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SYNTHESIS, PHYSICO-CHEMICAL INVESTIGATIONS AND BIOLOGICAL SCREENING OF METAL (II) COMPLEXES WITH HYDRAZONE SCHIFF BASE DERIVED FROM 5-FLUORO-3-HYDRAZONOINDOLIN-2-ONE AND ISOPHTHALALDEHYDE

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Keywords:

Schiff base, Metal complexes, Spectral studies, Antimicrobial activity and DNA cleavage.

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ABSTRACT: New tetradentate Schiff base and its Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) complexes formed by the condensation of 5-fluoro-3-hydrazonoindolin-2-one with isophthalaldehyde. The coloured complexes were prepared of $[ML_2X_2]$, Where L = Schiff base hydrazone, M = Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II), X = Cl-. Physico-chemical characterization has been carried out to determine the structure of the complexes. All the synthesized compounds, were studied for their in vitro antibacterial, and antifungal activities, against two Gram-negative (*Shigella flexneri* and *Enterococcus aerogens*) and one Gram-positive (*Micrococcus luteus*,) bacterial strains and against three fungal strains (*Candida krusiae Candida parasilopsis* and *Malassesia pachydermatis*) by using cup-plate method. The DNA cleavage capacity of all the complexes was analysed by agarose gel electrophoresis method.

INTRODUCTION: The development of bioinorganic chemistry field has increased the interest in Schiff base complexes, as they may serve as models for biologically important compounds and bioinorganic processes. Biological activity of complexes derived from hydrazones has been widely studied and found to have numerous biological activities: antibacterial, antitumor, antimalarial and antituberculosis effects. [1] Schiff bases are a significant group of organic compounds that have biological activities and diverse applications because of their antibacterial, antivirus activities, metal complexation and other pharmacological effects.

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In recent years, Schiff bases and their complexes were established to have significant antitumor and biological activity. [2] Schiff base containing more than one hetero atoms beside π-electrons exhibit high inhibiting properties by providing electrons to interact with metal surface. [3] In this paper we report the preparation and structural characterization and biological screening of hydrazone Schiff base derived from 5-fluoro-3-hydrazonoindolin-2-one and isophthalaldehyde and its metal (II) complexes.

Experimental:

All the starting chemicals and solvents were of analytical grade and used without further purification. The hydrated metal salts were obtained from Loba.

Synthesis of Schiff base ligand:

0.002 mol, solution of 5-fluoro-3-hydrazonoindolin-2-one was added to 0.001 mol, solution of isophthalaldehyde in methanol, after addition reaction mixture is heated under reflux for

about 6-7 hours at 70°C. After completion of reaction precipitate of ligand was formed. The product filtere after cooling and purified with methanol to afford Schiff base ligand as shown in **Fig. 1**. The purity of ligand was checked by M. P. and TLC.

Synthesis of metal complexes:

Metal complexes of Schiff base were prepared by mixing 1 mmol of Schiff base with 1 mmol of Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) salts keeping ligand-metal ratio 1:1. The resultant mixture was than refluxed for 2-3 hours. Then, to the reaction mixture sodium acetate was added to adjust the pH to 6.0–7.0. The complex obtained in each time was cooled, filtered and washed with the ethanol many times to purify and removed the excess of ligand. Finally complexes were places in desiccators for drying. [4]

Analytical Methods:

Elemental analysis carbon, hydrogen and nitrogen analysis was carried out using a Heracus Carlo Erba 1108 CHN analyzer at STIC, Cochin. The IR spectra of the Schiff base and its Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) complexes were recorded in the region of 4000-250 cm^{-1} on a Perkin Elmer - Spectrum RX-IFTIR spectrophotometer. The electronic spectra of the Co(II), Ni(II) and Cu(II) complexes were recorded on an ELICO SL-164 double beam UV-visible spectrophotometer in the range of 200-900 nm in DMF (10^{-3} M) solution. Magnetic susceptibility measurements were made at room temperature on a Gouy balance using $Hg[Co(NCS)_4]$ as the calibrant.

Molar conductivity measurements were recorded on an ELICO CM-180 conductivity bridge in DMF solution (10^{-3} M) using a dip-type conductivity cell fitted with a platinum electrode, The ^{1}H-NMR spectra were recorded in DMSO-d_6 on a Bruker 500MHz spectrophotometer using TMS as an internal standard. The mass spectra were recorded on a JEOL GC mate mass spectrophotometer. The ESR spectrum of the Cu(II) complex in the polycrystalline state was recorded on a Varian-E-4X band EPR spectrophotometer using TCNE as the 'g'marker (g = 2.00277) at room temperature. The XRD patterns of the ligand and its Cu(II) complex were recorded on a Rigaku D_{max} X-ray diffractometer using Cu K α = 1.5404 radiation (λ A ˚).

