

Spectral and Antimicrobial Study of Cd (II) Complexes and Heterocyclic base Adducts Derived from Thiosemicarbazone Moiety

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ABSTRACT: The synthesis and characterization, spectroscopic, and biological studies of 5-chloro-2-hydroxy acetophenone thiosemicarbazone and its complexes with cadmium (II) chloride are reported here. The ligand was synthesized by condensation reaction of appropriate ketone and thiosemicarbazide compounds. The reactions were carried out in the presence of sulfuric acid. The ligand, complex and adducts were characterized by means of Elemental analysis, ESI-MS, Fourier Transform Infrared (FT-IR) spectroscopy, Nuclear Magnetic Resonance (^1H NMR and ^{13}C NMR) spectroscopy magnetism and conductivity. The metal complexes and their corresponding ligands were tested against bacterial parasites. It was found that the cadmium complexes synthesized are more biologically active than their corresponding ligand.

KEYWORDS: Metal salts, Bioactive metal complexes, antimicrobial activity, thiosemicarbazone

I. INTRODUCTION

Thiosemicarbazones are compounds constituting N,S donor atoms. On complexation with metal ion, they show heterodentate nature [1-6]. Thiosemicarbazones are good ligands with high biological activity. Their biological activity is due to ability to coordinate to metal centres. Thiosemicarbazone derivatives also have additional functional groups which can coordinate to metal ion. This suggests the biological activity may also depend on the non-coordinating groups. It is the important transition metal biologically. Cadmium is toxic element and is naturally present in the environment. The analysis of biosystems with cadmium ion is a problem due to toxic effect of this metal, associated with Hg and Pb most toxic environmental pollutants [7-9]. It is rapidly localized intracellularly in the liver and then bound to metallothionein forming a complex that is slowly transferred to the blood stream to be deposited in the kidneys. This metal competes with Zn and blocks active sites of metal-enzymes. As a relatively soft acid it removes Zn(II) in cysteine-coordinated zinc compounds or Ca(II) ions in bone cells. The development of chelating agents is essential for the treatment of cadmium in toxication. The ability to coordinate *via* sulphur is enhanced for cadmium as compared to Zn (II), whose toxic properties are related to strong Cd-S bond [10]. Moreover, chelating sulfur donors are under study as antidotes in cadmium(II) poisoning [11,12]. The increase in antitumor activity in presence of Cd (II) ions has been reported [13]. Cd (II) has stable filled d sublevel (10) and it shows few characteristics of transition metals despite its position in the d-block of the periodic table. It also forms stable complexes with O,N,S donor ligands like other elements. d^{10} configuration affords no crystal field stabilization. Hence stereochemistry of a compound depends on the size and polarizing power of the M(II) cation and the steric requirement of the ligands. The M^{2+} ion with its d^{10} configuration shows no stereochemical preferences arising from ligand field stabilization effects. Therefore they form a variety of coordination numbers ranging from two to six, and geometries depended on the interplay of electrostatic forces, covalence and the size factor. Both Zn (II) and Cd(II) favour 4-coordinate tetrahedral complexes though Cd(II), being the larger one, forms 6-coordinate octahedral complexes more readily than Zn(II). 4, 5 and 6 Coordination numbers are the common for both elements.

In this research work the synthesis, spectral characterisation and biological studies of six coordinate complexes of zinc (II) with 5-dichloro 2-hydroxy acetophenone N (4) methyl thiosemicarbazone have been reported.

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II. MATERIALS AND METHODS

The chemicals used are of A.R. grade. Room temperature magnetic susceptibility measurement was carried out by Faraday method. IR spectra were recorded in solid state in the range 4000-200 cm^{-1} range. NMR spectra were recorded in the mixture of CDCl_3 and DMSO-d_6 (1:1 v/v) with a Bruker AC-300F 300MHz spectrometer. The number of water molecules in the complexes were determined by TGA in the temperature range 25-800°C. Metal in the complex and adducts was estimated by standardized E.D.T.A using xylenol orange as an indicator and PH-10 buffer solution. Chloride in the complex was determined by Mohr's method.

