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SYNTHESIS, CHARACTERIZATION OF NOVEL MIXED METAL TARTRATEC OMPLEXES AND STUDY OF THEIR *IN VITRO* ANTIMICROBIAL ACTIVITY

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Key words:

Mixed Metal Complexes, Tartrate, Thermal Behavior, Antibacterial, Bioassays

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ABSTRACT: A series of six new mixed metal complexes of general formulation [MM12(L)n]xH2O, where M= Mn, Co, Ni, Cu, Zn, Cd,M1=Fe, L= Tartrate ligand, x=1,5,6 and n = 3, are synthesized using tartrate as a bidented ligand. The newly synthesized complexes were characterized using various analytical techniques such as IR, XRD, TGA, Elemental analysis, magnetic susceptibility study etc. IR spectral data suggested that the bidentate ligand has coordination to both the metal ions through oxygen as donor atoms. The thermal behavior (TGA) of the complexes was studied; suggest the decomposition patterns were in good agreement with recommended formulae of the complexes. XRD technique revealed the presence of crystalline nature of all the studied complexes. The antibacterial activity of the metal complexes along with ligand was assayed against three bacterial pathogens viz. *Bacillus Megatherium*, *Staphylococcus aureus*, *E-Coli*, *Pseudomonas* and *Proteus Vulgaris* by well diffusion method. The bioassays of all the metal complexes showed a greater inhibitory effect in the form of broad activity spectrum than the individual ligand, which indicate an increased antimicrobial activity after the coordination.

INTRODUCTION: The discovery of Cisplatin, that is a coordinated drug has generate the great interest in coordination chemistry [3] and has now become a multidisciplinary area having a wide range of applications in medicinal chemistry such as anti-bacterial, Antifungal, anticancer, antiviral and anti-inflammatory actions [5, 9, 14, 15]. The transition metal complexes can provide variety of properties based on their cationic nature, assorted three dimensional structural features, and the possibility to tune their redox potential through the choice of proper ligands [3, 8].

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It has been observed that the metal complexes shows enhanced biological activity than those of either ligands or the metal ions which has been reported with several transition metal ions and variety of ligands [4, 5, 6, 7]. The Literature survey reveals that the wide variety of Schiff based ligand metal complexes have been extensively studied over past few decades for their biological activity [9, 11, 12]. Ever since it has been accepted that many of these complexes may serve as models for biologically significant species [10].

Still a widespread research has been going on to search the complexes with improved action mechanism against bacteria, with reduced toxicity of the metal ion in the form of a complex. Although a wide variety of metal-ligand complexes were studied for their antimicrobial activity, the literature survey reveals that there has been not much work done yet on combination of mixed metal-ligand complexes to study their biological activity.

In search of new potentially active metal complex and based on the above considerations, we have synthesized and characterized mixed metal tartrates, containing tartrateas a ligand which may coordinate with both metal ions through carboxylate and carbonyl oxygen as observed in case of complexes with metal ions such as Mn (II), Fe(II) Co(II), Ni(II) [1, 2]. The interaction of metal ions with tartrate ligand shows improved antimicrobial activity compared to free ligands (not coordinated). In addition, the co-precipitation method of complex synthesis using water as a solvent is attractive offering reduced effluence, low cost and offer high yields in concert with simplicity in processing and management [1, 2].

We report here the synthesis, Characterization and antimicrobial activity study of mixed metal complexes which contain one fixed metal ion (Fe) along with a series of metal ions (Mn, Co, Ni, Cu, Zn, Cd) bonded together through bidentatetaratrate ligand. These mixed metal complexes were characterized by elemental analysis, IR, XRD, TGA and magnetic studies etc. The antimicrobial activity of the compounds was investigated systematically.

METHODOLOGY:

Materials:

All the reagents and the hydrated metal salts ($FeSO_4 7H_2O$, $MnSO_4 H_2O$, $CoSO_4 7H_2O$, $NiSO_4 6H_2O$, $CuSO_4 5H_2O$, $ZnSO_4 7H_2O$, and $CdSO_4$) are of analytical grade, purchased from Merck and were used as received. The double distilled water was used as solvent for preparation of various solutions of metal salts and final complexes as well.

Synthesis and Characterization of metal complexes:

A simple co-precipitation method was used to prepare the complexes. The two metals salts aqueous solutions i.e. $FeSO_4 7H_2O$and $MnSO_4 H_2O$ were mixed homogenously in a fixed molar ratio. The pH of the solution adjusted to slightly acidic condition by dilute H_2SO_4 to hydroxide formation of cations. In these mixture of metal salts the 10% aqueous solution of tartrate is the added slowly with constant stirring to get complete precipitation. The stirring is continued for next 30 minute by adding acetone in equal amount to the ligand solution. A colored product appeared on standing and cooling the above solution. The precipitated complex was, filtered washed with acetone and dried under normal atmospheric conditions and then stored in a desiccator over dried. All other metal complexes were prepared using this similar general procedure.

The Infrared absorption spectra of the ligand and metal complexes were recorded on a Shimadzuspectrophotometer from 4000 to 400 cm^{-1} using KBr pellets. The Powdered diffraction patterns of metal complexes were recorded using Regaku Mini flex diffractometer CuKα (λ=1.5405 A^o). The Gouy method were used to measure magnetic susceptibilities at room temperature using $Hg[Co(SCN)_4]$as calibrant. The magnetic moments were calculated using standard formulae. The elemental analysis of C and H were performed by microanalytical technique. The percentage of metals was anticipated as per standard monograph for chemical analysis. The thermoanalytical measurements were obtained with Perkin-Elmer (Delta series-TGA7) instrument.