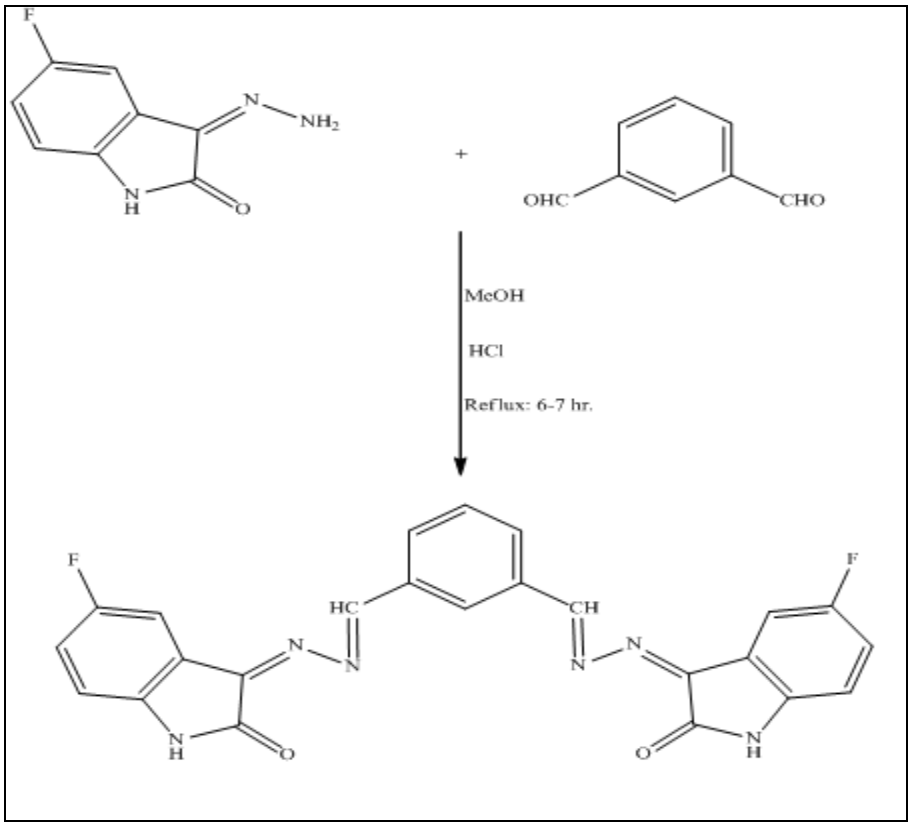

FIG. 1: SCHEMATIC REPRESENTATION OF SCHIFF BASE

Antimicrobial assay:

The synthesized compounds were evaluated for their antimicrobial activity against Gram positive bacterial strain, *Micrococcus luteus*, Gram negative bacterial strains, *Shigella flexneri* and *Enterococcus aerogens* and three fungal strains, *Candida krusiae, Candida parasilopsis* and *Malassesia pachydermatis* by cup-plate method. [5] Standard antibacterial drug (Ampicillin) and antifungal drug (Nystatin) were used for comparison under similar conditions. DMSO was used as solvent to dissolve the compounds and also used as control. Activity was determined by measuring the diameter of the zone of inhibition in (mm). 200 ml of nutrient agar growth medium was dispensed into sterile conical flasks; these were then inoculated with 20 µl of cultures mixed gently and poured into sterile petridish. After setting a borer with 6 mm diameter was properly sterilized by flaming and used to make three uniform wells in each petridish.