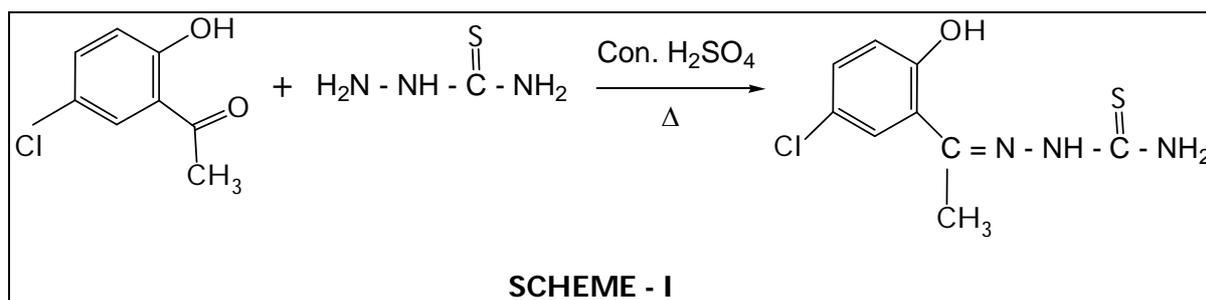
III . LITERATURE SERVE

Fifteen novel biologically active macrocycles have been synthesized by condensing orthophthaldehyde with different primary amines [14]. A series of new hydrazides have been synthesized by reaction of novel ligands acetic acid (3-chloro-4-hydroxyl benzylidene)-hydride, acetic acid (2-nitro-benzaylidene)-hydride with Cd (II) bromide [15]. New complexes of 2-amino 5-chloro benzophenone-4-R thiosemicarbazone (where R=H (HL1), C₆H₅ (HL2 with Pd (II), cd (II) and Hg (II) chloride salts were synthesized [16]. The complexes of Cd (II) and Hg (II) with thiosemicarbazone have been studied by microanalysis, thermal electrochemical and spectral [17]. Mixed ligand complexes of Zn(II), Cd(II), Hg(II) with cis-3,7-dimethyl 2,6-octadienthiosemicarbazone (CDOTSC :LH) and N Pthaloyl amino acids AH have been synthesized [18].

IV. EXPERIMENTAL

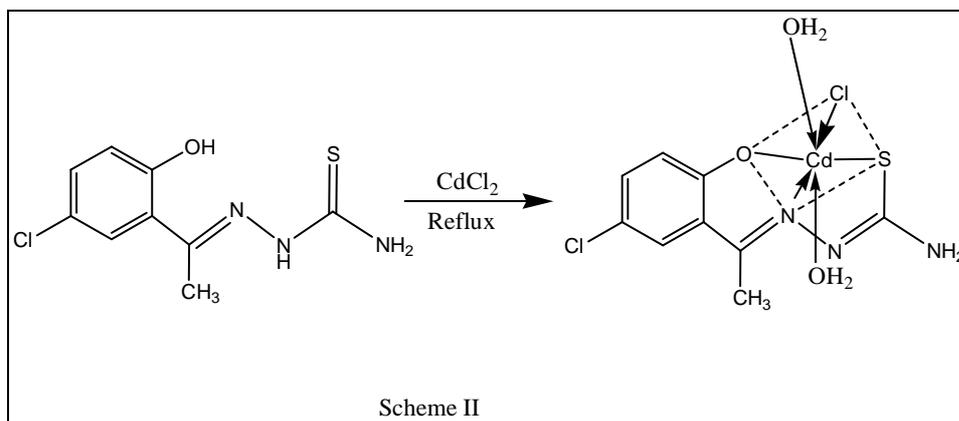
Synthesis of thiosemicarbazone :

Ethanol solution of 5-chloro 2-hydroxy acetophenone (0.01 mole) was added to ethanol solution of thiosemicarbazide (0.01 mole) in the mole ratio 1:1. The reaction mixture was refluxed for three hours. The pale yellow product obtained on cooling was filtered and washed with hot water then cold ethanol and finally with ether. The compound was then purified in ethanol and dried in vacuum.



