-IR	Raman	FT-IR	FT-IR	Assignments
3337	-	3360	3233	υsyNH
1628	1612	1636	1608	υC=N + υC=C
1335	1343	1350	1313	υNH + υasyC=S
820	834	800	798	υsyC=S + δNH
-	-	470	472	M-N

Table 3: Anti bacterial activity of ligand and their Cu(II) and Pd(II) complexes.

Bacterial species	Compound	Zone of inhibition in mm				
		Concentration in µg/ml				
		25	50	100	150	Std. 30
Escherichia coli	Ligand	9	12	16	28	34
	Cu(II)	8	11	16	24	32
	Pd(II)	8	10	18	26	31
Klebsiella pneumonia	Ligand	ND	ND	ND	ND	32
	Cu(II)	ND	8	14	20	32
	Pd(II)	10	13	18	24	33
Salmonella typhi	Ligand	ND	ND	ND	ND	32
	Cu(II)	ND	12	15	21	29
	Pd(II)	ND	10	14	23	31
Bacillus cereus	Ligand	ND	ND	ND	ND	36
	Cu(II)	10	12	18	28	33
	Pd(II)	8	11	19	28	34
Staphylococcus aureus	Ligand	ND	ND	ND	ND	35
	Cu(II)	12	14	20	32	36
	Pd(II)	10	12	20	30	33

Std. Streptomycin; Standard Error: ±2 mm

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6. References

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