The wells were loaded with 50 µl of different investigated compounds. The solvent DMSO, used for reconstituting the solvent for diluting the compounds, was similarly analyzed for control. The plates were incubated at 37°C for 24 hours. The above procedure was also adopted for fungal assays. The used medium was potato dextrose agar and incubated at 27°C for 48 hours. The zone of inhibition was measured with a scale in mm. [6]

DNA cleavage experiment:

The gel electrophoresis experiments were performed by incubation at 37°C for 2 hours as follows: 250 mg of agarose and dissolve it in 25 ml of TAE buffer (pH=8.0). The samples were electrophoresed for 45 min at 50 V on agarose gel using tris-acetic acid-EDTA buffer. After electrophoresis, the gel was stained using with ethidiumbromide (EB) and photographed under UV light. [7]

RESULTS AND DISCUSSION:

The analytical data for the ligand and the complexes together with some physical properties are summarized in **Table 1**. The analytical data of the complexes correspond to the general formula [MLX2], where L = Schiff base hydrazone, M =Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II), X = Cl^-. The formation of metal (II) complexes may proceed according to the equation given below:

$$MX_2 + L \rightarrow [MLX_2]$$

TABLE: 1 PHYSICAL AND ANALYTICAL DATA OF THE HYDRAZONE SCHIFF BASE AND ITS METAL (II) COMPLEXES

Compound	Molecular formula	Yield (%)	Found (Calculated) %					Ωm (Ω^{-1} cm^2 mol^{-1})	μ_{eff} (BM)
			C	H	N	M	Cl		
Ligand	$C_{24}H_{14}F_2N_6O_2$	79	63.16 (68.02)	3.09 (3.00)	18.41 (18.39)	-	-	-	-
$[CoLCl_2]$	$CoC_{24}H_{14}N_6O_2Cl_2F_2$	71	49.17 (49.08)	2.41 (2.40)	14.34 (14.31)	10.05 (9.95)	6.04 (5.93)	19.33	4.95
$[NiLCl_2]$	$NiC_{24}H_{14}N_6O_2Cl_2F_2$	68	49.19 (49.03)	2.41 (2.38)	10.02 (10.00)	10.01 (9.88)	6.05 (5.77)	28.81	2.98
$[CuLCl_2]$	$CuC_{24}H_{14}N_6O_2Cl_2F_2$	83	48.79 (48.77)	2.39 (2.37)	14.22 (14.15)	10.75 (10.72)	6.00 (5.93)	20.45	2.03
$[ZnLCl_2]$	$ZnC_{24}H_{14}N_6O_2Cl_2F_2$	60	48.64 (48.41)	2.38 (2.36)	14.18 (41.05)	11.03 (10.99)	5.98 (5.77)	17.92	-
$[CdLCl_2]$	$CdC_{24}H_{14}N_6O_2Cl_2F_2$	84	45.06 (45.01)	2.21 (2.19)	13.14 (13.00)	17.57 (17.39)	5.54 (5.44)	24.50	-
$[HgLCl_2]$	$HgC_{24}H_{14}N_6O_2Cl_2F_2$	85	39.60 (39.54)	1.94 (1.92)	11.55 (11.52)	-	-	15.11	-

Molar conductance:

Molar conductance of the complexes were measured in DMF at a concentration of 0.001 M. The observed conductance values indicating that the complexes are non-electrolyte. [8]

Electronic spectral studies:

Electronic spectra of Co(II), Ni(II) and Cu(II) complexes were recorded in DMF medium and spectral bands of the complexes are summarized in **Table 2**. The Co(II) complex exhibited three bands at 16077 and 21186 cm^{-1} corresponding to v_2 and

v_3 transitions attributed to $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ (v_2) and $^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)$ (v_3). However, v_1 band is not observed because of its proximity to strong v_3 transition. Magnetic measurements for the Co(II) complex has μ_{eff} value of 4.51 BM.

The three well-separated absorption bands were observed at λ_{max} ~ 9649, ~ 15479 and ~ 25252 cm^{-1} which are attributed to the $^3A_{2g} \rightarrow {}^3T_{2g}$, $^3A_{2g} \rightarrow {}^3T_{1g}$ (F), $^3A_{2g} \rightarrow {}^3T_{1g}$ (P), transitions respectively, in the spectra of the Ni(II) suggest the octahedral geometry. The complex shows μ_{eff} value of 3.00 BM.