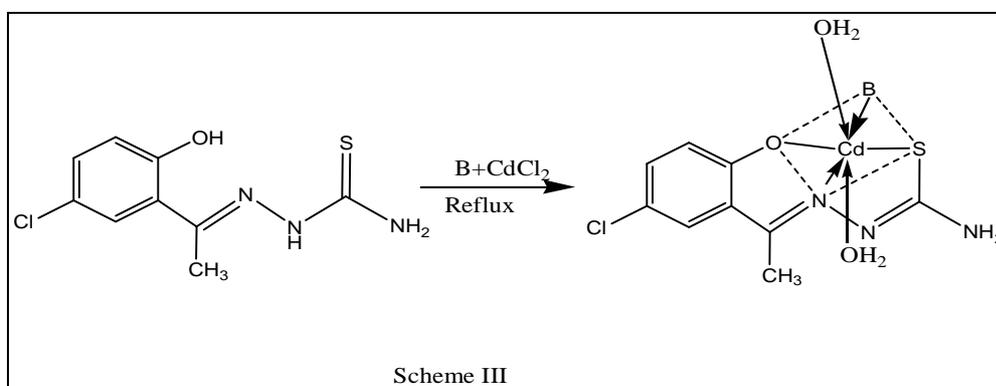
Synthesis of complex M:L:

The complex of the type $\text{M.L.Cl} \cdot (\text{H}_2\text{O})_2$ was synthesized by refluxing ethanol solutions of thiosemicarbazone (0.01 mole), $\text{CdCl}_2 \cdot 2.5 \text{H}_2\text{O}$ (0.01 mole) in the mole ratio 1:1 for two hours. The light yellow product obtained was filtered and washed with hot water, cold absolute ethanol and then ether and dried in vacuum.



Synthesis of adducts:

The adducts of the type Cd.L.B (B is heterocyclic base like pyridine, 2-chloro pyridine, 3-chloro pyridine, 4-chloro pyridine) was synthesized by adding slowly ethanolic solutions of CdCl₂.2.5H₂O (0.01 mole) and heterocyclic base (0.01 mole) to the hot ethanolic solution of thiosemicarbazone(0.01 mole) in the ratio 1:1:1 and refluxing reaction mixture for four hour. The yellow adduct obtained was filtered and washed with hot water, cold ethanol and diethyl ether and dried in vacuum.



(B= pyridine 2-chloro pyridine,3-chloro pyridine,4-chloro pyridine)

TABLE 1 PHYSICAL PROPERTIES:

Compounds	Colour	Empirical Formula	Molar conductance Ohm ⁻¹ cm ² mole ⁻¹	Magnetic Moment B.M.
L	Yellow	C ₉ H ₁₀ N ₃ OSCl	-	-
Cd.L.Cl.(H ₂ O) ₂	Yellow	C ₉ H ₁₂ N ₃ O ₃ SCl ₂ Cd	45.4	Diamagnetic
Cd.L.Py.(H ₂ O) ₂	Yellow	C ₁₄ H ₁₇ N ₄ O ₃ SClCd	38.7	Diamagnetic
Cd.2-Cl.py.(H ₂ O) ₂	Yellow	C ₁₄ H ₁₆ N ₄ O ₃ SCl ₂ Cd	33.6	Diamagnetic
Cd.3-Cl.py.(H ₂ O) ₂	Yellow	C ₁₄ H ₁₆ N ₄ O ₃ SCl ₂ Cd	37.8	Diamagnetic
Cd.4-Cl.py.(H ₂ O) ₂	Yellow	C ₁₄ H ₁₆ N ₄ O ₃ SCl ₂ Cd	36.7	Diamagnetic

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Physical data of ligand and its complexes is presented in Table 1. The complex and all adducts are in soluble in ethanol and soluble in DMF in which conductivity measurements were carried out (25⁰C), showing all complexes are non electrolyte in nature [19]. The magnetic moment measurement was carried out at room temperature by Faraday method showed diamagnetic behavior expected for d¹⁰ configuration.

