

Synthesis, Characterization, Antibacterial and Antifungal Studies of Hetero Binuclear Metal Complexes of Zn (II) Fe(II) and Mn(II) Via

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ABSTRACT

Binuclear Schiff base complexes of Zn(II), Fe(II) and Mn(II) were prepared by inter-complex reaction between the corresponding metal complexes of 3-ethoxy Salicylaldehyde and 2-amino 3-hydroxy pyridine. The complexes were characterized by elemental analysis and estimation of metals. Thermal behavior of the complexes was studied by TG-DTA analysis. Structures of the complexes were elucidated by spectroscopic methods like, infrared spectroscopy, UV-visible spectroscopy, mass spectrometry and ¹H NMR spectroscopy. The powder X-ray diffraction study suggested crystalline nature of the complexes with tetragonal geometry. Magnetic moments and electronic spectra reveal tetrahedral structure of the complexes. Antibacterial activity of complexes were studied against Gram-positive bacteria, *Staphylococcus aureus*, *Bacillus subtilis* and Gram-negative bacteria, *Salmonella typhi*, *Escherichia coli* by agar cup method. Their antifungal activity was also tested against *Aspergillus niger*, *Penicillium chrysogenum*, *Fusarium moneliforme* and *Aspergillus flavus* by poison plate method using potato dextrose agar medium at one percent concentration. All complexes show considerable antimicrobial activity.

Keywords : Schiff Base, Inter-Complex Reaction, Binuclear Complex, Biological Activity

I. INTRODUCTION

Mixed metal complexes differ from traditional complexes in the sense that they are having at least two different or same metals associated with two different ligands (metal organic ligands) the presence of more than one type of ligands in a complex

increases chances of variation in properties expected for the complex. This makes the researcher interested in the synthesis of mixed metal complexes with varying properties.[1] Synthesis and characterization of mixed metal complexes is gaining importance day by day. The increased interest in this research area has motivated many researchers to get involved in

this field. In recent years many publication are devoted to synthesis and characterization of mixed metal as well as ligands complexes. [2-6] The Schiff base complexes were also used as drugs and they possess a wide variety of antimicrobial activity against bacteria, fungi and it also inhibits the growth of certain type of tumors.[7-8] The complexes formed by coordination with metal ions, have the tendency to coordinate further or react with other complexes, then they may act as metal organic ligand (MOL). The donor atoms are unable to coordinate with the same metal ions due to steric factors. This unutilized functionality is drawn on another metal ion forming poly nuclear complex [9-10]

Here a complex containing some unutilized functionality in ligand is considered as a ligand and named as metal organic ligand (MOL). This MOL when allowed to react with metal ions result in the formation of mixed metal complexes. We report here, a novel approach of synthesizing mixed metal complexes. It has been hypothesized that the coordinated ligands of two metal chelates can be reacted to obtain a new metal chelate. In the present work, we have allowed to react two such complexes under the conditions that permit coordinated NH_2 to react with the coordinated CHO group. Here an ionic bonds of the precursor do not dissociate and metal-ligand bonding in both the complexes remained intact [11]. Due to the reaction between coordinated amino and aldehyde groups, Schiff base were formed. The imine nitrogen of the Schiff base was allowed to coordinate with the metal nearby while the deficiency created at the metal ion on aldehyde end was sufficed by aquo-ligands liberated during imine formation. The resultant binuclear complex thus has one of the metal ions in di aquo form. When the metal ion in the reacting complexes was different, the resultant complex was mixed metal complex [12].

II. EXPERIMENTAL

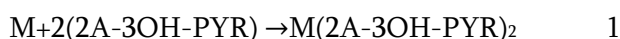
Reagents: 2-amino 3-hydroxy pyridine and 3-ethoxy salicylaldehyde (>99.0%) were purchased from S.D. Fine Chemicals. Nickel acetate, copper acetate, cobalt acetate sodium hydroxide and solvents (>99.0%) were purchased from E-Marck Ltd, Mumbai (India). The purification was done according to the needs through known procedures.

