Synthesis of Acetyl Resorcinol and Investigation of the Anti Microbial Potentials of Some of its Metal Complexes.

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***Abstract*: The chemical synthesis of acetyl resorcinol with glacial acetic acid and resorcinol in presence of zinc chloride as catalyst to obtain acetyl resorcinol was the adopted procedure. The resulting acetyl resorcinol are complexed with the following metals Cr^{3+}, Mn^{2+}, Fe^{2+} and Co^{2+} ions. The acetyl resorcinol and its complexes were characterized using IR Spectrometry, Gas Chromatography/ Mass Spectrometry (GC/MS) as well as melting point. The IR Spectrum revealed the functional groups present. The IR absorption shows characteristic behaviour in the sense that, OH group found in 4 – Acetyl resorcinol in the region v(OH):3,303.35cm^{-1} was absent in the complexes due to deprotonation and co- ordination between metal ion and oxygen of phenol. The type of ligands involved were bidentate and polydentate ligands. The GC/MS studies showed the molecular weight (mass) of the complexes formed. The Mass Spectroscopy of all the compounds show molecular ion peaks indicating molecular weight of the various compounds hence showing that chelation has taken place. Interestingly, all the synthesized compounds were obtained in good yield (74.20% - 90.10%) The antibacterial of the acetyl resorcinol and its metal complexes were screened against *Salmonella enteric, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa.* It was observed that the growth of micro-organisms were inhibited in acetyl resorcinol, Co complex, Mn Complex. The Fe Complex inhibited the growth of *Staphylococcus aureus* but could not inhibit others while Cr Complex inhibited the growth of *Escherichia coli* but could not stop others. This study shows that chemical synthesis and complexation have taken place and the knowledge gained will help to advance the course of bioinorganic and inorganic chemistry.**

***Keywords*: Acetyl resorcinol, Antibacterial, Infrared spectrometry, Spectrum, Metal complexes**

I. INTRODUCTION

Over the past few decades, the bacterial resistance to antibiotic has become one of the most important problems of infections treatment. Although there are antimicrobial agents having different structures and mechanism which are frequently used in the treatment of microbial infections even then these agents are associated with resistance. It is necessary to synthesize new classes of antimicrobial compounds possessing different mechanism or chemical properties from those that are used commonly. Derivatives 4,6- diacetyl resorcinol for example

show potential antibacterial activity [1]. Resorcinol is a simple and important aromatic chemical (1,3-benzenediol) that has been chemically incorporated into various compounds to enhance their pharmacological profile [2]. Resorcinol is a benzenediol that is benzenedihydroxylated at positions 1 and 3. It has a role as an erythropoietin inhibitor and a sensitizer [3].

Resorcinol is a very white crystalline solid that becomes pink on exposure to light if not completely pure. Burns, although ignition is difficult, density approximately 1.28 g / cm^3. Irritating to skin and eyes, Toxic on skin absorption, used to make plastics and pharmaceuticals.

Resorcinol is a 1,3-isomer (or meta-isomer) of benzenediol with formula $C_6H_4(OH)_2$ or general formula ($C_6H_6O_2$). The IUPAC name is 1,3-dihydroxybenzene; other names are 1,3-benzenediol, m-benzenediol, m-dihydroxy benzene, m-hydroquinone, 3-hydroxyphenol, and resorcin. Resorcinol can be regarded as a phenol derivative in which a hydrogen atom is substituted by a hydroxyl group in the meta- position to the OH [4].

Transition metal complexes are cationic, neutral or anionic species in which a transition metal is coordinated by ligands [5]. Research has shown significant progress in utilization of transition metal complexes as drugs to treat several human diseases. Transition metals exhibit different oxidation states and can interact with a number of negatively charged molecules. This activity of transition metals has started the development of metal-based drugs with promising pharmacological application and may offer unique therapeutic opportunities [6]. The advances in inorganic chemistry provide better opportunities to use metal complexes as therapeutic agents. The mode of action of metal complexes on living organism is differs from non-metals. These complexes show great diversity in action [7]. Medicinal inorganic chemistry can exploit the unique properties of metal ions for

the design of new drugs. This has, for instance, led to the clinical application of chemotherapeutic agents for cancer treatment, such as cisplatin [8]. The use of transition metal complexes as therapeutic compounds has become more and more pronounced. These complexes offer great diversity in their action; they do not only have anti-cancer properties but have also been used as anti-inflammatory, anti-infective and anti-diabetic compounds.