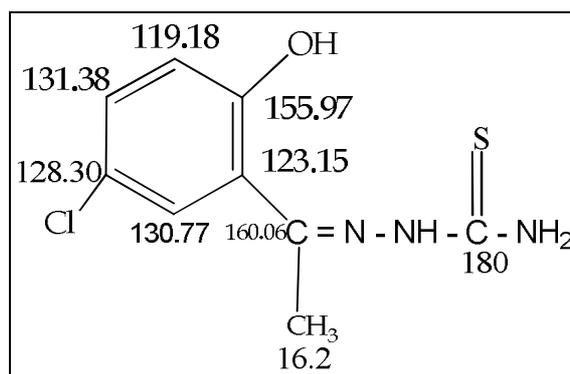
¹H-NMR

Signals at 13, 3.40 ppm are assigned to – OH, - CH₃ protons respectively.

Thiosemicarbazone does not show any peak corresponds to S-H proton, indicating it exists in thioketo form. Absence of ²NH proton signal suggests enolisation of ²NH – C = S group to ²N=C-SH. Little low field position of ⁴NH (7.8 ppm) could be attributable to the deshielding caused by – N = C< of the system N=CSH = NH). Aromatic protons show multiples at 7.00, 7.25 and 7.50 ppm range.

¹³C-NMR (DMSO-D₆): δppm

119.18 (C = C), 131.38 (C = C), 128.27 (C = C – Cl), 130.77 (C = C), 123.15 (C = C), 155.97 (C = C – OH), 160.06 (C = N), 180.0 (C=S), 16.2 (C-CH₃).



ESI-MS m/z, ion M⁺ (Calcd) found

C₉H₁₀N₃O₃SCl (243.70) 243.11, C₉H₁₂N₃O₃SCl₂Cd (425.16) 425.90, C₁₄H₁₇N₄O₃SClCd (468.81) 468.11, C₁₄H₁₆N₄O₃SCl₂Cd (503.26) 503.88, C₁₄H₁₆N₄O₃SCl₂Cd (503.26) 503.89, C₁₄H₁₆N₄O₃SCl₂Cd (503.26) 503.91. Mass spectral data confirmed the structure of the thiosemicarbazone and complexes as indicated by molecular ion peak (M + 1) corresponding to their molecular weights.

V. ANALYTICAL DATA

1. **L**: % C 44.91 (44.35), % H 4.88 (4.14), % N 17.92 (17.24), % O 6.06 (6.57) % S 13.71 (13.16)
2. **Cd-L.Cl.(H₂O)₂**: % Cd 26.84 (26.34), % Cl 8.61 (8.34), % C 25.04 (25.42), % H 2.02 (2.84), % N 9.10 (9.88), % O 11.78 (11.28) % S 8.10 (7.54).
3. **Cd-L.Py.(H₂O)₂**: % Cd 23.10 (23.89), % C 35.12 (35.87), % H 3.08 (3.65), % N 12.17 (11.95), % O 10.91 (10.24) % S 6.05 (6.84).
4. **Cd-2-Cl.py.(H₂O)₂**: % Cd 22.88 (22.25), % C 33.90 (33.41), % H 3.92 (3.27), % N 11.71 (11.13), % O 10.91 (10.24) % S 6.05 (6.84).
5. **Cd-3-Cl.py.(H₂O)₂**: % Cd 22.87 (22.25), % C 33.88 (33.41), % H 3.87 (3.27), % N 11.65 (11.13), % O 10.72 (10.24) % S 6.09 (6.84).
6. **Cd-4-Cl.py.(H₂O)₂**: % Cd 22.70 (22.25), % C 33.77 (33.41), % H 3.68 (3.27), % N 11.65 (11.13), % O 10.89 (10.24) % S 6.12 (6.84).