The Cu(II) complexes showed a broad visible band, λ_{max} at ~ 14285-16949 cm^{-1} is assignable to $^2Eg_2 \rightarrow T_{2g}$ transition. This, together with the measured μ_{eff} value of ~ 1.74 BM suggests the octahedral geometry.[9]

TABLE 2: ELECTRONIC SPECTRAL BANDS AND LIGAND FIELD PARAMETERS OF THE Co(II), Ni(II) AND Cu(II) COMPLEXES IN DMF (10^{-3} M) SOLUTION

Complexes	Transitions in cm^{-1}			Dq (cm^{-1})	*B'* (cm^{-1})	β	β%	v_2/v_1	LFSE (k cal)
	v_1	v_2	v_3						
$[CoLCl_2]$	7366	16077	21186	871	1010	0.97	2.78	2.18	14.93
$[NiLCl_2]$	9649	15479	25252	964	785	0.75	24.46	1.60	33.08
$[CuLCl_2]$	14285-16949			1561	-	-	-	-	26.77

IR spectral studies:

The IR spectral data of the hyrazone Schiff base and its metal (II) complexes are presented in **Table 3**. In the spectrum of the ligand, a band corresponding to the azomethine group (–HC=N) and ν(C=O) were found at 1577 cm^{-1} and 1681 cm^{-1}. On complexation, this band shifted to a lower wave number range of 1508–1570 cm^{-1} and 1650-1676. This indicated the involvement of N-atom of the azomethine (–HC=N) group and carbonyl oxygen in complex formation[10,11] and the band at 3207, 1606 cm^{-1} in the spectra of all the complexes were assigned to ν(N–H) and ν(C=N) of the ketimine moiety remain almost unaffected, indicating the non participation of these groups in coordination. [12] Therefore, the IR spectral data indicated that the coordination of hydrazone Schiff base to metal ion occurred through the N-atom of the azomethine (–C=N) group and the O-atom of carbonyl (C=O) group. Assignment of the proposed coordination sites was further supported by the appearance of medium bands at 453–499 cm^{-1} and 351–379 cm^{-1} due to M–N and M–Cl stretching frequencies, respectively. [13, 14]

TABLE 3: IR SPECTRAL BANDS OF THE LIGAND AND ITS METAL COMPLEXES (CM^{-1})

Tentative assignments	L	$[CoLCl_2]$	$[NiLCl_2]$	$[CuLCl_2]$	$[ZnLCl_2]$	$[CdLCl_2]$	$[HgLCl_2]$
Indole ring NH	3207	3207	3207	3207	3207	3207	3207
v(C=O) ring	1681	1676	1668	1653	1650	1673	1650
v(C=N) ring	1606	1606	1606	1606	1606	1606	1606
v(C=N) aldemine	1577	1530	1526	1508	1567	1570	1564
v(N-N)	928	998	1000	1024	1045	1068	1077
v(M-N)	-	463	474	482	499	453	458
v(M-Cl)	-	351	356	363	369	372	379

Mass spectral studies:

The mass spectrum of the hydrazone Schiff base shows a molecular ion peak at m/z 456, which is equivalent to its molecular weight. The mass spectrum of the Zn(II) complex showed a molecular ion peak at m/z 592, which is the same as that of the molecular weight of the complex. This supports the suggested structure for the complex.

^{1}H-NMR spectral studies:

The ^{1}H NMR spectrra of the prepared hydrazone Schiff base and its Zn(II) metal complex were measured and interpreted. The azomethine (–HC=N) proton is seen at 8.5 δ (singlet), but in case of Zn(II) complex the peak was observed at 9.3 δ (singlet). The azomethine proton signal in the spectrum of the corresponding complex is shifted

downfield compared to the free ligand, suggesting the deshielding of the azomethine group due to the coordination with the metal ion. [15] The peak appeared at 9.6 δ (singlet) is due to the hydrogen of –NH in the ligand, but in case of Zn(II) complex the peak was observed at 9.6 δ (singlet). [16] The phenyl multiplet is seen at 6.5 - 7.4 δ, but in Zn(II) complex multiplet is seen at 6.9 - 7.8 δ. [17]

ESR spectra of Cu(II) complex:

The ESR spectrum of Cu(II) complex offer information of status in studying the metal ion environment. The ESR spectra of complexes have been recorded on X - band at frequency 9.1 GHZ under the magnetic field strength 3000 G in DMF at room temperature and their $g_{||}$, $g_{\perp}$, g_{av}, and G values have been calculated. The $g_{||}$ and $g_{\perp}$ values were found to be 2.1549 and 2.0387, respectively. The g_{av} and G value were calculated to be 2.0774 and 4.2340.[18]

Powder X-ray diffraction studies:

In the present study, X-ray diffraction study was carried out using Rigaku D_{max} X-ray diffractometer. The X-ray was produced using a sealed tube and the wavelength of X-ray was 0.154 nm (Cu K α radiation). The X-ray was detected using a fast counting detector based on silicon strip technology (Rigaku Lynx Eye detector). X-ray absorption fine structure studies was carried out using a conventional Siefert sealed X-ray tube with Tungsten target operating 20 kV and 40 mA. After this process, the scanning of the X-ray films was completed on Carl-Ziess microdensitometer coupled with computer to convert the data into IFEFFIT analysis. There are 11 reflections (2θ) between 4.664 to 68.435 with maxima at 2θ = 40.648 corresponding to the value of d=2.217.