Antibiotic resistance has been growing at an alarming rate and consequently the activity of antibiotics against Gram-negative and Gram-positive bacteria has dropped dramatically day by day [9]. In this sense there is a strong need to synthesis new substances that not only have good spectrum of activity, but having new mechanisms of action. Inorganic compounds particularly metal complexes have played an important role in the development of new metal-based drugs [9]. A significantly rising interest in the design of metal complexes as drugs and diagnostic agents is currently observed in the area of scientific inquiry, specifically termed medicinal inorganic chemistry. At this end the main focused on research undertaken over the past few decades which has sought to possess preclinical pharmacological screenings like anti-bacterial, anti-fungal, anti-inflammatory, anti-cancer, DNA- interaction and anti-tumor action of synthetic metal complexes [9]. Metal ions play important roles in biological processes and the field of knowledge concerned with the application of inorganic chemistry to therapy or diagnosis of disease in medicinal inorganic chemistry [10]. The introduction of metal ions or metal ion binding components into a biological system for the treatment of diseases is one of the main subdivisions in the field of bioinorganic chemistry [11]. Metals is that they easily lose electrons to form positively charged ions which tend to be soluble in biological fluids, it is in this cationic form that metals play their role in biology. Metal ions are electron deficient, whereas most biological molecules such as proteins and DNA are electron rich. The attraction of these opposing charges leads to a general tendency for metal ions to bind and interact with biological molecules [12,13].

This research work focuses on synthesis, characterization and investigation of the microbial potential of acetyl resorcinol and some of its metal complexes.

II. MATERIAL AND METHOD

2.1 The Apparatus and Reagents

The reagents used for this research were of analytical standard. They include; Zinc chloride (Sigma - Aldrich, Germany), Acetic Acid (Sigma-Aldrich, Germany),Resorcinol (Sigma-Aldrich, Japan), Hydrochloric Acid (Sigma Aldrich, Germany),Methanol (ReildeHaen, Germany), Ethanol (Reil deHaen, Gemany),Cobalt (II)Acetate Tetrahydrate (Sigma-Aldrich, Germany), Iron (II) Acetate Tetrahydrate (Sigma-Aldrich, Japan), Manganese(II) Acetate Tetrahydrate (Sigma Aldrich, Japan), Chromium (III) Acetate Hydrate (Sigma Aldrich Germany), Potassium hy droxide(May and Baker, M&B, England), Ether (Sigma Aldrich, Germany), Dimethyl Sulphuroxide (DMSO) (Sigma Aldrich, Japan)

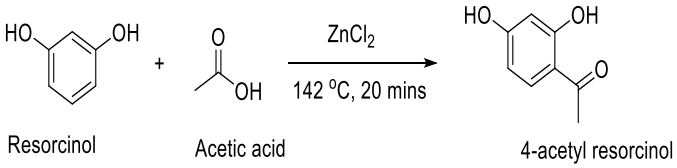
The electronic equipments: Fourier Transform Infrared (FTIR) (Nicolet Is5, Thermo Fisher Scientific USA), Electronic weighing balance (Ohuas, Adeventurer),Beakers(Pyrex),Conical flasks(Pyrex),Bunsen burner (Fisherbrand),Waterbath (Grant), Filter paper (Fisher brand),Stuart MP 3, Agilent 7977 Gas Chromatograph,5973D Inert Mass Spectrometer (Thermo Scientific USA),Conductivity meter (HACH HQ40D), Elemental Analyzer CE -440 (Exeter Analytical Inc.UK).

*2.3 The bacteria Species***:**

Salmonella enteric, Escherichia coli, Staphylococcus aureus and *Pseudomonas aeruginosa*, were obtained from the Reference Laboratory Section of Conig-Simonne Laboratories, Awka, Anambra State, Nigeria. The organisms were maintained on Nutrient Broth for 24 hours.