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The calculated and experimental percentages are matched and confirmed 1:1 mole ratio of metal ion, thiosemicarbazone for complex and 1:1:1 mole ratio for metal ion, thiosemicarbazone and heterocyclic base for all adducts.

VI INFRARED SPECTROSCOPIC DATA (cm⁻¹)

- 1.L:** ν (- OH) 3147; ν (C = N) 1624; ν (- C - S) 758 (s), 1374 (m); ν (N - N) 1049; ν (²N-H) 2943; ν (C - O) 1281.
2 Cd-L.Cl.(H₂O)₂: ν (C = N) 1555; ν (C = N-N=C) 1520, ν (C-S) 706, 1308, ν (N-N) 1152, ν (M - N) 462, ν (M-O) 532, ν (M-S) 331, ν (C - O) 1212, ν (H₂O) 3420,3540.
3 Cd.L.Py.(H₂O)₂: ν (C = N) 1562; ν (C = N-N=C) 1532, ν (C-S) 710, 1311; ν (N-N) 1154, ν (M - N) Base 259, ν (M - N) 460, ν (M - O) 532, ν (M-S) 334, ν (C - O) 1214, Band due to HB 1422, ν (H₂O) 3450,3522.
4. Cd.2-Cl.py.(H₂O)₂: ν (C = N) 1566; ν (C = N-N=C) 1533, ν (C-S) 716, 1317, ν (N-N) 1162, ν (M - N) Base 262, ν (M - N) 473, ν (M - O) 545, ν (M-S) 342, ν (C - O) 1229, Band due to HB 1426, ν (H₂O) 3460,3533.
5. Cd.3-Cl.py.(H₂O)₂: ν (C = N) 1577; ν (C = N-N=C) 1548, ν (C-S) 722, 1326, ν (N-N) 1172, ν (M - N) Base 275, ν (M - N) 470, ν (M - O) 553, ν (M-S) 352, ν (C - O) 1236, Band due to HB 1438, ν (H₂O) 3470,3542.
6. Cd.4-Cl.py.(H₂O)₂: ν (C = N) 1592; ν (C = N-N=C) 1559, ν (C-S) 733, 1338, ν (N-N) 1188, ν (M - N) Base 276, ν (M - N) 482, ν (M - O) 566, ν (M-S) 365, ν (C - O) 1244, Bands due to HB 1444, ν (H₂O) 3480,3555.

The band due to ν (N-H) appears at 3250 cm⁻¹ for thiosemicarbazone and disappeared in the spectra of all the complexes. The band at 1624 cm⁻¹ due to ν (C = N) in thiosemicarbazone shifted to lower side upon coordination suggest bonding through azomethine nitrogen. The coordination of azomethine nitrogen is confirmed by a band in the range 400-485cm⁻¹, assignable to ν (Cd-N) for the complexes. A shift in the ν (N- N) bands from 1049 cm⁻¹ to higher region is due to the increase in double bond character off-setting the loss of electron density via donation to the metal. This also confirms the coordination through azomethine nitrogen. The coordination via thiolate sulfur is indicated by decrease in ν (C=S). The bands at 330-370 cm⁻¹ assignable to ν (Cd-S) in the complexes confirm sulfur coordination. The absence of band in the region 2600-2800 cm⁻¹ suggests the coordination through thiolato sulphur. The new peaks observed in the range 530-570 cm⁻¹ are due to the Cd-O (phenolic) bond. This is indicated by decrease in ν (C-O).