The XRD pattern of the complexes is reported in **Fig.2** and data are given in **Table 4**. The interplanar spacing (d) was analyzed by Bragg's law, $2d\sin\theta=n\lambda$ and calculated by Debye Scherer's formula. The observed and calculated values of d and are quite consistent. The $h^2+k^2+l^2$ values of the complex were found to be 33, 36, 56, 66, 73, 81, 123, 133,162, 191. The presence of forbidden numbers 123 and 133 indicates the Cu(II) complex belongs to hexagonal system. [19]

TABLE 4: X-RAY DIFFRACTION DATA OF Cu(II) COMPLEX

2θ	θ	Sin θ	Sin^2 θ	$h^2+k^2+l^2$	h k l	d Value		a in Å
						Cal	Abs	
4.664	2.332	0.0406	0.0016	1	1 0 0	18.9236	18.9327	18.92
56.856	13.428	0.2322	0.0539	32.5716 (33)	5 2 2	3.3157	3.3170	18.92
28.214	14.107	0.2437	0.0594	35.8804 (36)	6 0 0	3.1591	3.1604	18.92
35.507	17.753	0.3049	0.0929	56.1573 (56)	6 4 2	2.5252	2.5261	18.92
38.576	19.288	0.3303	0.1091	65.9005 (66)	5 5 4	2.3310	2.3320	18.92
40.648	20.324	0.3473	0.1206	72.8632 (73)	6 6 1	2.2619	2.2177	18.92
42.881	21.4405	0.3655	0.1336	80.7022 (81)	9 0 0	2.1065	2.0173	18.92
53.736	26.868	0.4519	0.2042	123.3624 (123)	-	1.7037	1.7044	18.92
56.014	28.007	0.4695	0.2205	133.1821 (133)	-	1.6397	1.6404	18.92
62.284	31.142	0.5171	0.2674	161.5397 (162)	9 9 0	1.4888	1.4894	18.92
68.435	34.217	0.5623	0.3162	190.9940 (191)	-	1.3692	1.3698	18.92

Antimicrobial evaluation of ligand and its metal complexes:

The ligand and its metal complexes was examined for antimicrobial assay against three bacterial and three fungal strain using the well diffusion method. The values of the tested compounds are shown in **Table 5**. It was observed from these studies that metal chelates had a higher activity than the free ligand against both bacterial and fungal strains. The complexes Zn(II) and Hg(II) exhibited significant antibacterial activity against all bacterial strains. These complexes also exhibited profound activity against all fungal strains.

TABLE 5: THE ANTIMICROBIAL ACTIVITY OF LIGAND AND ITS METAL (II) COMPLEXES EVALUATED BY (MM).

Compounds	Zone of inhibition in mm					
	Antibacterial activity			Antifungal acivity		
	Micrococcus luteus	*Shigella flexneri,*	*Enterococcus aerogens*	*Candida krusiae*	*Candida parasilopsis*	*Malassesia pachydermatis*
L	12	8	14	7	13	10
$[CoLCl_2]$	20	25	22	28	19	17
$[NiLCl_2]$	19	28	16	15	21	26
$[CuLCl_2]$	18	20	26	18	27	23
$[ZnLCl_2]$	35	33	28	34	36	30
$[CdLCl_2]$	22	28	20	30	25	21
$[HgLCl_2]$	33	30	26	35	37	29
Ampicillin	40	36	32	-	-	-
Nystatin	-	-	-	39	42	34

DNA cleavage efficiency:

The degree to which the five complexes could function as DNA cleavage agents was examined using Calf-thymus DNA as the target. The efficiency of cleavage of these molecules was probed using agarose gel electrophoresis. [20] DNA cleavage activity of prepared compounds (Co(II), Ni(II), Cu(II), Cd(II), Zn(II) and Hg(II) (lanes SA_1-SA_6 respectively)) were analyzed by monitoring the conversion of supercoiled DNA (Form I) to nicked DNA (Form II) and linear DNA (Form III). SA_4 has displayed partial cleavage of DNA and all the other samples have shown complete cleavage of DNA (**Fig. 3**). [21]