All samples studied under static atmosphere of air using 30-60 mg samples and providing heating rate upto10 deg/min in the range of ambient to 600^oC. Thermo gravimetric analysis data for the metal complexes concealed that nearly 15% of the total mass of the metal complexes reduced between 45^0C to 250^0C, followed by substantial decomposition up to 600^0C that corresponds to the decomposition of the ligand leaving metal oxides as residue. The results obtained through all above instrumental analysis indicate the formation of binuclear metal complexes with adequate degree of purity for the bioassays experiments.

Above synthesized and well characterized complexes were for screened their antibacterial activity against some gram positive and gram negative bacteria. The obtained results were summarized and discussed below.

RESULTS AND DISCUSSION: All the synthesized metal complexes are powdered solid, colored and stable towards air and moisture at room temperature with good percentage yield (**Table 1**). There decomposition temperature is quite high, due to predicted neutral polymeric nature they are less soluble in common organic solvents.

The physical data of the compounds are summarized in the **Table 2**. Analytical date show that metal-metal chelates have1:2:3 stoichiometry respectively. So the general molecular formula of the synthesized complexes can be given as $[MM^1{}_2(L)n]xH_2O$, where M= Mn, Co, Ni, Cu, Zn, Cd, M^1=Fe, L= Tartatrate ligand, x=1,5,6 and n = 3

TABLE 1: COMPLEX COMPOSITIONS

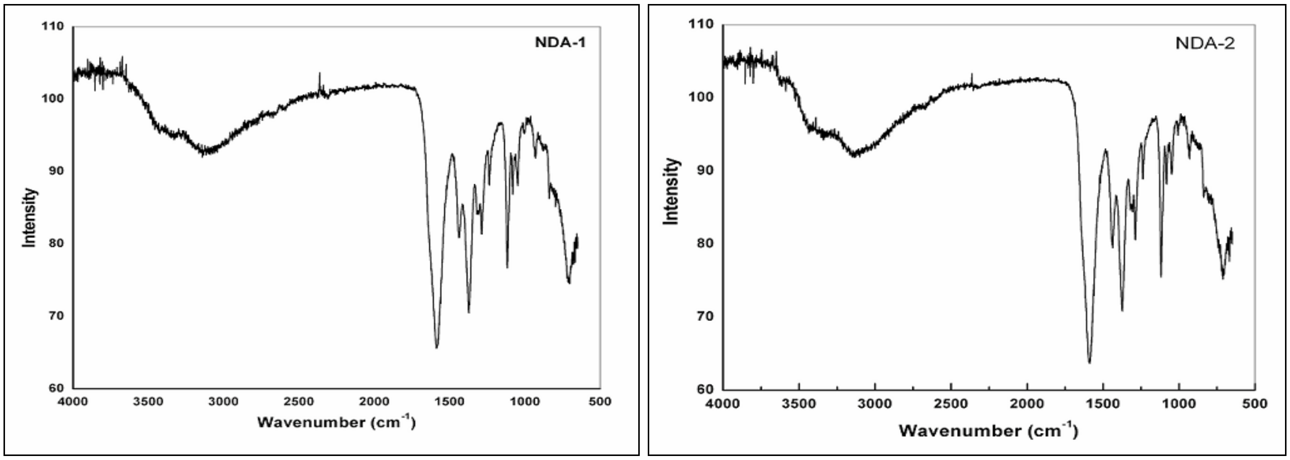
Complex	Symbol	Molecular Wt.	Amount of $FeSO_47H_2O$ (gm)	Amount of metal salt (gm)	Tartrate solution added	% Yield of complex
$MnFe_2(C_4H_4O_6)_35H_2O$	NDA1	700.64	11.84	$MnSO_4H_2O$ 3.66	10%	65.23%
$CoFe_2(C_4H_4O_6)_36H_2O$	NDA2	722.63	5.92	$CoSO_47H_2O$ 2.99	10%	71.05%
$NiFe_2(C_4H_4O_6)_35H_2O$	NDA3	704.41	11.85	$NiSO_46H_2O$ 5.60	10%	56.19%
$CuFe_2(C_4H_4O_6)_36H_2O$	NDA4	727.24	11.84	$CuSO_45H_2O$ 5.21	10%	61.85%
$ZnFe_2(C_4H_4O_6)_3H_2O$	NDA5	639.07	11.84	$ZnSO_47H_2O$ 5.96	10%	68.15%
$CdFe_2(C_4H_4O_6)_35H_2O$	NDA6	758.1	12.95	$CdSO_4$ 5.97	10%	72.92%