VII TGA ANALYSIS DATA

- 1.Cd-L.Cl.(H₂O)₂:** First step, 118 °C, Mass loss 8.86 % second step, 210 °C, Mass loss, 24.23 % Third Step 355 °C, Mass loss, 50.22 % Fourth Step, 670°C, Mass loss 65.5 %, Residue 782 °C, % of CdO, 30.86 (30.11).
2.Cd.L.Py.(H₂O)₂: First step, 115 °C, Mass loss 7.07 % second step, 220 °C, Mass loss, 22.32 % Third Step 355 °C, Mass loss, 77.02 % Fourth Step, 669 °C, Mass loss, 62.00 %, Residue, 779 °C, % of ZnO, 27.96 (27.34).
3. Cd.2-Cl.py.(H₂O)₂: First step, 113 °C, Mass loss 7.88 % second step, 230 °C, Mass loss, 27 % Third Step 365 °C, Mass loss, 48.40 % Fourth Step, 660 °C, Mass loss 62.5 %, Residue 778 °C, % of ZnO, 22.88 (22.25).
4 Cd.3-Cl.py.(H₂O)₂: First step, 116 °C, Mass loss 7.90 % second step, 235 °C, Mass loss, 28.25 % Third Step 360 °C, Mass loss, 49.02 % Fourth Step, 666 °C, Mass loss, 63.27 %, Residue, 779 °C, % of ZnO, 22.90 (22.25).
5 Cd.4-Cl.py.(H₂O)₂: First step, 117 °C, Mass loss 7.91 % second step, 240 °C, Mass loss, 28 % Third Step 365 °C, Mass loss, 48.28 % Fourth Step, 669 °C, Mass loss 62.70 %, Residue 800 °C, % of ZnO, 22.82 (22.25).

The hydrated layer was removed below 103°C. The two coordinated water molecules were eliminated in the complexes at relatively temperature less than 120°C. The two coordinated water molecules in complexes were removed in one step at a temperature less than 120 °C. The Thermal analysis decomposition proceeded in four steps. The compounds showed stability upto about 260°C. The complexes containing heterocyclic bases are more stable than other complex. This shows heterocyclic bases in coordination increases the thermal stability. There are three steps after the removal of water molecules in complexes. In the second step the loss is about 25%. The organic molecule was lost upto 650°C. The mass lost corresponding to this step is about 60%. The decomposition was complete and metal oxide was formed at a temperature about 780 °C. The stability of thiosemicarbazone increases on complexation. The metal complexes are more stable than thiosemicarbazone.

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VIII. BIOLOGICAL ACTIVITY (AGAR PLATE DIFFUSION METHOD)

Table.4 Minimum Inhibitory concentration L , Cd (II) complexes and standered

Compound	<i>Staphylococcus aureu</i>		<i>Bacilus subtilis</i>		<i>Escherichia Coli</i>		<i>Peudomonas aeruginosa</i>	
	Gram positive				Gram negative			
	2 µg/ml	2.5 µg/ml	2 µg/ml	2.5 µg/ml	2 µg/ml	2.5 µg/ml	2 µg/ml	2.5 µg/ml
L	0.70	0.365	0.72	0.42	0.72	0.41	0.74	0.41
Cd-L.(H ₂ O) ₃	0.61	0.32	0.63	0.21	0.63	0.22	0.66	0.23
Cd-L.Py.(H ₂ O) ₂	0.61	0.31	0.63	0.25	0.64	0.21	0.63	0.24
Cd-2-Cl.py.(H ₂ O) ₂	0.66	0.32	0.65	0.22	0.66	0.24	0.68	0.26
Cd-3-Cl py.(H ₂ O) ₂	0.67	0.34	0.67	0.23	0.69	0.23	0.67	0.25
Cd-4-Cl py.(H ₂ O) ₂	0.60	0.31	0.61	0.23	0.62	0.21	0.65	0.22
CdCl ₂ .2.5H ₂ O	0.20	0.15	0.22	0.17	0.14	0.23	0.12	0.25
Standard	0.14	0.12	0.15	0.13	0.24	0.13	0.15	0.12

