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Spectral And Biological Evaluation Of Co (III) Complexes Derived From 5- Amino 2(4'thiazolyl) [1]hbezimidazole (ATBZ) and Heterocyclic Bases

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ABSTRACT: Six coordinate Co (III)-complex and adducts were synthesized by the reaction of $CoCl_2.6H_2O$ with and 5-aminothiabendazole as chelate ligand in ethanol and heterocyclic bases like pyridine, bipyridine, α-picoline,β-picoline. The synthesized compound ATBZ was elucidated by elemental analysis, ^{13}C, ^{1}H NMR.The synthesized complex and adducts were characterized by elemental analysis,IR,TGA,magnetic measurement and conductivity. Screenings of antimicrobial activity of synthesized compounds were also carried out against different bacterial and fungal species such as *Pseudomonas putida*, *Escherichia coli*, *Aspergillus Nigar*, The synthesized compounds showed potent activity bacterial and fungal species.

KEYWORDS: 5-aminothiabendazole, $CoCl_2.6H_2O$, antimicrobial assay.

I .INTRODUCTION

The word 'cobalt' has been derived from the German 'Kobalt', from Kobold meaning 'globin', a word used by miners for the ore of cobalt [1]. Cobalt is required in the active centre of coenzymes called cobalamins. Cobalamins are pharmaceutical agents which are treated in pathologies arising from a lack of vitamin B_{12} [2]. In 1952 [3] the biological activity of cobalt complexes was reported. There has been interest in Co (III) complexes of bidentate mustards, which appear to act as hypoxia-selective agents [4]. Some Co (II) complexes have been found to be active against leukaemia, lymphoma cell lines [5] and bacterial strains [6]. Moreover Co (II) complexes possess in vivo insulin-like properties [7], antifungal [8] and antioxidant activity [9]. Dixit et al. have reported that, the Co (II) octahedral complexes exhibited good activity against both Gram (+) and Gram (-) bacterial strains but not higher than the free ligand alone [10].

Complexes of Cobalt in +2 and +3 oxidation states have been prepared and investigated with focus on the reactivity of the metal ions in the transmethylation reaction and reversible absorption of molecular oxygen [11, 12]. Planer Cobalt (II) complexes of Schiff bases and related ligands have been used as catalysts for activation of molecular oxygen [13-18]. The study of Co (II) complexes of thiosemicarbazones is important because of their antitumour [19], antimicrobial [20] and electrical properties [20, 21].

Sulfur and/or nitrogen heterocycles occur in the nature in the form of alkaloids, vitamins, pigments and as constituents of plant and animal cells. The utility of thiazoles in curative treatment has been firmly established. They exhibit anti-bacterial, anti-hypertensive, anti-anginal, anti-arrhythmetic, anti-histaminic, narcotic antagonist activities [21]. Thiazole nucleus is found in many antibiotics and vitamins.
The benzimidazole compounds are important group of fungicides. They have pronounced ability to control a large number of fungal diseases. Benomyl, thiabendazole and thiophanate methyl are examples of this fungicide class.

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Because of their systematic activity, they can help to control some diseases after infection due to their activity. Benzimidazole fungicides are used to prevent post-harvest rots and in soil-drench treatments [23].

The fungicidal properties of 2-(4-thiazolyl)-*1H*-benzimidazoles in plants have already been reported with protective and curative action. It is used to control of *Aspergillus, Botrytis, Ceratocystis, Cercospora, Colletotrichum, Corticium, Diaporthe, Diplodia, Fusarium, Gibberella, Gloeosporium, Oospora, Penicillium, Phoma, Rhizoctonia, Sclerotinia, Septoria, Thielaviopsis, Verticillium spp., etc*.[24] in asparagus, avocados, bananas, barley, beans, cabbage, celery, chicory, cherries, citrus, cotton, some cucurbits, flax, mangoes, mushrooms, oats, onions, ornamentals, pawpaws, pome fruit, potatoes, rice, soyabeans, strawberries, sugar beet, sweet potatoes, tobacco, tomatoes, turf, vines and wheat. Also used for control of storage diseases of fruits and vegetables and for control of Dutch elm disease. It is commonly used as an anthelminitic in human and veterinary medicine too [25].