TABLE 2: ELEMENTAL ANALYSIS

Complex	Formula weight (gm)	C		H		Fe		Metal		Magnetic Moment (μ)B.M.
		Obs	Cal	Obs	Cal	Obs	Cal	Obs	Cal	
$MnFe_2(C_4H_4O_6)_35H_2O$	700.64	20.36	20.55	3.49	3.13	15.52	15.93	7.14	7.69	9.25
$CoFe_2(C_4H_4O_6)_36H_2O$	722.63	19.24	19.92	3.64	3.32	14.20	15.75	7.96	8.13	8.12
$NiFe_2(C_4H_4O_6)_35H_2O$	704.41	19.78	20.44	3.70	3.12	16.44	15.85	8.72	8.33	7.19
$CuFe_2(C_4H_4O_6)_36H_2O$	727.24	19.61	19.80	3.14	3.30	15.87	15.65	8.16	8.73	7.68
$ZnFe_2(C_4H_4O_6)_3H_2O$	639.07	23.20	22.53	20.16	21.90	16.25	17.47	10.81	10.22	6.81
$CdFe_2(C_4H_4O_6)_35H_2O$	758.1	19.10	18.99	2.53	2.90	14.13	14.82	15.43	14.64	7.06

IR Spectra:

The IR spectral assessment of the ligand and its metal complexes had been used to discover the binding mode of the metal ions at donor sites of the ligand. IR spectra of metal-metal tartratesare given in **Fig. 1** and all the characteristic absorption bands of the complexes are represented in **Table 3**.



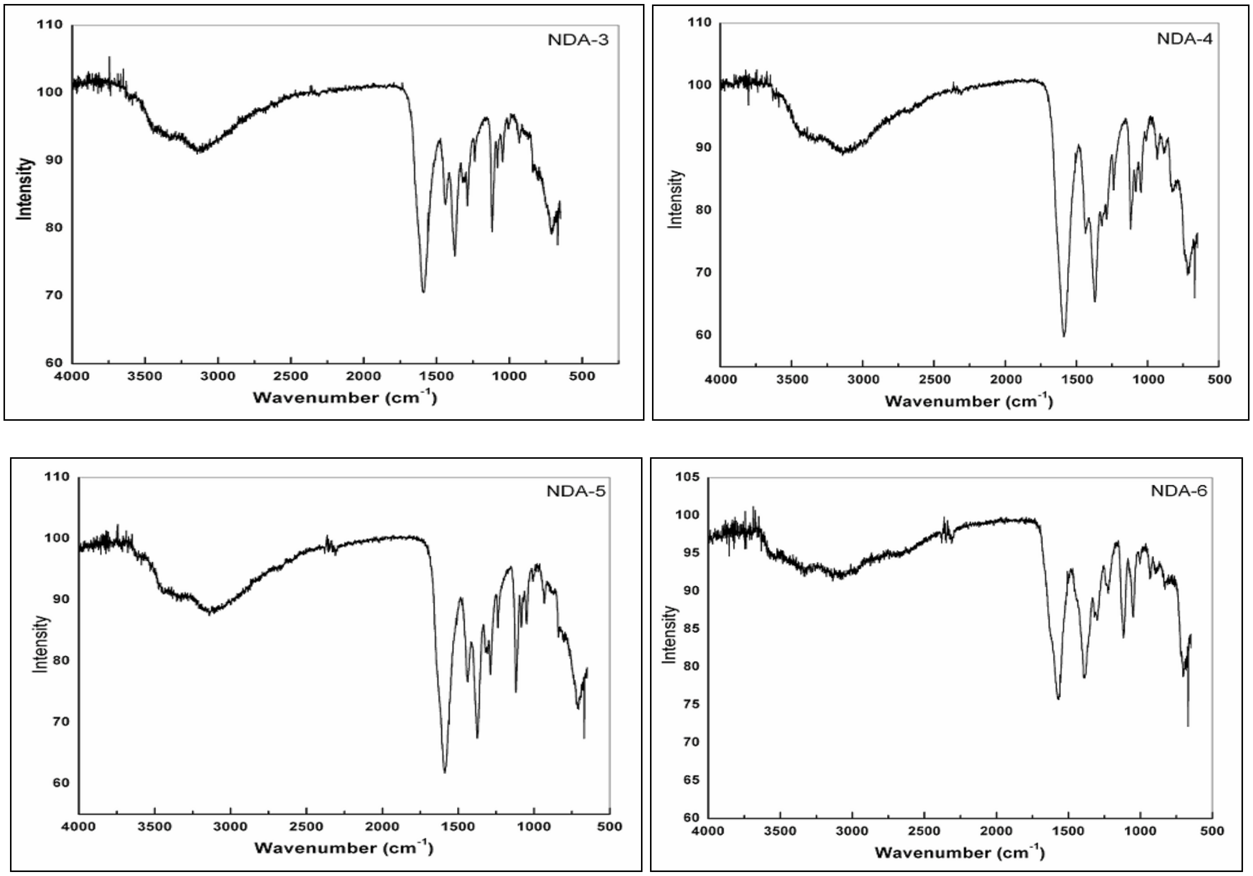


FIG. 1: IR SPECTRA

TABLE 3: IR SPECTRAL DATA

Fe-Mn Complex (NDA1)	Fe-Co Complex (NDA2)	Fe-Ni Complex (NDA3)	Fe-Cu Complex (NDA4)	Fe-Zn Complex (NDA5)	Fe-Cd Complex (NDA6)	Assignment
3431	3438	3442	3446	3447	3543	HOH
3336	3339	-	3338	3339	3336	
-	3144	3142	3144	3145	3098	
1588	1591	1590	1583	1590	1572	C=O
1440	1437	1439	1435	1436	-	C=O, C-C
1376	1375	1377	1368	1375	1389	C-O
1316	1320	-	1321	1310	1303	C-C
1285	1287	1287	1285	1285	-	
1236	1236	1237	1234	1240	1224	C-O
1119	1118	1120	1119	1120	1120	C-O
1080	1079	1082	1084	1084	-	
1047	1048	1047	1048	1048	1051	C-O(alcohol)
1006	1008	1006	1008	1005	1001	
932	930	934	935	933	929	O-C=O, C-O
-	-	-	879	-	898	
838	838	-	828	836	832	
711	712	714	716	710	707	C-C
-	668	667	667	667	667	H-O-H
-	650	-	649	-	-	M-O, C-C