2.4 Synthesis of 4-Acetyl Resorcinol:

To a mixture of freshly fused and powdered $ZnCl_2$ (10 g) in dry acetic acid (15 ml) contained in a conical flask, was added quickly dry resorcinol (10 g) while stirring. The mixture was gently heated on a flame to 142^{o}C for 20 minutes. The viscous red solution was allowed to cool to room temperature. 80 ml of HCL (1:1) (i.e., 40 ml of acid and 40 ml of water) was added to syrupy mass and stirred. After a few minutes an orange-red crystalline material separated out. The crude product was crystallized from cold methanol to give 4-acetylresorcinol [14]. Yield 90.10 %, melting point 145^{0}C.



2.5 Synthesis of 4 – Acetyl Resorcinol Metal Complexes:

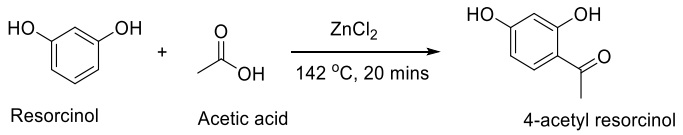
A portion (5 g) of 4-acetyl resorcinol was dissolved in hot 20 ml methanol. To this hot solution, 1.940 g of Chromium (III) Acetate Hydrate,2.081 g of Iron (II) Acetate Tetrahydrate, 2.198 g of Cobalt (II) Acetate Tetrahydrate, 2.049 g of Manganese (II) Acetate Tetrahydrate in 20 ml distilled water was added. The pH of the solution was adjusted to 7 by alcoholic KOH (10 % v/v). The resulting mixture was refluxed over water bath for 2-4hrs. The solid product was filtered, washed with cold methanol and cold ether and dried [14]. Yield:87.90 %,74.20 %, 85.20 %, 86.10 % for Cr, Fe, Co and Mn Complexes respectively. The IR and GC-MS were determined. The melting point, molar conductivity and elemental composition was equally determined.

2.6 Instrumental analysis:

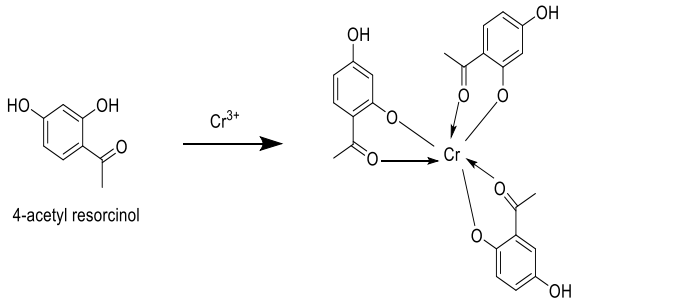
The molar conductivity measurement of the samples was carried out with a 0.003 moles solution of the sample in

absolute ethanol, at 25±0.5^{0}C. The conductivity was determined using conductivity meter (HACH HQ40d). The FT-IR Spectra of the samples were obtained in the 400-4000 cm^{-1} range using Fourier Transform Infrared (FTIR) (Nicolet Is5, Thermo Fisher Scientific USA), equipped with KBr optics and complimentary ATR diamond accessories. The acquired interferogram was converted into a spectrum by Fourier Transformation. In other to achieve good balance ripple size and resolution, the Happ Genzel function was used for Apodization (used in Hann window in fast FT analyzer to smooth the discontinuities at the beginning and end of the sampled time record). Agilent micro lab Expert FTIR Spectrometer software was used to acquire and process the data. The C, H, O and M(Metals) Contents of the samples were determined by flash combustion, using elemental analyzer (CE-440 Elemental Analyzer, Exeter Analytical Inc, UK). Sample weight used for the determination ranged from 1.0-1.5mg. The combustion and reduction were 975 and 600^{0}C respectively while the oven temperature was 81^{0}C. The chromatographic column Parapak PQS column, while the detector was thermal conductivity detector. The combustion was calculated from the second stage after pyrolysis and subsequent formation of Carbon monoxide (CO). The instruments used for the determination were Agilent 7977 Gas Chromatograph, coupled to 5973D Inert Mass Spectrometer (with triple axis detector) with electron-impact source.