5-Aminothiabendazole (ANTBZ) acts as both acid and base, thus it is possible to make compounds which are neutral, cationic or anionic in nature, as well as report biological activity of metal complexes. The potential N, N'-donor chelating agent are quite rare. In present paper we report synthesis and characterization of derivatives of 5-Aminothiabendazole, and differentiate fungi toxic activity with those of nitrothiabendazole.

Benzimidazole and many of its derivatives exhibit a variety of biological actions, including antibacterial, antiviral, anticancer and antifungal activity [26]. Benomyl, thiabendazole and thiophnate methyl are main examples of this fungicide class. Because of their systematic activity, they can help to control some diseases after infection.

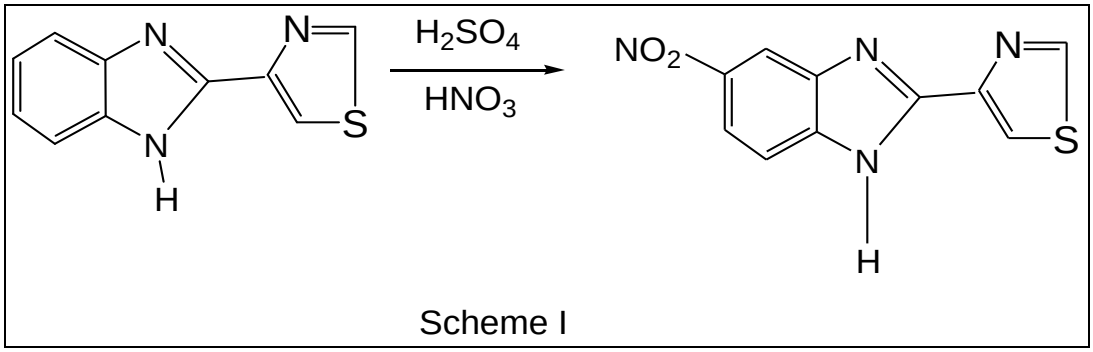
In present work 5-Aminothiabendazole,is selected as chelte ligand because of structural similarity to chelating agents such as 2,2' bipyridine and 1,10 phenanthroline.

II. MATERIALS AND METHODS

Thiabendazole (A.R.Grade), Cobalt Chloride (A.R.Grade), zinc dust, methanol, formic acid, chloroform, sodium bicarbonate, super saturated solution of NaCl (A.R.Grade).

Synthesis of Thiabendazole to 5-Nitrothiabendazole

Ice cold conc.H_2SO_4 was added to thiabendazole with constant stirring .The reaction mixture was warmed at 50º C for 10 min. till thiabendazole dissolved completely. In ice bath below - 4º C. Nitrating mixture (ice cold 1.5 ml conc.H_2SO_4 and 10.2 ml of conc. HNO_3) was added with constant stirring. After complete addition the reaction mixture was kept aside for 45 min (i.e. at R.T.25º C). The reaction mixture was then warmed at 85-90º C for 90 min. The reaction mixture was then cooled at room temperature. Crushed ice was then added with constant stirring, very faint yellowish white precipitate was then separated out. Sodium bicarbonate was then added to it till the effervesces of CO_2 completely stopped and precipitate became neutral. The precipitate was then filtered off, washed with water and finally with diethyl ether and dried under IR lamp.