Measurements: Elemental analysis (C, H, N & O) was done using Perkin Elmer, series II, 2400 CHNS/O Analyzer. The metal content of the complexes were determined by EDTA titration method after decomposition of the metal complexes with an acid mixture of HClO_4 , H_2SO_4 and HNO_3 (1:1.5:2.5) The amount of Zn(II), Fe(II) and Mn(II) from hetero-dinuclear complex of Zn(II), Fe(II) and Mn(II) viz $\text{ZnFe}(\text{SB})_2(\text{H}_2\text{O})_2$,

$\text{FeZn}(\text{SB})_2(\text{H}_2\text{O})_2$, $\text{MnFe}(\text{SB})_2(\text{H}_2\text{O})_2$, $\text{MnZn}(\text{SB})_2(\text{H}_2\text{O})_2$, $\text{ZnMn}(\text{SB})_2(\text{H}_2\text{O})_2$, $\text{FeMn}(\text{SB})_2(\text{H}_2\text{O})_2$ was determined by various method. All chemicals used were of analytical grade and used without purification. All metal salts were purchased from SD fine chemicals. Elemental analysis (C, H, N, O) were performed on Perkin Elmer-2400. IR spectra were recorded on FTIR spectrophotometer model RZXC Perkin-Elmer in the range (4000-400 cm^{-1}), ^1H NMR spectra were recorded on Bruker Avance II at 400 MHz using tetramethylsilane as an internal standard. Electronic spectra was recorded on Shimadzu 1800 spectrophotometer using DMSO as solvent. Mass spectra were recorded on Waters, Q-TOF Micro Mass (LC-MS). Magnetic data at room temperature were collected on Guoy balance. Mercury (II) tetrathiocyanato cobalt acetate was used as a calibrant. Diamagnetic contributions were calculated for each compound by Pascal's constants. TGA/DT analysis was performed in an inert nitrogen atmosphere on Perkin Elmer STA 6000. Heating rate was 10°/min. x-ray diffractogram was scanned on Bruker AXD.

Synthesis of Metal Complexes:

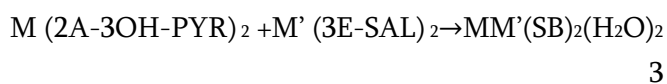
The method used in the synthesis of metal complexes consists of following three steps. In the first step, 2-amino-3-hydroxy pyridine (2A-3OH-PYR), (0.404gm) in absolute alcohol (~20mL) was prepared and a solution of zinc/Iron / manganese acetates (0.998g/0.0.497g/0.498g) in rectified spirit (20mL), were mixed, stirred for an hour to obtain a four coordinated complex, $M(2A-3OH-PYR)_2$ in solution as shown in equation-1,



In the second step, 3-ethoxy salicylaldehyde(3E-SAL), (0.665 g) in absolute alcohol (~20ml) was prepared and a solution of zinc/Iron / manganese acetates (0.998g/0.0.497g/0.498g) in rectified spirit (~20ml), were mixed and stirred for an hour to obtain a four coordinated complex, $M'(3E-SAL)_2$ in solution. The reaction is shown in equation 2.



In third step, a solution of $M(2A-3OH-PYR)_2$ was added to the refluxing solution of $M'(3E-SAL)_2$. The reaction mixture was refluxed for 6-hours in a water bath to obtain the product under slightly alkaline condition created by sodium hydroxide. The precipitate was then filtered, washed with ethanol and dried over fused $CaCl_2$. The third step of the reaction is depicted in equation 3.



All complexes were prepared by the above discussed method. The homodinuclear complex, $Zn_2(SB)_2(H_2O)_2$, $Fe_2(SB)_2(H_2O)_2$ and $Mn_2(SB)_2(H_2O)_2$ were obtained when M and $M' = Zn(II)$, $Fe(II)$ and $Mn(II)$,

respectively. The melting points of all the complexes were found to be higher than $300^\circ C$.

III. RESULT AND DISCUSSION

IR Spectra : The IR Spectra of reactant complexes and dinuclear complexes displayed some similarities and dissimilarities, Significant IR bands are shown in Table A. The spectra of the reactant complex $M(2H-3AP)_2$ Showed a strong absorption at 1551 cm^{-1} frequency¹ which was assigned to coupled vibrations of NH_2 bending and stretching (12-13) absorptions at 3330 cm^{-1} were attributed to NH_2 asymmetric and symmetric stretching frequency respectively. A weak band at 556 cm^{-1} was observed in the complex which was assigned to the M-N stretching.