Shift in the positions of strong intensity band at 1750 cm-1 in the d-tartaric acid to lower wave numbers atν_{asy} (OCO) 1591-1572 Cm-1 andν_{sy} (OCO)1458-1375 Cm-1 for complexes reveals the coordination of both COO- groups present in the tartaric acid molecules to metallic ions. Furthermore, it was reported that the bidentate co-ordination of the carboxylate group with metal results in lowering of both ν (COO) frequencies due to drainage of electron density from carboxylate group to the metal ion. Thus on the basis of difference between the antisymmetric and symmetric (C=O) stretching frequencies of tartrate complexes, bidentate nature of attachment of ligand to metal ion can be predicted. The IR spectra show a strong and wide absorption band at 3430 Cm-1 assigned to the –OH vibration frequency of water and secondary alcohol groups. The presence of intermolecular hydrogen bonding in all compounds was confirmed by band width of IR spectra.

Magnetic Susceptibility:

The magnetic susceptibility of the complexes measured at room temperature and the corresponding magnetic moment values were given in **Table 2**. As the complexes are bimetallic the overall magnetic moment values reported were total of μ_{eff} of Fe (II) and μ_{eff} of metal (II). As all the complexes showing overall μ_{eff} (in BM) values give an evidence for paramagnetic nature of complexes

XRD Spectra:

All tartrates are isolated as powder rather than a single crystal, due to which single crystal XRD studies are not possible for such complexes. However the powdered diffraction pattern reveals information about polycrystalline nature of the complexes (**Fig.2**). The observed d spacing values and particle size data are given in **Table 4**.

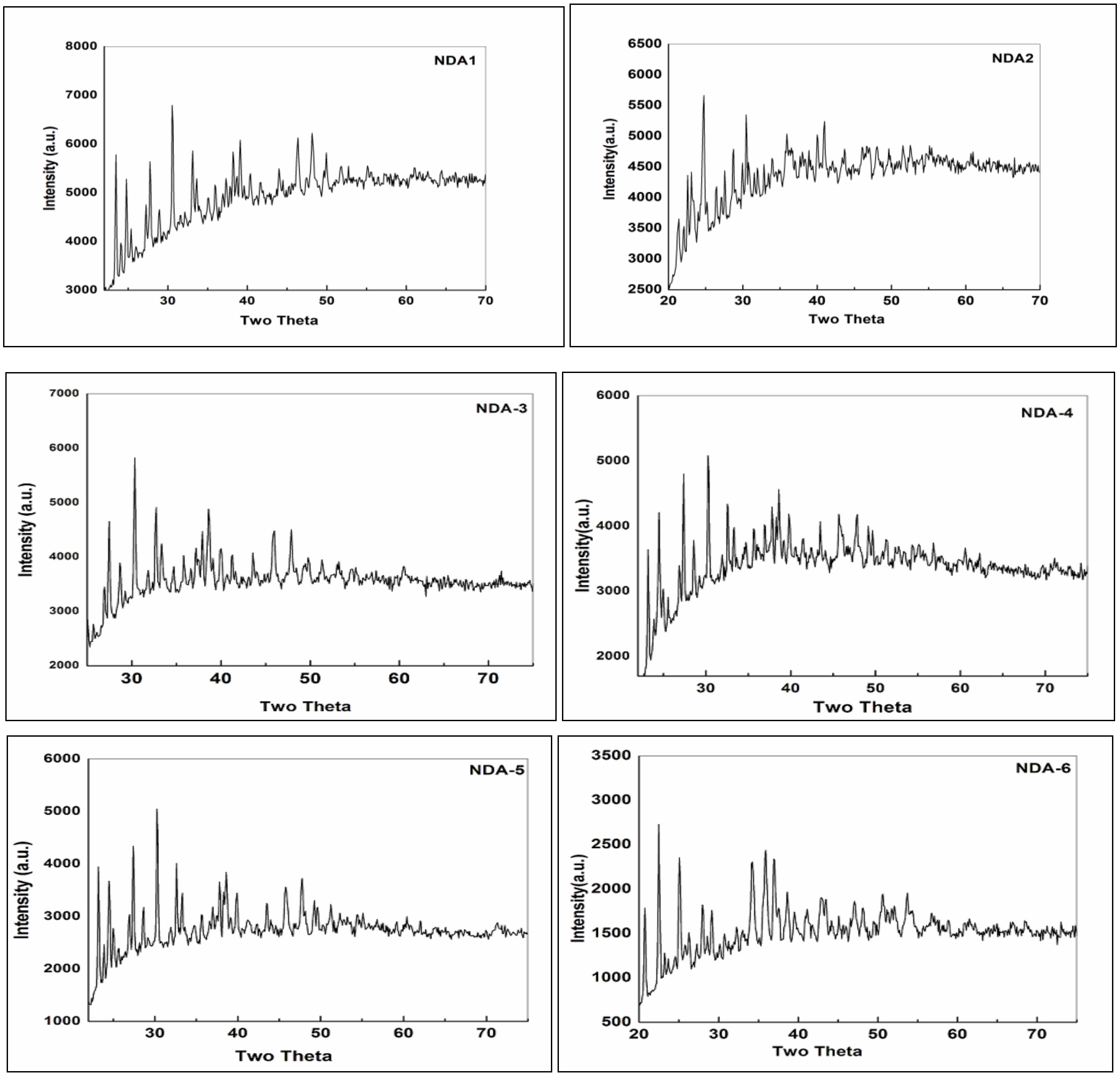


FIG. 2: XRD

TABLE 4: OBSERVED D SPACING VALUES (A^O)