Scheme I

Synthesis of 5-Nitrothiabendazole To 5-Aminothiabendazole

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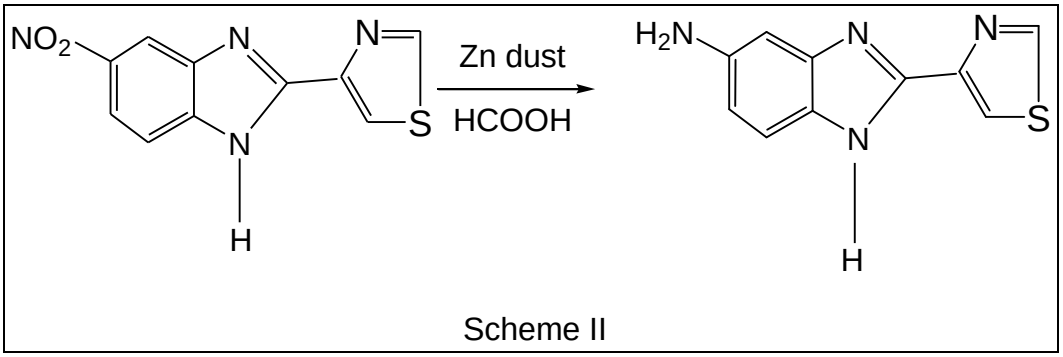
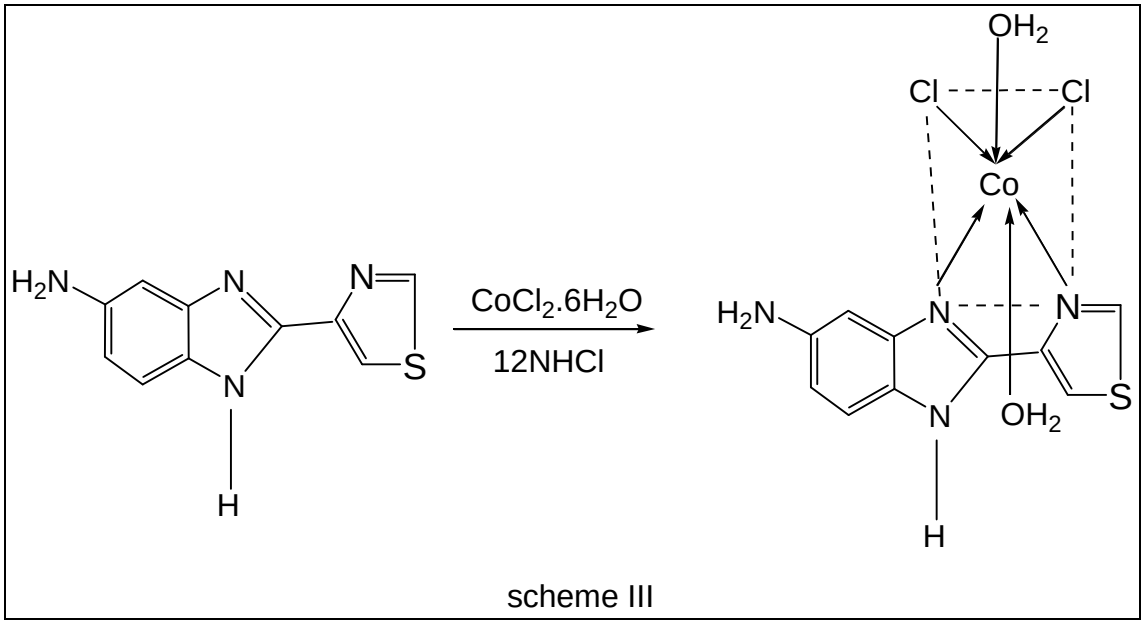
Methanol was added to homogeneous mixture of nitro thiabendazole and Zn dust. Formic acid was then added slowly with constant stirring. The solution was filtered. The filtrate was then warmed to evaporate the organic solvent completely. Few drops of chloroform and hot supersaturated solution of NaCl were added to remove the formic acid completely. Sticky residue formed washed to convert to the powdered residue, filtered, dried under IR lamp and stored in vacuum.



Scheme II

Synthesis of complex:

The complex of the type $Co.L.Cl_2.(H2O)_2$ was prepared by mixing 5-Amino TBZ in 40 ml of boiling ethanol containing 0.2ml of 12N HCl, The mixture was slightly warmed to ensure complete dissolution of the ligand. To the above mixture 10 ml of methanolic solution of cobalt (II) chloride was added. The solution was warmed for about 15 minutes. When it turned to a deep brown solution, the mix was refluxed for 2 hrs on a steam bath. The brown complex which separated out by centrifugation, washed with ethanol, dried under IR lamp and stored in vacuum.



scheme III

Synthesis of adducts:

This adduct of the type $Co.L.Cl.(H_2O)_2B$ (where B is heterocyclic bases pyridine,α-picoline,β- picoline,γ-picoline) was prepared by mixing 5-Amino TBZ in 40 ml of boiling methanol containing 0.2ml of 12N HCl, The mixture was slightly warmed to ensure complete dissolution of the ligand. To the above mixture 10 ml of methanolic solution of