IR spectra of reactant complex $M'(3E-S)_2$ exhibited a broad band and strong peak at 1530 cm^{-1} which was assigned to C=O stretching in the complex. A weak band at 456 cm^{-1} observed in the spectra was due to M-O stretching frequency. Both the complexes showed a band in the region of 3330 cm^{-1} & 3365 cm^{-1} arising due to aromatic ring vibrations the spectra of both the reactant complexes did not show a broad band in the region of 3400 cm^{-1} which indicated the absence of any coordinated water molecule.

In the spectra of resulting dinuclear complexes viz $MM'(SB)_2(H_2O)_2$ peak due to C=O stretching (1530 cm^{-1}) NH_2 bending and NH_2 stretching (1551 cm^{-1}) was found to be absent. New stronger bonds appearing at $560-570 \text{ cm}^{-1}$ and $450-485 \text{ cm}^{-1}$ were assigned to M-N and M-O stretching frequencies. A band seen at C-O stretching at 1203 cm^{-1} a sharp and strong peak between $1600-1619 \text{ cm}^{-1}$ which may be attributed to C=N stretching was in accordance with proposed structure of the complex.

Table 1 : FT-IR Spectral frequencies of Complexes

System	VC=N cm ⁻¹	VO-H cm ⁻¹	VM-O cm ⁻¹	VM-N cm ⁻¹
M(3H-2AP) ₂			543	429
M'(3E-S) ₂			582	
ZnMn(SB) ₂ (H ₂ O) ₂	1608	3416	540	465
ZnFe(SB) ₂ (H ₂ O) ₂	1605	3434	523	467
MnFe(SB) ₂ (H ₂ O) ₂	1634	3427	542	457
MnZn (SB) ₂ (H ₂ O) ₂	1601	3427	549	440
FeZn(SB) ₂ (H ₂ O) ₂	1603	3419	521	485
FeMn(SB) ₂ (H ₂ O) ₂	1599	3423	547	463

Electronic Spectra and Magnetic Studies

All the complexes showed absorption peaks in the near UV region and these high intensity bands were due to $\pi \rightarrow \pi^*$ transition in the aromatic group of ligand. In heterodinuclear ZnMn (SB)₂ (H₂O)₂ complex, it was difficult to find the effective magnetic moment per each ion. Where as the total effective magnetic moment were high. The higher value of the effective magnetic moment suggest the presence of some ferromagnetic interaction at room temperature & is characterized by two weak bands at region, 654-650 nm, 638-635nm assigned to $^3T_{1g}(F) \rightarrow ^3A_{2g}(F)$ transition. The effective magnetic moment at room temperature for ZnFe(SB)₂ (H₂O)₂ was found to be 5.13 BM for each ions. (14) and characterized by weak band at region 430-425nm, 390-385nm assigned to $^4T_2F \rightarrow ^4T_1F$ & $^6A_{1g} \rightarrow ^4A_{1g}$ respectively. The spectra of MnFe(SB)₂(H₂O)₂ is characterized by two weak band at region, 430-425nm & 312-310 nm assigned to $^4T_2F \rightarrow ^4T_1F$ & $^6A_{1g} \rightarrow ^4A_{1g}$ respectively and charge transfer transitions indicating tetrahedral geometry around the metal ions. The spectra of MnZn (SB)₂(H₂O)₂ is characterized by two weak band at region, 480-475nm & 324-320 nm assigned to $^6A_{1g} \rightarrow ^4T_2g$ & $^6A_{1g} \rightarrow ^4A_{1g}$ respectively and charge transfer transitions indicating tetrahedral geometry around the metal ions. The effective magnetic moment at room temperature for MnZn (SB)₂(H₂O)₂ was found to be 5.2 BM for each ions. (15) The spectra of FeZn(SB)₂(H₂O)₂ is characterized by two weak band at region, 461-459nm & 392-390 nm assigned $^6A_{1g} \rightarrow ^4A_{1g}$ and charge transfer transitions indicating tetrahedral geometry around the metal ions. The effective magnetic moment at room temperature for FeZn (SB)₂(H₂O)₂ was found to be 5.09 BM for each ions. The spectra of FeMn(SB)₂(H₂O)₂ is characterized by two weak band at region, 409-407nm & 387-385 nm assigned $^6A_{1g} \rightarrow ^4A_{1g}Eg(G)$ & $^6A_{1g} \rightarrow ^4A_{1g}$ respectively and charge transfer transitions indicating tetrahedral geometry around the metal ions. FeMn(SB)₂(H₂O)₂, it was difficult to find the effective magnetic moment per each ion. On the basis physic-chemical and spectral study, Following structure may be proposed for the complexes.