(Std-Amphiciline)

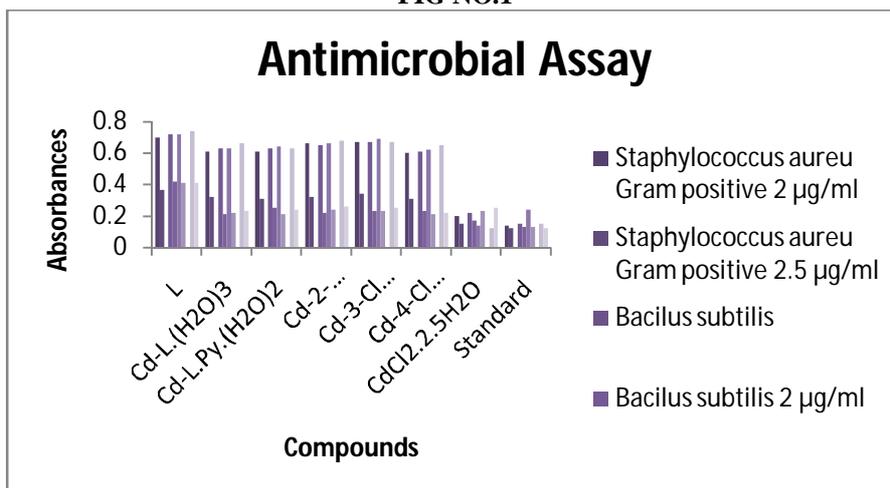
The bacterial assay was carried out by the agar plate diffusion method. Activity was measured by measuring the absorbance. As compared to complex and thiosemicarbazone, adducts showed good activity against bacterial species than free ligand. The minimum inhibitory concentration was determined by liquid dilution method [20]. The solutions of thiosemicarbazone and complexes with 2 µg/ml, 2.5 µg/ml and 3 µg/ml concentrations were prepared in the solvent DMF. The solutions of standard drug ampicillin and metal salt were also prepared in the same concentration. Inoculums of the overnight culture were prepared. 0.2 ml of the inoculums was added to the test tubes containing the solutions of the compounds of different concentrations. After the addition of sterile water to each of the test tubes, these were incubated for 24 hours and observed for turbidity. The absorbance of the turbid solutions was measured at 520 nm. The same method was used for standard [21]. It was observed that at 2 µg/ml the absorbance is more. Less absorbance at 2.5 µg/ml and no absorbance observed at 3 µg/ml. The inhibition is more at 2.5 µg/ml. The metal salt solution showed better inhibition than ligand and complexes. The inhibitory concentration is 2.5 µg/ml. Thus increase in coordination number in coordination and coordination of metal ion to ligand enhances microbial activity. The free metal ion was found more effective than thiosemicarbazone and complexes.

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FIG NO.1



The above bar graph indicates antimicrobial assay of the thiosemicarbazone and the complexes. The absorbance in the complex is less than the complexes containing heterocyclic bases. This shows better inhibitory activity by complex than the complexes containing heterocyclic bases. The presence of metal ion in the complexes enhances microbial activity. The absorbances in the metal salt solutions and standard Amphotericin drug are less. The free metal ion is more effective than the complexes.

IX. CONCLUSION

The synthesized thiosemicarbazone is tridentate ONS donor. The spectral data indicates octahedral geometry for complexes and adducts. The complex and adducts are diamagnetic. Two water molecules were found in coordination. The compounds found thermally stable. The decomposition proceeded in several steps. The complexes showed growth inhibitory activity against gram +ve bacterial species. The absorbances are found more at 2 µg/ml concentration than 2.5 µg/ml. This suggested increase in concentration increase the activity. The metal salts showed more inhibitory activity.

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