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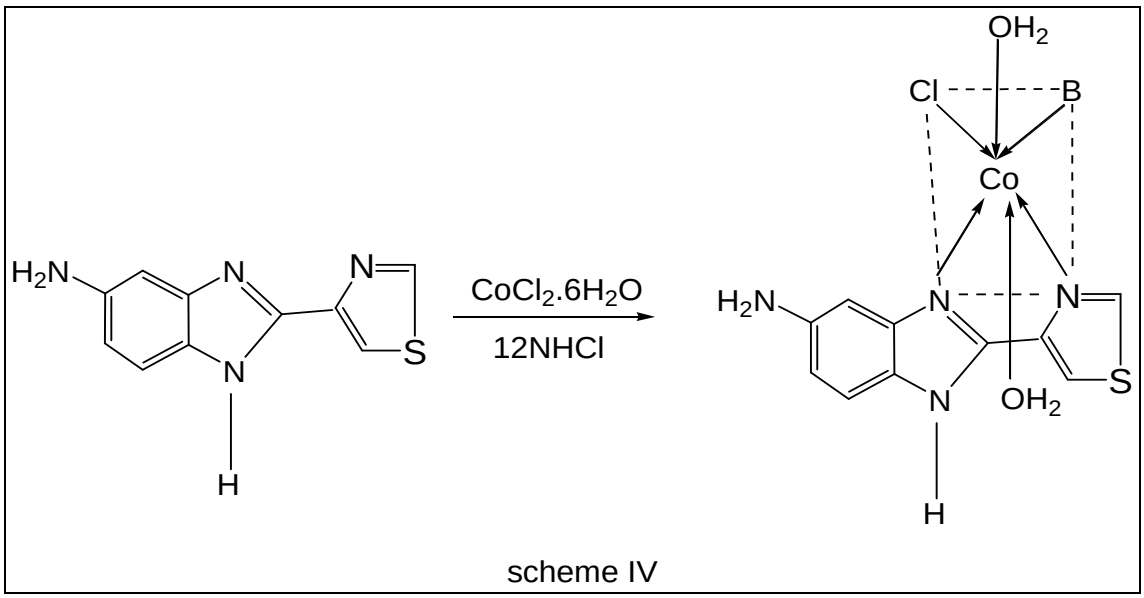
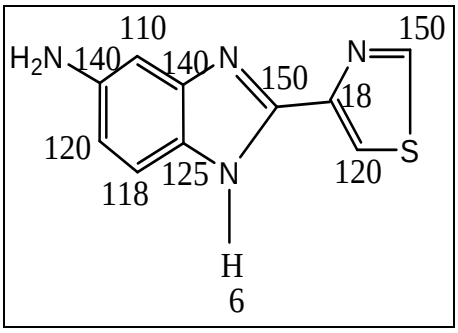
cobalt (II) chloride and heterocyclic bases in the ratio 1:1:1 in ~25 ml of methanol. The mix was refluxed for 2 hrs on a steam bath. The brown adducts which separated out by centrifugation, washed with ethanol, dried under IR lamp and stored in vacuum.



scheme IV

(B= pyridine, α-picoline, β- picoline, γ-picoline)

The ^{1}H-NMR signals at 4.2 δ-ppm corresponds to-NH_2, at 8.00 δ-ppm corresponds to N=C-H ,at 7.20 δ ppm corresponds to C=C-H-S,at 5.2 δ-ppm corresponds to N-H.The aromatic protons show multipletes at 6.4,6.8,7.40 δ-ppm.13C-NMR (DMSO-D6):δ-ppm 110 (C=C),140 (C=C),120 (C=C),118 (C=C),125 (C=C),140 (C=C),150 (N=C),150 (N=C),18 (C=C),120 (C=C).6 (N-H).



ESI-MS m/z for ligand (L) 216.58 (216.25) , ESI-MS m/z for Co.L.Cl_2.$(H_2O)_2$ 382.80 (382.11), ESI-MS m/z for Co.L.Cl.$(H_2O)_2$.py 425.32 (425.75),ESI-MS m/z for Co.L.Cl. $(H_2O)_2$.α-pico 440.10 (439.78), ESI-MS m/z for Co.L.Cl.$(H_2O)_2$.β-pico 439.18 (439.78), ESI-MS m/z for Co.L.Cl.$(H_2O)_2$.γ-pico 439.35 (439.78). Mass spectral data confirmed the structures of ligand and adducts as indicated by molecular ion peak (M+1) corresponding to their molecular weights.