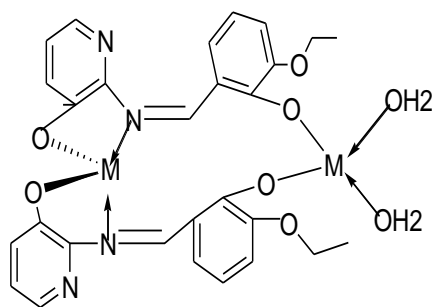


Fig-1 Proposed structure for the complexes where M & M' = Zn(II), Fe(II) & Mn(II)

Table 2 : Physicochemical and analytical data of metal complexes

System	Mol .Wt g/mole	Color	% Yield	μ f per ion B. M.	Elemental Analysis			% Found (Calculated)			
					C	H	N	O	Zn (II)	Fe(I)	Mn(I)
ZnMn(SB) ₂ (H ₂ O) ₂	666	Yellowish brown	75		52.99 (53.79)	3.3 6 (3.73)	8.00 (8.19)	19.99 (20.18)	9.25 (9.79)		8.25 (8.20)
ZnFe(SB) ₂ (H ₂ O) ₂	667	Yellowish brown	81		50.00 (50.44)	3.00 (3.30)	8.11 (8.40)	9.20 (9.60)	9.05 (9.80)	8.25 (8.36)	
MnFe(SB) ₂ (H ₂ O) ₂	657	Yellowish brown	74		50.11 (50.37)	3.20 (3.34)	8.40 (8.52)	9.28 (9.73)		8.11 (8.36)	8.20 (8.49)
MnZn(SB) ₂ (H ₂ O) ₂	666	Brown	83		46.99 (47.31)	3.10 (3.34)	9.00 (9.06)	19.12 (19.45)	8.12 (8.24)		9.25 (9.80)
FeZn(SB) ₂ (H ₂ O) ₂	667	brown	75		50.21 (50.37)	3.12 (3.29)	8.0 (8.39)	9.31 (9.58)	9.60 (9.79)	8.20 (8.36)	
FeMn(SB) ₂ (H ₂ O) ₂	657	brown	76		46.11 (46.24)	3.00 (3.05)	8.40 (8.81)	21.09 (21.18)		8.20 (8.49)	8.21 (8.36)

Mass and $^1\text{H-NMR}$ Spectra of the Complexes:

Mass Spectra

The molecular weights of all the binuclear complexes is exactly equal to that calculated theoretically from the proposed structures. These results are further supported by the conclusions drawn from the elemental analysis which agree with the molecular formula assigned to these complexes.

Formation of dinuclear metal complexes and their structure is confirmed by $^1\text{HNMR}$ spectral study of representative metal complexes. The result obtained was used to interpret the proton environment and number of protons present in the sample. The $^1\text{HNMR}$ spectra of complex are presented in Fig 2 where as the characterization of particular protons are presented in Table 3

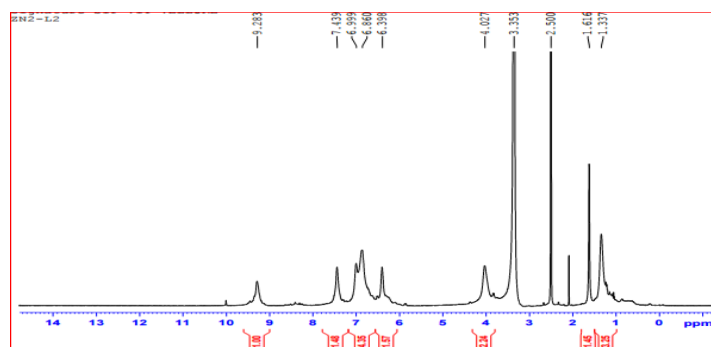


Fig:2 $^1\text{HNMR}$ Spectrum of $\text{ZnMn}(\text{SB})_2(\text{H}_2\text{O})_2$