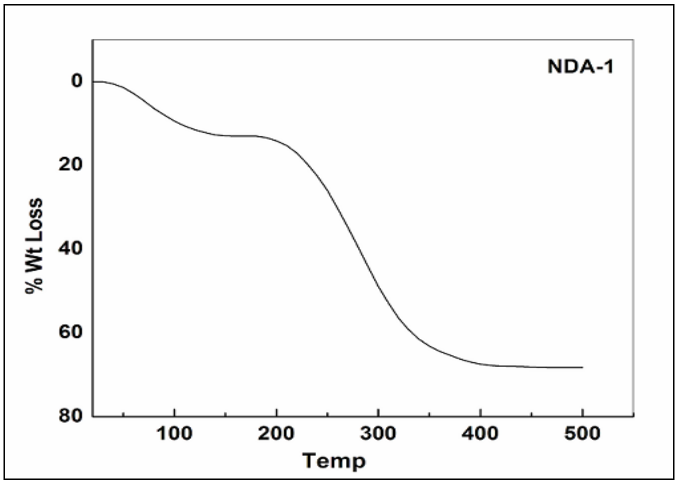
Fe-Mn Complex (NDA1)	Fe-Co Complex (NDA2)	Fe-Ni Complex (NDA3)	Fe-Cu Complex (NDA4)	Fe-Zn Complex (NDA5)	Fe-Cd Complex (NDA6)
1.05221	0.80585	3.037141	0.92387	0.92387	0.95623
1.53325	0.77081	0.990907	2.20678	1.30478	0.79707
3.75926	0.80524	0.843971	3.37844	2.49536	0.92289
7.105095	0.90636	0.787172	1.016988	19.8477	15.6882
0.881231	4.75632	1.472267	0.865363	2.859239	1.436599
0.802021	1.272623	4.01638	0.779063	0.981719	0.885922
0.808301	0.823537	1.22933	1.372647	0.865363	0.778914
1.760167	0.791545	0.93307	2.86891	0.78486	0.777536
1.028	1.129274	0.77214	1.48151	1.385135	0.844318
0.85703	1.632901	0.94808	0.95538	2.86891	1.320823
0.98766	11.3556	3.17443	0.77253	1.31429	2.327204
3.79213	3.08533	8.647741	0.90154	0.95114	1.98661
2.991363	1.20117	1.741651	2.19567	0.77575	1.06968
1.18806	0.82178	0.837623	10.23351	0.92646	0.77926
0.791204	0.96556	0.79016	1.712881	2.23206	0.975
0.837979	5.203081	3.895608	0.883466	19.91254	2.08559
69.09591	1.37951	0.92236	0.809694	1.692409	1.834461
0.83701	0.843325	0.84385	3.278165	0.863508	0.996524
0.88167	0.771657	2.96912	1.01413	3.278165	0.776931
4.90366	5.533994	1.472998	0.82153	0.97919	1.483311
1.098352	0.85126		1.47436	0.81106	1.45655
0.813833	2.22851		2.46672	1.58646	0.77083
1.169106	1.272825		1.585555	2.5138	0.87564
	0.85234		1.182323	1.678584	4.153069
			6.69477	0.884093	0.780641
			0.84845	1.190874	7.61976

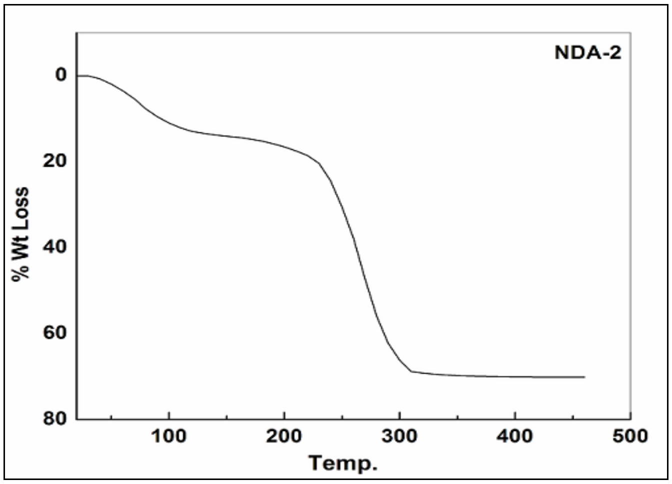
Observed Particle Size: (D=0.89λ/BCosθ)

Fe-Mn Complex (NDA1)	Fe-Co Complex (NDA2)	Fe-Ni Complex (NDA3)	Fe-Cu Complex (NDA4)	Fe-Zn Complex (NDA5)	Fe-Cd Complex (NDA6)
680.49	656.16	646.83 A^o	685.64 A^o	696.08 A^o	692.57 A^o

Thermogravimetric analysis:

The TGA decomposition curves of all the complexes are show in (**Fig.3**) and the data summarized in **Table 5**.