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Table I Physical properties

Compounds	% yield	Empirical Formula	Molar conductance $Ohm^{-1}cm^2mole^{-1}$	Magnetic Moment B.M.
L	80.2	$C_{10}H_8N_4S$	-	-
$Co.L.Cl_2.(H_2O)_2$	82.3	$C_{10}H_{12}N_4SCoCl_2O_2$	90.2	Diamagnetic
$CoL.Cl.py.(H_2O)_2$	88.4	$C_{15}H_{17}N_5SCoCl\ O_2.py$	96.2	Diamagnetic
$CoL.Cl.\alpha$-pico.$(H_2O)_2$	88.0	$C_{16}H_{19}N_5SCoCl\ O_2.\alpha$-pico	90.0	Diamagnetic
$CoL.Cl.\ \beta$-pico.$(H_2O)_2$	85.2	$C_{16}H_{19}N_5SCoCl\ O_2.\beta$-pico	50.0	Diamagnetic
$CoL.Cl.\ \gamma$-pico.$(H_2O)_2$	87.6	$C_{16}H_{19}N_5SCoCl\ O_2.\gamma$-pico	30.2	Diamagnetic

The yield of the compounds is good. The compounds are insoluble in polar and non polar solvents and soluble in DMF in which conductivity measurement was carried out. The conductivity data indicate all compounds are non electrolyte [27]. The magnetic moment measurement was carried out at room temperature in polycrystalline state by Faraday method. The complex and adducts are found diamagnetic [28].

Table II Analytical data

Compounds	Elemental Analysis Found (Calculated) %				
	Metal%	%C	%H	%N	%S
L	-	55.92 (55.54)	3.15 (3.73)	25.20 (25.91)	14.97 (14.30)
$Co.L.Cl_2.(H_2O)_2$	15.70 (15.42)	30.10 (30.94)	3.71 (3.17)	14.04 (14.66)	8.91 (8.39)
$CoL.Cl.py.(H_2O)_2$	13.21 (13.54)	42.90 (42.31)	3.74 (4.02)	17.01 (16.45)	7.14 (7.53)
$CoL.Cl.\alpha$-pico.$(H_2O)_2$	13.10 (13.40)	43.85 (43.69)	4.25 (4.35)	15.27 (15.92)	7.07 (7.29)
$CoL.Cl.\beta$-pico.$(H_2O)_2$	13.18 (13.40)	43.85 (43.69)	4.25 (4.35)	15.52 (15.92)	7.82 (7.29)
$CoL.Cl.\ \gamma$-pico.$(H_2O)_2$	13.88 (13.40)	43.85 (43.69)	4.95 (4.35)	15.33 (15.92)	7.97 (7.29)

.Elemental analysis data are consistent with 1:1 ratio for metal ion, ligand and 1:1:1 ratio for metal ion ligand and heterocyclic base for all adducts. The metal in the complex and adducts was determined by E.D.T.A using xylenol orange as an indicator