Table 3

Chemical Shift ' δ 'ppm	Numbers of Protons	Multiplicity (Splitting)	Assignment
1.337	3H	S	Methyl hydrogen of Ethoxy group
4.027	2H	S	Methylene hydrogen of Ethoxy group
6.398-7.439	12H	M	Hydrogen of aromatic ring and heterocyclic pyridine
9.283	1H	S	Imine proton

Thermogravimetric studies

The Simultaneous TG/DT analysis of a representative $\text{ZnMn}(\text{SB})_2(\text{H}_2\text{O})_2$ Complex was studied.

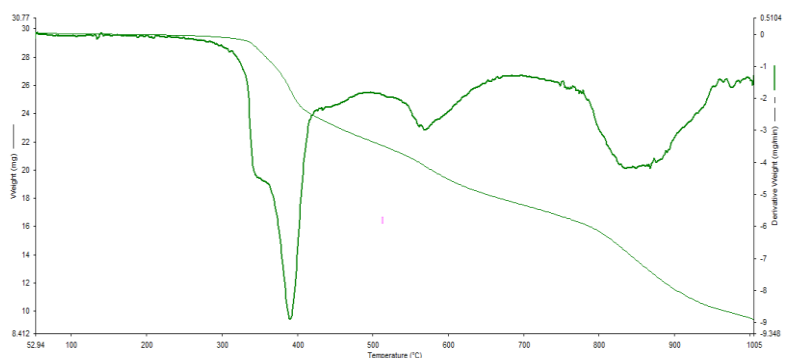


Fig. 3 : ZnMn (SB)₂(H₂O)₂

The thermogram of ZnMn (SB)₂ (H₂O)₂ complex is presented in (Fig3). The curve indicates the absence of lattice water and presence of coordinated water molecules in the complex.

TG curve of ZnMn (SB)₂ (H₂O)₂ complex (Fig3) shows loss of 6.8% (calc.7.1%) within temperature range 50-300 oC corresponds to elimination of two coordinated water molecules. An endothermic peak observed in DTA curve at 300C supports it and attributed to the removal of two coordinated water molecules. The second step decomposition takes place up to 420 oC. This decomposition suggests removal of organic part of the complex as four benzene ring fragments which is confirmed by an endothermic peak at 3900C in DTA curve .

In the third step sudden decomposition within temperature range 420-810 oC with weight loss of 29% (calc.30%) corresponds to decomposition of actual coordinated part of the complex above which the residue attains constant weight corresponding to metal oxide as a final product.

Table 4.

Metal complex	Method	Step	Decomp. Temp.	Order of Reaction	Ea(KJ mol ⁻¹)	ΔS(KJ mol ⁻¹)	ΔG(KJ mol ⁻¹)	Zx10 ⁻⁴ (S ⁻¹)	Correlation Coefficient(r)
ZnMn (SB) ₂ (H ₂ O) ₂	H-M	I	530	0.7	156.33	-140.8041801	168.066922	64.004326	0.999
	C-R				134.65	-58.34849809	139.514052	129.53072611	0.999
	H-M	II	800	0.7	82.76	-148.1036324	95.10773515	26.60742927	0.999
	C-R				94.06	-136.6998783	105.4501293	104.8461038	0.999

The thermal kinetic parameters ΔS, Ea and Z for non- isothermal decomposition of complexes have been calculated by Coats-Redfern method from TG-DTA curves (Fig. 3) and are presented in Table 4.

Generally, with decreasing value of ΔE, the value of Z increases, and higher value of activation energy suggest higher stability.[19] In the present complexes, the value of Ea decrease with the increasing value of (Z) i.e.

frequency factor indicating that the activated complexes have more ordered or more rigid structure than the reactants or intermediate and that the reactions are slower than normal.