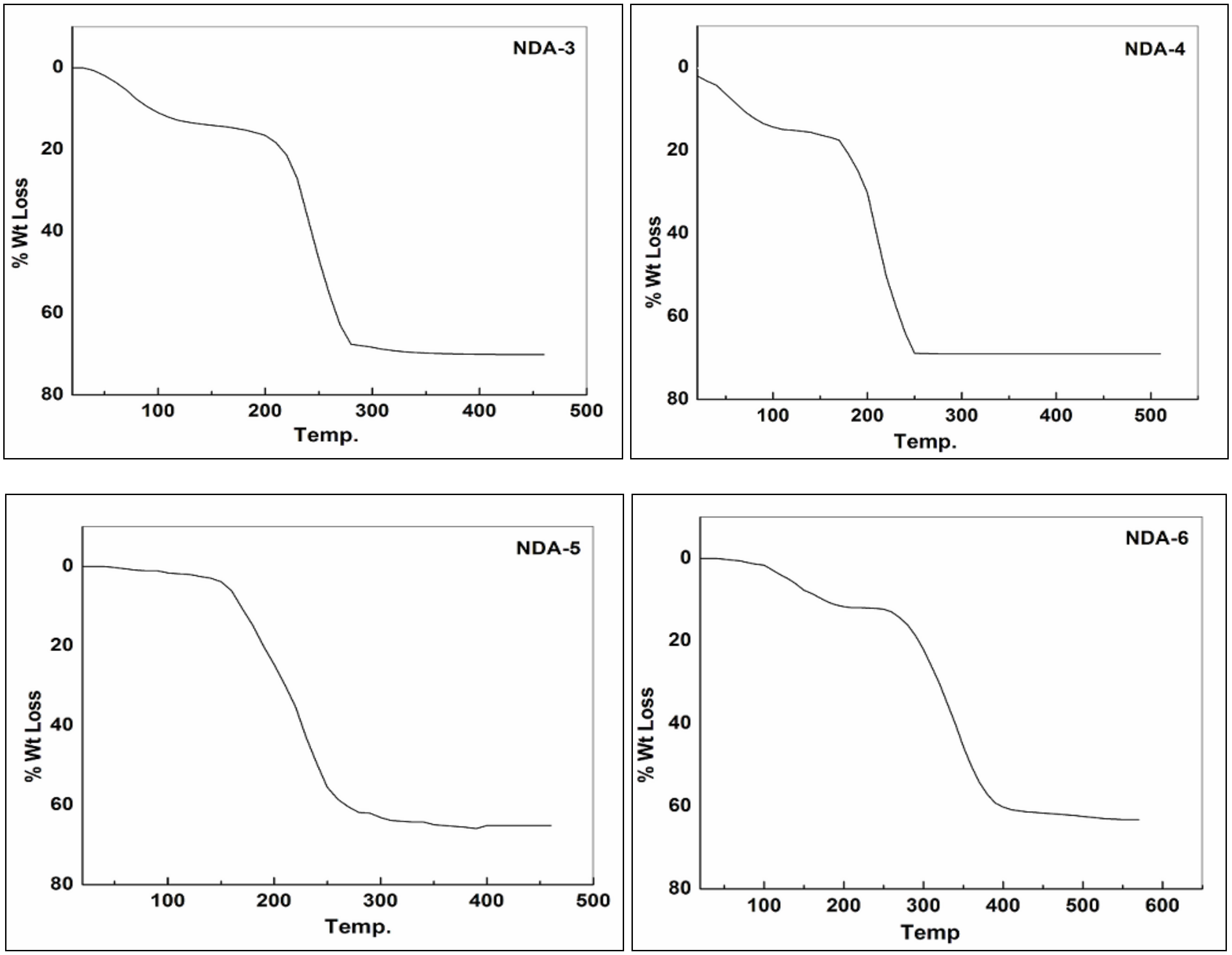


FIG.3: TGA

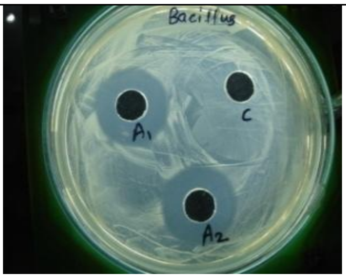
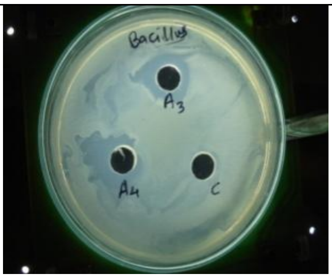
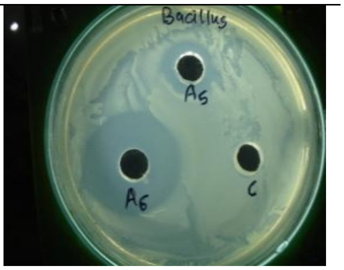
TABLE 5: TGA RESULT