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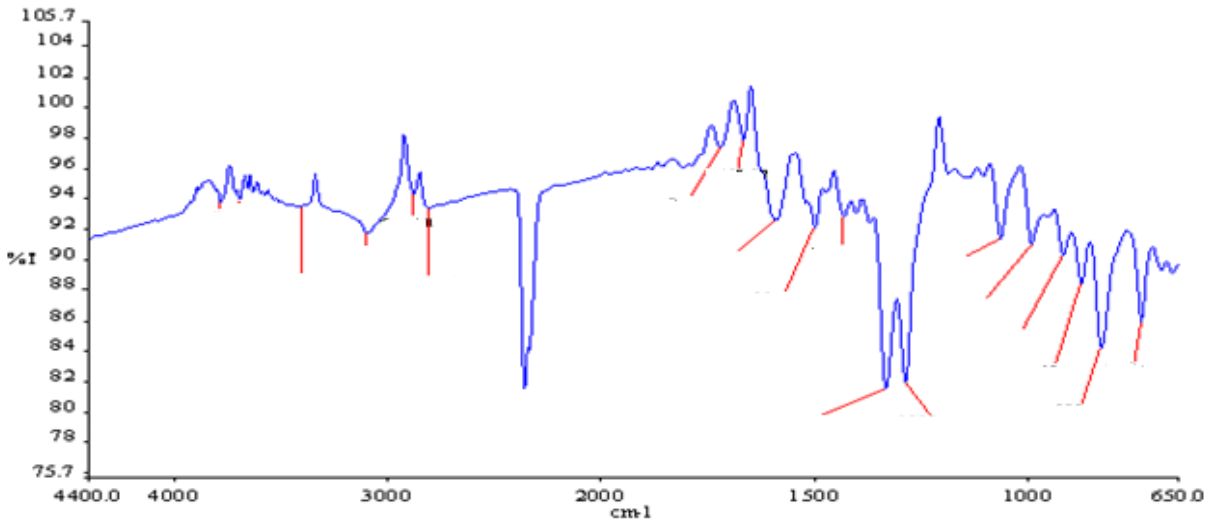
III IR SPECTRAL DATA

1. **L:** ν(C=N) 1722ν(N=C-S)1668
2. **Co.L.Cl$_2$.(H$_2$O)$_2$:** ν(C=N),ν(N=C-S) 1555,ν(H$_2$O) 3569,3646
3. **CoL.Cl.py.(H$_2$O)$_2$**ν(C=N)1638,ν(N=C□-S□□ 1531□ ,ν(H$_2$O□ □) 3546,3569,□ν□(M-N)□ □□ base,260,Bands due to heterocyclic base 1430.
4. **CoL.Cl.α-pico.(H$_2$O)$_2$:**ν(□ □C=N□)1635□ν□ □(N=C□-S□) 1534□, ν□ □(H$_2$O□ □)□ 3542,3555,,□ν(M-N)□ □□ □base,255, Bands due to heterocyclic base 1460.
5. **CoL.Cl.β-pico.(H$_2$O)$_2$:**ν(□ □ C=N□1651),□ν(□ □ N=C□S) □1522□ν(H$_2$O□ □) 3571,3550,□ν(M-N)□ □□ □ base,265, Bands due to heterocyclic base 1420.
6. **CoL.Cl.γ-pico.(H$_2$O)$_2$:**ν(□ □C=N)□□ 1648□,ν□ □(N=C□-S) □1540□,ν□□ □ (H$_2$O□ □) 3581,3588,,□ν□(M-N)□ □ base, 265, Bands due to heterocyclic base 1435.



IR spectrum of L

The position of IR bands is useful to detect the bonding sites of all ligand molecules interacted with the metal. The coordination of nitrogen shifted ν (C = N),□ ν (□N=C-S□) to lower wave numbers. The band shifted from 1722.-1668 cm^{-1} in uncomplexed compound spectra to about 1500-1800 cm^{-1} in the spectra of complexes. . The coordination of N atom of heterocyclic base is confirmed by ν (CoN) band in 250-270 cm^{-1} range. The bands of coordinated heterocyclic bases have also been observed in IR spectra of all complexes.

IV.THERMO GRAVIMETRIC ANALYSIS

The TGA curves of complexes were recorded between the temperatures 30 °C to 800 °C

1. **Co.L.Cl$_2$.(H$_2$O)$_2$**: First step, 115.30 °C, Mass loss 9.42 % second step, 140.29 °C, Mass loss, 18.00 % Third Step 230.43 °C, Mass loss, 25.02 % Fourth Step, 360.14 °C, Mass loss 55.5 %, Residue 800 °C, % of CoO, 19.93 (19.62).

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2. **CoL.Cl.py.$(H_2O)_2$**: First step, 115.40 °C, Mass loss 8.46 % second step, 142.40 °C, Mass loss, 15.52 % Third Step 245 °C, Mass loss, 22.02 % Fourth Step, 362.29 °C, Mass loss, 52.01 %, Residue, 793.57 °C, % of CoO, 17.82 (17.60).

3. **CoL.Cl.α-pico.$(H_2O)_2$** : First step, 116.29 °C, Mass loss 8.21 % second step, 140.29 °C, Mass loss, 18.00 % Third Step 225.43 °C, Mass loss, 25.02 % Fourth Step, 360.14 °C, Mass loss 55.5 %, Residue 800 °C, % of CoO, 22.01 (17.04).