Powder X-ray diffraction data. The X-ray diffractogram, of a representative complexes of Zn(II),Fe (II),Mn (II) metals were scanned in the range 0-60° at wavelength 1.54 Å. The X-ray diffraction pattern of the complex with respect to major peaks having relative intensity greater than 10% have been indexed by using computer program.[20] The above indexing program gives hkl planes, unit cell parameters and volume of the unit cell. The diffractogram and associated data gives 2θ values for each peak, relative intensity and inter planer spacing (d-values). On the basis of X- ray diffraction analysis Zn(II) complex crystallize in monoclinic system with 2 molecules per unit cell having probable space group P. having unit cell volume 0.9324gcm⁻³The lattice parameters were a=15.41619Å , b=8.588565Å, c=7.546127Å, β =122° α= γ=90°

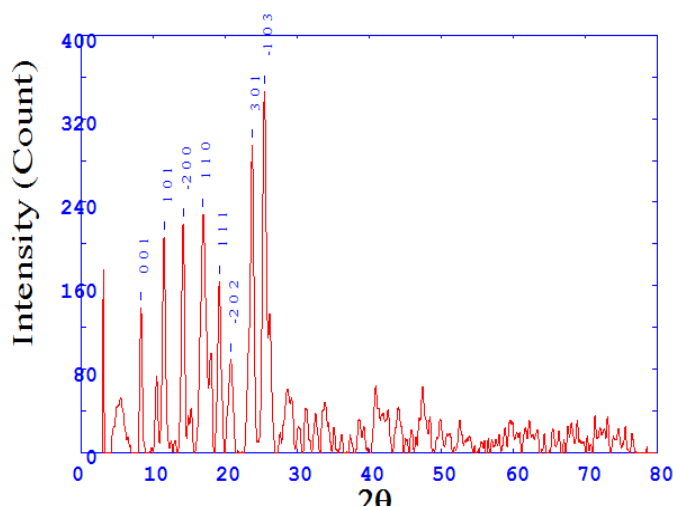


Fig.4: FeZn(SB)₂(H₂O)₂

Table 5 : Report for Antibacterial Testing.

Medium-Nutrient Agar

Dose of compound -1%

Method –Agar cup method

cup size-10mm

Sr. No.	Test Compound	Inhibition Zone (nm)			
		Escherishia coli	Salmonella typhi	Staphylococcus aureus	Bacillus subtilis
	Penicillin	14 mm	20 mm	36 mm	28 mm
01	ZnMn(SB) ₂ (H ₂ O) ₂	-ve	-ve	18	15
02	ZnFe(SB) ₂ (H ₂ O) ₂	-ve	-ve	-ve	20
03	MnFe(SB) ₂ (H ₂ O) ₂	-ve	-ve	-ve	36
04	MnZn(SB) ₂ (H ₂ O) ₂	15	-ve	18	14
05	FeZn(SB) ₂ (H ₂ O) ₂	-ve	-ve	15	14
06	FeMn(SB) ₂ (H ₂ O) ₂	-ve	-ve	-ve	34