Reaction of 4 – Acetyl resorcinol(ligand) with the following metals (Cr, Fe, Co and Mn) to form Complexes:

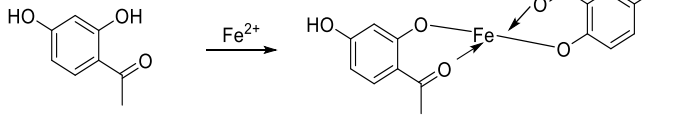


Resorcinol Acetic acid 4-acetyl resorcinol



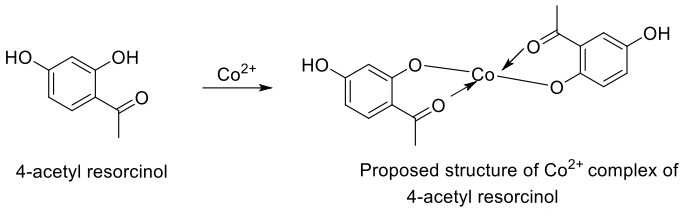
4-acetyl resorcinol

Proposed structure for Cr(III) complexes of 4-acetyl resorcinol



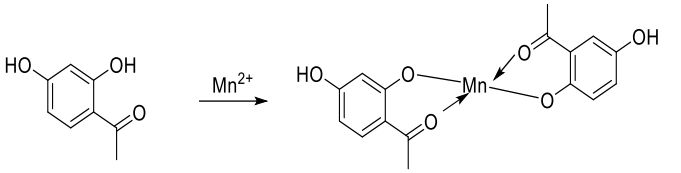
4-acetyl resorcinol

Proposed structure of Fe^{2+} complex of 4-acetyl resorcinol



4-acetyl resorcinol

Proposed structure of Co^{2+} complex of 4-acetyl resorcinol



4-acetyl resorcinol

Proposed structure of Mn^{2+} complex of 4-acetyl resorcinol

III. RESULT AND DISCUSSION

The ligand and complexes are stable at room temperature; maintain their characteristic coloration, soluble in organic solvent like methanol and ethanol. The physical properties, molar conductivity, melting point are shown in Table 3.0. Interestingly, in Table (3.0) Acetyl resorcinol gave good yield (74.20 – 90.10) indicating that the method of synthesis is viable. When compared with the work of [14] it was evidence that the yield of this work is good. The melting point is relatively high when compared with equally relatively high melting point found in the work of [14]. The elemental composition of the ligand and complexes when compared with the work of [14] is relatively good.

The Characteristic infrared frequencies of the ligand and metal complexes are listed in the Table 3.1. Infrared Spectrum of the 4 – Acetyl resorcinol shows two broad band 3,362.46 cm^{-1} and at 3,303.35 cm^{-1} that may be attributed to H- bonded two phenolic OH Stretching frequencies v(O-H). In addition, there are four strong bands at 1,625.72 cm^{-1},2821.46 cm^{-1},690.27 cm^{-1} and 1,534.11 cm^{-1} which are assigned to the v(C=O), v(C-H), aromatic ring and v(C=C) respectively. This indicate that 4-Acetyl resorcinol is formed [14].

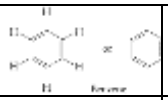
The absorption of carbonyl group v(C=O) was recorded in all the metal complexes indicating coordination of carbonyl group with metal ions. The band at 3,303 cm^{-1} observed due to v(OH) in 4- Acetyl resorcinol disappeared in Cr(III) Complex, Mn(II) Complex,Fe(II) Complex and Co(II) Complex indicating deprotonation of OH and coordination between metal ion and oxygen of phenol. All the metal complexes show absorption of v(OH) at 3,361.71 cm^{-1},3,346.35 cm^{-1}, 3,353.10 cm^{-1} and 3,331 cm^{-1} respectively. There is strong indication for the formation of v(C=C) and v(C-H) bonds in all the complexes with their values ranging from (I,460.11 – 1,520.09) cm^{-1} for v(C=C) and (2,800.42 – 2,945.95) cm^{-1} for v(C-H) respectively [14].