4. **CoL.Cl.β-pico.$(H_2O)_2$** : First step, 117.28 °C, Mass loss 8.20 % second step, 142.14 °C, Mass loss, 15.52 % Third Step 235 °C, Mass loss, 25.02 % Fourth Step, 365.29 °C, Mass loss, 60.01 %, Residue, 773.57 °C, % of CoO, 16.72 (17.04).

5. **CoL.Cl. γ-pico.$(H_2O)_2$**: First step, 115.25 °C, Mass loss 8.19 % second step, 149.29 °C, Mass loss, 17.00 % Third Step 231.43 °C, Mass loss, 26.02 % Fourth Step, 367.14 °C, Mass loss 58.5 %, Residue 800 °C, % of CoO, 22.01 (17.04).

The coordinated water molecules were eliminated from their complexes at relatively higher temperature than those in the case of the lattice water molecules. The coordinated water molecules in complex and adducts were removed in one step. The two water molecules were removed at a temperature less than 120 °C The TGA data of complex and adducts indicated that the decomposition proceeded in several steps. There are three steps after the removal of two water molecules, first at a temperature > 140 °C, second > 225 °C and third > 360 °C. The decomposition was complete and CoO formed at a temperature > 750 °C.

V. ANTIMICROBIAL ASSAY

The antimicrobial activity was carried out by agar well diffusion method. The activity was confirmed by measuring the diameter of the inhibition zone (in mm) showing by the hanging drop method. Activity was measured in 10^{-3}M concentration. The results of antibacterial and antifungal studies are given in Table III and IV

Table III Antimicrobial Assay of L, Co (III) adducts

Compound	Pseudomonas putida	Escherichia coli	Aspergillus Nigar	Candida Albicans
L	10	11	10	10
Co.L.Cl_2.$(H_2O)_2$	16	15	14	15
CoL.Cl.py.$(H_2O)_2$	15	13	13	14
CoL.Cl.α-pico.$(H_2O)_2$	14	12	13	14
CoL.Cl.β-pico.$(H_2O)_2$	13	12	12	12
CoL.Cl. γ-pico.$(H_2O)_2$	14	13	12	13
Standered	17	16	16	17
$CoCl_2.6H_2O$	18	19	20	18

(Zone in mm)

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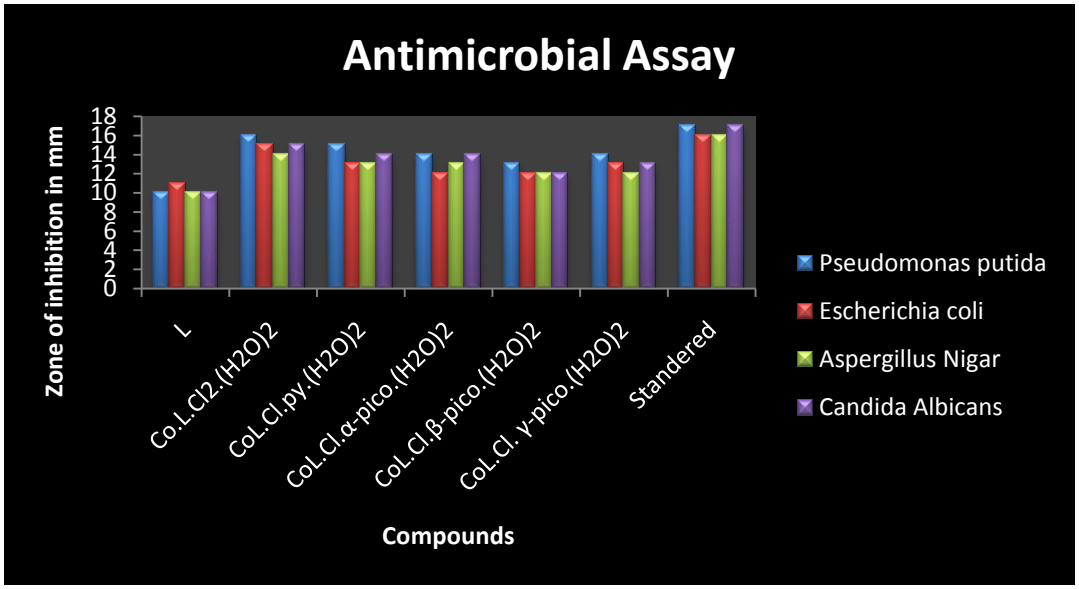
Fig III Antimicrobial Assay Bar Graph