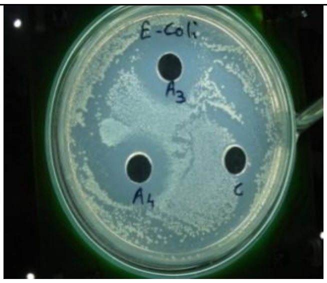
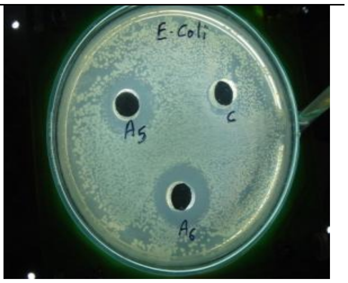
Complex	% Mass loss		Temp Range^{O}C
	Observed (%)	Calculated (%)	
$MnFe_2(C_4H_4O_6)_3 5H_2O$	12.60	12.85	30-135
	66.76		210-390
$CoFe_2(C_4H_4O_6)_3 6H_2O$	14.50	14.95	40-115
	23		115-235
	69.30		235-380
$NiFe_2(C_4H_4O_6)_3 5H_2O$	12.61	12.78	30-120
	18.90		120-210
	68.03		210-280
$CuFe_2(C_4H_4O_6)_3 6H_2O$	13.24	14.85	30-90
	17.32		90-170
	68.90		170-250
$ZnFe_2(C_4H_4O_6)_3 H_2O$	2.84	2.82	30-132
	63.62		132-310
$CdFe_2(C_4H_4O_6)_3 5H_2O$	11.64	11.87	35-201
	12.90		201-260
	60.47		260-400

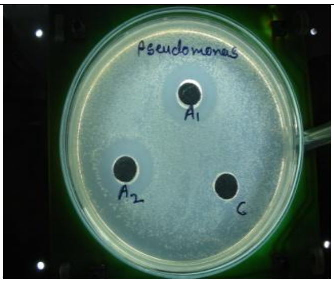
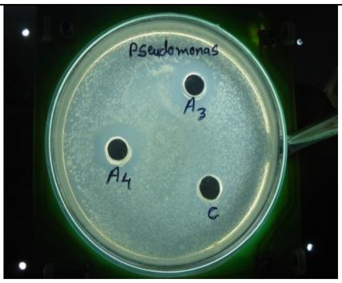
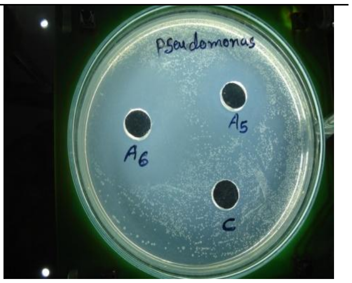
The thermogram of all compounds showed a continuous mass loss between 35to220^{o} C indicating the water molecules loss from the complexes at this temperature. These mass losses are in agreement with theoretical values. The mass loss in the range of 235 to 500^{0}C corresponds to decomposition of complexes and formation of respective ferrites (i.e. $MFe_2O_{4,}$ where M= Mn, Co, Ni, Cu, Zn & Cd).

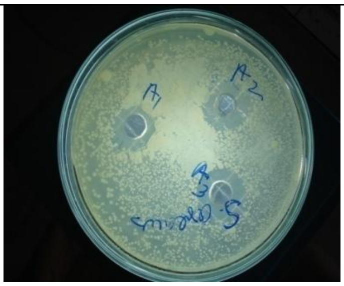
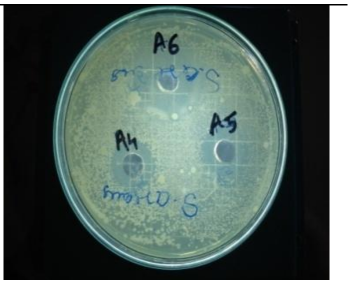
Antibacterial Activity

The synthesized metal complexes are screened for their *in vitro* biological activity. The ligand and the metal complexes were assayed against Gram-positive bacterial strains such as *Bacillus Megatherium*, *Staphylococcus aureus and* Gram-negative bacterial strains include *E-Coli*, *Pseudomonas*, *Proteus vulgaris.* A Well diffusion method was employed for the assessment of antibacterial activity. Each of the compounds was dissolved in sterile dilute hydrochloric acid and solutions of the 0.1% concentration were prepared individually. In a typical process, a well was made on agar medium inoculated with microorganism. The well was filled with the test solution using a micropipette and the plate was incubated 24 hrs for bacteria at 37°C. During this period, the test solution diffused and the growth of the inoculated microorganism was affected. The complexes potentiality was assisted by measuring the diameter of zone of inhibition in mm. The bactericidal investigation data of the compounds are summarized in **Table 6**.

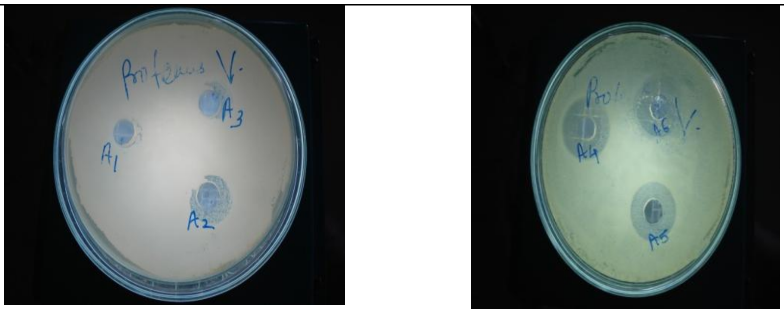
Antibacterial activity against *Bacillus Megatherium*			
Complexes	**A_1& A_2**	**A_3& A_4**	**A_5& A_6**