However, there are evidence of the formation of M-O bond in all the complexes ranging from (419.01 –614.26) cm^{-1}. The aromatic ring absorption band range from (690.27 – 906.43) cm^{-1}. The IR absorption Frequencies are summarized in the table 3.1

Table 3.0 shows Physical Characteristics, analytical data and elemental composition of Acetyl Resorcinol and its Metal Complexes

S/N	Compounds	Yield (%)	Melting Point(°C)	Colour	Molar Conductivity Ω^{-1} cm^{-2} mol^{-1}	Molecular Weight(m/z)	Elemental Composition %			
							C	H	O	M
1	Acetyl Resorcinol $C_8H_8O_3$	90.10	145	Orange	5.9	152	63.46	4.81	12.60	-
2	Chromium Complex $CrC_{24}H_{21}O_9$	87.90	>270 Dec	Gray	7.3	504.9	56.69	4.39	11.20	11.09
3	Iron Complex $FeC_{16}H_{14}O_6$	74.20	>300 Dec	Brown	1.8	357.9	32.53	3.55	12.10	18.18
4	Cobalt Complex $CoC_{16}H_{14}O_6$	85.20	250 - 251	Purple	8.2	360.9	39.40	3.20	14.25	13.54
5	Manganese Complex $MnC_{16}H_{14}O_6$	86.10	256 - 257	Brown	6.8	356.9	47.34	5.20	13.34	12.35

Table 3.1 Important IR absorption Frequencies (in cm^{-1}) of Acetyl Resorcinol Ligand and some of its complexes

S/N	Compounds (Ligand and Complexes)	V(C=O)	V(O-H)	V(C-H)		V(M-O)	V(C=C)
1	Acetyl Resorcinol $C_8H_8O_3$	1,625.72	3,362.46 3,303.35	2,821.46	690.27	-	1,534.11
2	Chromium Complex $CrC_{24}H_{21}O_9$	1,631.95	3,361.71	2,800.43	692.27	422.77	1,460.11
3	Manganese Complex $MnC_{16}H_{14}O_6$	1,621.48	3,346.35	2,831.44	795.96	496.43	1,470.12
4	Iron Complex $FeC_{16}H_{14}O_6$	1,622.05	3,353.10	2,823.43	845.96	442.58	1,486.10
5	Cobalt Complex $CoC_{16}H_{14}O_6$	1,642.40	3,331. 36	2,945.95	907.06	614.26-419.01	1,520.09

The results of mass spectrometry of the compound are shown in (table 3.3), depicting the m/z results of the ligand and complexes as well as that of the fragments. These m/z results confirmed the structure of the ligand and metal complexes. For instances the m/z acetyl resorcinol fragment (151), Cr complex of acetyl resorcinol (504.9), Fe complex of acetyl resorcinol (357.9), Mn complex of acetyl resorcinol (356.9) and Co complex of acetyl resorcinol (360.9).

The microbial potential of the ligand and the complexes was determined by screening against some selected bacteria such as Salmonella enterica (G-), Escherichia coli(G-), Pseudomonas aeruginosa(G-) and Staphylococcus aureus(G+). It was observed that the growth of micro-organism was inhibited in acetyl resorcinol(ligand), Co complex, Mn complex. Fe complex inhibited the growth of Staphylococcus aureus but could not inhibit others while Cr complex inhibited the growth of Escherichia coli but could not stop others.

Table 3.2: Antimicrobial Susceptibility Screening of Extracts

Test Bacteria/ Compounds	Mean Inhibition Zone Diameters (Izd) In Millimetres±Standard Deviation																						
	Salmonella enteric				Escherichia coli				Staphylococcus aureus				Pseudomonas aeruginosa				+ve ctrl				- vectrl			
	x	y	z	Mean± SD	X	Y	z	Mean± SD	ⅹ	Y	Z	Mean±SD	ⅹ	y	z	Mean± SD	X	Y	z	Mean± SD	ⅹ	y	z	Mean± SD
ACETYL RESORCINOL	30	32	31	31±1.00	30	30	31	30.33±0.58	35	37	37	36.33±1.15	29	29	30	29.33±0.58	50	48	46	48±2.00	-	-	-	-
COBALT COMPLEX	14	15	14	14.33±0.58	22	22	23	22.33±0.58	24	23	23	23.33±0.58	13	13	13	13±0.00	45	45	45	45±0.00	-	-	-	-
IRON COMPLEX	-	-	-	-	-	-	-		30	32	34	32±2.00	-	-	-	-	60	64	62	62.0±2.00	-	-	-	-
MANGANESE COMPLEX	14	17	16	15.67±1.53	20	19	21	20±1.00	14	14	14	14±0.00	12	12	13	12.33±0.58	50	48	46	48±2.00	-	-	-	-
CHROMIUM COMPLEX	-	-	-	-	15	14	16	15±1.00	-	-	-	-	-	-	-	-	51	48	46	48±2.04	-	-	-	-