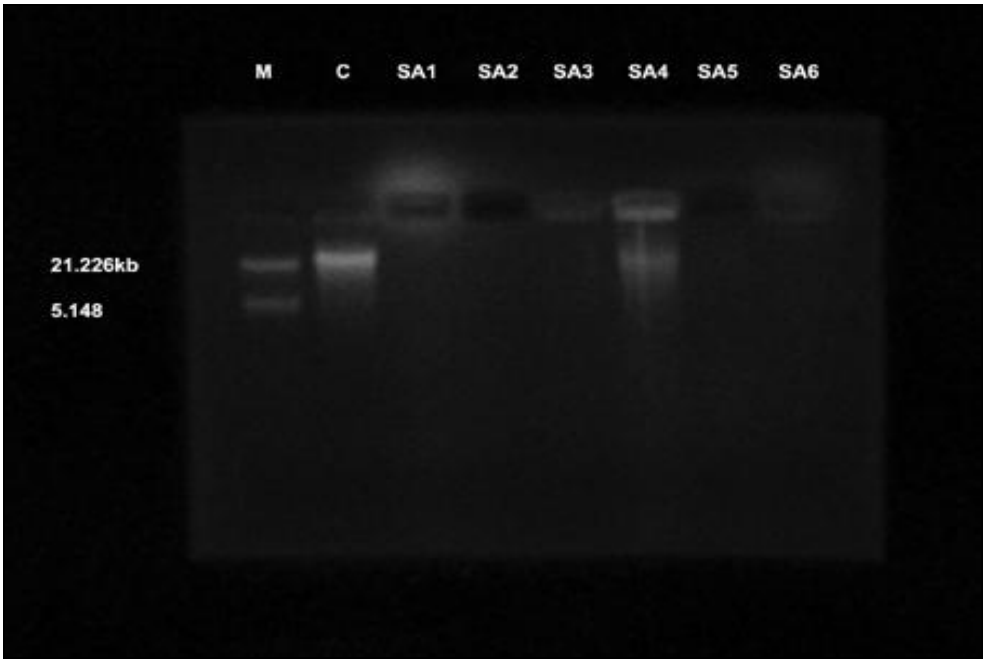

FIG. 3: GEL PICTURE SHOWING THE CLEAVAGE ANALYSIS OF SAMPLES

CONCLUSION: In this paper, the preparation, physico-chemical investigations and biological Screening of hydrazone Schiff base and its metal (II) complexes were reported. The complexes were formed in 1:1 (metal:ligand) ratio, as confirmed by the spectral analysis. The results of different analytical and spectroscopic analyses revealed that the complexes have octahedral geometry **(Fig. 4**). The hydrazone Schiff base acts as a tetradentate ligand and binds to metal ions through the carbonyl oxygen and the azomethine nitrogen. The Microbial activity of the ligand and its complexes have been studied on three bacterial , *Micrococcus luteus, Shigella flexneri, Enterococcus aerogens* and *three fungal strains Candida krusiae, Candida parasilopsis* and *Malassesia pachydermatis* by well Fusion method and found that the metal complexes are more active on microorganisms than the

hydrazone Schiff base ligand. The DNA cleavage activity of the metal (II) complexes showed good DNA cleavage efficiency.

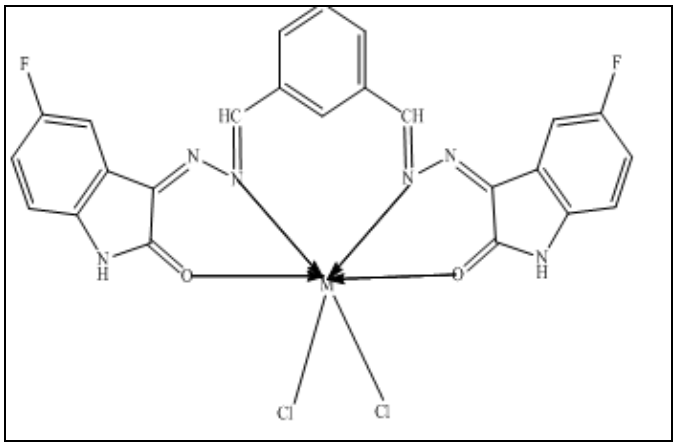

FIG. 4: PROPOSED STRUCTURE OF M =Co(II), Ni(II), Cu(II), Zn(II), Cd(II) AND Hg(II) METAL COMPLEXES

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