% activity index was calculated by the formula

$$\% \text{ Activity Index} = \frac{\text{Zone of inhibition of test compound}}{\text{Zone of inhibition of standard (diameter)}} x\,100$$

Table IV
% Activity index of L, Co (III) complexes and standard

Compound	Pseudomonas putida	Escherichia coli	Aspergillus Nigar	Candida Albicans
L	58.82	68.75	62.5	58.82
$Co.L.Cl_2.(H_2O)_2$	94.12	93.75	87.5	88.24
$CoL.Cl.py.(H_2O)_2$	88.23	81.25	81.25	82.35
$CoL.Cl.\alpha\text{-pico}.(H_2O)_2$	82.35	75.00	81.25	82.35
$CoL.Cl.\beta\text{-pico}.(H_2O)_2$	76.47	75.00	75.00	70.59
$CoL.Cl.\ \gamma\text{-pico}.(H_2O)_2$	82.35	81.25	75.00	76.47
Standered	100	100	100	100
$CoCl_2.6H_2O$	105.88	118.75	125.00	105.88

(Std-amphiciline,bicip)

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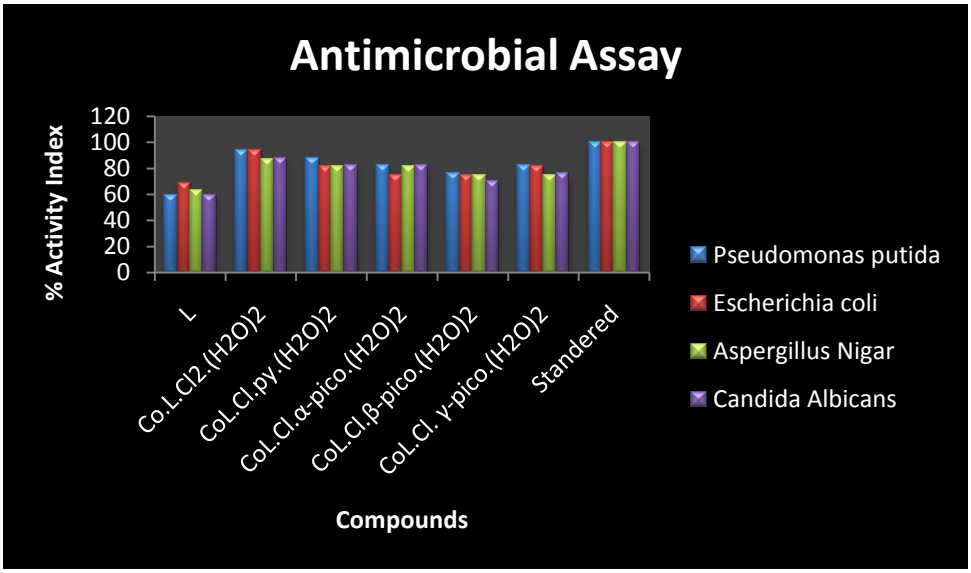
Figure IV: % Activity Index Bar Graph



The complex and adducts showed maximum activity against bacterial and fungal species than free ligand. The Co $L.Cl_2(H2O)_2$ exhibited high activity against all the bacterial and fungal species. Thus the coordination of metal ion to ligand is responsible for high biological activity. The most probable reason for this difference is chelation which reduces the polarity of the central metal atom because of the partial sharing of its positive charge with donor groups and possible π-electron delocalization within the whole chelating ring. As a result of this, the lipophilic nature of the central metal atom increases, which favors the permeation of the complexes through the lipid layer of the cell membrane [29].

All spectral characterizations confirmed that the complex and adducts have octahedral geometry, with 5-aminothiabendazole acting as NN bidentate ligand and N–atom of heterocyclic base occupying the fourth coordination site about the Co (III) atom. The TGA curves indicated coordinated water molecules in complex and adducts.

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