Antibacterial activity against *E-Coli*			
Complexes	**A_1& A_2**	**A_3& A_4**	**A_5& A_6**

Antibacterial activity against *Pseudomonas*			
Complexes	**A_1& A_2**	**A_3& A_4**	**A_5& A_6**

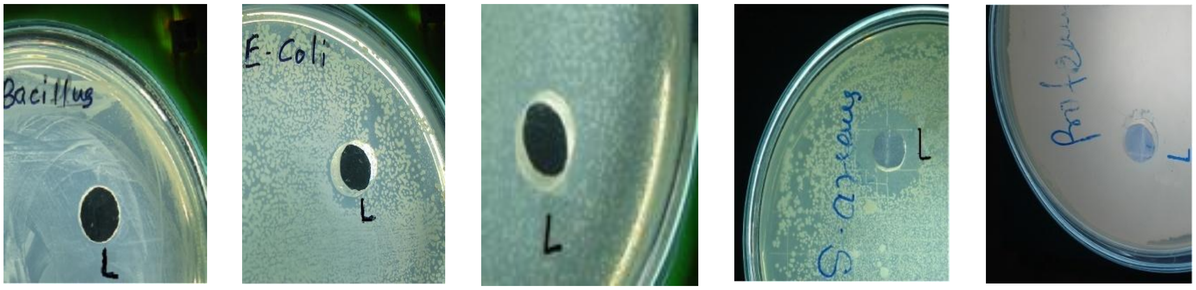
Antibacterial activity against *Staphylococcus aureus*		
Complexes	**A_1, A_2& A_3**	**A_4, A_5& A_6**

Antibacterial activity against *Proteus Vulgaris*



Complexes **A_1, A_2& A_3** **A_4, A_5& A_6**

Antibacterial activity of ligand (L)



Bacteria ***Bacillus Megatherium*** ***E-Coli*** ***Pseudomonas*** ***Staphylococcus aureus*** ***Proteus Vulgaris***

FIG.3: ANTIBACTERIAL ACTIVITY PLATES

TABLE 6: ANTIBACTERIAL ACTIVITY (zone of inhibition in mm)

Complexes	Symbol	*Bacillus Megatherium* (Gram positive)	*Staphylococcus aureus*(Gram positive)	*E-Coli* (Gram negative)	*Pseudomonas* (Gram negative)	*Proteus Vulgaris* (Gram negative)
$MnFe_2(C_4H_4O_6)_35H_2O$	A_1	24	15	22	22	12
$CoFe_2(C_4H_4O_6)_36H_2O$	A_2	25	20	22	21	17
$NiFe_2(C_4H_4O_6)_35H_2O$	A_3	19	21	25	21	16
$CuFe_2(C_4H_4O_6)_36H_2O$	A_4	27	18	23	20	22
$ZnFe_2(C_4H_4O_6)_3H_2O$	A_5	15	20	20	27	21
$CdFe_2(C_4H_4O_6)_35H_2O$	A_6	32	28	21	41	23
Ligand (tartrate)	L	-	15	10	-	10

The antimicrobial results obtained revealed that both free ligand and its metal complexes show moderate to better antibacterial activity. As per the references this increased antimicrobial activity of metal complexes may be due to chelation of metal ions with ligand due to which delocalization of pi-electrons over the whole chelate ring takes place and thus increase its lipophilicity. This improved lipophilicity enhances the penetration of complexes into the lipid membranes of microorganisms and also disturb their respiration process. Due to which the further synthesis of proteins were get blocked, cause restriction in extension of growth of the organisms. On the whole assessment of observed data gave knowledge that the metal complexes are more biologically active in comparison with the ligand against all bacteria.

CONCLUSION: We report the successful synthesis of six mixed Fe(II)-M(II) coordinated complexes (Where M= Mn, Co, Ni, Cu, Zn, Cd) prepared by co-precipitation technique in the ratio of Fe_2ML_3.

The results obtained can be summarized as follows:

i) The elemental analysis of and C, H data obtained were in agreement with the predicted formula.

ii) The x-ray diffraction data suggest polycrystalline nature of complexes.

iii) Presence of water molecules have been confirmed by thermogravimetric analysis.

iv) μ_{eff} values calculated using magnetic susceptibility, suggest paramagnetic nature of complexes.

v) Spectral, thermal and other analytical data revealed the ligand coordinate to metal ions in bidentate fashion through O,O donor sites, and thus forming a continuous polymeric structure with each metal ion surrounded by ligand in octahedral manner to give the stable complexes.

vi) The antibacterial examination of the compounds led to the conclusion that the mixed metal tartrate complexes exhibited moderate activity compared to free ligand.

vii) Comparable antibacterial sensitivity test of the ligand and metal complexes showed that they bear strong activity against *Pseudomonas* and *Bacillus Megatherium.*

viii) Among all synthesized complexes, $CdFe_2(C_4H_4O_6)_35H_2O$ complex exhibits increased activity against both Gram positive and Gram negative bacteria. All the other tested complexes exhibited mild to moderate antibacterial activity.

ix) The highest antimicrobial activity showed by mixed metal complex $CdFe_2(C_4H_4O_6)_35H_2O$ against bacteria *Pseudomonas* (Gram negative).

x) Least activity observed with complex $MnFe_2(C_4H_4O_6)_35H_2O$ against bacteria *Proteus vulgaris* (Gram negative).

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