KEY: xyz= Inhibition zone Diameter in Millimeter

IV. CONCLUSION

Acetyl resorcinol and its complexes gave high percentage yield showing that the method of analysis is viable. The complexes of acetyl resorcinol have relatively high melting points. The IR spectral studies indicated the presence of Iron, Chromium, Manganese, Cobalt metals, Carbonyl group (C=O) showing acetyl resorcinol, carbon to carbon, carbon to hydrogen bond, aromatic ring and hydroxyl group. The GS/MS studies confirmed the number of molecular components in each complex. The fragmentation pattern of the moieties in all the prepared compounds also confirmed the suggested structures of the complexes. The screening of antibacterial of acetyl resorcinol and its complexes with selected micro-organisms showed that acetyl resorcinol and Manganese complex inhibited the growth of the micro-organisms than the other complexes.

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Conflicts of Interest:

The authors declare that they have no conflicts of interest.

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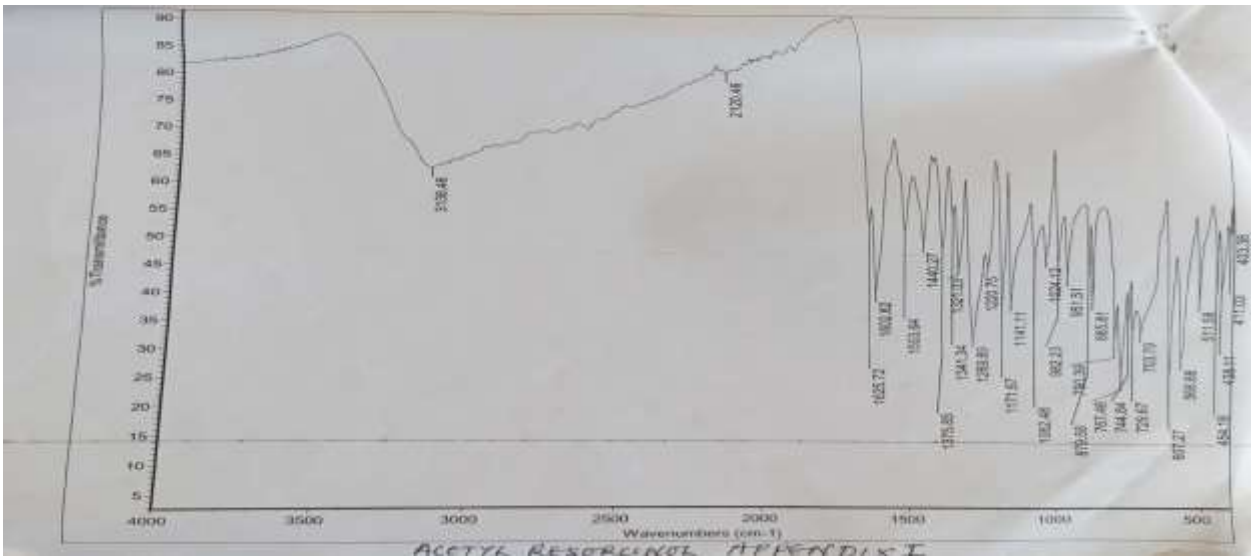
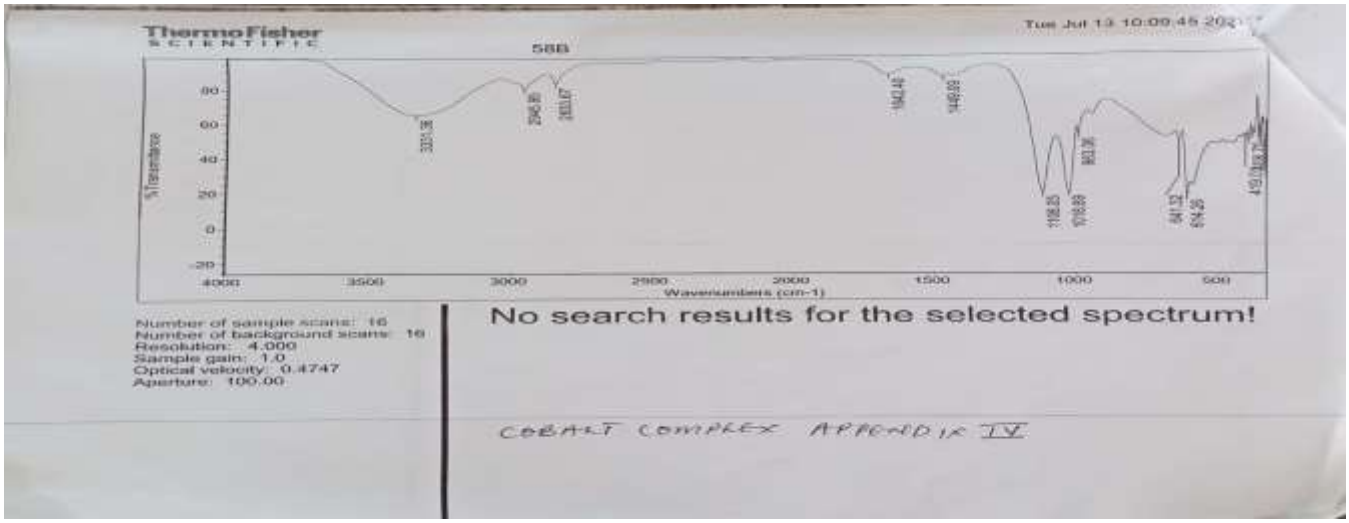
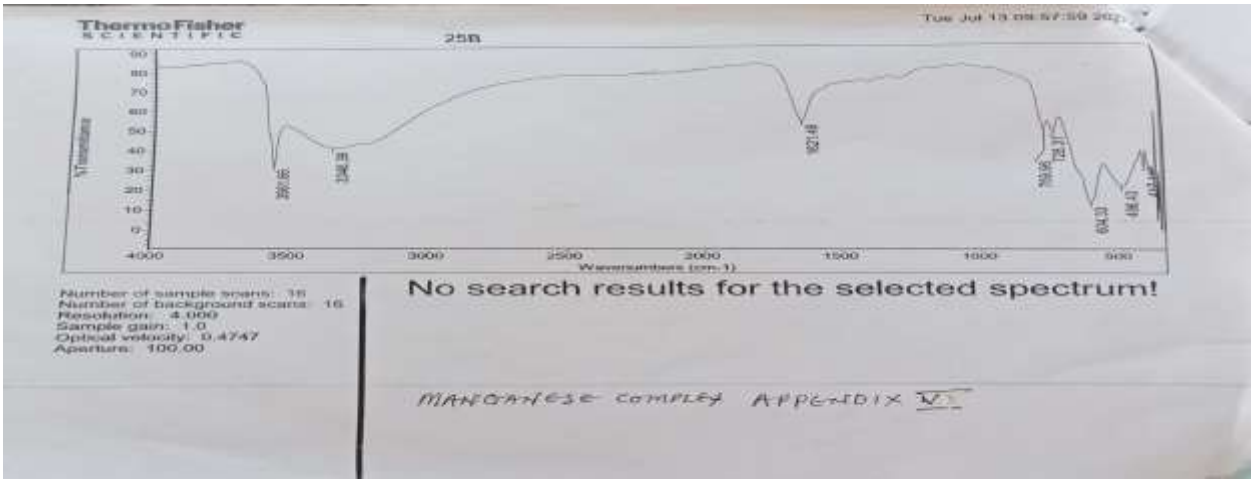


ACETYL RESORCINOL IR RESULT



COBALT COMPLEX IR RESULT



MANGANESE COMPLEX IR RESULT