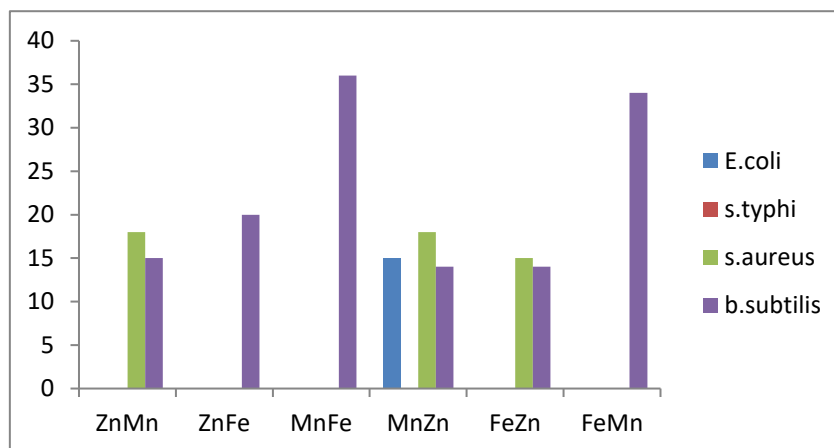


Table 6 : Report for Antifungal Testing

Test compound	Inhibit			
	Aspergillus niger	Penicillium chrysogenum	Fusarium moneliforme	Aspergillus flavus
<u>Griseofrin</u>	-ve	-ve	-ve	-ve
ZnMn(SB) ₂ (H ₂ O) ₂	+ve	+ve	RG	RG
ZnFe(SB) ₂ (H ₂ O) ₂	+ve	+ve	RG	RG
MnFe(SB) ₂ (H ₂ O) ₂	+ve	RG	RG	RG
MnZn(SB) ₂ (H ₂ O) ₂	RG	RG	RG	+ve
FeZn(SB) ₂ (H ₂ O) ₂	+ve	+ve	+ve	+ve
FeMn(SB) ₂ (H ₂ O) ₂	RG	RG	RG	+ve

Complex: +ve growth = Antifungal activity absent -ve growth = Antifungal activity present
 RG = reduced growth (more than 50% reduction in growth observed)

Antimicrobial activity of the complexes:

In vitro antibacterial activity of the compounds

The antimicrobial activity of the ligand and the complex were tested against the standard microbial strains, Escherichia coli, Salmonella typhi, staphylococcus aureus, Bacillus subtilis by agar cup method at fixed concentration of 1% in DMSO. The test was performed on nutrient agar Cup of 10 mm diameter were borered in the agar plate with stirile cork borer. All solutions were prepared in DMSO(1%) was add on cup, One cup for DMSO as blank and other for standard reference penicillium was also placed on the seeded nutrient agar. Then the plates

were shifted to incubator at 37° c and incubated for 24 hours. Activity measured in diameter (mm). The results obtained are presented in (Table 5).

The complexes individually show varying degrees of inhibiting effects on the growth of the bacterial species. The metal complex ZnMn(SB)₂ (H₂O)₂ shows activity against gram negative bacteria Staphylococcus aureus, Bacillus subtilis however the activity of these complex is slightly less than that of standard drug.. The complex ZnFe(SB)₂ (H₂O)₂ shows activity against gram negative bacteria Bacillus subtilis however the activity of these complex is slightly less than that of standard drug . The complex MnFe(SB)₂(H₂O)₂ shows

activity against gram negative bacteria *Bacillus subtilis* however the activity of these complex is higher than that of standard drug. The complex $\text{MnZn}(\text{SB})_2(\text{H}_2\text{O})_2$ shows activity against gram negative bacteria *Staphylococcus aureus*, *Bacillus subtilis* however the activity of these complex is slightly less than that of standard drug. also these complex show activity against gram positive bacteria *E.coli* however the activity of these complex is higher than that of standard drug. $\text{FeZn}(\text{SB})_2(\text{H}_2\text{O})_2$ shows activity against gram negative bacteria *Staphylococcus aureus*, *Bacillus subtilis* however the activity of these complex is slightly less than that of standard drug. $\text{FeMn}(\text{SB})_2(\text{H}_2\text{O})_2$ shows activity against gram negative bacteria *Bacillus subtilis* however the activity of these complex is higher than that of standard drug.

In vitro antifungal activity of the compounds

Compound were screened in vitro against *Aspergillus niger*, *Penicillium chrysogenum*, *fusarium moneliforme*, *Aspergillus flavus*, by poison plate method with potato dextrose agar media. The compound were tested at the 1% concentration in DMSO and compared with control.

Gresiofulvin was prepared as standard reference plate. The fungal suspension was spot inoculated on the plates prepared using compound with nicrom wire loop. The plates were incubated at room temperature for 48 hours. [21,22] The result obtained are presented in Table. 6. The complexes shows more than 50% reduction in fungal growth in some fungal species studied as well as in some fungal species antifungal activity is absent.

IV. CONCLUSION

In the present work we have been synthesized metal organic ligands and their binuclear metal complexes. The synthesized compounds were characterized by various analytical techniques. Magnetic study reveals the paramagnetic nature of complexes. Solution conductivity suggests the nonelectrolytic nature of

complexes. The XRD pattern indicate the crystalline nature of the complexes. ^1H NMR, mass spectra and UV. Study are in good agreement with the proposed structure of the complex. All the complexes shows high antibacterial activity and moderate to high antifungal activity.

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